High resolution monitoring of marine protists based on an observation strategy integrating automated on board ship filtration and molecular analyses

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Abstract

Information on recent ~~photosynthetic~~ biomass distribution and biogeography of photosynthetic marine protists with adequate temporal and spatial resolution is urgently needed to better understand consequences of environmental change for marine ecosystems. Here we introduce and review a molecular-based observation strategy for high resolution assessment of ~~marine~~ these protists in space and time. ~~The observation strategy~~It is the result of extensive technology developments, adaptations and evaluations which are documented in a number of different publications and the results of recently accomplished field testing, which are introduced in this review. The observation strategy is organized ~~in~~ atfour different levels. At level 1, samples are collected ~~in~~ at high spatio-temporal resolution using the remote-controlled automated filtration system AUTOFIM. Resulting samples can either be preserved for later laboratory analyses, or directly subjected to molecular surveillance of key species aboard the ship via an automated biosensor system or quantitative polymerase chain reaction (level 2). Preserved samples are analyzed at the next observational levels in the laboratory (level 3 and 4). This involves at level 3 molecular fingerprinting methods for a quick and reliable overview of differences in protist community composition. Finally, selected samples can be used~~subjected~~ to generate a detailed analysis of taxonomic protist composition via the latest Next Generation Sequencing Technology (NGS) at level 4. An overall integrated dataset of the results based on the different analyses provides comprehensive information on the diversity and biogeography of protists, including all related size classes. At the same time the cost effort of the observation is optimized in respect to analysis effort and time.

Keywords

Molecular observation strategy, Marine protists, Next Generation Sequencing, Automated Sampling, Molecular fingerprinting, Quantitative PCR

1 Introduction

It is expected that marine ecosystems will be affected by climate change in multiple ways, including rising atmospheric CO_2 levels, shifts in temperature, circulation, stratification, nutrient input, oxygen content, and ocean acidification. In summary, these changes will strongly impact marine biota and ecosystems with consequences for abundance, diversity, spatial distribution, biogeography, or dominance of marine species (Doney et al., 2012). Marine plankton, comprising prokaryotic and eukaryotic microbes (bacteria and protists) as well as small or juvenile metazoans, is of utmost importance for the functioning of marine ecosystems. It is traditionally divided by its size into three classes: The microplankton (~~>~~20-200 µm), the nanoplankton (20-2 µm), and the picoplankton (<2 µm). Within these groups of organisms, phytoplankton as the photosynthetic active part of the plankton accounts for roughly half of global net primary productivity (NPP) (Field et al., 1998) and is fundamental for any marine ecosystem function or service. As a consequence, changes in phytoplankton community structures and biogeography as a response to climate change are currently topical issues ~~driving topics~~ in marine ecology. Moreover, marine phytoplankton is very well suited to serve as an indicator of climate change (Nehring, 1998), because its dynamics are closely coupled to environmental conditions (Acevedo-Trejos et al., 2014). Despite the necessity and advantage of using marine phytoplankton to assess consequences of climate change, the task is also challenging in various ways. Marine phytoplankton distribution displays high spatial heterogeneity or "patchiness" (Mackas et al., 1985) and a pronounced seasonality as a consequence of physical and chemical oceanographic processes (Boersma et al., 2016; Bresnan et al., 2015). Furthermore, there are difficulties with the taxonomic surveillance of species in the pico-, or nano-fraction, related to their cell size and insufficient morphological features (e.g. Caron et al.,1999). As a consequence it is very challenging to provide information on composition, occurrence, and dynamics of phytoplankton with adequate spatial and temporal resolution. Together with the difficulties to financially support and maintain long time series these challenges might account for the relatively small number of marine phytoplankton long-term time series worldwide. Among them, one long lasting time series, the Helgoland Roads Time Series, is maintained by the Alfred Wegener Institute Helmholtz Centre for Polar- and Marine Research at the island Helgoland in the German Bight (North Sea). The dataset comprises information on abundance of phytoplankton on a daily basis since 1962 (Kraberg et al., 2015; Wiltshire et al., 2009). However, it does not provide information on the abundance of the smallest phytoplankton species and is restricted to one sampling point. The latter restriction is overcome by a second major long term marine observation programme that is operated by the Sir Alistair Hardy Foundation for Ocean Science in Plymouth, UK: the **C**ontinuous **P**lankton **R**ecorder (CPR) Survey's marine observation programme (McQuatters-Gollop et al., 2015). Together with its sister surveys it provides large-scale information on marine plankton distribution, mainly in the North Atlantic and the North Sea since the first surveys in 1931. Unfortunately, the CPR-approach is restricted to zooplankton and larger ~~bigger~~ phytoplankton e.g. diatoms. Again, the ecological relevant picophytoplankton ~~of the pico- and nanoplankton~~ fraction is omitted. However, the smaller phytoplankton is to a certain degree included in the surveys of the FerryBox project implemented by the Helmholtz Centre Geesthacht in the North Sea. A FerryBox is an autonomous device located on ships of opportunity that has the capability to autonomously generate information on the plankton composition and a number of other parameters for the North Sea (Petersen, 2014). Here, phytoplankton is

characterized on the basis of the pigment composition present, which is estimated via multi-channel fluorescence measurements. All phytoplankton groups and size fractions are included in this analysis, but this approach is only suited for the identification of larger taxonomic algal groups. Furthermore, spectrally similar groups (e.g. diatoms and dinoflagellates) cannot be distinguished by this method. Thus the FerryBox project lacks information on species composition of phytoplankton.

Overall, these long term monitoring programmes and other current marine plankton observation approaches have already given important information and indication on climate related change in the marine plankton community. Nevertheless, each of them is limited in one or the other way: (i) the ongoing long time series are mainly limited to one or small numbers of sampling points; (ii) they do not provide a holistic view of changes at the base of marine food webs, because they neglect the pico- and most of the nanophytoplankton ~~fractions~~; (iii) broad taxonomic knowledge is required for the identification of taxa at species level; (iv) fluorescent characterization of phytoplankton is restricted to the identification of larger taxonomic groups; (v) they are costly if larger numbers of samples need to be processed.

To address these ~~In respect to these~~ shortcomings and challenges of current observation approaches, it is of utmost importance to develop efficient automated high throughput approaches and observation strategies that allow reliable surveillance of all phytoplankton size classes with adequate spatio-temporal resolution. Over the past decade numerous publications demonstrated the power of ~~a large variety of~~ molecular methods for the observation of marine plankton organisms, especially of those that are missing distinct morphological features (Metfies et al., 2010; Wolf et al., 2014a; Wollschlaeger et al., 2014). Previous publications have shown the power of the analysis of ribosomal genes (rRNA-genes) to gain new insights into the phylogeny and biogeography of prokaryotic and eukaryotic micro-organisms (Comeau et al., 2011; Sunagawa et al., 2015). The genes coding for the rRNA are particularly well suited for phylogenetic analysis and taxonomical identification, because they are universally present in all cellular organisms. Furthermore, rRNA genes are of relatively large size and contain both highly conserved and variable regions with no evidence for lateral gene transfer (Woese, 1987). The continually growing number of available algal 18S rDNA-sequences, e.g. in the Ribosomal Database Project (Quast et al., 2013), and phylogenetic analysis makes it possible to design hierarchical sets of probes that specifically target the 18S-rDNA of different taxa (Metfies and Medlin, 2007; Thiele et al., 2014). The probes can be used in combination with a wide variety of hybridization based methods, such as RNA-based nucleic acid biosensors (Diercks et al., 2008a; Ussler et al., 2013) quantitative PCR (Bowers et al., 2010; Toebe et al., 2013) or fluorescence *in situ* hybridization (FISH) (Thiele et al., 2014) to identify marine microbes. Other methods, such as molecular fingerprinting approaches and Next Generation Sequencing provide information on variability and composition of whole microbial communities. The molecular fingerprinting method **A**utomated **R**ibosomal **I**ntergenic **S**pacer **A**nalysis (ARISA) is a quick, cost-effective and meaningful method to determine overall variability in phytoplankton community composition (Kilias et al., 2015) that is independent of the size or morphology of target organisms. In contrast, Next Generation Sequencing (NGS) of ribosomal genes allows high resolution, taxon-specific assessments of protist communities, including their smallest size fractions and the rare biosphere (de Vargas et al., 2015; Kilias, 2014).

Here, we introduce a combined molecular-based observation strategy that allows observation of current phytoplankton composition, distribution, and dynamics at adequate spatial and temporal scales. The resulting data sets can be used to estimate possible alterations related to climate or environmental change. Our strategy is the result of technical developments and the integration of latest sampling- and molecular tools in an advanced

molecular-based observation approach that will optimize marine microbial observation in general, while phytoplankton was in the focus of our developments. In the future our molecular observation strategy is intended to cut down surveillance costs and provide information on marine microbial biodiversity with unprecedented resolution. It is a development of the Helmholtz Young Investigators Group PLANKTOSENS (Assessing Climate Related Variability and Change of Planktonic Foodwebs in Polar Regions and the North Sea) carried out within the framework of COSYNA (Coastal Observing System for Northern and Arctic Seas). Here, we review major published results that lead to the development of the molecular observation strategy and demonstrate the applicability of newly developed sampling technology within the observation strategy. Special emphasis was put on observation of Arctic pico-phytoplankton that constitutes a major contribution to pelagic Chl *a* biomass during summer (Metfies et al., 2016).

2 Material and Methods

2.1 Sampling

Water samples analyzed in this study were collected during expeditions PS85 (June 2014) and PS96 (May/June 2015) of RV Polarstern to the Arctic Ocean. Samples from deeper water layers containing the deep chlorophyll maximum (DCM) were taken with a rosette sampler equipped with 24 Niskin bottles (12 L per bottle) and sensors for Chl *a* fluorescence, temperature and salinity (CTD). Samples collected via CTD were taken during the up-casts at the vertical maximum of Chl *a* fluorescence determined during the down-casts. The sampling depths varied between 10–50 m. Two litres~~er~~ of water subsamples were taken in PVC bottles from the Niskins. Particulate organic matter for molecular analyses was collected by sequential filtration of one water sample through three different mesh sizes (10 µm, 3 µm, 0.4 µm) on 45 mm diameter Isopore Membrane Filters at 200 mbar using a Millipore Sterifil filtration system (Millipore, USA). Subsequent to sampling the filters were stored at -20°C until further analyses.

Additional samples were collected from a depth of ~ 10 m with the **Auto**mated **fi**ltration device for **m**arine microorganisms (AUTOFIM), which is coupled to the ship's pump system. Fitting and programming of the device does not require special expertise if it is done according to the manufacturer's protocol. All steps related to the filtration process, including application of Lysis Buffer RLT (Qiagen, Germany), were carried out automatically by AUTOFIM. ~~Here~~In this study, two litres~~er~~ of sea water were collected and filtrated on a filter with 0.4 µm pore size at 200 mbar. Subsequent to filtration, particulate organic matter on the filter was re-suspended with 600 µl Lysis Buffer RLT (Qiagen, Germany) and stored at -80°C until further processing in the laboratory. The filtration device was cleaned after each filtration step by rinsing the device with fresh-water.

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2.2 Environmental parameters

Standard oceanographic parameters (salinity, temperature, Chl *a* fluorescence, turbidity, chromophoric dissolved organic matter, dissolved oxygen, pH, nutrients) were measured at the sampling sites by the FerryBox-System (Petersen, 2014) deployed on board RV Polarstern. The measurement interval was 1 min, and the water intake of the system was identical to the water supply of AUTOFIM. To prevent biofouling of the sensors, the FerryBox performed a cleaning cycle including an acid wash and freshwater rinsing once per day.

2.3 DNA isolation

Isolation of genomic DNA from the field samples was carried out using the E.Z.N.A TM SP Plant DNA Kit Dry Specimen Protocol (Omega Bio-Tek, USA) following the manufacturer's protocol. The resulting DNA-extracts were stored at -20 °C.

2.4 DNA quality

The integrity of the genomic DNA isolated from water samples collected with AUTOFIM was assessed using the Agilent DNA 7500 kit (Agilent Technologies, USA) according to the manufacturer's protocol. A volume of 1µl DNA was applied to the flow cell.

2.5 ARISA

PCR-amplification and subsequent determination of the size of the PCR fragments, and statistical analyses related to ARISA were accomplished as described previously in the studies contributing to the development of the molecular observation strategy (e.g. Kilias et al., 2015). This included the determination of variability in the length of the ~~intergenic spacer region~~ internal transcribed spacer 1 (ITS1) amplified via a specific primer set from genomic DNA extracted from field samples.

2.6 454-Pyrosequencing

Sequencing of protist communities via 454-pyrosequencing was based in all studies reviewed in this manuscript on amplification of~~-~~ a ~ 670 bp fragment of the 18S rDNA containing the hypervariable V4 region. Sequence library preparation and data analysis was described previously in the studies contributing to the development of the molecular observation strategy (~~e.g.~~ Kilias et al., 2013; Metfies et al., 2016). Thus, for more detailed information, the reader is referred to these publications~~.~~

2.7 Quantitative PCR-assay

The quantitative PCR was carried out in a nested two~~-~~step approach. We used this nested approach, because it minimized the variability between technical replicates of q-PCR data obtained from analyses of field samples. The applicability of the nested approach was evaluated by a comparison of q-PCR data with manual counts of *Phaeocystsi pouchetii* in field samples (data not shown). In the first step total eukaryotic 18S rDNA was amplified from a positive control (genomic DNA *Phaeocystis pouchetii*), a negative control (no template) and genomic DNA isolated from field samples using the universal primer-set 1F-(5´-AAC TGG TTG ATC CTG CCA GT-3´) / 1528R- (5´-TGA TCC TTC TGC AGG TTC ACC TAC-3´) (modified after Medlin et al., 1988). PCR-amplifications were performed in a 20 µl volume in a thermal cycler (Eppendorf, Germany) using 1x HotMasterTaq buffer containing Mg^{2+}, 2.5 mM (5´Prime); 0.5 U HotMaster Taq polymerase (5´Prime, Germany); 0.4 mg/ml BSA; 0.8 mM (each) dNTP (Eppendorf, Germany); 0.2 µM of each primer (10 pmol/µl) and 1µl of template DNA (20 ng/µl). The amplification was based on 35 cycles, consisting of 94°C for 1 min, 54°C for 2 min and 72°C for 2 min, followed by 1 min denaturation at 94°C and finalized by a final extension of

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10 min at 72°C. Subsequently PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). In the second step a qPCR-assay was carried out using a species specific primer-set 82F-(5´-GTG AAA CTG CGA ATG GCT CAT-3´) / P1np- (5´-CGG GCG GAC CCG AGA TGG TT-3´) for *Phaeocystis pouchetii*. The quantitative PCR-assays were performed in triplicate in a 20 µl volume in a 7500 Fast Real-Time PCR-System (Life Technologies Corporation; Applied Biosystems, USA) using 1x SYBR Select Mastermix (Life Technologies, USA); 0.2 µM of each primer (10 pmol/µl) and 2µl of the purified 18S rDNA PCR-fragment. The amplification was based on 40 cycles, consisting of 95°C for 10 min, 95°C for 15 sec, 66°C for 1 min. The quantitative PCR-assay was calibrated with a dilution series of a laboratory culture of *Phaeocystis pouchetii* (Figure 4). Based on this calibration CT-values were transformed into cell numbers using the following equation:

CT= -2.123 ln (cell numbers) + 38.788.

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3 Results and Discussion:

3.1 Overview Molecular Based Observation Strategy

The molecular based observation strategy that we present here is organized in 4 different levels (Figure 1). At level 1, samples are collected in high spatio-temporal resolution using the remote-controlled automated filtration system AUTOFIM (Figure 2). The sampling system can either be deployed on a fixed monitoring platform or aboard a ship (research vessel or ship of opportunity) without the need of highly trained personal. Samples can be preserved with a preservation buffer (e.g. DNAgard, Biomatrica, USA) for later laboratory analyses, or directly subjected to molecular surveillance of key species aboard the ship via an automated biosensor system or quantitative polymerase chain reaction (level 2). Direct analyses aboard ships provide near real time information on abundance and distribution of phytoplankton key species that can be used to optimize phytoplankton sampling for detailed high resolution analyses of overall phytoplankton composition during an ongoing sampling campaign. The resulting preserved samples will be analyzed at the next observational levels in the laboratory (level 3 and 4). This involves at level 3 molecular fingerprinting methods that provide a quick and reliable overview of differences in protist community composition of the samples in a given observation area or time period. Furthermore, this information can be used to select representative samples for detailed analysis of taxonomic protist composition via latest next generation sequencing at level 4. An overall integrated dataset of the results based on the different analyses provides comprehensive information on the diversity and biogeography of protists, including all related size classes. At the same time, the cost effort of the observation is optimized in respect to analysis effort and time. Sampling based on the autonomous filtration device is more cost efficient, because labor costs and the requirement of ship space and time are reduced.

The development of the Molecular Observation Strategy was based on extensive method development and evaluation. Overall, it included: (i) the development of an automated remote controlled filtration system (Figure 2, (ii) the evaluation and application of **A**utomated **R**ibosomal **I**ntergenic **S**pacer **A**nalysis (ARISA) (Kilias et al., 2015), (iii) the implementation of Next Generation Sequencing (454-pyrosequencing; Illumina) for

marine protists (e.g. Wolf et al., 2013) and (iv) the development and evaluation of molecular probe based methods such as molecular sensors (Wollschlaeger et al., 2014) or quantitative PCR (qPCR). Most of the field work presented here in this publication was accomplished in the Arctic Ocean with special emphasis on the area of the "Deep-Sea Long-Term Observatory Hausgarten" established by the Alfred Wegener Institute for Polar- and Marine Research in 1999 to carry out regular observations of the ecosystem in the eastern Fram Strait (Soltwedel, 2005). In the following, the different parts of the observation strategy are presented in detail.

3.1.1 Automated remote controlled filtration system

The remote controlled **auto**mated **fi**ltration system for **m**arine microbes (AUTOFIM) is the core of the observation strategy. The filtration system (Figure 2) can be operated autonomously aboard research vessels or ships of opportunity. AUTOFIM allows filtration of a sampling volume up to five litres from the upper water column. In total, 12 filters can be taken and stored in a sealed sample archive. Prior to storage, a preservative such as Lysis Buffer RLT (Qiagen, Germany) is applied to the filters preventing degradation of the sample material, that can be used for molecular or biochemical analyses. Exchanging the sample archive is a quick and easy task, which makes it feasible for lay persons from the ships´ staff to take care of the automated filtration. This would circumvent the need to provide support of an additional specifically trained personal for filtration in the field. Filtration can be triggered after defined regular time intervals or remote controlled from a scientist at the research institute. Additionally, it could also be event-triggered if the filtration system would be operated in connection with *in situ* sensor systems (Petersen, 2014). Overall, AUTOFIM provides the technical background for automated high spatio-temporal resolution collection of marine particles e.g. for molecular analyses. During expedition PS92 of RV Polarstern to the Arctic Ocean in summer 2015, AUTOFIM was used for the first time to collect samples from the upper water column at a depth of ~ 10 m, which is the depth of the inlet of the ships water pump system. Subsequent to filtration, samples were preserved with a preservation buffer and stored at -80°C until further analyses in the laboratory.

3.1.2 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

ARISA provides information on variability in protist community structure in larger sample sets at reasonable costs and effort. In an ARISA-analysis the community is characterized by its community profile, which is based on the composition (presence/absence) of differently sized DNA fragments. The DNA fragments are a result of the amplification of the internal transcribed spacer region of the ribosomal operon, which displays a high degree of taxon-related variability in its length.~~ARISA provides information on variability in protist community structure in larger sample sets at reasonable costs and effort, while~~ ARISA-profiles reflect taxon specific differences observed in NGS-data sets (Kilias et al., 2015). In the developmental phase of the molecular observation strategy, this method was used in a number of different studies to better understand variability of Arctic marine protist communities in relation to environmental conditions and ocean currents. Based on ARISA analyses we identified large scale patterns of protist biogeography that were tightly connected to ambient water masses, ocean currents and sea ice coverage (Kilias et al., 2014a; Metfies et al., 2016; Wolf et al., 2014b). We suggest ~~to use~~using ARISA as part of the molecular observation strategy to identify biogeographic or biodiversity patterns ~~at meso- or large-~~in large sample sets, e.g. collected via AUTOFIM. Identification of pattern in phytoplankton biogeography or biodiversity requires analyses of large samples sets, because ~~However,~~

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spatial heterogeneity of marine phytoplankton is ~~large~~considerable, while the vertical dimension is of ~~from~~ particular importance, since differences in vertical abundance and composition of phytoplankton impact primary production, export processes and energy transfer to higher trophic levels (Leibold, 1990). Vertical distribution of marine protists is determined by opposing resource gradients and mixing conditions (Mellard et al., 2011). In respect to this it was necessary to evaluate how representative samples from 10 m depth might be for the photic zone in the underlying water column. This would be important in case AUTOFIM would be applied to study large scale biogeographic patterns of marine protists. Acknowledging the potential of ARISA to quickly generate meaningful information on variability between protist samples, we used this methodology to assess the similarity of phytoplankton community composition in samples from the upper water column collected with AUTOFIM and in samples collected in deeper water layers via CTD at the same location. The ARISA patterns obtained from deeper water layers (20m; 50m) ~~horizons~~ are highly similar to those obtained from the samples collected with AUTOFIM. The samples collected with AUTOFIM at stations PS92/19 and PS92/43 clustered together with the individual samples collected at other depths at the same location ~~deeper horizons~~ (5m; 20m; 50m) and with the integrated signal from the CTD sampling all three depths at this location ~~the same station~~ (Figure 3). This result suggests that qualitative information on phytoplankton community composition based on sampling with AUTOFIM ~~might be~~can be considered as being representative for the photic layer of the water column. This might be attributed to the observation that geography and ambient water masses have a major impact on qualitative composition of marine plankton communities on a larger scale, with plankton communities being partially structured according to the basin of origin (de Vargas et al., 2015; Metfies et al., 2016).

3.1.3 Next Generation Sequencing (454-pyrosequencing; Illumina)

Sequencing of ribosomal genes is a valuable approach to describe the taxonomic composition of protist communities including the small size fractions. Technical progress in this field has been tremendously rapid over the last 5-10 years. Around ten to fifteen years ago, sequencing of 18S rDNA clone libraries was the gold standard to assess marine eukaryotic and prokaryotic communities (Hugenholtz, 2002). Around six years ago, first studies reported the use of 454 pyrosequencing for assessment of prokaryotic diversity (Turnbaugh et al., 2009). The massively parallel 454-pyrosequencing was found to generate~~d~~ several hundred thousands of ribosomal sequence per sample and had the potential to uncover more organisms, even rare species from large scale biodiversity surveys (Sunagawa et al., 2015). We assessed the validity of 454 pyrosequencing by evaluating the sequence data sets with results obtained via other methods, such as 18S clone libraries, HPLC and microscopic counts. The samples analyzed in the course of this evaluation originated from the same Niskin-bottle of a respective CTD-cast. In our data sets pyrosequencing data were in good agreement with information on community composition generated by high pressure liquid chromatography (HPLC) or clone libraries (Kilias et al., 2013; Wolf et al., 2013). During the past six years, we used 454-pyrosequencing to determine the variability of protist community structure in Fram Strait, in the area of the "Deep-Sea Long-Term Observatory Hausgarten", and the central Arctic Ocean (Kilias et al., 2014a; Metfies et al., 2016). Overall, our data revealed that *Phaeocystis pouchetii* is an important contributor to Arctic protist communities, particularly to the pico-eukaryote community composition. In 2009 the species constituted up to 29.6% of the sequence assemblage retrieved from pico-eukaryote samples in that area ~~collected in the area of the "Deep-Sea Long-Term Observatory Hausgarten"~~ (Kilias et al., 2014b). A larger survey of Arctic protist community composition in 2012

including Fram Strait and larger parts of the Central Arctic Ocean confirmed these observations and identified *Phaecystis pouchetii* again as an important contributor to Arctic pico-eukaryote Chl *a* biomass~~.~~ ~~which~~The latter constituted between 60-90% of Chl *a* biomass during summer 2012 in the Arctic Ocean (Metfies et al., 2016). This comprehensive sequence based information on phytoplankton community composition was very well suited to serve as a basis for the development of molecular probes that can be used for molecular surveillance with molecular sensors or quantitative PCR (qPCR).

3.1.4 Development and evaluation of molecular probe based methods~~: such as~~ molecular sensors or qPCR

Molecular Sensors are ~~chip~~ chip-based formats that allow parallel identification and quantification of multiple taxa in a single experiment. The identification is based on solid phase hybridization of molecular probes, immobilized to the surface of the sensor chips that bind to the rRNA or rDNA of the target species (Diercks et al., 2008a; Diercks et al., 2008b; Ussler et al., 2013). ~~Quantitative or real time PCR (qPCR) is a standard PCR with the advantage of detecting the amount of DNA formed after each cycle with either fluorescent dyes or fluorescently tagged oligonucleotide probes.~~ Quantitative or real time PCR (qPCR) is a PCR-based method that utilizes fluorescent dyes or fluorescently-labelled molecular probes to quantify nucleic after each PCR cycle. It~~Quantitative PCR~~ is a useful tool for quantitation of nucleic acids, respectively species in a given environment (Toebe et al., 2013). An automated molecular sensor (Diercks et al., 2008a) and qPCR are intended to be part of the molecular observation strategy in order to generate near real time information on the occurrence of key species on board ship and to complement NGS-based information on phytoplankton community composition with quantitative information on the occurrence of selected key species (Figure 1). These approaches are necessary because of biases related to the amplification of the 18S rDNA gene via PCR, and uncertainties in respect to copy number of the gene in the genome of different species (e.g. Zhu et al.,2005), which make it difficult to deduce species abundance based on NGS. ~~I~~W~~n respect to this, w~~e developed new molecular probes for relevant taxa that were major contributors in our NGS-libraries or that were known from published literature to occur in the observation areas (North Sea and Arctic Ocean). The molecular probes were either used in combination with molecular sensors (Wollschlaeger et al., 2015), qPCR or fluorescent *in situ* hybridization (FISH) (Thiele et al., 2014). The data on species abundance obtained from ~~results~~ of molecular sensors targeting either 18S rDNA or 18S rRNA were evaluated with the results obtained from microscop~~y~~ic counts~~, HPLC and flow cytometry~~ (Wollschlaeger et al., 2014). The molecular sensor targeting 18S rRNA shows a robust linear relationship between molecular sensing signal and cell counts via microscopy. The positive evaluation results for the rRNA based nucleic acid biosensor suggest an excellent ~~high~~ potential of the method to be used as module in a Molecular Observation Strategy. Here, the ~~related~~ regular quantitative molecular monitoring would benefit from advantages like reduced effort (time, costs and labor), and the high potential for automation of the methodology (Wollschlaeger et al., 2014). In the current study we demonstrate the potential of quantitative PCR to better understand the biogeography and abundance of *Phaeocystis pouchetii* in Arctic Waters using a specific primer set for qPCR. The qPCR values were calibrated against defined numbers of laboratory cultures (Figure 4) to allow quantification of *Phaeocystis pouchetii* via this method. During expedition PS85 of RV Polarstern in June 2014, we used qPCR on board ship to determine the abundance of *Phaeocystis pouchetii* on a transect through Fram Strait at ~79°N (Figure 4). The results of our survey suggest that abundance of *Phaeocystis pouchetii* in Fram Strait is determined by water mass properties such as salinity, ice coverage and water

temperature, ~~while~~ salinity is positively correlated with abundance of *Phaeocystis pouchetii*. The abundance of *Phaeocystis pouchetii* was higher in Atlantic Waters, which are characterized by higher salinities in the range of 33-34 PSU~~,~~ than in Polar Waters of Fram Strait which are characterized by salinities around 31 PSU. In Atlantic Waters the average cell number of *Phaeocystis pouchetii* was ~ 3.5 times higher than the average cell number in Polar Waters of Fram Strait. Furthermore, Chl *a* biomass appears to be correlated with abundance of *Phaeocystis pouchetii*. Our findings are in agreement with previous studies that reported blooms of *Phaeocystis pouchetii* in waters around Svalbard with cell abundances in a similar range as observed in this study (Wassmann et al., 2005). In 2012, we carried out a large scale study to survey the biogeography of marine protists in the Arctic. This survey included a comprehensive NGS based analysis of community composition along 79°N in Fram Strait in June and later in the season in Nansen Basin and Amundsen Basin. Overall, the findings of 2014, suggesting a positive correlation of Atlantic water properties, e.g. higher salinity and lower ice coverage with high abundance of *Phaeocystis pouchetii* are in agreement with the previous study of 2012. This study also ~~suggested this~~found a positive correlation in agreement with the findings of 2014, even though sequence abundance of *Phaeocystis pouchetii* was more evenly distributed in Fram Strait in 2012~~that year~~ (Metfies et al., 2016). This might be attributed to the complex current system in the area. Overall, qPCR carried out on board ship provided a near real time overview of the distribution of a protist key species during expedition PS85.

4 Conclusions

Here we introduce for the first time an integrated hierarchically organized molecular based observation strategy that combines autonomous sampling with molecular analyses. It is ~~as~~ a valuable tool to survey phytoplankton abundance and biodiversity in the desired high spatial and temporal resolution as well as at different levels of taxonomic resolution. The observation strategy is based on a combination of ship based automated filtration, online measurements of oceanographic parameter, and different molecular analyses. On one hand, our approach provides near real time information on phytoplankton key species abundance in relation to environmental conditions already on board ship. On the other hand, it provides detailed information on variability in the total phytoplankton community composition based on comprehensive, laboratory-based molecular analyses such as molecular fingerprinting methods and NGS. This information can be subsequently correlated with information on the physical and chemical marine environment and has ~~strong~~excellent potential to complement other hierarchically organized observation strategies as described e.g. for the detection of marine hazardous substances and organisms (Zielinski et al., 2009). In summary, our molecular observation strategy is a significant contribution to refine regular assessment of consequences of ongoing environmental change for marine phytoplankton communities with respect to adequate spatial, temporal, and taxonomic resolution.

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assistance. Annegret Müller and Uwe John are acknowledged for excellent technical support of the fragment analysis.

Figure Legends:

Fig. 1: A:Overview of the smart observation strategy which is organized in four different levels: –level 1: samples are collected underway or at monitoring sites using the remote-controlled automated filtration system AUTOFIM; level 2: direct molecular surveillance of key species aboard the ship via an automated biosensor system or quantitative polymerase chain reaction; level 3: preserved samples are analyzed via molecular fingerprinting methods (e.g. ARISA) that provide a quick and reliable overview of differences in protist community composition of the samples in a given observation area or time period; level 2: detailed analysis of taxonomic protist composition in selected samples via latest next generation sequencing. B-E: Schematic diagrams illustrating the analyses used in the smart observation strategy.

Fig. 2: A: AUTOFIM installed on board RV Polarstern (1: Sample reservoir; 2: Filtration; 3: Archive for preserved filters. B: Filtration-module (1:Filter stacker; 2:Filtration cap).

Fig. 3: ~~MetaMDS Plot of ARISA fingerprints generated from samples collected via CTD and AUTOFIM. Samples were collected during expeditions PS92 and PS 94 of RV Polarstern to the Arctic Ocean.~~ MetaMDS Plot (non metric multidimensional scaling plot) of ARISA fingerprints generated from samples collected via Niskin bottles coupled to a CTD-rosette and AUTOFIM.The closer the samples are located to each other in the metaMDS-plot, the more similar are the ARISA-profiles of the samples. The label of the samples gives information on the cruise leg (PSXX) and the station (/XX). Samples were collected during expeditions PS92 and PS 94 of RV Polarstern to the Arctic Ocean during summer 2015. The samples collected during PS94 serve as an outgroup in this analysis.

Fig. 4: ~~Assessment of *Phaeocystis pouchetii* in Fram Strait. A: Calibration of Phaeocystis pouchetii specific qPCR assay with laborartory cultures. B: Abundance of Phaeocystis pouchetii in Fram Strait. C: Principal component analysis including environmental parameter and *Phaeocystis pouchetii*.~~ Assessment of *Phaeocystis pouchetii* in Fram Strait. A: Calibration of *Phaeocystis pouchetii* specific qPCR assay with a dilution series of laboratory cultures. The CT value is significantly correlated with cell numbers. B: Abundance of *Phaeocystis pouchetii* in Fram Strait. The dots and the associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII(PS85) of RV Polarstern in summer 2014, while cell numbers/liter are reflected by different colours. C: Principal component analysis including environmental parameters (temperature, salinity, Chl *a* biomass and sea ice coverage) and abundance of *Phaeocystis pouchetii*. Triangles and associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII (PS85) of RV Polarstern in summer 2014. HG4 indicates the central station of the "Deep-Sea Long Term Observatory Hausgarten" in Fram Strait. The Eigenvalues indicate the proportion of variance explained by different dimensions in the diagram. The black bars in the histogram reflect the x-axis and the y-axis. Here ~ 80% of variance is explained in this two-dimensional diagram of the PCA (x-axis: 50.29%; y-axis: 30.08%).

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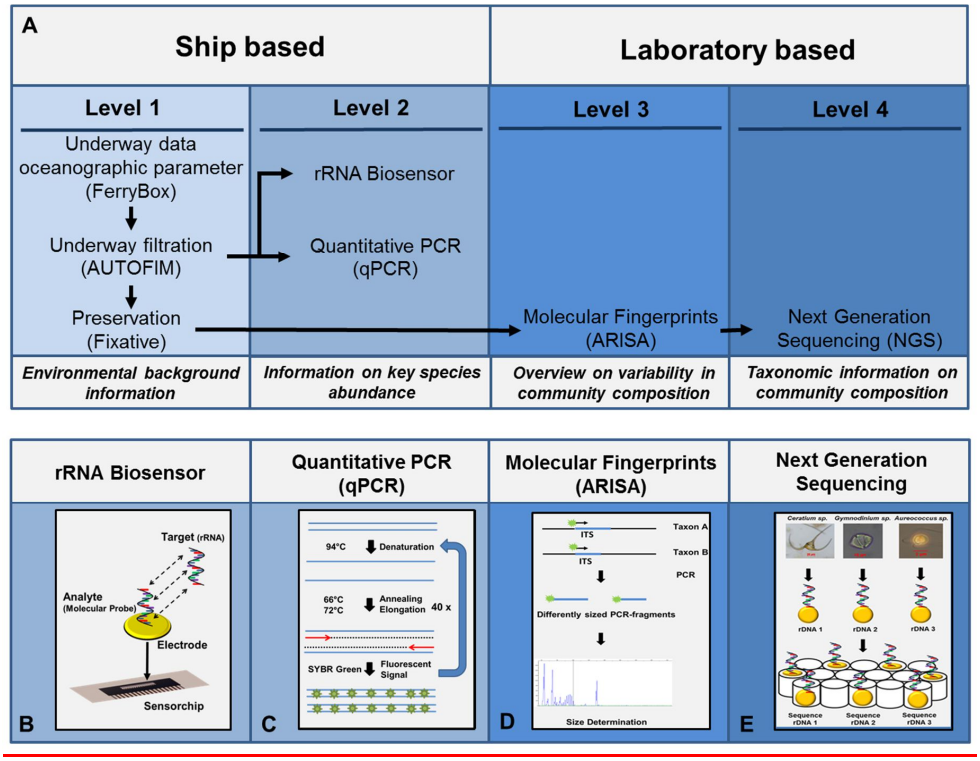
Fig. 1


A
Ship based
Laboratory based
Level 1
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Underway data oceanographic parameter (FerryBox)
Underway filtration (AUTOFIM)
Preservation (Fixative)
rRNA Biosensor
Quantitative PCR (qPCR)
Molecular Fingerprints (ARISA)
Next Generation Sequencing (NGS)
Environmental background information
Information on key species abundance
Overview on variability in community composition
Taxonomic information on community composition
rRNA Biosensor
Quantitative PCR (qPCR)
Molecular Fingerprints (ARISA)
Next Generation Sequencing
Target (rRNA)
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Electrode
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66°C
72°C
Annealing
Elongation
40 x
SYBR Green
Fluorescent Signal
ITS
Taxon A
Taxon B
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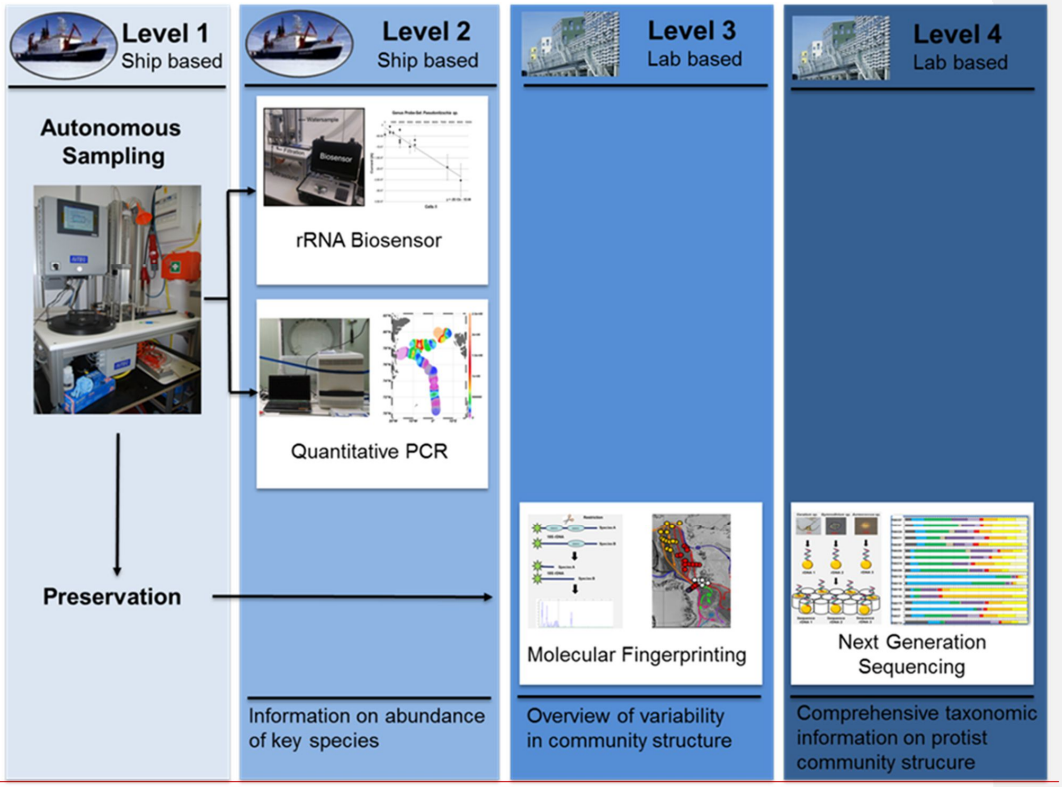

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Figure 2

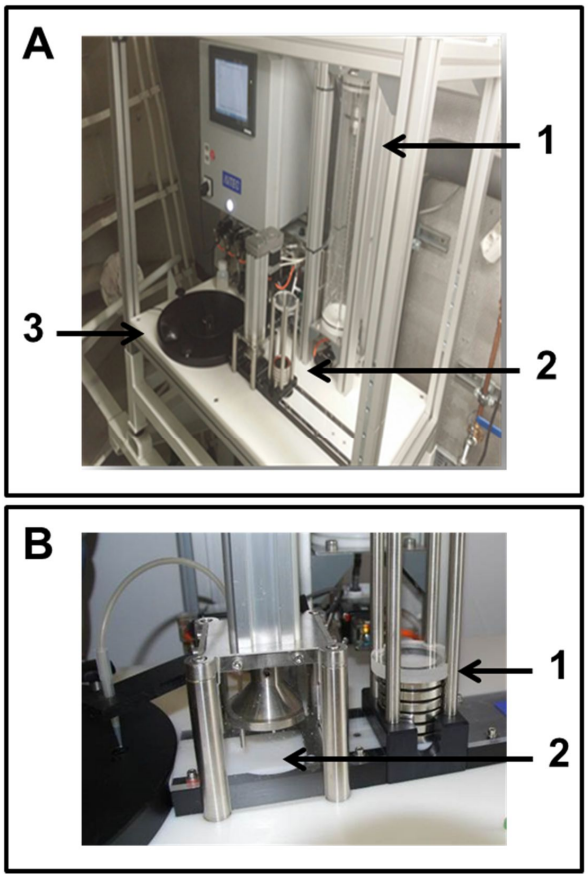

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Fig. 3

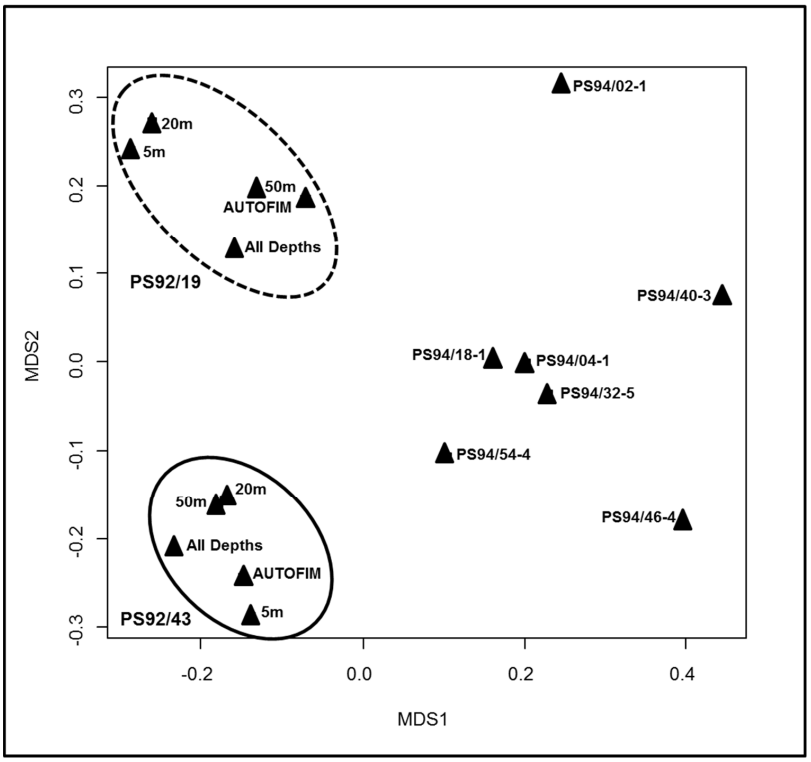

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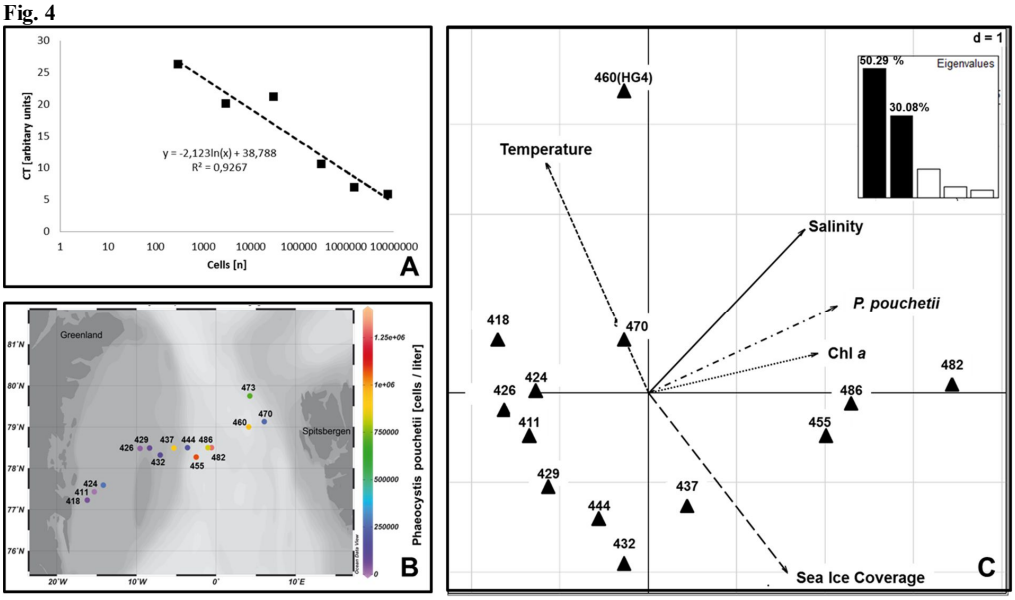
Fig. 4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P. pouchetii
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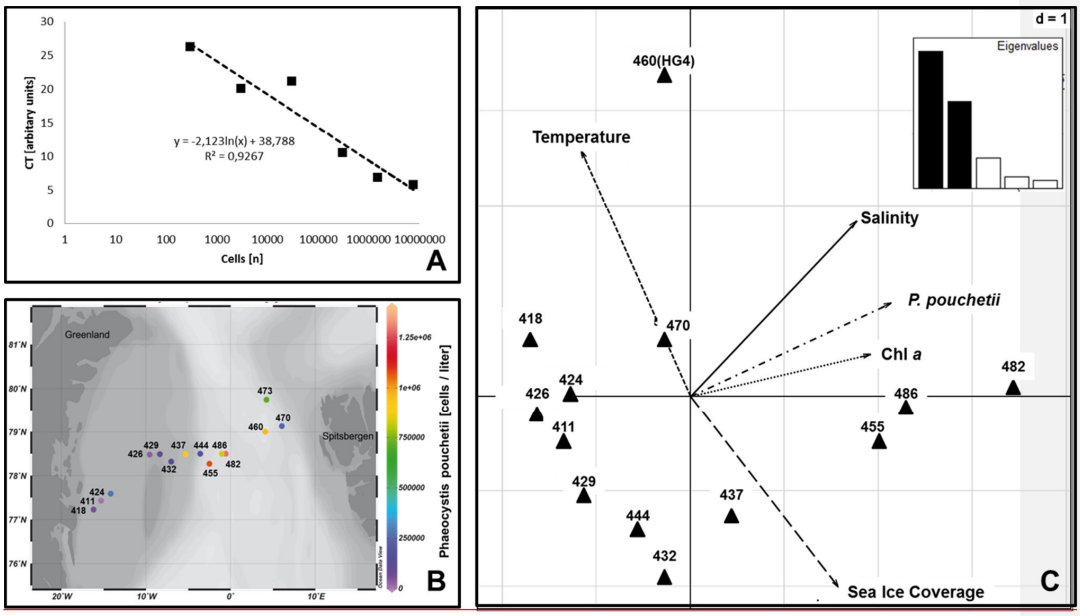
Sea Ice Coverage
C



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Author's Reply (AR) to Anonymous Referee # 1 (R1)

R1: L30 The observation strategy is organized AT four different levels
AR: The sentence was changed according to the reviewer's suggestion.

R1: L30 At level 1, samples are collected AT…
AR: The sentence was changed according to the reviewer's suggestion.

R1: L35 protist mentioned for first time here and the first sentence relates only to photosynthetic microbes.
AR: The term protist is introduced now in the first sentence of the abstract (L 24 Information on recent biomass distribution and biogeography of photosynthetic marine protists)

R1: L36 replace subjected with used
AR: The sentence was changed according to the reviewer's suggestion.

R1: L36-37 via THE latest next-generation sequencing TECHNOLOGY
AR: The sentence was changed according to the reviewer's suggestion.

R1: L63 Microplankton should have an upper limit
AR: An upper limit was added (L63(20-200 µm))

R1: L66 Unclear what "currently driving topics" means. Do you mean its a topical issue in marine ecology?
AR: L66 The term "driving topics" was replaced by "topical issues"

R1: L74 reference difficulties in assessing pico/nano sized fraction needed.
AR: An exemplary reference was added (Caron et al., 1999).

R1: L87- 88: I would say currently restricted to mostly monitoring larger phytoplankton (it does record coccolithophores that are nano-sized).
AR: The sentence was changed according to the reviewer's suggestion (L88-89 Unfortunately, the CPR-approach is restricted to zooplankton and larger phytoplankton e.g. diatoms. Again, the ecological relevant picophytoplankton fraction is omitted).

R1: L105 suggest an alternative starter: TOADDRESS THESE ...
AR: The sentence was changed according to the reviewer's suggestion (L107 To address these shortcomings and challenges of current observation approaches….)

R1: L108 remove "a large variety of", for THE observation
AR: The sentence was changed according to the reviewer's suggestion (L109-110 Over the past decade numerous publications demonstrated the power of molecular methods for the observation of marine plankton organisms).

R1: L121 end the sentence with something like "to identify protists".
AR: The sentence was changed according to the reviewer's suggestion.

R1: L126 define NGS
AR: The sentence was changed according to the reviewer's suggestion (L128 . In contrast, Next Generation Sequencing (NGS) of ribosomal genes)

R1:L154: Here, two LITRES OF water.
AR: The sentence was changed according to the reviewer's suggestion (L152 Two liter of water subsamples were taken in PVC bottles…)

R1: L153-157 - Does the fitting of AUTOFIM require expertise?
- Which parts of this process is carried out manually or required scientific supervision and which automatically by AUTOFIMS?
-Was this done by AUTOFIM ?
AR: A sentence to clarify these questions was added (L157-160 Fitting and programming of the device does not require special expertise if it is done according to the manufacturer's protocol. All steps related to the filtration process, including application of Lysis Buffer RLT (Qiagen, Germany) were carried out automatically by AUTOFIM.)

R1: L153-157 How is it cleanded (if at all?) between samples?
AR: A sentence to clarify this question was added (L162-163 The filtration device was cleaned after each filtration step by rinsing the device with fresh-water.)

R1: L180-184: More information needed on bioinformatic methods or reference to methods. Methods for any specific comparisons in the results need to be elaborated, e.g. "our data sets pyrosequencing data were ingood agreement with information on community composition generated by high pressure liquid chromatography (HPLC) or clone libraries..". Explain here how HPLC comparison
AR: Originally we had submitted a version of this manuscript with detailed information on the methods. The editor had an issue with the description of previously published material and method information. Considering the editors requirements we now cite the relevant publications that contain detailed descriptions of the methodology and comparisions (Kilias et al. 2013 and Metfies et al.2016).

R1: L184 remove e.g if these articles have sufficient information.
AR: The sentence was changed according to the reviewer's suggestion (L191).

R1: L186 Why was a nested approach used, this may have implications for the quantification step- how was this overcome?
AR: Information was added to clarify this question (L194-197 We used this nested approach, because it minimized the variability between technical replicates of q-PCR data obtained from analyses of field samples. The applicability of the nested approach was evaluated by a comparison of q-PCR data with manual counts of Phaeocystsi pouchetii in field samples (data not shown).)

R1: L195-202 What controls did you use?
AR: Information was added to clarify this question (L197-199 In the first step total eukaryotic 18S rDNA was amplified from a positive control (genomic DNA Phaeocystis pouchetii), a negative control (no template) and genomic DNA isolated from field samples using the universal primer-set…).

R1. L203: where does this equation come from?
AR: The equation comes from the calibration with a dilution series of a laboratory culture of Phaeocystis pouchetii described in line 201-202 and illustrated in figure 4a.

R1: L235-245 So can it be deployed without any experts on it from start to finish?
AR: This is elaborated in line 254-255 of the revised manuscript.

R1: L238- Name the preservative
AR: The name of the preservative used in this study is given (L255 Prior to storage, a preservative such as Lysis Buffer RLT (Qiagen, Germany)).

R1: L248 How did you ensure the piping in the ship pump apparatus was clear of microbial biofilm and/or residual water?
AR: AUTOFIM is deployed in close proximity to the inflow of the same ships pump system that continuously supplies water to a flow through sensor system (FerryBox) which is installed on RV Polarstern. This fact insures that AUTOFIM does not filter residual water of the ships pump system. The piping of the ship pump system is cleaned in regular intervals to avoid microbial biofilms.

R1: L249 "...at meso- or large in large sample sets". Sentence confusing- meso or large-scale- you mean geographically large or large sample numbers? Re-word if its due to large sample numbers explain why?
AR: "at meso- or large" was removed from the sentence.

R1: L258-263 - alter to....marine phytoplankton is CONSIDERABLE
- "...dimension is OF particular importance
-The word "However" is used but i cannot see a connection between the first sentence and the second. Clarification needed.
AR: The sentence was rephrased according to the reviewer's suggestions (L280-282 Identification of pattern in phytoplankton biogeography or biodiversity requires analyses of large samples sets, because spatial heterogeneity of marine phytoplankton is considerable,)

R1: L270: - What do you mean by "deeper horizons"? do you mean greater depths?
AR: "deeper horizons" was replaced by "deeper water layers"
- I think you need to explain the connection between autofim stations and ctd stations-where they geographically close or was it just depth?
AR: It was clarified in the text that the AUTOFIM-filters were collected at the same station/location as CTD-samples (L291).
- I would mention that 5m- 50m is within the photic zone.
AR: The term "photic zone" was added to the text (L286-287).
- Also in what way were they similar- taxonomic assemblage or together with other factors?
AR: Additional information on the technical background of ARISA was added at the beginning of the ARISA-paragraph to clarify this question (L268-272 ARISA provides information on variability in protist community structure in larger sample sets at reasonable costs and effort. In an ARISA-analysis the community is characterized by its community profile, which is based on the composition (presence/absence) of differently sized DNA fragments. The DNA fragments are a result of the amplification of the internal transcribed spacer region of the ribosomal operon, which displays a high degree of taxon-related variability in its length).

R1: L271 typo in individual
AR: Fixed

R1: L272 ..and WITH the integrated signal FROM THE CTD SAMPLING at all three depths....
AR: The sentence was changed according to the reviewer's suggestions (L293-295 The samples collected with AUTOFIM at stations PS92/19 and PS92/43 clustered together with the individual samples collected at other depth at the same location (5m; 20m; 50m) and with the integrated signal from the CTD sampling all three depths at this location (Figure 3).)

R1: L277: According to THE basin of origin
AR: The sentence was changed according to the reviewer's suggestion.

R1: L282- 284: This is extra information so can go.
AR: The authors think that this information should stay in the text because it is not redundant and illustrates the quick technological progress in the field.

R1: L284: "...parallel 454-pyrosequencing WAS FOUND TO generate..."
AR: The sentence was changed according to the reviewer's suggestion (L307).

R1: L286-7: What sequence data sets? CTD, AUTOFIM or both?
AR: Information to clarify this question was added to the text (L311-312 The samples analyzed in the course of this evaluation originated from the same Niskin-bottle of a respective CTD-cast.)

R1: L290: to determine THE variability
AR: The sentence was corrected according to the reviewer's suggestion (L314)

R1: L295: Repetitive. Replace "collected in the area of the "Deep-Sea Long-Term Observatory Hausgarten"" with "in that area".
AR: The sentence was changed according to the reviewer's suggestion (L319)

R1: L304: Alter to "Development and evaluation of molecular probe based methods: molecular sensors and qPCR"
AR: The heading was changed according to the reviewer's suggestion (L328)

R1: L307: "...the surface of the sensor chips THAT BIND to EITHER the rRNA (transcriptome) or rDNA (genome) of the target species."
AR: The sentence was corrected according to the reviewer's suggestion.

R1: L306:308: Mention that it is also quantitative and how this is achieved- a diagram of these methods would help readers understand.
AR: In line 329 it says already that the method is quantitative, while the detection principle is described and illustrated in the references provided in L331-332.

R1: L308-310: Alter to "Quantitative or real time PCR (qPCR) IS A PCR-BASED METHOD THAT UTLITISES FLUORESCENT DYES OR FLUORESCENTLY-TAGGED DNA PROBES TO QUANTIFY SPECIES BYDETECTING THE AMOUNT OF DNA FORMED AFTER EACH PCR CYCLE". This way the reader can link species abundance with DNA quantity. L310: Also useful for quantifying species.
AR: The sentence was re-phrased (L334-336 Quantitative or real time PCR (qPCR) is a PCR-based method that utilizes fluorescent dyes or fluorescently-labelled molecular probes to quantify nucleic after each PCR cycle. Itis a useful tool for quantitation of nucleic acids, respectively species in a given environment)

R1: L314-317: reference needed for this sentence
AR: A reference was added (Zhu et al.,2005).

R1: L317: May be use "As such" or another term instead of "In respect to this,"
AR: "In respect to this" was removed.

R1: L322: What are you measuring"from microscopy, HPLC and flow cytometry". Cell counts, pigments? Add this in. How did you related pigments to cell counts- give a reference to the method?
R1: L323: What about the other measurements?
AR: The sentence was re-phrased (L347-349 The data on species abundance obtained from of molecular sensors targeting either 18S rDNA or 18S rRNA were evaluated with the results obtained from microscopic counts(Wollschlaeger et al., 2014).)

R1: L324 replace high potential with good potential
AR: "high potential" was replaced by "excellent potential"

R1: L325: What do you mean by "the related regular monitoring". Is it qpcr/molecular sensors? If so i suggest Here, additional quantitative molecular monitoring

AR: The sentence was changed (L352-354 Here, the regular quantitative molecular monitoring would benefit from advantages like reduced effort, and the high potential for automation of the methodology (Wollschlaeger et al., 2014))

R1: L326: reduced effort in what? Change high potential to excellent or good potential.
AR: The effort was specified (L353 (time, costs and labor))

R1: L333: delete while
AR:"While" was deleted

R1: L335:PSU, than.... Delete comma.
AR: Comma was deleted

R1: L343 reference needed for the 2014 findings
AR: The 2014 findings are presented in figure 4 of this manuscript.

R1: L345: This study also suggested this positive correlation. Suggest This study also found a positive correlation in agreement with XYZ, et al 2014.
AR: The sentence was re-phrased (This study also found a positive correlation in agreement with the findings of 2014, even though sequence abundance of Phaeocystis pouchetii was more evenly distributed in Fram Strait in 2012(Metfies et al., 2016))

R1: L352: hierarchically organized molecular based. I would add that its a combined autonomous sampling and molecular testing platform.
AR: The information was added (L379-380 Here we introduce for the first time an integrated hierarchically organized molecular based observation strategy that combines autonomous sampling with molecular analyses.)

R1: L360 change strong to excellent/good
AR: "strong" was changed to "excellent"

R1: Figures I think map figure would be really helpful to allow readers to understand spatial Comparisons
AR: This manuscript reviews the findings of ~ 10 publications, that all contain maps of the respective research area, references for the maps are provided. Figure 4 contains a map for the newly published data on Phaeocystis pouchetii abundance in Fram Strait in summer 2014.

R1: Also for section 3.1.4 for readers who are not familiar with molecular methods a diagram of how a qpcr/molecular sensors work or a photograph of the one you have would be good- you could alter fig 1 as its quite small and provide a clearer picture of these?
AR: Figure 1 was revised and contains now diagrams that explain the background of the analyses used in the observation strategy.

R1: Fig. 2: Would be good to see basic diagram of its layout and its connected and its modules.
AR: The layout and technical details of the device is not in focus of this manuscript. These informations will be published elsewhere.

R1: Fig. 3: Define Meta MDS. Methodology needs to be referred to in methods. I would explain the labelling system for expeditions and stations. Where/when were the other PS stations and what did they represent?
AR: The figure legend was extended to provide this information. The method used to generate the metaMDS plot is described in Kilias et al. which is cited in the material and methods section.

R1: Fig. 4: A- i would explain the significance of that graph to non-expert readers, to say the assay worked and provided a good relationship between DNA quantity and cell abundance. B- What do the numbers next to the points in the map represent? C: parameter needs to be plural,. What does the inset graph show? What do the numbers represent by the triangles? I suggest explain the plot.

AR: The figure legend was rephrased and more elaborate information to answer the questions of reviewer 1 were added. (Assessment of Phaeocystis pouchetii in Fram Strait. A: Calibration of Phaeocystis pouchetii specific qPCR assay with a dilution series of laboratory cultures. The CT value is significantly correlated with cell numbers. B: Abundance of Phaeocystis pouchetii in Fram Strait. The dots and the associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII(PS85) of RV Polarstern in summer 2014, while cell numbers/liter are reflected by different colours. C: Principal component analysis including environmental parameters (temperature, salinity, Chl a biomass and sea ice coverage) and abundance of Phaeocystis pouchetii. Triangles and associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII(PS85) of RV Polarstern in summer 2014. HG4 indicates the central station of the "Deep-Sea Long Term Observatory Hausgarten" in Fram Strait. The Eigenvalues indicate the proportion of variance explained by different dimensions in the diagram. The black bars in the histogram reflect the x-axis and the y-axis. Here ~ 80% of variance is explained in this two-dimensional diagram of the PCA (x-axis: 50.29%; y-axis: 30.08%).)

Author's Reply (AR) to Anonymous Referee # 2 (R2)

R2: A bit more thorough overview of related technology could be relevant, however. For instance the ESP (Environmental Sample Processor) has an automated filtration unit directly connected to the possibility of using qPCR or hybridization for microbial species identification under water (although not as part of a ferry box system as far as I know).

AR: L122 The ESP is cited in the manuscript in line 120 (Ussler et al., 2013).

R2: Firstly, the authors should explain what they mean by "underlying water column". They did not present vertical CTD profiles of the water column, so it is difficult to know if the samples taken from distinct depths using the Niskin bottles were all taken from the same layer.

AR: L286-287 This sentence was rephrased (In respect to this it was necessary to evaluate how representative samples from 10 m depth might be for the photic zone in the underlying water column.)

R2: Their results show that at the (only) two stations sampled for the comparison, the AUTOFIM sample communities at 10m were associated with the communities collected using Niskin bottles from the same water layer. Their discussion around this result (around lines 270-274) is a bit unclear referring how the AUTOFIM samples is representative of that of "deeper horizons". What do the authors mean by this? I assume they mean that the AUTOFIM samples are representative for the upper mixed layer, because they do not have data from deeper layers. This part should be rephrased/clearified.

AR: L291-295 This part was rephrased (The ARISA patterns obtained from deeper water layers of the photic zone (20m; 50m) are highly similar to those obtained from the samples collected with AUTOFIM. The samples collected with AUTOFIM at stations PS92/19 and PS92/43 clustered together with the individual samples collected at other depth at the same location (5m; 20m; 50m) and with the integrated signal from the CTD sampling all three depths at this location.)

R2: The automated biosensor system used - it is a bit unclear to me exactly what this is. Do

they refer to ferry box data or to analyses performed on the filtered seawater collected using the AUTOFIM? If the latter, which sensors/analyses do they refer to? Or do the refer to the molecular sensors of Wollschlaeger et al. (2014)?
AR: Yes, we are referring to the molecular sensor experiment described and evaluated in Wollschlaeger et al., 2014.

R2: In the introduction, when the authors refer to the molecular tools available (line 105 and onwards), they mainly refer to their own work. But there are several studies that would be relevant to include in such an overview. I suggest the authors refer also to other studies from Arctic waters, in particular the Canadian Arctic has been explored using similar and relevant molecular tools.
AR: L114 In this manuscript we cited Sunagawa et al., 2015, which is one of the most relevant manuscripts published in the field during the past two years. In addition to this we complemented our citations with Comeau et al., 2011, a description of the Arctic microbial community structure before and after the record sea ice minimum in 2007.

R2: line 152: Were the particles for molecular analyses added a buffer? Or perhaps stored in -80 prior to DNA extraction?
AR: L155-156 Information to clarify this was added to the text (Subsequent to sampling filters were stored at -20°C until further analyses.

R2: line 165: Is the use of the E.Z.N.A DNA extraction kit correct? And was it used for both the AUTOFIM and Niskin bottle samples? If so, why was the the Qiagen lysis buffer added to the filters collected using AUTOFIM?
AR: Yes, the E.Z.N.A. DNA extraction kit is correct and it was used for both the AUTOFIM and Niskin bottle samples. We used the Qiagen lysis buffer, because it is on one hand exchangeable with the E.Z.N.A. lysisbuffer and on the other hand preservation with QLT-buffer left the possibility to use the AUTOFIM-samples for quantification with our automated biosensor system.

R2: line 178: ITS1 is the internal transcribed spacer 1. It is also an "intergenic spacer region", but the use of that term without explaining the ITS1 abbreviation is a bit confusing.
AR: L185 "intergenic spacer region" was replaced by "internal transcribed spacer"

R2: first paragraph of 3.1 is mostly repeating what is already pointed out in the introduction. This section could be reduced.
AR: Paragraph 3.1 describes the observation strategy that is not mentioned in the introduction. Thus it did not get clear to the authors, which parts of the paragraph reviewer 2 is referring to.

R2: line 259: Rephrase sentence, the word "scale" lacking? line 261: "from" should be replaced with "of" (particular importance).
AR: L 278-284 This sentence was rephrased (We suggest to use ARISA as part of the molecular observation strategy to identify biogeographic or biodiversity patterns in large sample sets, e.g. collected via AUTOFIM. Identification of pattern in phytoplankton biogeography or biodiversity requires analyses of large samples sets, because spatial heterogeneity of marine phytoplankton is considerable, while the vertical dimension is of particular importance,....)

R2: line 297-299, incl Metfies et al 2016: Is the % cells of Phaeocystis due to % reads or quantitative counts? If it refers to % reads, the statement is a bit strong.

AR: L320-323 The statement is based on measurements of Chl a biomass subsequent to fractionated filtration. The sentence was rephrased to clarify the uncertainty (A larger survey of Arctic protist community composition in 2012 including Fram Strait and larger parts of the Central Arctic Ocean confirmed these observations and identified Phaecystis pouchetii again as an important contributor to Arctic pico-eukaryote Chl a biomass. The latter constituted between 60-90% of Chl a biomass during summer 2012 in the Arctic Ocean (Metfies et al., 2016).)

R2: Fig. 1 text: This text should explain the different levels of the observation strategy in greater detail, so that it is not necessary to check the manuscript text to identify the different parts.

AR: Information on the different levels of the observation strategy was added (Overview of the smart observation strategy which is organized in four different levels: level 1: samples are collected underway or at monitoring sites using the remote-controlled automated filtration system AUTOFIM; level 2: direct molecular surveillance of key species aboard the ship via an automated biosensor system or quantitative polymerase chain reaction; level 3:.preserved samples are analyzed via molecular fingerprinting methods (e.g. ARISA) that provide a quick and reliable overview of differences in protist community composition of the samples in a given observation area or time period; level 2: detailed analysis of taxonomic protist composition in selected samples via latest next generation sequencing.

R2: Fig. 3 text: Samples collected via CTD ... imprecise, the samples were collected using Niskin bottles.

AR: The sentence was rephrased (Fig. 3: MetaMDS Plot (non metric multidimensional scaling plot) of ARISA fingerprints generated from samples collected via Niskin bottles coupled to a CTD-rosette and AUTOFIM.The closer the samples are located to each other in the metaMDS-plot, the more similar are the ARISA-profiles of the samples. The label of the samples gives information on the cruise leg (PSXX) and the station (/XX). Samples were collected during expeditions PS92 and PS 94 of RV Polarstern to the Arctic Ocean during summer 2015. The samples collected during PS94 serve as an outgroup in this analysis.)

R2: Fig. 4 text: This text also does not explain the figure very well. Do the numbers represent station numbers? The eigenvalues histogram in 4C is not explained - what do the black vs white histograms signify? What values are at the y axis?

AR: The figure legend was rephrased (Assessment of Phaeocystis pouchetii in Fram Strait. A: Calibration of Phaeocystis pouchetii specific qPCR assay with a dilution series of laboratory cultures. The CT value is significantly correlated with cell numbers. B: Abundance of Phaeocystis pouchetii in Fram Strait. The dots and the associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII(PS85) of RV Polarstern in summer 2014, while cell numbers/liter are reflected by different colours. C: Principal component analysis including environmental parameters (temperature, salinity, Chl a biomass and sea ice coverage) and abundance of Phaeocystis pouchetii. Triangles and associated numbers represent sampling sites and associated station numbers of expedition ARKXXVIII(PS85) of RV Polarstern in summer 2014. HG4 indicates the central station of the "Deep-Sea Long Term Observatory Hausgarten" in Fram Strait. The Eigenvalues indicate the proportion of variance explained by different dimensions in the diagram. The black bars in the histogram reflect the x-axis and the y-axis. Here ~ 80% of variance is explained in this two-dimensional diagram of the PCA (x-axis: 50.29%; y-axis: 30.08%).)

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