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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 # 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

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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

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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 $\sim$ 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 $\sim$ 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-AC1-supplement.pdf

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