

## ***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.***

**Anonymous Referee #1**

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L30 The observation strategy is organized AT four different levels. L30 At level 1, samples are collected AT.... L35 protist mentioned for first time here and the first sentence relates only to photosynthetic microbes. Ensure it is defined earlier or use another consistent term. L36 replace subjected with used L36-37: via THE latest next-generation sequencing TECHNOLOGY... L63: Microplankton should have an upper limit. L66 Unclear what “currently driving topics” means. Do you mean its a topical issue in marine ecology? L74 reference difficulties in assessing pico/nano sized fraction needed. L87-88: I would say currently restricted to mostly monitoring larger phytoplankton (it does record coccolithophores that are nano-sized). L105 suggest an alternative starter: TO

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ADDRESS THESE ... L108 remove “a large variety of”, for THE observation L121 end the sentence with something like “to identify protists”. L126 define NGS L154: Here, two LITRES OF water. Does the fitting of AUTOFIM require expertise? L153-157: Which parts of this process is carried out manually or required scientific supervision and which automatically by AUTOFIMS? How is it cleaned (if at all?) between samples? L155: Was this done by AUTOFIM 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 in good agreement with information on community composition generated by high pressure liquid chromatography (HPLC) or clone libraries.”. Explain here how HPLC comparison L184 remove e.g if these articles have sufficient information L186: Why was a nested approach used, this may have implications for the quantification step- how was this overcome? L195-202: What controls did you use? L203: where does this equation come from? L235-245: So can it be deployed without any experts on it from start to finish? L238- Name the preservative L248- How did you ensure the piping in the ship pump apparatus was clear of microbial biofilm and/or residual water? 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? L260 alter to....marine phytoplankton is CONSIDERABLE L260-1 “...dimension is OF particular importance,” L258-263: The word However is used but i cannot see a connection between the first sentence and the second. Clarification needed. L270: What do you mean by “deeper horizons”? do you mean greater depths? I think you need to explain the connection between autofim stations and ctd stations-where they geographically close or was it just depth? I would mention that 5m- 50m is within the photic zone. Also in what way were they similar- taxonomic assemblage or together with other factors? L271 typo in individual L272 ..and WITH the integrated signal FROM THE CTD SAMPLING at all three depths.... L277: According to THE basin of origin L282- 284: This is extra information so can go. L284: “...parallel 454-pyrosequencing WAS FOUND TO generate...” L286-7: What sequence data sets?

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CTD, AUTOFIM or both? L290: to determine THE variability L295: Repetitive. Replace "collected in the area of the "Deep-Sea Long-Term Observatory Hausgarten"" with "in that area". L304: Alter to "Development and evaluation of molecular probe based methods: molecular sensors and qPCR" L307: "...the surface of the sensor chips THAT BIND to EITHER the rRNA (transcriptome) or rDNA (genome) of the target species." L306:308: Mention that it is also quantitative and how this is achieved- a diagram of these methods would help readers understand. L308-310: Alter to "Quantitative or real time PCR (qPCR) IS A PCR-BASED METHOD THAT UTILISES FLUORESCENT DYES OR FLUORESCENTLY-TAGGED DNA PROBES TO QUANTIFY SPECIES BY DETECTING 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. L314-317: reference needed for this sentence L317: May be use "As such" or another term instead of "In respect to this," 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? L323: What about the other measurements? L324 replace high potential with good potential L325: What do you mean by "the related regular monitoring". Is it qpcr/molecular sensors? If so i suggest Here, additional quantitative molecular monitoring L326: reduced effort in what? Change high potential to excellent or good potential L333: delete while L335: PSU, than.... Delete comma. L343 reference needed for the 2014 findings L345: This study also suggested this positive correlation. Suggest This study also found a positive correlation in agreement with XYZ, et al 2014. L352: hierarchically organized molecular based. I would add that its a combined autonomous sampling and molecular testing platform. L360 change strong to excellent/good

Figures I think map figure would be really helpful to allow readers to understand spatial comparisons 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? Fig. 2: Would be good to see basic diagram of its layout and its

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connected and its modules. 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? 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.

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