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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

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General comments 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 Tvärminne, 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

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Interactive Discussion

Discussion Paper



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). 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. 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. 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. 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. Specific comments Introduction 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. 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

C9742

BGD

12, C9741–C9746, 2016

[Interactive
Comment](#)

[Full Screen / Esc](#)

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[Interactive Discussion](#)

[Discussion Paper](#)



or where they not included into the analysis? The term ‘microzooplankton’ traditionally refers to a specific size fraction (20-200 μm) which also includes copepod nauplii. If only ciliates are included into this category, it would be more appropriate to term the category ‘Ciliates’.

Material & methods – 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. It is mentioned that the strobilid Lohmaniella oviformis was included into the category ‘Strobilid < 20 μm ’ 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? – 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? Results General Comment: 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. 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. 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 microzooplankton groups/species to available prey items (e.g. phytoplankton). In addition, is bacteria data e.g. from flow cytometry available the account for bacteria-microzooplankton interactions? Figure 3+4a: Instead of showing percent contributions of each species/genera/group in sep-

C9743

BGD

12, C9741–C9746, 2016

Interactive
Comment

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Interactive Discussion

Discussion Paper



arate 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. 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. 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. 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). 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 μm. 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. 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.

Discussion 4.1.1: Changes in MiZP diversity are discussed within the framework of temperature increases. Temperature is treated as an additional explanatory variable to relate changes in MiZP to thermal conditions. Such explanations need to be treated

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Discussion Paper



with caution, since this relates back to increases in temperature during the summer season and reflect rather different succession phases than direct temperature effects. Overall, effects of temperatures are considered within the present ms at some points without reasoning why temperature changes are expected to change zooplankton communities and diversity and why this is an important aspect in the context of OA. 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 were 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. 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 μm). 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. 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 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 conclusion 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. 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*? Conclusions The conclusions need to be mitigated according to the data and arguments provided.

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12, C9741–C9746, 2016

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