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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.

Anonymous Referee #2

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The manuscript entitled "High resolution monitoring of marine protists based on an observation strategy integrating automated on board ship filtration and molecular analyses" by Katja Metfies and colleagues introduce the possibility of high resolution, automated sampling of seawater that can later be analysed to assess microbial diversity and abundance based on molecular tools. The automated sampling and filtration equipment that has been developed and tested allow for new sampling possibilities from ships to assess e.g. biological responses to environmental variability.

Comments: In general the manuscript is well-written and clear, discussing the possi-

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bilities of the new methodology. In particular the AUTOFIM system provides a highly relevant sampling possibility for research vessels allowing automated sampling and conservation of filtered organic material for molecular analyses. Some parts of the methodology have been tested previously, and the current manuscript gives a nice compilation of those studies. 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 hybridisation for microbial species identification under water (although not as part of a ferry box system as far as I know).

Regarding the test performed to assess how representative the 10m sample taken by AUTIFIM is for the underlying water column: 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. Assuming that the samples collected for this test was from the same layer: 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.

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)?

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.

Typos and minor comments: lines 97-99: This statement should include references.

line 152: Were the particles for molecular analyses added a buffer? Or perhaps stored in -80 prior to DNA extraction?

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?

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.

The first paragraph of 3.1 is mostly repeating what is already pointed out in the introduction. This section could be reduced.

line 259: Rephrase sentence, the word "scale" lacking? line 261: "from" should be replaced with "of" (particular importance)

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.

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.

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

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

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do the black vs white histograms signify? What values are at the y axis?

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