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Interactive comment (in bold, italic) on “Shifts in the microbial community in the Baltic Sea with increasing CO<sub>2</sub>” by K. J. Crawford et al.

Also supplied as pdf with Figs embedded in the text (see supplement)

***We thank the reviewers for taking the time to review this manuscript and for the pertinent and constructive comments they have raised. Wherever possible we have incorporated their suggestions and if not I hope that we have clearly explained our reasoning.***

#### **Anonymous Referee #2**

The authors present mesocosm experiment in which they test the effect of ocean acidification on microbial community, by increasing CO<sub>2</sub> levels. Since the ongoing climate change this research is of high importance and data presented in this msc are very valuable. The authors focused on the phytoplankton size fraction <math>\leq 20 \mu\text{m}</math>, as well as to heterotrophic prokaryotes and viruses. The experiment set up is well explained and the msc is in general well written. I do recommend the msc to be published after major revision. Key points that I would recommend to be answered:

1. All though, the target organisms are of key importance for ecosystem functioning, I do not agree that the results are relevant if not being in correlation to the whole phytoplankton community, at least presented as Chl a concentration. If those data exist I highly recommend including them in the msc.

***We believe abundance and cell size of the different phytoplankton groups are of key importance as Chlorophyll a consisted mainly of algal groups smaller than 20  $\mu\text{m}$  cell diameter (Paul et al. 2016). Already at the start of the experiment less than 5% was larger than 20  $\mu\text{m}$  diameter. At day 5 even 70% was smaller than 2  $\mu\text{m}$ . Therefore adding Chl a concentration will not add to the current study. We made this clear in the Discussion of the revised manuscript.***

2. In Material and Methods it is stated that the samples of surrounding water were taken, but there are not presented in results or at least discussed. It is essential to discuss those data. I would recommend including those data into the graphs reporting about the microbial community changes.

***We chose not to include them in the main figures for 2 reasons. Firstly they are not directly comparable, this location is subject to water movement and during the experiment distinctly different water masses with different physical and biological signatures moved into the surrounding water. Secondly, and due to the previous reasoning, including the phytoplankton abundances in the surrounding waters makes the figures more difficult to read. Occasionally the abundances are much greater than in the mesocosms and it is then harder to discern differences between the mesocosms (see Supplementary Table S1 and Fig S1). Overall, microbial temporal dynamics are largely comparable, with a few exceptions: i.e., phytoplankton Nano I and II show much higher abundances in the outside water whilst all the picoplankton abundances are lower in the surrounding waters. We will add a description on the outside water microbial dynamics in the Results section of the revised manuscript, and add discussion on what may have caused the differences.***

3. Also, I am not sure if I have understood it correctly – but it seems that CO<sub>2</sub> was added to all mesocosms? In that way you do not have control and any change in microbial community could have been because of some other factor?

***We realize that the text was not clear and have improved this in the revised manuscript, i.e., all mesocosms were sparged with water so that a similar water treatment occurred, but no CO<sub>2</sub> was added to the mesocosms that served as present-day controls.***

4. What about the temperature?

***The temperature was similar for all mesocosms as well as the surrounding water and therefore can only potentially have influenced the dynamics of the microbial populations but not the extent of change between the different mesocosms. We present temperature now briefly at the start of the Results section (of the revised manuscript).***

5. What is the usual phytoplankton development dynamics in the Baltic Sea?

***We now briefly commented on this at the start of the Discussion.***

6. Also, I do not see any shifts in microbial community, but changes abundances during the experiment.

***We acknowledge that and clarify in the Discussion of the revised manuscript that the extent of temporal dynamics differed for the high pCO<sub>2</sub> mesocosms (and not the dynamics***

***itself). Also we changed the title of the manuscript to “Shifts in the size structure of the microbial community in the Baltic Sea with increasing CO<sub>2</sub>” to make this clear.***

7. Since every experiment need to be repetitive and results comparable with other study site and experiment set up it would be necessary to know the community structure of microbial community. I am aware of the difficulty of taxonomical recognition of size fraction below 20µm, but it is essential. Flow cytometry in an excellent tool, but in an experiment set up of this range I do not believe it is enough. I did like the way how the investigated groups were divided, but the next step in research should be the taxonomical identification.

***We agree that taxonomic identification would be a next step but would need flow cytometry sorting and genomics, which goes beyond the scope of the current study. Linking to taxonomic identification by phytoplankton pigment composition analysis is only partly possible as a few large cells may obscure the share of a certain group as compared to total Chl a. Paul et al. (2015) showed that the smaller fraction (<2 µm) was likely to be chlorophytes and prasinophytes. This is mentioned in the Discussion (section 4.1).***

8. Not all organisms in the same size fraction have same physiological response to environment drivers.

***We recognise this and discussed this in relation to the Pico I and Syn data. We will add a similar line of reasoning for potential differences within a group even as not all are necessarily the same species.***

9. Also, the authors in the discussion, do not discuss their data, but cite different authors and their research. If you did not go to the species level – those data cannot be discussed.

***We checked the Discussion and amended where needed.***

10. The analyzed groups were good explained except the cyanobacteria. The authors distinguish Synechococcus, but no Prochlorococcus. Since the oligotrophy Prochlorococcus could develop in the environment and it can be separated by flow cytometry. Then stated that the prokaryotes include bacteria, archea and unicellular cyanobacteria (together marked as HP) – what cyanobacteria could it be?

***Prochlorococcus can indeed be distinguished by flow cytometry, but was not present during this experiment. Therefore cyanobacteria and Synechococcus are used interchangeably during the manuscript. We will make a statement that Prochlorococcus was not observed (Results section).***

11. The tittle also does not represent the results – there is not shift in microbial community presented.

**We have altered the title to “Shifts in the size structure of the microbial community in the Baltic Sea with increasing fCO<sub>2</sub> “ to more accurately reflect the results.**

12. It is not easy to follow the results and discussion with given abbreviations (M1, M5. . .) for mesocosm experiments. It is not clear enough. Maybe a table would be a good way to explain the abbreviations.

**The notation used is consistent across all manuscripts in this special issue and the mesocosms with their mean fCO<sub>2</sub> are presented in Fig. 1. However, we see Reviewer’s point of view and will include a Table with the necessary information. See Attachment 1.**

**Table 1. fCO<sub>2</sub> concentrations (µatm) as an average for the duration of the experiment following CO<sub>2</sub> addition and specification of this CO<sub>2</sub> level as low, medium or high. \*denotes mesocosms sampled for grazing and viral lysis assays**

<b>Mesocosm</b>	<b>M1*</b>	<b>M5</b>	<b>M7</b>	<b>M6</b>	<b>M3*</b>	<b>M8</b>
<b>CO<sub>2</sub> Level</b>	<b>LOW</b>	<b>LOW</b>	<b>INTERMEDIATE</b>	<b>INTERMEDIATE</b>	<b>HIGH</b>	<b>HIGH</b>
<b>Mean fCO<sub>2</sub> (µatm) days 1-43</b>	<b>365</b>	<b>368</b>	<b>497</b>	<b>821</b>	<b>1007</b>	<b>1231</b>

13. The discussion needs to be rewritten. The results would need to be discussed in more detail. The cited literature and results are maybe not the best choice for the results presented.

**It is not clear which examples the reviewer is referring to. However, we have tried to improve the discussion according to Reviewer’s comments.**

**Paul, A. J., Bach, L. T., Boxhammer, T., Czerny, J., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., Riebesell, U., Road, M., Lismore, E. and Way, E.: Effect of elevated CO<sub>2</sub> on organic matter pools and fluxes in a summer , post spring-bloom Baltic Sea plankton community, *Biogeosciences* , 1–60, 2015.**