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# *Interactive comment on* "Micro- and mesozooplankton community response to increasing $CO_2$ levels in the Baltic Sea: insights from a large-scale mesocosm experiment" *by* S. Lischka et al.

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Received and published: 27 April 2016

Interactive comment on "Micro- and mesozooplankton community response to increasing CO2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment" by S. Lischka et al.

Anonymous Referee #1 Received and published: 15 February 2016

General comments

Ref #1: The manuscript by Lischka et al. presents relevant data on the impact of pCO2 on plankton communities in the Baltic Sea. The data was obtained during a mesocosm study in Tværminne, Sweden, using natural plankton communities during a summer situation. The focus of the present study was on micro- and mesozooplankton communities and their vulnerability to changes in ocean pH. In addition, ambient temperature and chlorophyll a (as a proxy for phytoplankton biomass) were considered as additional factors in order to relate these to changes in micro- and mesozooplankton abundances. While the overall aim of the present study as well as the experimental approaches addressed are of great relevance, the manuscript has some considerable shortcomings. The ms is written in a very descriptive manner presenting many details on specific taxonomic groups/species/genera while a thorough elaboration of the main results and conclusions is missing. The way the data is presented should be re-considered in order to concentrate on the main important results instead of including too many details (e.g. showing both abundance data of each specific group and the percent contribution of major taxonomic groups each in a separate graph).

Author response: We thank referee #1 and appreciate the very constructive and helpful comments that will help improving the scientific merit of our manuscript substantially. In response to the general comment, we agree to focus better on our main results, re-consider the figures presented and more thoroughly interpret our data with respect to trophic interactions (s.b.). Please find our detailed point-by-point response to all comments including suggested modifications in the following.

Ref #1: The authors should consider converting abundance data into carbon biomass in order to relate micro- and mesozooplankton biomass developments to each other and to allow comparisons with previous studies addressing similar research questions.

Author response: We had considered estimating carbon biomass but refrained, because due to time constraints we were not able to do an adequate amount of size/volume measurements of each species/stage from each sample. Without reasonably accurate size/volume measurement, respectively, we think carbon biomass

estimations would be far too imprecise and potentially misleading and, therefore, we preferred to show abundance data instead of biomass estimation.

Ref #1: While the statistical analyses performed are of good quality, biotic factors influencing micro- and mesozooplankton succession patterns need further considerations. So far, the study addresses each zooplankton group separately rather than relating both zooplankton groups to each other and considering predator-prey relationships.

Author response: We agree with referee #1. In a revised version we will consider predator-prey relationships between MiZP and MZP more closely by doing some correlations between potential predators and prey. However, unfortunately, we must point to the fact that MZP was not exactly sampled synchronously (i.e. not always on the same day) with the MiZP limiting possible correlations between the two groups to a relatively small number of concurrent observations.

Ref #1: Total chlorophyll a is used as a single factor to explain relationships between autotroph and heterotroph fractions in the plankton but the study would benefit substantially from taking e.g. different size fractions or taxonomic groups of phytoplankton as potential prey items for microzooplankton into consideration and by addressing predator-prey relationships between micro- and mesozooplankton.

Author response: This comment is quite similar to the previous. We appreciate the suggestion of referee #1 and will accommodate for it in a revised version by including in the suggested correlations also specific phytoplankton groups.

Ref #1: While the authors stress the relevance of microbial food webs and the link to classical food webs at the very end of the discussion section, trophic interactions are scarcely addressed so far. With regard to ocean acidification, especially such interactions between taxonomic groups/species need to be considered, in order to account for direct and indirect effects on plankton communities and their vulnerability to future OA conditions.

Author response: See our response to the two preceding referee comments. We will consider trophic interactions more closely in a revised version.

Specific comments Introduction

Ref #1: The introduction should focus more strongly on trophic interactions between autotrophs and heterotrophs as well as on the links between micro- and mesozooplankton under present and future OA conditions.

Author response: We will include a paragraph focusing on trophic interactions and links between micro- and mesozooplankton under present and future OA conditions.

Ref #1: L. 84: It is mentioned that the category 'microzooplankton' comprised ciliates only. What about other microzooplankton groups (e.g. radiolaria, heterotrophic dinoflagellates)? Where those groups not present at all or where they not included into the analysis? The term 'microzooplankton' traditionally refers to a specific size fraction (20-200 $\mu$m) which also includes copepod nauplii. If only ciliates are included into this category, it would be more appropriate to term the category 'Ciliates'.

Author response: Other microzooplankton groups such as heterotrophic dinoflagellates were present but not part of this analysis. Data on heterotrophic dinoflagellates are shown in Spilling et al. 2015. Radiolarians were not present. With respect to the termination we agree with referee #1 and will change what we termed 'microzooplankton' to category 'Ciliates'.

Material & methods

Ref #1: Myrionecta rubra is listed as a 'phototrophic' ciliate. In fact, it is more precise to term it 'mixotrophic' because this species can switch from autotrophic to heterotrophic feeding modes.

Author response: We will change 'phototrophic' to 'mixotrophic'.

Ref #1: It is mentioned that the strobilid Lohmaniella oviformis was included into the

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category 'Strobilid < 20 $\mu$m' due to uncertainties in a more detailed identification. Usually, L. oviformis is one of the few ciliate species that shows distinct morphological characteristics even in Lugol-preserved samples. Since L. oviformis often plays a key role in temperate marine systems, it would be helpful to have this species separated from other Strobilids. Any chance to achieve such a separation from the analyzed data still?

Author response: Unfortunately, a clear separation of Lohmaniella from other Strobilids < 20 $\mu$m is not possible anymore. However, most of these small Strobilids probably were Lohmaniella. So, we suggest adding a sentence mentioning this.

Ref #1: The authors mention that 3 different phases (I-III) were defined according to temperature variations. The temperature changes presented here are in fact auto correlated with changes in succession/seasonality patterns since temperatures in the mesocosms reflect natural thermal conditions with ongoing season. Why was temperature chosen to define different phases of the experiment instead of using e.g. chlorophyll a as a proxy for seasonal succession patterns?

Author response: Variation in chlorophyll a pretty much coincided with temperature fluctuations but was not as pronounced. Thus it was more obvious to define the different phases by the pronounced temperature phases that started with a warmer phase, followed by a cooling and a subsequent warming. However, data analysis in the present study did not follow this phase definition but was done on the complete dataset.

Results General Comment:

Ref #1: The authors should consider converting abundance data into carbon biomass in order to relate micro- and mesozooplankton biomass developments to each other and to allow comparisons with previous studies addressing similar research questions.

Author response: Please see our response above.

Ref #1: Figure1: It would be helpful if the 3 different phases of the experiment would

be mentioned within Figure 1. Further, adding temperature and total chlorophyll a as additional y-axes will help to improve the interpretation of the results.

Author response: We will mention the 3 different phases in the figure caption. However, we think, including temperature and chlorophyll a as additional y-axes would overload the graph as it would result in 12 extra lines. Therefore, in a revised version we could split the plot into 6 different subplots separated by fCO2 and include temperature and chlorophyll a as additional y-axes.

Ref #1: Figure 2: Is there data available to include e.g. specific phytoplankton size fraction or succession patterns into the graphs to show responses of individual micro-zooplankton groups/species to available prey items (e.g. phytoplankton).

Author response: Principally, these data are available and were mostly included in the overview paper to this study (Paul et al. 2015) and some others are shown in Spilling et al (2015). In general we agree with the referee's comment, but this suggestion would again result in an overloaded graph as we would have to include data of all different fCO2 treatments. Alternative could be subplots as suggested above or to do some correlation plots to show potential relations between predator and prey. We will try this out and, if meaningful, present respective plots in a revised version.

Ref #1: In addition, is bacteria data e.g. from flow-cytometry available the account for bacteria-microzooplankton interactions?

Author response: Bacteria data are presented in the manuscript by Hornick et al. (2016). In a revised version of our manuscript, we can pay particular attention to bacteria/microzooplankton interactions, for example look for correlations and/or if meaningful include those in respective figures.

Ref #1: Figure 3+4a: Instead of showing percent contributions of each species/genera/group in separate graphs, it is recommended to sort the data by CO2-treatment and create stack plots showing the relative shares of species/genera/group

over the course of the experiment.

Author response: We will change Figure 3 accordingly.

Ref #1: The diversity data (H) could be included into the individual graphs by adding an additional y-axis to the plot (showing H values over the course of the experiment). This would facilitate the interpretation of the results.

Author response: Fig. 4a is meant to visualize the significant change in Shannon diversity with the daily change in fCO2. In Fig. 3, percent contribution of specific groups is plotted against the mean fCO2 in a treatment. Including H values over the course of the experiment into the individual graphs by adding an additional y-axis wouldn't result in the same resolution of change in H, therefore we would like to keep Fig. 4a as it is. But we will try out what gain the addition of H values in a new Fig. 3 would bring and, if meaningful, present H values over time in Fig 3 also.

Ref #1: Figure 4b: This graph illustrates the relationship between the mean temperatures during specific phases of the bloom and microzooplankton diversity (H). The factor temperature was not manipulated within the present study and thus reflects the natural thermal conditions in the seawater with ongoing season. The changes in microzoo diversity point rather at changes in H at different succession phases of the plankton community rather than temperature-induced changes. Such changes in successional phases could rather be explained by chlorophyll a development than temperature. Why was temperature chosen as a factor characterizing these phases. It seems not convincing that the observed changes in diversity are in fact related to temperature changes.

Author response: Chlorophyll a was included in the initial model but was not significant and therefore removed during model selection.

Ref #1: Figure 5: Similar to Figure 1 it would be helpful to include the 3 different phases of the experiment to Figure 5. In addition, temperature, chlorophyll a and total ciliate abundance/biomass should be added (additional y-axes).

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Author response: We will include the 3 different phases in the figure caption. However, as mentioned above, we think, including temperature, chlorophyll a and total ciliate abundance as additional y-axes would overload the graph as it would only make sense to include them per fCO2 treatment resulting in 18 extra lines. To overcome this problem we will try out with subplots (s.a.) and show them if reasonable.

Ref #1: Figure 6+7a: The ms would benefit considerably if potential prey items could be included into the graphs (e.g. specific phytoplankton and ciliate size fraction/groups/species) which might explain some of the succession patterns found in mesozooplankton groups. It seems that e.g. total copepods could be nicely related to Strombidium cf. epidemum or Strobilidium sp. < 20 $\mu$m.

Author response: As mentioned above already, in general we agree with the referee's comment, but, again, this suggestion would result in an overloaded graph. An alternative could be to do some correlation plots (copepods vs Strombidium for example) to show potential relations between predator and prey. We will try this out and, if meaningful, present respective plots in a revised version.

Ref #1: Figure 7b: Similar to Figure 3+4, stack plots showing the relative contributions of mesozooplankton species within the different CO2-treatment would allow a better interpretation of the data.

Author response: We will prepare stacked plots in a revised version.

Ref #1: Figure 8 a+b: Since Bosmina seemed to be the most relevant cladoceran species in this study, it is suggested to reduce the number of graphs dealing with cladocerans and focus predominately on Bosmina.

Author response: We will adhere to this comment and reduce the amount of figures showing cladocerans focusing on Bosmina.

Discussion 4.1.1:

Ref #1: Changes in MiZP diversity are discussed within the framework of tempera-

ture increases. Temperature is treated as an additional explanatory variable to relate changes in MiZP to thermal conditions. Such explanations need to be treated with caution, since this relates back to increases in temperature during the summer season and reflect rather different succession phases than direct temperature effects.

Author response: We agree with referee #1 and will change the text accordingly pointing to a more general effect of temperature with the natural succession of MiZP during the summer season in line with Rose et al. (2009).

Ref #1: Overall, effects of temperatures are considered within the present ms at some points without reasoning why temperature changes are expected to change zooplankton com- munities and diversity and why this is an important aspect in the context of OA.

Author response: We mentioned in the introduction (p 20029, L17–23) that temperature can have a general effect on MiZP abundance and community composition and can also govern the dynamics of crustacean species. OA happens concurrently with ocean warming, i.e. it is important not only to estimate how CO2 changes may affect plankton communities but also temperature changes. Though it is not possible to manipulate temperature in the large mesocosms, we wanted to use the natural temperature variability over the experimental period to get an estimate on the importance of temperature changes on the plankton communities.

Ref #1: 4.1.2: The authors point at significant responses of the mixotroph ciliate Myrionecta rubra to all factors included into this analysis. While the significant responses are undoubted, the magnitude of changes in M. rubra abundance in relation to a higher pCO2 need to be taken into consideration when stressing the overall benefit of OA to this ciliate species. M. rubra showed extremely high numbers at the beginning of the experiment and strong declines thereafter. From day 20 onwards this species showed significantly higher abundances in the high compared to the low CO2 treatments. However, compared to initial values, M. rubra abundances where overall rather low and the

results seem to be over-interpreted. The argument that increased CO2 will strongly stimulate growth in M. rubra needs to be re-considered.

Author response: We agree with referee #1 and will reconsider and tone down our argumentation accordingly. Growth stimulation of M. rubra at higher CO2 levels seems to be of some importance only in the post-bloom phase.

Ref #1: Further, it is stated that in the absence of cryptophytes, M. rubra sustains a larger biovolume while when cryptophytes are present the biovolume is reduced. This contradicts to observations from this study where high abundances of cryptophytes were observed during phase 1 (L. 474) of the experiment when the community was dominated by M. rubra (<10 $\mu$m). In addition, highest abundances of cryptophytes were also found during phase II and II (L. 477). As a suggestion, the authors could consider to correlate cryptophyte abundances with the different size classes of M. rubra in order to analyse predator-prey relationship in this species in more detail.

Author response: We will pick up this suggestion and do the suggested correlation to get a better insight into possible predator-prey relationships.

Ref #1: So far, arguments provided on e.g. higher CO2 –mediated photosynthetic rates and potential relationships with cryptophyte availability (L. 491ff, L. 499 ff) are quite speculative. Overall, the whole section on benefits of M. rubra from OA seems overinterpreted and vague.

Author response: We agree with referee #1 that this paragraph contains some speculations but think that they are not completely unfounded as outlined in the text and though speculative may be part of an explanation of observed differences in chlorophyll a during phase II and III. In a revised version we suggest to cut this section to a minimum but keep the main statements that we think could be likely explanations.

Ref #1: 4.2: While the relevance of the microbial loop and the central role of heterotrophic protists as a trophic link to higher trophic levels is stressed within the con-

clusion section at the very end of the ms, the microzooplankton- mesozooplankton relationship is not considered at all in the discussion section. This is astonishing since direct interactions between these two zooplankton groups are of substantial importance and changes in e.g. prey items in relation to OA are likely to be directly transferred to the next trophic level. The lack of a solid interpretation of data with regard to predator-prey relationships is thus considered as a major shortcoming of the present study.

Author response: Please see above our response to the respective comments to the results section. We will analyze predator-prey relationships in more detail in a revised manuscript and discuss results accordingly.

Ref #1: 4.2.3: Feeding modes of cladocerans are nicely described within this section. It is stressed that cladocerans can effectively feed on bacteria and flagellates thus effectively channeling carbon from the microbial loop to higher trophic levels. The authors state in L. 654 that this is in contrast to copepod-dominated systems where an intermediate trophic levels is missing thus concluding that OA might support cladoceran growth and enhance trophic transfer to higher trophic levels. This is not a convincing argument since copepod-dominated systems can highly depend on secondary production from the microbial loop (by feeding effectively e.g. on ciliates and heterotrophic dinoflagellates) instead of relying only on phytoplankton production following the classical food web model. The section does not consider any effects of cladocerans on the MiZP community within the mesocosms. Any indication for a suppression of MiZP abundance by Bosmina?

Author response: This comment is in line with some previous comments and also asks for more detailed analyses of possible trophic interactions. As mentioned above already, we will deal with this and look at predator-prey relationships more closely and modify this part of the discussion accordingly.

Ref #1: Conclusions The conclusions need to be mitigated according to the data and arguments provided.

Author response: Will be considered in a revised version.

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