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Interactive comment on "High resolution monitoring of marine protists based on an observation strategy integrating automated on board ship filtration and molecular analyses" *by* Katja Metfies et al.

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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 $\sim$ 80% of variance is explained in this two-dimensional diagram of the PCA (x-axis: 50.29%; y-axis: 30.08%).)

Please also note the supplement to this comment:
http://www.ocean-sci-discuss.net/os-2016-23/os-2016-23-AC2-supplement.pdf

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