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Revision 2 of our manuscript bg-2015-618

November 30, 2016

Dear Corina,

please find enclosed the second revision of our manuscript for the BG Special Issue "*Effects of rising CO2 on a Baltic Sea plankton community: ecological and biogeochemical impacts*" entitled „*Ciliate and mesozooplankton community response to increasing CO2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment*".

We have revised our manuscript according to your Decision letter. Please find all details on realized changes in the response to all points raised. Herein also page/line numbers of revised mansucript versions where changes have been performed are stated.

We have also attached revisions of our original point-by-point-response letters to the two reviewers. These include complemented replies to some general comments made by Ref. #1 and Ref. #2 as well as indications of pages/line numbers in the different manuscript versions refering to the performed changes. Specifically, in these response letters, the heading „**Author's initial response (prior first resubmission)"** indicates our response prior invitation to resubmit a revised manuscript version. The heading "**Changes performed in revision 1"** describes the changes that were done on the original manuscript, and under heading "**Author response (added 25./28.11.2016)"** we provide requested information indicating what has been changed in revision 1 and state page/line numbers of the different revisions.

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Please don’t hesitate to contact me in case further questions should arise.

We hope to have revised our manuscript to your satisfaction and would appreciate if it could be considered now for publication in Biogeoscience.

Thank you very much.

Best regards,

Silke

Associate Editor Decision: Reconsider after major revisions (27 Sep 2016) by Prof C.P.D. Brussaard
Comments to the Author:

Both reviewers raised the issue of not having all zooplankton groups included. Authors' reply that these groups were not part of the analysis but can be found in other papers is scientifically not strong. It is for the reader of the paper unclear why these were left out and the focus was only on the ciliates.
- Author response: To make it clear for the reader straight from the beginning, in revision 2, we have included two sentences in the introduction mentioning that our manuscript focuses on ciliates and mesozooplankton and refer to Bermudez et al. (2016, part of this special issue) that includes protozoa other than ciliates (L90–92 in track-changed version of revision 2; L89/90 clean version of revision 2). We agree, it is a bit unfortunate to have these data separate. However, this procedure was agreed upon among all participants of the study prior to the manuscript writing and publication process.

Why were these data not requested from partners in this mesocosm study, to be included in the analysis in the current paper? The focus is different than the paper(s) referred to, and as such would produce a nice complementary study. The goal that communities are studied does not hold by excluding important groups such as the heterotrophic dinoflagellates.
- Author response: We have picked up the suggestion made by Ref. #1 and included analyses of different kinds of possible predator/prey relationships to provide insight on possible CO2 related changes in trophic interactions. The performed Pearson correlations included among a variety of others also heterotrophic dinoflagellates for which a positive correlation with M. rubra was found (Table 2, Supplemental material). We refer to the paper by Bermudez et al. (2016) that includes detailed analyses of different phytoplankton and protozoan communities and link our results to the findings on Dinophyta described herein. During phase II, Dinophyta showed a negative trend with increasing CO2 consistent with the significant CO2 related increase in numbers of M. rubra during that time. Furthermore, Crawfurd et al. reported a high grazing pressure of microzooplankton during that time. In revision 2, we have added text in the respective section 4.1.3 and discuss these possible predator/prey relationships in relation to increased CO2 (L 482–486 in tracked-changed version of revision 2; L479–483 of clean version of revision 2).

Not estimating carbon biomass (as strongly argued for by Ref #1) because of time constraints is not a good argument for a paper with the objective to look at zooplankton community and species/taxon in response to CO2 enrichment. This aspect is very important for answering the research question, and needs to be addressed/discussed. Best would be to include size/biovolume after all. Either way, please check current conclusions and edit (toned down) where needed; holds in particular for conclusions on zooplankton biomass development and carbon flux.
- Author response: In revision 2, we have addressed the issue discussing possible differences in our results if estimated biomass was used instead of abundance data. Mostly this problem applies to conclusions drawn from significant findings determined for Myrionecta and Bosmina (no need to bother about this in case of non-significant results). In case of Myrionecta, the CO2 effect was found for small cells of more or less the same size, i.e. calculated biomass data should scale proportionally with abundance but the overall result/conclusion should not change. In case of Bosmina, the significant brood-chamber ratios are independent of abundance or biomass. The pronounced abundance increase would probably be smaller if biomass was calculated. However, given that abundances in some mesocosms more than doubled, the increase would still exist. Thus, our conclusions drawn for carbon flux still basically remain the same. We have included a respective paragraph in the discussion section 4.2.4 (L631–638 in track-changed version of revision 2; L623–630 in clean versision of revision 2).
Moreover, we are really convinced that carbon biomass estimates from literature size/mas relationships would result in a too high inaccuracy with the potential to rather falsify than

substantiate our results and conclusions drawn. Notably this applies to estimations of developmental stages (nauplii, copepodids) for which size/mass relationships are mostly not available. Fig. 7a and 7b indicate that copepod nauplii and developmental stages mostly dominated the copepod community. Applying size/mass relationships established for adult copepods can obviously not yield to some meaningful results.

There is the issue of plotting against temperature instead of Chlorophyll because Chl was not found significant. This needs improved discussion why it was not significant, and please include more/clearer discussion as to how the authors this affects the results (since succession of the predators depends on changes in prey community and growth, and predation pressure by higher trophic levels).
- Author response: We have included a paragraph in section 4.1.2 (L449–460 in track-changed version of revision 2; L447–458 in clean version of revision 2) discussing this issue. What we see in our data is that H increases towards the end of the experiment (i.e. the dominance of single species decreased, which was mainly due to the fact that Myrionecta was not so much dominating anymore, Fig. 3a, c) when chlorophyll a decreased/was low. That implies, that other predator prey relationships other than ciliate/chlorophyll became more important and that the contribution of species that were less important during the beginning of the experiment increased (mostly Strombidium sp.). Together with a decreasing importance of Myrionecta, this resulted in a higher Shannon index.

How do the temporal changes in zooplankton relate to CO2 elevations, i.e. the goal of this study? The predator-prey relationships form an important aspect and actual grazing rates are available from other partners in this project. These may provide arguments in favour or against conclusions currently drawn from correlations in the revised manuscript.
- Author response: We picked up on this comment in section 4.1.3 where we discuss the significant findings found for Myrionecta rubra. According to Crawfurd et al., microzooplankton grazing pressure was responsible for the losses of PICO III in phase II. This is in support with the strong positive correlation we found for M. rubra and Cryptophytes and Dinophyta from Chemtax analysis and cell counts. Accordingly, we have added a sentence to this paragraph (L481–486 in track-changed version of revision 2; 479–483 in clean version of revision 2).

The Discussion on predator-prey relationships deals largely with the earlier finding that the microbial loop is of particular importance during the summer (at present only reference to older publications while the current special issue has papers dealing with this topic; I recommend to include those as well).
- Author response: We have revised this discussion section and now better incorporate results from other publications of this special issue (e.g. Crawfurd et al., Hornick et al., L597, L602–608, L613–615 in track-changed version of revision 2; L594, 599–605, 610–612 in clean version of revision 2). Please see also our response to the following comments that address similar issues.

The authors do include in the revised manuscript discussion on the paper referred to by Ref #2, however, the comments by Ref #2 are more general: enhanced flow through the microbial loop tends to decrease food web efficiency. This needs to be addressed better (check also papers on topic in the current special issue).
- Author response: We have revised the respective paragraph and discuss the statement put forward by Wikner and Andersson (2012) in better connection with results of the current special issue. Results from Crawfurd et al., Hornick et al. and Paul et al. also suggest increased importance of the microbial loop, i.e. to some extent agree with Wikner and Andersson. However, in the presence of consumers that are able to exploit microbial production such as cladocerans, food web efficiency must not necessarily be diminished. Respective text additions can be found in L623–627 of the track-changed version of revision 2; and in L617–622 of clean version of revision 2.

Some other points:

1) Please indicate in the reply to reviewers what exactly has been edited/changed and state page and line numbers of the revised manuscript (this holds for the original as well as the current review comments).

- Author response: We have amended our original point-by-point-response to Ref. #1 and Ref. #2 and included references to line numbers and pages of the different manuscript versions where changes have been performed.

2) Check whether all points raised by reviewers are considered and state clearly why what has been done to address them (e.g. not all general comments by Ref #1 were addressed).

- Author response: We have checked all comments made by the two reviewers and tried to better make clear why we did what and where, in particular we included response to general comments made by Ref. #1 that were not adequately addressed earlier. We also included reference to line numbers and versions where changes were done. We have included a revised point-by-point-response to both reviewers in the submission of this revised draft manuscript.

3) Some figures still state microzooplankton instead of ciliates.

- Author response: We have corrected Fig. 1 and changed “microzooplankton” into “ciliates”.

4) State genus/species names italic (e.g. Tables).

- Author response: We have checked all tables and figures and made corrections accordingly (Fig. 1, 2b, 2c, 3a, 6, 7a, 7b, 9).

5) Please provide the correct information when referring to published papers by partners in this field campaign; e.g. Picoflagellates in the paper of Crawfurd et al. (special issue) are not all flagellated and should instead be cited as originally stated (picoeukaryotic phytoplankton, Picoeuk I etc.).

- Author response: We have corrected notations for pico- and nanoeukaryotic cells.

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.

- Author's initial response (prior first resubmission): 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.

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 (added 25.11.2016): We have reconsidered our results and conclusions and widened data elaboration and discussion with respect to possible changes in trophic relationships resulting from CO2 elevations. Please see our response to the specific comments for details on the changes/additions performed.

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 initial response (prior first resubmission): 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's initial response (prior first resubmission): 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.

- Changes performed in revision 1: In the revised version we did several correlations between MiZP, MZP and phytoplankton groups and also bacterial data were included to reveal potential predator-prey relationships. We used Pearson correlation to describe the strength of all specific pairs. In the main document we have included in Table 2 the strongest and most important correlations and show the respective pairplots and Pearson correlations in the supplemental material (Fig. S1–S2).

- Author response (added 25.11.2016): Furthermore, we have included sections 2.3.3 (p7 L10–15 track-changed version of revision 1; L224–230 in track-changed version of revision 2; L222–228 of clean version of revision 2;), 3.2.6 (p12 L5–13 track-changed version of revision 1; L386–395 in track-changed version of revision 2; L385–393 in clean version of revision 2) and 4.2.4 (p17 L11–p18 L36 track-changed version of revision 1; L589–615 in track-changed version of revision 2; L586–622 in clean version of revision 2) to explain the approach, report and discuss the main results.

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's initial response (prior first resubmission): 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.
- Changes performed in revision 1: See our response just above. Different phytoplankton taxonomic groups were included in the pairwise correlations addressing predator/prey relationships (Table 2, supplemental material).
- Author response (added 25.11.2016): As mentioned above, we have included respective text passages to explain the approach, report and discuss the main results (please see above for details on where these changes were included).

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's initial response (prior first resubmission): See our response to the two preceding referee comments. We will consider trophic interactions more closely in a revised version.
- Changes performed in revision 1: See our response to the previous two referee comments. However, as a main focus of the manuscript is on community changes as such, we haven't extended the predator/prey considerations too much.
- Author response (added 25.11.2016): We have further revised and amended the discussion. Please see our response to the last Editor Decision letter where line numbers of performed changes in revision 2 are indicated.

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's initial response (prior first resubmission): We will include a paragraph focusing on trophic interactions and links between micro- and mesozooplankton under present and future OA conditions.
- Changes performed in revision 1 We have included a paragraph with some more details on the food web structure in the Tvärminne region (L73–82).
- Author response (added 25.11.2016): Changes can be found in L73–82 of clean version of revision 1; p3 L2–9 track-changed version of revision 1; L69– 78 of clean version of revision 2; L69–78 of track-changed version of revision 2.

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 µm) which also includes copepod nauplii. If only ciliates are included into this category, it would be more appropriate to term the category 'Ciliates'.

- Author's initial response (prior first resubmission): 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. 2016. Radiolarians were not present. With respect to the termination we agree with referee #1 and will change what we termed 'microzooplankton' to category 'Ciliates'.

- Changes performed in revision 1: In the revised manuscript, we have changed the category 'microzooplankton' to 'ciliates'.

- Author response (added 25.11.2016): This issue was picked up again in the Editor Decision letter. Please see our response included in the Decision letter.

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's initial response (prior first resubmission): We will change 'phototrophic' to 'mixotrophic'.

- Changes performed in revision 1: We use 'mixotrophic' now.

Ref #1: 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?

- Author's initial response (prior first resubmission): Unfortunately, a clear separation of Lohmaniella from other Strobilids < 20 µm is not possible anymore. However, most of these small Strobilids probably were Lohmaniella. So, we suggest adding a sentence mentioning this.

- Changes performed in revision 1: We have included an according sentence in the M&M and discussion section (L191–193).

- Author response (added 25.11.2016): As mentioned above, most Strobilids were probably Lohmaniella. A respective sentence was incldued in L191–193 (clean version of revision 1), on p6 L2–4 (track-changed version of revision 1), L181–183 (track-changed version of revision 2), and L179–181 (clean version of revision 2).

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's initial response (prior first resubmission): 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.

- Author response (added 25.11.2016): Please note also that phase definition was agreed upon by all project participants prior to the manuscript writing and publication process and therefore is consistently used throughout the different publications of this SI. Details on phase definition can be found in Paul et al. 2015.

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's initial response (prior first resubmission): Please see our response above.

- Author response (added 25.11.2016): We have further responded to this comment in the Reply to the Editor's Decision letter.

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's initial response (prior first resubmission): 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.

- Changes performed in revision 1: We mention the three different phases in the caption now. In addition, Chlorophyll a succession, temperature and fCO2 development is shown now in separate plots below total microzooplankton cell number. Note: In response to a comment to Fig 5 (s. below), in Fig. 1, we have also included mesozooplankton total abundance in order not to show Chl a, temperature and fCO2 development double.

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 microzooplankton groups/species to available prey items (e.g. phytoplankton).

- Author's initial response (prior first resubmission): 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 (2016) and Crawfurd 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. An 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.

- Changes performed in revision 1: As mentioned earlier, we have included correlation plots done in the supplemental material. Additionally, to acknowledge for the strong correlations found for the most important ciliate (Myrionecta) and cladoceran (Bosmina) species, we now also show succession patterns for Myrionecta/ Cryptophytes/ Cyanobacteria and Bosmina/ Cyanobacteria, respectively, in the new Fig. 9.

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

- Author's initial response (prior first resubmission): Bacteria data are presented in the manuscript by Crawfurd et al. (2016) and 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.

- Changes performed in revision 1: We have considered bacteria data in the Pearson correlations (Table 2, Supplemental material).

- Author response (added 25.11.2016): Interpretation of the respective results was included in discussion section 4.2.4 (see above).

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's initial response (prior first resubmission): We will change Figure 3 accordingly.
- Changes performed in revision 1: Fig. 3 has been re-arranged to show the percent contribution of different groups as stacked bar plots for each mesocosm and CO2 treatment, respectively.

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's initial response (prior first resubmission): 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.
- Changes performed in revision 1: Shannon index over time is shown now in Fig. 3b (for the different fCO2 mesocosms) and 3c (for the 4 defined different temperature phases). For the reasons mentioned above, Fig. 4a and 4b were not changed as they are meant to depict the statistical results.

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's initial response (prior first resubmission): Chlorophyll a was included in the initial model but was not significant and therefore removed during model selection.
- Changes performed in revision 1: The reason why temperature was chosen to characterize phases was explained earlier in response to a previous comment.
- Author response (added 25.11.2016): We reply further on this comment in the reply to the

Editor's letter. Chlorophyll was included in the statistical analyses but was not significant, whereas temperature was. Though maybe not intuitive, this can not be ignored either. Please see our response in the reply to the decision letter where we discuss possible reasons and mention where respective text passages have been added about this issue.

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).
- Author's initial response (prior first resubmission): 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 subplots (s.a.) and show them if reasonable.
- Changes performed in revision 1: Fig. 5 has been included now in Fig 1, s. comment above. The 3 different phases are mentioned in the figure caption.

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 µm.
- Author's initial response (prior first resubmission): 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.
- Changes performed in revision 1: As mentioned already, correlation plots can be found in the supplemental material, the most strongest Pearson coefficients are shown in Table 2, Fig. 9 visualizes relations between the most important species found in this study (Myrionecta, Bosmina, Cryptophytes, Cyanobacteria).

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's initial response (prior first resubmission): We will prepare stacked plots in a revised version.
- Changes performed in revision 1: Fig. 7b has been changed to a stacked bar plot.

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's initial response (prior first resubmission): We will adhere to this comment and reduce the amount of figures showing cladocerans focusing on Bosmina.

- Changes performed in revision 1: We have removed Fig. 8b (percent contribution of different cladoceran species) and only show total abundance of Bosmina. The occurrence of the other cladoceran species and the percent contribution of cladocerans is now only mentioned in the text. For the same reason, we have removed Fig. 9b (ratio of Podon sp. With empty and full brood chamber).

Discussion 4.1.1:

- Changes performed in revision 1: As general information, we have included additional subsection headings to the first paragraphs of the section "Ciliates" (4.1) and "Mesozooplankton" (4.2): "Ciliate succession" (4.1.1) and also "Mesozooplankton succession" (4.2.1). Therefore, all further section numbering has changed.

Ref #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 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's initial response (prior first resubmission): 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).

- Changes performed in revision 1: At the end of this paragraph (now 4.1.2, L476) we have included a sentence pointing to a more general temperature effect in line with Rose et al. (2009).

- Author response (added 25.11.2016): The respective sentence can be found on p13 L28/29 (track-changed version of revision 1), in L446–448 of track-changed version of revision 2, and in L444–446 of the clean version of revision 2.

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 communities and diversity and why this is an important aspect in the context of OA.

- Author's initial response (prior first resubmission): 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.

- Changes performed in revision 1: We have included a new sentence in the introduction pointing to the ongoing ocean warming concurrently with ocean acidification and the potential to impact species by providing suboptimal temperature conditions (L101–103).

- Author response (added 25.11.2016): The respective sentence can be found on p3 L24–26 (track-changed version of revision 1), in L98–100 of the track-changed version of revision 2, and in L96–98 of the clean version of revision 2.

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's initial response (prior first resubmission): 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.

- Changes performed in revision 1: We have considerably shortened this paragraph and toned down our argumentation in particular we point out that CO2 stimulation leading to higher abundances was only important during the post-bloom phase of Myrionecta.

- Author response (added 25.11.2016): Changes and reductions performed in section 4.1.3 can be seen in the track-changed version of revision 1. On p14 L17–20, we included the sentence mentioned above that growth stimulation of M. rubra was only important during the post-bloom phase (L502–505 of clean version of revision 1). In revision 2, the paragraph starts on p14, the respective sentence can be found in L486–489 of the track-changed version of revision 2, and in L484–486 of the clean version of revision 2.

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 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.

- Author's initial response (prior first resubmission): We will pick up this suggestion and do the suggested correlation to get a better insight into possible predator-prey relationships.

- Changes performed in revision 1: We have done the suggested correlation (supplemental material, Table 2, Fig. 9, all three Myrionecta size classes showed a strong correlation with cryptophyte occurrence.) and as mentioned above shortened this paragraph and rephrased to correct for the confusion with respect to the contradictions the referee had raised.

- Author response (added 25.11.2016): In particular, the sentence "In the absence of cryptophytes, they sustain a larger cell volume but exposure to cryptophytes stimulates incorporation and cell devision of M. rubra resulting in a decreased average cell but increased population size (hence biomass)" was removed (L470–473 original manuscript version). All performed reductions and changes done in section 4.1.3 can be followed from p13 L30–32 to p14 L1–20 of the track-changed version of revision 1. In the clean version of revision 1, section 4.1.3 goes from L480–505. In the track-changed version of revision 2, this section goes from L461–489, and in the clean version of revision 2 section 4.1.3. goes from L459–486.

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's initial response (prior first resubmission): 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.

- Changes performed in revision 1: Section was condensed and argumentation consolidated through correlations with cryptophyte abundances, s. above.

- Author response (added 25.11.2016): To accommodate further suggestions on this issue

pointed out in the Decision letter of the Editor, we have better included results from other publications of this SI. In particular, we mention that losses of picoeukaryotes III were mainly due to grazing of microzooplankton which is supporting our conclusion for M. rubra (please see more detailed response in our reply to the Decision letter). The added sentence can be found in L481–484 of the track-changed version of revision 2, and in L479–482 of the clean version of revision 2.

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 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.

- Author's initial response (prior first resubmission): 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.

- Changes performed in revision 1: We have inserted a new paragraph "Predator/ prey relationships" (4.2.4) and moved much of the former conclusion to this section. As the foucs of this manuscript is not on trophic interaction/ predator/ prey relationships in the strict sense but more on effects of CO2 on the zooplankton community in general, we have focused this paragraph on predator/ prey interactions of the species that turned out to be key species of our study (Bosmina sp., Myrionecta rubra). We have consolidated our argumentation with further references on "who eats whom" and included the paper mentioned by referee #2 by Wikner and Andersson. Beyond that we have no evidence for CO2 effects on predator/ prey relationships and therefore, don't want to extent the discussion on that topic much further. Furthermore, we have inserted a paragraph in the introduction where we give some information on the food web in the Tvärminne region (s.a.).

- Author response (added 25.11.2016): The new paragraph (4.2.4) can be found on p17/18 of the track-changed version of revision 1 (L12–33, L1–3), from L611–640 of the clean version of revision 1, from L589–638 in the track-changed version of revision 2, and in L586–630 of the clean version of revision 2. Further changes that have been made in response to the Editor Decision letter are detailed in our reply to the Decision letter.

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's initial response (prior first resubmission): 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.

- Changes performed in revision 1: The strongest Pearson correlation for Bosmina was in fact found for cyanobacteria. For ciliates, no particular strong relations were found that suggested feeding pressure of Bosmina. Somewhat higher correlations were found between Bosmina and Myrionecta (-0.5, -0.6, not shown) and (small) Strombidium (0.6, not shown). However, for Myrionecta this correlation seemed rather be connected with general species-specific succession patterns but rather not with feeding pressure. If required, we can provide these correlations plots in the supplemental material, too.

- We have shortened section 4.2.3 and base our argumentation in support of an indirect food effect on Bosmina abundances in three of the elevated CO2 mesocosms on the strong positive correlation found for Bosmina and Cyanobacteria and the CO2 mediated differences in Cyanobacteria during phase II.

- The remaining part of this reviewer's comments relates more to the conclusion and therefore is dealt with below.

- Author response (added 25.11.2016): Changes performed in section 4.2.3 are indicated in the track-changed version of revision 1 (p16 L8 – p17 L10). Most importantly we have cut down on description of general cladoceran biology and C/N content (in original manuscript version: L587–591, L593/594, L598/599, L599–604, L608/609, L613–618). In the clean version of revision 1, this paragraph goes from L567–610, in the track-changed version of revision 2 from L548–588, and in the clean version of revision 2 from L545–585. Mentioning of the strong positive correlation between Bosmina and Cyanobacteria is on p16 L24/25 of the track-changed version of revision 1 (respectively L587/588 of the clean version of revision 1, L567/568 of the track-changed version of revision 2, and L564/565 of the clean version of revision 2). Furthermore, section 4.2.4 on predator/prey relationships was revised

and includes some discussion on this topic, too (see details on revisions in our reply to the Decision letter of the Editor).

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

- Author's initial response (prior first resubmission): Will be considered in a revised version.

- Changes performed in revision 1: Conclusions have been customized accordingly.

- Author response (added 25.11.2016): The conclusions have been changed and mitigated considerably. In particular, we toned down our main conclusions with respect to Myrionecta and Bosmina (p17/18 in track-changed version of revision 1, L643–655 of clean version of revision 1).

References:

Crawfurd, K. J., Riebesell, U., and Brussaard, C. P. D.: Shifts in the microbial community in the Baltic Sea with increasing CO2, Biogeosciences Discussions, 2016.

Hornick, T., Bach, L. T., Crawfurd, K. J., Spilling, K., Achterberg, E. P., Brussaard, C. P. D., Riebesell, U., and Grossart, H.-P.: Ocean acidification indirectly alters trophic interaction of heterotrophic bacteria at low nutrient conditions, Biogeosciences Discuss., doi:10.5194/bg-2016-61, in review, 2016.

Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated CO2 on organic matter pools and fluxes in a summer Baltic Sea plankton community, Biogeosciences, 12, 6181-6203, doi:10.5194/bg-12-6181-2015, 2015.

Rose, J.M., Feng, Y., Gobler, C.J., Gutierrez, R., Hare, C.E., Leblanc, K., Hutchins, D.A. (2009) Effects of increased pCO2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Mar Ecol Prog Ser 388:27–40

Spilling, K., Paul, A. J., Virkkala, N., Hastings, T., Lischka, S., Stuhr, A., Bermudez, R., Czerny, J., Boxhammer, T., Schulz, K. G., Ludwig, A., and Riebesell, U.: Ocean acidification decreases plankton respiration: evidence from a mesocosm experiment, Biogeosciences Discuss., doi:10.5194/bg-2015-608, in review, 2016.

Biogeosciences Discussions

RC: Referee comment #2

S. Lischka, L.T. Bach, K.-G. Schulz and U. Riebesell

Micro- and mesozooplankton community response to increasing CO2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment

General comments

Ref #2: The ms. is interesting since it is one of the few studies where CO2 effects on whole plankton communities have been studied in ca. 55 m3 mesocosms. This provides a more realistic setting than single species experiments in smaller experimental units and allows for community effects to be realized.

- Author response (added 28.11.2016): We appreciate the general acceptance of our approach to study the response of whole plankton communities to varying CO2 in the large-scale mesocosms.

At the same time, the large mesocosm approach used provides some interpretation problems. With no replicate mesocosms in each of the manipulations, statistical analysis is difficult. The fact that the temporal variability of most species during the experiment greatly exceeds the minor differences between the CO2 manipulations makes difficult to detect any patterns caused by CO2. This problem has been partly but not wholly circumvented by using GAMM and GLM models.

- Author response (added 28.11.2016): We agree that temporal variability is much higher than the differences attributed to CO2. To factor this in, we applied mixed effect modeling (GAMM, GLM) to be able to separate different factor effects and to account for correlations inherent in the data, in this particular case time correlations. By including the factor time as random effect, correlations between data of different time points are accounted for in the estimated sigma. Accordingly, from our understanding, the significant CO2 effects described cannot be attributed to temporal variability but depict “true” CO2 effects.

Also, as with many community studies, it is very difficult to distinguish between direct and indirect (food web) effects, and many of the conclusions remain speculations.

The strongest evidence is found for (statistically significant) effects of temperature on microzooplankton abundance, and CO_2 effects on certain microzooplankton taxa. Indirect effects on cladocerans, instead, remain on a weak ground. Also, the suggested changes in the food web efficiency (enhanced carbon transfer to higher trophic levels) due to increase of cladocerans are not fully warranted and are not supported by data (see detailed comments).

- Author's initial response (prior first resubmission): We thank referee #2 and appreciate the constructive criticism and comments very much that will certainly help to improve our manuscript substantially. As a general response from our side, we just like to point out that we are aware of the complexity and limitations of such community mesocosm studies in particular the difficulty to assign certain changes to specific factors. Please find our detailed response to all points raised including suggested modifications in the following.

- Author response (added 28.11.2016): With respect to the conclusions drawn from our study, we have revised the manuscript accordingly. Most importantly we have analyzed predator/prey relationships in more detail and based on that consolidated and/or toned down the conclusions drawn. This applied mostly to the observed abundance changes of Myrionecta rubra and Bosmina sp., and the ratio of empty/filled brood chambers of Bosmina, respectively. Please find further details on amendments performed in our response to the detailed comments of reviewer #2 below.

Detailed comments

Abstract

Ref #2: The abstract is clear, but some of the conclusions are speculative and probably do not merit mentioning in the abstract (see below).

- Author's initial response (prior first resubmission): The abstract will be modified in consideration of all revisions applied to the manuscript.

- Changes performed in revision 1: The abstract has been modified and shortened, in particular we have toned a bit down our main conclusion with respect to Bosmina.

- Author response (added 28.11.2016): In revision 2, we have further mitigated conclusions mentioned in the abstract and included that in general increased regenerative production seems to results in lower trophic transfer efficiency but in the presence of organisms able to prey on bacterial production the opposite may be the case (L18–20 of track-changed version of revision 2; L18–20 of clean version of revision 2).

1. **Introduction**

Ref #2: The Introduction is generally well laid out and informative. It gives a proper justification for the study.

Where is "Storfjarden" and "Tvarminne"? (page 20029 / line 2, line 6)

- Author's initial response (prior first resubmission): Tvärminne and the Storfjärden

area is an open archipelago area on the eastern side of the Hanko peninsula on the south-west coast of Finland. A map showing the study site and mesocosm moorings is included in Paul et al. (2015). We will include this information in a revised version of the mansucript.

- Changes performed in revision 1: We have included this information in the introduction (L66/67).

- Author response (added 28.11.2016): The included information can be found in L67/68 of the clean version of revision 1, L31/32 of the track-changed version of revision 1, in L64/65 of the track-changed version of revision 2, and in L64/65 of the clean version of revision 2.

2. Methods

Ref #2: The field, laboratory and statistical methods are generally valid. Lack of replicates however creates difficulties in statistical analysis of data.

- Author's initial response (prior first resubmission): We are aware of this problem, however, a rash of particularly logistic, financial and time constraints make a more elaborate experimental design to allow disentangling multiple factor effects on a community level almost impossible to conduct in practice. Despite these potential shortcomings, we think that our approach allows for some valuable insights into possible effects of increased pCO2 concentrations on the plankton community level under at least close to in situ conditions that were otherwise not possible to obtain under at least semi-controlled conditions.

3. **Results**

Ref #2: The results are presented in a clear manner, but are a bit too exhaustive. The most interesting phenomena are swamped under a load of detailed descriptions of population variations, many of which are impossible to explain.

- Author's initial response (prior first resubmission): This comment is more or less consistent with referee #1. We will consider this comment carefully and rephrase the text to focus better on the most interesting and important results and shorten the amount of too detailed description of population variations.

- Changes performed in revision 1: We have condensed the results section and removed text passages that were not of major importance and sometimes a bit repetitive.

- Author response (added 28.11.2016): Listing each single change and deletion in all detail here with the respective reference to line numbers and pages would probably be not much

helpful and rather confusing. Therefore, we kindly ask to follow all changes and deletions performed in the track-changed version of revision 1.

Ref #2: To clarify the temporal patterns, and relate them to the minor differences between CO2 manipulations, it would be useful to show the CO2 development in each of the mesocosms.
- Author's initial response (prior first resubmission): This is a similar comment as given by referee #1 who suggested to include temperature, chlorophyll a and Shannon diversity, respectively into Fig. 1, 3 and 4. We would like to point out again, that this will increase the number of (sub-) plots. We will try out if including the CO_2 development results in an adequate gain of data visualization and based on this decide whether to show such plots or stick to the original plot.
- Changes performed in revision 1: We have included the CO2 development in Fig. 1.

Ref #2: 3.1.4: I would also like to see the temporal development in the Shannon index H.
- Author's initial response (prior first resubmission): Same reply as already given to referee #1: 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.
- Changes performed in revision 1: In Fig. 3b and 3c we show now the development of the Shannon index H over time as a function of the fCO2 and temperature phases (s. comments to referee #1). Fig. 4 is unchanged for the reason mentioned just above.

Ref #2: 3.1.5: Please add a short written summary of the most important findings of the statistical tests. At least those that you will also deal with in Discussion and mention in the Abstract
- Author's initial response (prior first resubmission): We will do that.
- Changes performed in revision 1: We have extended section 3.1.5 and described the most important statistical findings in more detail.
- Author response (added 28.11.2016): The details on performed changes can be followed in the track-changed version of revision 1 (p8/9). We have included significant results and the respective p-values (L284–296 of track-changed version of revision 2; L282–294 of clean version of revision 2).

4. **Discussion**

Ref #2: 4.1.1: Page 20044, lines 16-20. ("While… respectively") - An unclear sentence

- Author's initial response (prior first resubmission): To make it clearer, we will rephrase this sentence towards: "We found no significant relation between microzooplankton total abundance and fCO_2 concentration but total abundance was significantly affected by temperature. Moreover, there seemed to be a trend with respect to species diversity H towards a higher dominance of single species with increasing temperature and fCO_2, respectively."

- Changes performed in revision 1: Sentence was changed accordingly, L455/456 (clean version of revision 1).

- Author response (added 28.11.2016): In the track-changed version of revision 1, modifications to this sentence can be found on p13, L1–3, the same changes can be found in L427–430 of the track-changed version of revision 2, and in L425–428 of the clean version of revision 2.

Ref #2: 4.1.1: Page 20045, lines 2-3. Mentioning that "significant relations were determined for all factors" is not very helpful. rather pinpoint the most significant and meaningful findings.

- Author's initial response (prior first resubmission): We will consider this comment carefully in a revised version and better detail the most significant and meaningful findings.

- Changes performed in revision 1: After further careful consideration of this comment, we didn't change this sentence or part in order not to extent this section on the other ciliate species too much that were of minor importance compared to Myrionecta rubra –.

Ref #2: 4.1.2: May Myrionecta… This chapter is very speculative. I would condense this to minimum – or reject it totally.

- Author's initial response (prior first resubmission): This comment is consistent with referee #1 and we agree in principal (see our response to referee #1). In a revised version we suggest to cut this section to a minimum but keep the main statements that we think could provide some likely explanations.

- Changes performed in revision 1: This section was condensed, s. response to referee #1. Also, we changed the heading to "Myrionecta rubra".

- Author response (added 28.11.2016): Due to the addition of a further heading (4.1.1 Ciliate succession), the initial section 4.1.2 has changed to 4.1.3.

Ref #2: 4.2: mesozooplankton. There is not much relevant discussion on the cause-effect

relationships in this chapter. If no significant relations were found, I would not expand the discussion by adding a chapter on each of the Results chapters. E.g., you can easily delete chapter 4.2.2 Mollusks.

- Author's initial response (prior first resubmission): We agree with referee #2 that this section has some potential for shortening. We suggest the following for a revised version of the manuscript: We would like to keep the more general part that puts the mesocosm community in relation with the natural succession of MZP in Tvärminne/Storfjärden as this parts helps the reader to classify our study compared with the natural succession. As we are not presenting accompanying field data, we think this is helpful information for the wider context. Further, we will condense section 4.2.1 (copepods) to the most important points and omit section 4.2.2 (Mollusks).

- Changes performed in revision 1: In the revised version, we have shortened section 4.2.1 by about the half and removed section 4.2.2.

- Author response (added 28.11.2016): Details on performed modifications can be followed in the track-changed version of revision 1 (p15). Mostly we have deleted/rephrased information on state of the art knowledge about zooplankton response to ocean acidification (L540–554 initial submitted manuscript). The respective paragraph can be found from L547–567 in revision 1, from L523–547 in the track-changed version of revision 2, and from L520–544 in the clean version of revision 2. Note: due to insertion of a new heading "4.2.1 Mesozooplankton succession" and deletion of the section "Mollusks", the numbering of the section "Copepods" changed to 4.2.2.

Ref #2: 4.2.3: The long speculation on the "Cladocera-OA effect" is also far too stretched. The data do not show any effect of chl a on cladoceran abundance. Finding evidence in some imaginary phenomena ("missed peaks between samplings") is not a good strategy either. (Page 20052, lines 6-9).

- Author's initial response (prior first resubmission): We agree and will cut this section substantially. But in the same line as we argued above with respect to the discussion on Myrionecta rubra, we think that our considerations are not completely unfounded and shouldn't be completely neglected pointing out. The abundance differences in at least 3 of the elevated CO_2 mesocosms were substantial and together with the considerations on the reproductive biology and food preferences of Bosmina suggest for some most likely indirect cause-effect patterns related to CO_2 conditions that our experimental approach could not reveal. Therefore, in a revised version we would like to keep a revised part of the discussion and agree to substantially cut it down and focus on the most important and most justified statements.

- Changes performed in revision 1: We have shortened this part substantially and changed the part that argued for an indirect CO2 effect through chlorophyll a on Bosmina abundance. Rather, we found strong positive correlation between Bosmina and Cyanobacteria occurrence in connection with a CO2 mediated difference of Cyanobacteria during phase II. Therefore, together with the significant effect found for the ratio of empty to full brood chambers of Bosmina we argue in support of an indirect CO2 effect on Bosmina abundance through Cyanobacteria.

- Author response (added 28.11.2016): Reduction of text was mostly done on general information on cladoceran biology such as reproductive biology, life cycle and feeding (all details can be followed in the track-changed version, p16 L4–27). We discuss our findings of a strong positive correlation between Bosmina and Cyanobacteria in section 4.2.4 (Predator/prey relationships) that was added as a new section in revision 1 (p17 of track-changed version of revision 1; L589–638 of the track-changed version of revision 2; L586–630 of the clean version of revision 2).

Ref #2: 4.2.3 The finding of correlation between empty-filled brood chamber ratio and CO_2 and chl a is interesting, but, again, too many variables covary. All in all, if all phenomena on cladocerans are mediated through food, it is very speculative to say that CO_2 will have any effect. There are simply too many open issues between the relationship between CO_2 increase and Bosmina food conditions in the Baltic Sea.

- Author's initial response (prior first resubmission): We agree with the reviewer's concern of being too speculative here (again). In line with our argumentation above, we suggest to substantially tone down our statements and underline the more speculative nature where appropriate.

- Changes performed in revision 1: As just mentioned, we have shortened this section and consolidated our argumentation by looking more closely at predator/ prey relationships. We are discussing the possibility of an indirect CO2 effect on Bosmina mediated through food and we think that we have some evidence for this. If an indirect CO2 effect via food should exist, why would it be speculative to say that CO2 has any indirect effect? We agree that there are still many more open issues and our results are only a small contribution to shed some light on possible food web relationships and corresponding changes with CO2, and yet we think our data allow such discussion. However, we agree that we cannot be a 100% sure about that so we have toned down the respective parts.

- Author response (added 28.11.2016): This comment is quite similar to the previous. Please find the reference to the respective changes performed to this section above (see also in our response to the Editor decision letter).

5. Conclusions

Ref #2: The authors suggest that an increasing amount of filter feeding cladocerans (Bosmina) enhances carbon transfer to higher trophic levels due to enhanced usage of organisms of the microbial loop. Yes, filter feeders, like Daphnia, use bacteria and nanoflagellates for food, but Bosmina are not non-selective filter feeders, and many copepods also feed on flagellates. This complicates the picture. Also, Wikner & Andersson (2012, Global Change Biology 18: 2509-2519) claim that channeling more energy through microbial loop *decreases* the food web efficiency, and, hence, transfer of energy towards the higher trophic levels, including fish. If the authors want to retain this part, they should at least back up their conclusions with references, and include a description of the food web, clarifying who is eating whom, and how carbon will be channeled in each case. Actually, it is not obvious that Bosmina are much eaten by fish. Instead, it is possible that small cladocerans are suitable food for mysids and predatory cladocerans, like Cercopagis pengoi. Studies exist for the Baltic Sea for such interactions. How does this affect the conclusions on the trophic efficiency?

- Author's initial response (prior first resubmission): We will carefully consider the reasoning above and re-evaluate our logic. In particular we will take into account influence of other environmental drivers on carbon flux and the balance between auto- and heterotrophic processes in dependence on the mentioned publication by Wikner and Andersson (2012) and further consolidate the conclusions we will finally arrive at with references and a more detailed food web description.

- Changes performed in revision 1: We have inserted a new paragraph "Predator/ prey relationships" (4.2.4) and moved much of the former conclusion to this section. As the foucs of this manuscript is not on trophic interaction/ predator/ prey relationships in the strict sense but more on effects of CO2 on the zooplankton community, we have focused this paragraph on predator/ prey interactions of the species that turned out to be key species of our study (Myrionecta rubra, Bosmina sp.). We have consolidated our argumentation with further references on "who eats whom" and included the mentioned paper by Wikner and Andersson. Beyond that we have no evidence for CO2 effects on predator/ prey relationships and therefore, don't want to extent the discussion on that topic much further. Furthermore, we have inserted a paragraph in the introduction where we give some information on the food web in the Tvärminne region (s. response to referee #1).

- Author response (added 28.11.2016): With respect to the paragraph on predator/prey relationships, please see our response to previous comments for the links to where in the

different manuscript versions the respective changes have been included as well as our response to similar comments posed in the Editor Decision letter. The paragraph describing general aspects of the Baltic Sea food web was included in revision 1 and can be found on p2/3 L31–9 of the track-changed version of revision 1; from L71–83 in the clean version of revision 1; from L67–78 in the track-changed version of revision 2, and from L67–78 in the clean version of revision 2.

Ref #2: However, despite some shortcomings, there are valuable parts in this ms. If nothing else, the study shows that some CO2 effects can be seen at community level, but that the effects are complex and difficult to study in any type of experiment. This is useful information as such.

References:

Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated CO2 on organic matter pools and fluxes in a summer Baltic Sea plankton community, Biogeosciences, 12, 6181-6203, doi:10.5194/bg-12-6181-2015, 2015.

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Ciliate and mesozooplankton community response to increasing CO_2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment

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Abstract.

Community approaches investigating ocean acidification (OA) effects suggest a high tolerance of micro- and mesozooplankton to carbonate chemistry changes expected to occur within this century. Plankton communities in the coastal areas of the Baltic Sea frequently experience pH variations partly exceeding projections for the near future both on a diurnal and seasonal basis. We conducted a large-scale mesocosm CO_2 enrichment experiment (~ 55 m^3) enclosing the natural plankton community in Tvärminne/ Storfjärden for eight weeks during June–August 2012 and studied community and species/ taxon response of ciliates and mesozooplankton to CO_2 elevations expected for this century. Besides the response to fCO_2, we also considered temperature and chlorophyll *a* variations in our analyses. Shannon diversity of ciliates significantly decreased with fCO_2 and temperature with a greater dominance of smaller species. The mixotrophic *Myrionecta rubra* seemed to indirectly and directly benefit from higher CO_2 concentrations in the post-bloom phase through increased occurrence of picoeukaryotes (most likely Cryptophytes) and Dinophyta ~~Cryptophytes~~at higher CO_2 levels. With respect to meszooplankton, we neither detected significant effects for total abundance nor for Shannon diversity. The cladocera *Bosmina* sp. occurred at distinctly higher abundance for a short time period during the second half of the experiment in three of the CO_2-enriched mesocosms except for the highest CO_2 level. The ratio of *Bosmina* sp. with empty to embryo/ resting egg bearing brood chambers, however, was significantly affected by CO_2, temperature, and chlorophyll *a* . An indirect CO_2 effect via increased food availability (Cyanobacteria) stimulating *Bosmina* sp. reproduction can not be ruled out. Although increased regenerated primary production diminishes trophic transfer in general, the presence of organisms able to graze on bacteria such as cladocerans may positively impact organic matter transfer to higher trophic levels. ~~Filter-feeding cladocerans may effectively transfer microbial loop carbon to higher trophic levels.~~Thus, under increasing OA in cladoceran dominated mesozooplankton communities, the importance of the microbial loop in the pelagic zone may be temporarily enhanced and carbon transfer to higher trophic levels stimulated.

1 Introduction

Since the industrial revolution, anthropogenic CO_2 emissions have increased at an unprecedented rate and cause a concomitant increase of CO_2 concentration in the surface oceans. Thereby, ocean carbonate chemistry is altered with the main changes being reduced carbonate ion concentrations [CO_3^{2-}] and increased proton concentrations [H^+] causing a pH decrease. This phenomenon is nowadays well recognized as ocean acidification (OA). Ocean pH has decreased by approx. 0.1 units already and projections suggest a further decrease of 0.14–0.43 units by the end of the century (IPCC, 2013). The Baltic Sea, one of the largest brackish water systems, is sensitive to CO_2 changes because it naturally has low alkalinity and thus carbonate buffer capacity. Models project a drop of 0.5 pH units for the Baltic Sea by the year 2100 (Hjalmarsson et al., 2008; Havenhand, 2012; Omstedt et al., 2012). Eutrophication specifically affects coastal areas and can add to the fCO_2 fluctuations by provoking low oxygen partial pressure due to increased degradation processes, respectively respiration. Therefore, diel and seasonal variations of carbonate chemistry parameters particularly of coastal areas of the Baltic Sea are already huge today and the amplitude of fluctuations has even increased since the beginning of the industrialization and concomitant eutrophication (Omstedt et al., 2009; Melzner et al., 2013; Jansson et al., 2013). Consequently, zooplankton in the coastal Baltic naturally experiences large pH fluctuations on a daily and seasonal basis and possibly are at least to some extent adapted to these highly variable abiotic conditions (Melzner et al., 2013; Almén et al., 2014).

Ocean acidification is suspected to have severe consequences for marine organisms and acts synergistically with the concurrent temperature increase due to greenhouse gas emissions (Riebesell et al., 2009). Until now, most attempts to test for sensitivities of marine organisms to OA were conducted as single species experiments under controlled laboratory conditions. Such an approach can not account for community interactions in natural environments, and thus application of results to natural environments is limited. Laboratory experiments suggest calcifying organisms to be most vulnerable to OA because the formation and preservation of calcareous structures is hindered (e.g. Riebesell et al., 2000; Hoegh-Guldberg et al., 2007; Lischka et al., 2011). Non-calcareous micro- and mesozooplankton is generally considered quite robust to elevated CO_2 concentrations. Effects on the microzooplankton level seem to be of more indirect nature through changes in primary production, phytoplankton community composition and stoichiometry (Suffrian et al., 2008; Feng et al., 2009; Rossoll et al., 2012). Mesozooplankton is often dominated by copepods (Longhurst, 1985) which are relatively insensitive to fCO_2/pH changes expected for this century and direct negative effects usually do not occur unless exposed to much higher fCO_2 levels projected only much later (Kurihara et al., 2004; IPCC, 2013). More recent evidence suggests, however, that nauplii stages may be the weak point in copepod's life cycles (Cripps et al., 2014). As for the microzooplankton, studies on copepods and cladocerans suggest CO_2 effects may be more indirectly mediated to the zooplankton level through CO_2 induced changes in the biochemical and/or stoichiometric composition of their food (Urabe et al., 2003; Rossoll et al., 2012).

Holistic approaches studying CO_2 effects on entire natural plankton communities including zooplankton are still rare. In a preceding similar mesocosm experiment, Aberle et al. (2013) and Niehoff et al. (2013) found no effects on Arctic micro- and mesozooplankton communities, neither with respect to abundance of

single species or total numbers nor with respects change in community diversity. In terms of ciliates, these communities were dominated by large-sized forms (> 30 μm), in terms of mesozooplankton by copepods and cirripedia larvae.

The Tvärminne/Storfärden area is an open archipelago on the eastern side of the Hanko peninsula on the south-west coast of Finland. Among microzooplankton, ciliates and heterotrophic dinoflagellates dominate in summer in Tvärminne/Storfärden, among mesozooplankton rotifers, copepods and cladocera (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). In the Tvärminne/ Storfärden area during late summer and autumn, the microbial food web (MFW) is of particular importance when filter-feeding cladocerans mediate carbon transfer to higher trophic levels including fish (Koski et al., 1999, and references therein). Summer dynamics of the planktonic food web were described in more detail by Uitto et al. (1997). In general, omnivory dominates across all trophic groups, but the importance of herbivory and feeding on heterotrophs varies during summer. Earlier in summer, heterotrophic nanoflagellates (HNF) transfer carbon from picoplankton to ciliates, and ciliates constitute the link from nano- to metaozooplankton. Later in summer, HNF were largely bacteriovorous transferring bacterial carbon to ciliates and metazooplankton, when phytoplankton > 10 μm was grazed by metazooplankton and heterotrophic dinoflagellates. In July, < 10 μm phytoplankton increased and protists became the most important herbivores and the efficiency of the MFW in transferring bacterial carbon to metazooplankton was measured highest. However, the amount of carbon transferred to higher trophic levels depends also on the mesozooplankton species composition (Hansen et al., 1994). Elevated CO_2 concentrations can be beneficial for some phytoplankton groups, in particular picoeukaryotes. For micro- and mesozooplankton communities, so far no effects have been shown at least for CO_2 ranges projected to occur within this century (Aberle et al., 2013; Niehoff et al., 2013; Schulz et al., 2013).

As part of the KOSMOS Tvärminne mesocosm experiment, we examined CO_2 effects on the enclosed ~~micro-~~ciliate and mesozooplankton community. A map showing the study site and mesocosm moorings is included in Paul et al. (2015). Between June and August 2012, an fCO_2 gradient was set up in six approximately 55 m^3 mesocosms covering fCO_2 projections for this century or beyond (IPCC, 2013). Abundance and community composition was followed through enumeration of regularly taken water- and net samples. Per definition, ~~micro- and~~ mesozooplankton include ~~heterotrophic proto- and/ or~~metazoa ranging between ~~0.02–0.2 mm (20–200 μm) and~~ 0.2–20 mm (200–20,000 μm) in size~~, respectively~~ . In this study, we do not follow this classification strictly, as we also included the smaller juvenile life stages (< 200 μm) to the category 'mesozooplankton' (MZP) . ~~Within the category 'microzooplankton' (MiZP)~~ In case of protozoa we focus on ciliates only ~~and use the term 'ciliates' when referring to our study.~~ Other protozoa including Dinophyta and their response to CO_2 elevations are included in Bermúdez et al. (2016). Ciliates in our study include some species that can be facultative autotrophs or obligate mixotrophs (for instance *Myrionecta rubra*).~~, whereas all metazoa independent of their body size were assigned to the category 'mesozooplankton' (MZP).~~

Temperature can have a general effect on ~~MiZP~~ microzooplankton (MiZP) abundance and community composition and governs the dynamics of crustacean species (for instance affects productivity of cladocerans) in late summer in our study area (Nanazato and Yasuno, 1985; Koski et al., 1999; Rose et al., 2009; Aberle

et al., 2013). Furthermore, temperature changes towards a warming ocean are underway concurrently with ocean acidification with the potential to impact pelagic communities by providing suboptimal temperature conditions for species (IPCC, 2013). To consider possible impact of temperature variation and/or CO_2 driven chlorophyll *a* differences (Schulz et al., 2013), we also included temperature and chlorophyll *a* as explanatory variables in our statistical analyses.

2 Methods

To study the effect of elevated fCO_2 on a natural plankton community in the Baltic Sea, nine KOSMOS offshore pelagic mesocosms (**K**iel **O**ff-**S**hore **M**esocosms for future **O**cean **S**imulation) were deployed and moored on 12 June 2012 until the middle of August in the Tvärminne/ Storfjärden archipelago area at the south-west coast of Finland at 59°51.5' N and 23°15.5' E. The water depth at the mooring site was approximately 30 m. The mesocosm bags extended down to 17 m and were closed with 2 m long sediment traps at the bottom of the bags to enclose an isolated water body with its natural plankton community. After deployment, the mesocosm bags were initially kept open and submerged ~0.5 m below the surface to allow for a free exchange of the water and plankton community in the bags with the surrounding water masses. Organisms > 3 mm such as fish and cnidaria were excluded by 3 mm nets at the top and bottom openings of the bags during the first five days. These nets were removed on t_{-7} (i.e. seven days before the first CO_2 addition on t_0), the sediment traps were attached to the bottom, and the top ends of the mesocosm bags pulled up to 1.5 m above the surface to isolate the enclosed pelagic community from the Baltic Sea. The final volumes of the mesocosms ranged between 53.1 and 55.1 m^3 (Paul et al., 2015). The nine mesocosms were enriched with different amounts of CO_2 saturated seawater to set up an initial gradient of fCO_2 from 240 μatm (ambient, control mesocosms) up to ~1650 μatm. Three mesocosms (M2, M4, M9) were lost during the course of the experiment due to leakage. fCO_2 values in the six remaining mesocosms averaged over the sampling period (t_1–t_{43}) were 365 μatm (M1 control), 368 μatm (M5, control), 497 μatm (M7), 821 μatm (M6), 1007 μatm (M3) and 1231 μatm (M8). CTD profiles and samples for dissolved inorganic nutrients (silicate, phosphate, nitrate, nitrite, ammonium) and carbonate chemistry system parameters (DIC, TA, pH_T) were either taken daily or every second day. For more technical details about the experimental set-up, the CO_2 manipulations, and sampling procedures for various analyses see Paul et al. (2015). Sampling days were enumerated consecutively with t_{-3} indicating three days before CO_2 manipulation, t_0 as the day of the first CO_2 manipulation, and t_{1+X} as the days following the first CO_2 manipulation.

2.1 Microzoolankton sampling

Water samples for the enumeration of ciliates were taken every second day with a depth-integrating sampler (0–17 m), IWS (HYDRO-BIOS, Kiel, Germany), between 9:00 and 12:00 am from six mesocosms. After careful mixing, 250 ml of seawater were filled into brown-glass bottles and preserved in acidic Lugol's iodine (1% final concentration). 50 ml of the sample were transferred to Utermöhl sedimentation chambers. After 24

h settling time, ciliates were counted with a Zeiss Axiovert 100 inverted microscope at 200 x magnification Utermöhl (1958). At high cell numbers (> 400 cells), half the bottom plate area was counted. If less than 400 cells were found in the first half of the bottom plate area, the entire chamber was counted. Rare species were counted on the whole bottom plate. Ciliates were identified to the lowest possible taxonomic level (genus/ species) according to (Setälä et al., 1995), and according to descriptions found at the planktonic ciliate project (http://ciliate.zooplankton.cn/). 138 samples were analyzed in total. Abundances were calculated as cells l^{-1}.

2.2 Mesozooplankton sampling

Mesozooplankton samples from six mesocosms were taken with an Apstein net of 17 cm diameter and 100 μm mesh size. Zooplankton were sampled between 08:00 and 11:00 am by towing the net vertically from 17 m depth to the mesocosm surface. In total, at eleven sampling days, vertical net hauls were done from the mesocosms: prior to the CO_2 addition (t_{-3}, t_{-2}, t_{-1}), at the day of the first CO_2 addition (t_0), and after the first CO_2 addition (t_3, t_{10}, t_{17}, t_{24}, t_{31}, t_{38}, t_{45}). After collection, the samples were brought back to the laboratory in the Tvärminne zoological station (University of Helsinki) and preserved in 70% ethanol. Zooplankton abundance was calculated assuming 100% filtering efficiency of the net. The samples were divided with a Folsom plankton splitter (1:2, 1:4, 1:8, 1:16, 1:32) and the aliquots of the samples were counted. Organisms were counted and determined under a stereo microscope (WILD M3B) to the lowest taxonomical level possible. Abundant species/taxa (> 30 individuals in an aliquot) were only counted from subsamples, while less abundant species/ taxa were counted from the whole sample. Juvenile bivalves did not distribute equally in the Folsom splitter due to their relatively large mass and were therefore counted from the whole sample. Copepods (*Acartia* spp., *Eurytemora* spp., *Temora* spp.) were identified according to different stages (adult females, adult males, copepodite stages CI–CV). Copepod nauplii were counted but not determined to species level. The counting of the cladoceran species (*Bosmina* spp., *Evadne* spp., *Podon* spp.) was distinguished according to organisms with empty or filled brood chambers, respectively (i.e. organisms that had empty brood chambers or bore embryos/resting eggs, respectively, in their brood chambers) and categorized as 'empty' or 'filled'. For data analyses, the ratio between the number of organisms with 'empty' to 'filled' individuals was calculated for each mesocosm and sampling day, i.e. a small ratio stands for a higher proportion of reproducing organisms in the population in a particular mesocosm at a particular sampling day. A total of 66 samples were analyzed. Abundances were calculated as individuals m^{-3}.

2.3 Data analysis and statistics

To assure equally spaced data, some sampling days were excluded from statistical analyses. For the ciliate data this applied to t_{-3}, t_0, t_2 and t_4, and for the mesozoopankton this applied to t_{-3}, t_{-2}, t_{-1} and t_0. However, for demonstration purpose only, the data of these sampling days were included in the figures.

As explanatory variables, fCO_2, temperature and chlorophyll *a* were used to test for effects on different response variables (see below). Collinearity was checked prior to analyses. To account for the change in fCO_2 over time due to ingassing/outgassing as well as temperature and chlorophyll *a* changes over time, all

explanatory variables were used as continuous variable for each t-day included in the analyses. All analyses were carried out with R using the package nlme, mgcv, Hmisc and MASS. All plots were done in ggplot (Team, 2012).

The Shannon index (H) was calculated as a measure of diversity in each of the mesocosms and to estimate changes in the relative contribution of single species/groups in the whole ~~micro-~~ciliate/mesozooplankton community over time and in response to different abiotic parameters such as the fCO_2 levels. When all considered species/groups contribute equally to the community in terms of their abundances, H calculated on the natural logarithm becomes 2.3. The more a community is dominated by single species/group, the smaller the Shannon index gets. Calculations of H were performed in the vegan package of the R environment (Oksanen et al., 2012).

For the **ciliates**, 14 species/groups were included to calculate H: *Balanion comatum*, *Strombidium* cf. *epidemum*, *Mesodinium* sp., *Myrionecta rubra* ($\leqslant$ 10 μm), *M. rubra* (11–20 μm), *M. rubra* ($>$ 20 μm), *Rimostrombidium* sp., *Spathidium* sp., *Strobilidium* spp. ($\leqslant$ 20 μm), *Strobilidium* spp. ($>$ 20 μm), *Strombidium* sp., Tintinnids, cysts (*Strobilidium* sp., unidentified cysts), and ciliates sp. (*Euplotes* sp., *Lacrymaria* sp., *Strobilidium* sp., unidentified ciliates). *Lohmaniella* sp.could not be clearly separated from other Strobilids and was therefore included with Strobilidium spp. ($\leqslant$ 20 μm). Most of the Strobilids found, however, were probably *Lohmaniella* sp..

For the **meosozooplankton**, 17 species or taxonomic groups were included in the calculation of H: copepodite stages and larval stages of *Balanus* sp. (nauplii and cypris larvae) were summarized on the genus level (Copepoda: *Acartia* sp., *Eurytemora* sp., *Temora* sp., Harpacticoida sp., copepod nauplii; Cladocera: *Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.; Rotifera: *Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., Rotifera sp.; larvae of *Balanus* sp., juvenile bivalves, juvenile gastropods, and larvae of polychaets).

2.3.1 Ciliates

Statistical analyses were done on total cell numbers, the Shannon index H as well as the abundance of particular groups that showed distinct differences such as small size-class *Myrionecta rubra*, *Balanion comatum*, *Strombidium* cf. *epidemum*, and small *Strobilidium* sp.. Linear mixed effects modelling (LME) was applied on a Gaussian distribution to determine the effect of CO_2, temperature and chlorophyll a. Actually, count data should be modelled on a Poisson distribution, but model selection (s.b.) yielded in convergence problems in R for Poisson distribution. Therefore, we used a Gaussian distribution, which can also be applied on count data (Zuur et al., 2009). If preceding data exploration suggested interactions between the factors, respective interaction terms were included in the model. Model selection was based on the Akaike information criterion (AIC) by removing non-significant terms to find the simplest adequate model. However, missing values for chlorophyll a occurred for M3/t_{25} and for M5/t_{23}, these values were estimated as means of the preceding and following day. Chlorophyll a values were also missing for t_{41} and t_{43}. A polynomial fit curve applied on phase III (according to temperature variations, three experimental phases (I, II, III) were defined which

are thoroughly introduced in Paul et al. (2015). Phase III lasted from t_{31} until t_{43}.) resulted in no meaningful values, therefore these values were estimated as phase III means.

The different response variables were modelled as a function of the daily change in fCO_2, temperature and chlorophyll *a* and if suggested with interaction terms as mentioned above. To account for the time dependency and the nested nature of the data, GLM models (generalized mixed effects) were applied on a Gaussian distribution using fCO_2 (values on a continuous scale for each sampling day) and sampling day nested in mesocosm as random intercept. In case of violation of the assumptions for linear models yielding to non trustworthy p-values, the GLM model was re-applied as a GA(M)M (generalized additive (mixed) model) and a smoother for sampling day included to prove the validity of the GLM outcome. In some cases, some residual patterns mostly due to sampling day still remained even after applying the GAMM. But GAMM is as much as can be done with current hard- and software, and therefore, for highly significant p-values, our results should still be reasonably robust, and p-values that are not highly significant should be seen with some caution (Zuur et al., 2009).

2.3.2 Mesozooplankton

The statistical approach with respect to MZP corresponded with description in section 2.2.1. Total abundance, the Shannon index *H* as well as total abundance of species that suggested distinct differences such as *Bosmina* and the ratio of *Bosmina* with empty to individuals with full brood chambers (i.e. either bearing embryos or resting eggs in their brood chambers) were analyzed statistically. Missing values for fCO_2 occurred on t_{24}, t_{38} and t_{45}, and for temperature, and chlorophyll *a* on t_{38} and t_{45}. Missing observations for t_{24} and t_{38} were estimated by building the mean of values measured at t_{23}/t_{25} and respectively t_{37}/t_{39}. t_{45} was the last sampling day and hence it was not possible to estimate a mean from the preceding and following day. Therefore missing values for t_{45} were estimated from a polynomial fit curve applied on phase III values (Paul et al., 2015).

2.3.3 Predator/prey relationships

Pearson correlation was used to investigate possible trophic relationships between ciliates and MZP, respectively, and bacteria, nano- and ~~picoflagellates~~picoeukaryotes (total bacteria, low DNA bacteria, high DNA bacteria, Cyanobacteria, particle associated bacteria, *Synechococcus*, pico- and nano~~flagellates~~eukaryotes), and phytoplankton groups (Prasinophytes, Cryptophytes, Chlorophytes, Cyanobacteria, Diatoms, Euglenophytes, auto- and heterotrophic dinoflagellates, and heterotrophic dinoflagellates excluding *Ebria* sp.). For these correlations, data from Crawfurd et al. (2016) and Paul et al. (2015) were used.

3 Results

3.1 Ciliates

3.1.1 Ciliate total abundance

Total abundance of ciliates at experiment start (t_0) varied between 78,120 cells l^{-1} (M5) and 52,360 cells l^{-1} (M3) and more or less continually decreased from the beginning over time until t_{17} when a plateau was reached with low cell numbers between 7,080 (M8) and 10,940 (M3) until t_{33}. During the last five sampling days (t_{35}–t_{43}), total cell numbers were more variable again with some small ups and downs and reached minimum values between 900 cells l^{-1} (M6) and 3,580 cells l^{-1} (M8) on the last sampling day (Fig. 1).

3.1.2 Abundance of *Myrionecta rubra*

Myrionecta rubra was (by far) the most dominant ciliate species during the entire period (Fig. 2a). *M. rubra* occurred in three different size classes ($\leqslant$ 10 μm, 11–20 μm, > 20 μm) of which organisms of the smallest size range made up the highest numbers. On t_0 cell numbers of *M. rubra* of the smallest size class varied between 26,720 cells l^{-1} and 44,520 cells l^{-1}. Cell numbers stayed relatively high until t_{11}/t_{13} (16,600–37,400 cells l^{-1}) when they strongly declined to values below 10,000 cells l^{-1} on t_{17} and further decreased with some fluctuations until the end of the experiment to reach final values of between 130 cells l^{-1} and 1,740 cells l^{-1} among all mesocosms. Some striking difference, however, occurred between t_{25}–t_{35} when abundance in the three highest CO_2 mesocosms was higher compared to the two controls and the lowest CO_2 enriched mesocosm (mean: 4,518 cells l^{-1} (SD 1,082) and mean: 3,459 cells l^{-1} (SD 383), respectively). *M. rubra* of the medium size class also had maximum numbers on t_0 ranging from 17,600 cells l^{-1} to 25,680 cells l^{-1}. From the experiment start, numbers more or less continually decreased and reached minimum values of between 480 cells l^{-1} and 0 cells l^{-1} from t_{19} on. The largest *M. rubra* occurred only rarely but as in the other two size classes, highest numbers were found during the first few sampling days varying between 2,680–5,800 cells l^{-1} on t_0 and reaching very low numbers already on t_7/t_9 (1,080–280 cells l^{-1}). After t_{19}, *M. rubra* > 20 μm occurred only exceptionally.

3.1.3 Abundance of other species/genera/groups

Other dominant groups/species that contributed to the total cell numbers of ciliates were *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp. (< 20 μm and > 20 μm), *Mesodinium* sp., *Rimostrombidium* sp., *Strombidium* sp., Tintinnids, *Spathidium* sp., cysts, and ciliates that could not be identified (Fig. 2b, 2c). Among those, *Strombidium* cf. *epidemum* was most dominant and showed three peaks, around t_9/t_{11}, t_{23}, and t_{37}. On t_9/t_{11} some distinct difference occurred between control and CO_2 enriched mesocosm (mean: 1,250 cells l^{-1} (SD 180) and mean: 2,205 cells l^{-1} (SD 851), respectively). *Balanion comatum*, *Rimostrombidium* sp., *Strobilidium* sp. (< 20 μm), *Spathidium* sp., and tintinnids were of some importance during the first days of the experiment showing peaks in cell numbers between t_0 and t_{11}. Most interestingly, peak abundance of

Balanion comatum diverged with CO_2 concentration with higher mean cell numbers in the control and lowest enriched mesocosm compared to the three high CO_2 mesocosms (mean: 1680 cells l^{-1} (SD 139) and mean: 880 cells l^{-1} (SD 223), respectively). Likewise, small *Strobilidium* sp. developed some CO_2 related difference with mean abundance of 1,360 cells l^{-1} (SD 170) and 2,400 cells l^{-1} (SD 872) in the two controls and the CO_2 enriched mesocosms, respectively. *Mesodinium* sp., *Strobilidium* sp. > 20 μm, cysts and unidentifiable ciliates occurred always in relatively low cell numbers (mostly < 850 cells l^{-1}).

3.1.4 Percent contribution of numerically dominant species/genera/groups to total cell numbers

Fig. 3a shows the percent contribution of dominant species/ genera/ groups to the total cell numbers over time for each of the mesocosms. For better clarity, *Myrionecta rubra* size classes, *Strobilidium* sp. size classes together with *Rimostrombidium* sp., *Strombidium* spp. and cysts together with ciliates sp. were combined. *M. rubra* dominated the ciliate community in all mesocosms most of the time. During the first days of the experiment, *M. rubra* contributed $\sim$ 90% to the total cell numbers in all mesocosms and stayed above 50% until t_{21}. Minimum contributions occurred on t_{37} when *M. rubra* had a share of only 6–24%. After t_{37}, *M. rubra* proportions ranged between 18% and 67%. The second most important group was *Strombidium* sp. and among this *Strombidium* cf. *epidemum*. *Strombidium* sp. had highest shares during the second half of the experiment varying between 58% and 69% during t_{35}–t_{39}. All remaining groups usually had contributions below 15%.

The Shannon diversity index *H* ranged from 0.58–1.66 over the whole period of time (Fig. 3b). In general, it showed a slightly increasing trend varying between 1.04 and 1.23 on t_{-3} and, respectively 1.30 and 1.66 on t_{43} and was generally lower during higher temperature phases (I + II) (Fig. 3c).

3.1.5 Statistical analyses ciliates

GAMM's determined significant synergistic effects for total abundance of small size class *Myrionecta rubra* in response to fCO_2*temperature (p = 0.024) and fCO_2*chlorophyll *a* (p= 0.004). Total abundance of *Balanion comatum* was affected by temperature and fCO_2 ($p_{temperature}$ = 0.022; p_{fCO_2} = 0.03), total abundance of *Strombidium* cf. *epidemum* by chlorophyll *a* (p = 0.002), that of *Strobilidium* sp. showed synergistic responses to the combination of the factors fCO_2*temperature and fCO_2*chlorophyll *a*, respectively (p = 0.0005 and p = 0.0002, respectively), and for the Shannon index *H* a synergistic effect between fCO_2*temperature was determined (p = 0.0008). Depiction of the statistical results of *H* showed a non-monotonic relationship with a slightly increasing trend at lower fCO_2 and a decreasing trend the more the fCO_2 increased, as well as a decreasing trend with temperature (Fig. 4a, 4b). Statistical results are shown in more detail in Table 1. Model validation showed some residual pattern in all cases, but most of the obtained p-values are highly significant and are therefore reasonably trustworthy (Zuur et al., 2009). Only with respect to *Balanion comatum*, p-values should be seen with some caution as they are not highly significant.

3.2 Mesozooplankton

3.2.1 Mesozooplankton total abundance

After a sharp initial decrease, total abundance of mesozooplankton increased continuously until peak abundances were reached between t_{24} and t_{31} (Fig. 5). M7, M6, and M3 (497–1007 μatm) had highest peak values ranging between 130,276 ind. m^{-3} and 162,082 ind. m^{-3}, whereas abundance in M1 and M8 were somewhat lower with 111,980 ind. m^{-3} and 90,975 ind. m^{-3}, respectively. In M5, no abundance peak occurred but zooplankton developed a plateau between t_{24} until t_{38} of around 70–74,000 ind. m^{-3}. Towards the end of the experiment, zooplankton total abundance returned to about the initial values (29,325–44,824 ind. m^{-3} in M8 and M1, respectively).

3.2.2 Community composition

The mesozooplankton community was dominated by five taxonomic groups, i.e. cladocera (*Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.), copepoda (*Acartia* sp., *Eurytemora* sp., *Temora* sp., copepod nauplii, Harpacticoida, Cyclopoida, Copepoda sp.), crustacea (*Balanus* sp., inlcuding nauplii and cyprid larvae), mollusca (juvenile Bivalvia and Gastropoda) and rotifera (*Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., Rotifera sp.). The group 'others' comprises larvae of Bryozoans (cyphonautes), juvenile Polychaeta, and unidentifiable organisms (Fig. 6). Among these groups, cladocerans and copepods dominated the zooplankton community during the entire experimental period. Cladocerans contributed mostly between 50% and 95% to the total abundance. Copepods had their highest share half way through the experiment when they constituted 74–84% (t_{17}) of the whole community. Rotifera were a major part of the zooplankton only during the first days of the experiment with about 11% to 42% between t_{-1} and t_3. Among the group mollusca, gastropods always had a smaller share than bivalves with usually below 2% (max. 5%) contribution to the total abundance of this group. Juvenile bivalves mainly occurred from the start until day t_{10} and had maximum contributions of 17–45% to the total zooplankton community between t_{-2} and t_0. The group 'crustacea' comprises mainly larvae of *Balanus* sp. (nauplii and cyprids). Only very rarely a mysid was found and specimen of this order were also included in the group crustacea. The main occurrence of 'crustacea' was from t_{-1} until t_{10} contributing between 10% and 2% to the total zooplankton community during this time. The group 'others' always contributed less than 0.5% to the total abundance.

In all mesocosms, the Shannon diversity index was highest at the beginning of the experiment (T_3: 1.78–1.89) and decreased continuously with time reaching lowest values on the last sampling day (T_{45}: 0.23–0.5) indicating that towards the second half of the experiment and at the end, the dominance of single species/ groups increased.

3.2.3 Copepoda

Eurytemora sp. was the dominant copepod species in the zooplankton community over the entire period. *Acartia* sp. occurred regularly but in much lower abundances. *Temora* sp. occurred only in very low numbers

mainly during the first part of the experiment (Fig.7a). The abundances of *Eurytemora* sp. were relatively low at the beginning (82–2,496 ind. m^{-3}). Peak abundances were reached around day t_{17} and t_{24} (19,192–32,297 ind. m^{-3}) and then declined. During the course of the experiment, *Acartia* sp. varied in numbers between 117 ind. m^{-3} and 4624 ind. m^{-3} and did not show clear abundance peaks in most of the mesocosms. *Temora* sp. was present during the whole time (though not always in all mesocosms) but always in low abundances ranging between 330 ind. m^{-3} and 3 ind. m^{-3} among all mesocosms. Copepod nauplii occurred during the entire experiment duration with peak abundance between t_{10} and t_{24} (9,003– 33,555 ind. m^{-3}).

The three copepod species were determined to copepodite stages (CI–CV) and adult females and males (Fig.7b). *Eurytemora* sp. copepodites CI–CV were present in high proportions almost during the whole period of time with up to > 90%. Adult females and males had their minimum during the abundance peak of this species (t_{17}–t_{31}) but occurred during the entire study period indicating more or less continuous reproduction in all mesocosms. At the beginning and towards the end of the study, most of *Acartia* sp. were in the copepodite stage CI–CV. Adult females and males occurred during the whole period of time and had maximum proportions half way through the experiment (t_{17}, t_{24}). During this time, reproduction took place indicated by the following increase in copepodite stages during the second half of the study. The stage distribution of *Temora* sp. was similar to *Acartia* sp. with a peak of copepodite stages CI–CV during the first and the last sampling days. Most of the time, however, adult females and males dominated.

3.2.4 Cladocera

Four species of cladocera were found in the mesocosms: *Bosmina* sp., *Podon* sp., *Evadne* sp. and *Daphnia* sp. *Daphnia* sp. occurred only rarely in very low abundances (< 0.5% contribution to total cladocera, abundance range: 2.6–12.8 ind. m^{-3}). *Evadne* sp. had maximum abundances on t_3/t_{10} (184 ind. m^{-3}–3,893 ind. m^{-3}) and contributed up to 38% to this group during the first days of the experiment but decreased noticeably in importance later. *Podon* sp. dominated among the cladocerans at the beginning of the experiment accounting for more than 80% of the total abundance until day t_{10} (max. numbers: 43,688–15,272 ind. m^{-3}). By day t_{17} *Bosmina* sp. reached more than a 90% share until termination of the experiment. Peak abundance of *Bosmina* sp. occurred between t_{24}–t_{38} and was substantially higher in the medium range CO_2 mesocosms M7 (497 μatm), M6 (821 μatm) and M3 (1007 μatm) (138,394 ind. m^{-3}, 114,169 ind. m^{-3}, 127,080 ind. m^{-3}, respectively) compared to the two controls M1, M5 and the highest CO_2 mesocosm (M8, 1231 μatm) (72,020 ind. m^{-3}, 58,107 ind. m^{-3}, 63,182 ind. m^{-3}, respectively) (Fig. 8a, only *Bosmina* sp. is shown).

The counting of the two dominant cladoceran species *Podon* sp. and *Bosmina* sp. was divided into organisms with empty brood chambers and organisms bearing embryos/ resting eggs in their brood chambers to inspect for a possible direct or indirect effect of CO_2 on asexual/ sexual reproduction and subsequently a ratio was calculated, s.a. Mostly, the percent contribution of organisms with filled brood chambers varied between 40% and 10% in all mesocosms among the study period. Only during the very first days, *Bosmina* sp. with filled chambers had contributions of up to 67% (not shown). The ratio of *Bosmina* brood chambers varied during peak occurrence (t_{24}–t_{31}) between 3.47 (M8) and 17.18 (M7) (Fig. 8b). During times of high *Podon*

sp. abundances, the share of this organism with full brood chambers varied roughly between about 25% and 50%. *Podon* actively reproduced during the first days of the experiment indicated by a low ratio of organisms with empty/ full brood chambers (0.79–2.77), whereas lowest reproductive activity occurred on t_{17}/t_{24} (5.09–33.10) (not shown).

3.2.5 Statistical analyses mesozooplankton

For total abundance of mesozooplankton we determined no significant relationship with fCO_2 or any of the other explanatory variables (temperature, chlorophyll *a*) (Table 1).

The cladocera *Bosmina* sp. showed distinct abundance peaks in M7, M6, and M3 with approx. 110–130 ind. 10^3 m^{-3} higher numbers between t_{24} and t_{31} compared to the two control mesocosms and M8. The GLM model revealed neither a significant relation of the total abundance of *Bosmina* sp. with fCO_2 nor temperature. Chlorophyll *a* concentration was determined to significantly affect the *Bosmina* occurrence but model validation showed heterogeneity of the residuals mostly due to experiment day. Running the GAMM model with a smoother on experiment day did not confirm this result.

GAMM analysis on the ratio between *Bosmina* with empty brood chambers to organisms with full brood chambers yielded in significance of all three main terms as well as in a significant interaction term between fCO_2 and chlorophyll *a* ($p = 0.01$). Some minor residual structure remained after GAMM on the *Bosmina* ratio that should be kept in mind with respect to resulting p-values (Zuur et al., 2009).

According to a GAMM applied on the Shannon diversity index *H*, neither of the factors significantly affected MZP species diversity.

3.2.6 Predator/prey relationships

Pearson correlation coefficients larger than $\pm$ 0.7 are listed in Table 2 and shown in the supplementary material (Fig. S1−S2). *Myrionecta rubra* and *Bosmina* sp. turned out to be of particular importance in this study. Therefore, in the following, we focus on correlations of these two species with particular phytoplankton and bacteria groups, respectively. *M. rubra* positively correlated with Cryptophytes and heterotrophic Dinoflagellates, whereas the species negatively correlated with Cyanobacteria and low DNA bacteria. Pearson correlation for the different size classes of *M. rubra* were very similar when determined for all fCO_2 levels (0.8; 1.0; 0.9) or low (0.8; 0.9; 0.8) and high (0.8; 1.0; 0.9) levels separate, respectively. *Bosmina* sp. showed a strong positive correlation with Cyanobacteria (0.7). Fig. 9 depicts the succession of the two species in relation to the mentioned potential prey organisms during the course of the experiment.

4 Discussion

4.1 Ciliates

4.1.1 Ciliate succession

The ciliate abundance and species succession in our experiment corresponded well with description by Kivi (1986) on annual succession of protozooplankton in Tvärminne/Storfjärden. In May, shortly after the chlorophyll maximum, this author observed the highest protozoan biomass whereas a minimum was found in June/July two weeks after the spring bloom (mostly ciliates and heterotrophic dinoflagellates). Dominant ciliates during the summer month were *Lohmaniella* spp. or small *Strombidium* spp. (35 μm). *Myrionecta rubra* was always present with maximum abundance in late spring. *Lohmaniella* spp. also occurred in the present study but was classified with *Strobilidium* spp. ($\leqslant$ 20 μm) due to difficulties with clear identification. However, most of the Strobiliids $\leqslant$ 20 μm probably belonged to *Lohmaniella* spp. In our study, the ciliate community was dominated by the primarily photoautotrophic ciliate *M. rubra* (=*Mesodinium rubrum*) Lohmann (1908); Jankowski (1976) (Mesodiniidae, Litostomatea) most of the time (Lindholm, 1985). Only towards the end of our experiment, heterotrophic ciliates became more important in the ciliate community when small Strombidiids such as *Strombidium* cf. *epidemum* occurred with similar abundances as *M. rubra*. *M. rubra* is also a common species in the Baltic Sea with maximum reported densities of 26,600 cells l^{-1} in the Arkona Basin usually above the thermocline and associated with the euphotic layer (Setälä and Kivi, 2003). Maximum total ciliate densities in the entrance of the Gulf of Finland varied between 10–50,000 cells l^{-1} in 1988 and 1990, respectively, and hence are in the same range as in our study, and also consisted of the same typical species/groups (Setälä and Kivi, 2003).

4.1.2 Changes in ciliates species diversity

Previous studies on sensitivities of MiZP communities towards ocean acidification are inconsistent. For example Rose et al. (2009) report on significant changes in MiZP abundance and community composition in the open North Atlantic Ocean between their single factor (only temperature) and two factor (temperature and CO_2) experiments and conclude that a combination of direct and indirect (bottom-up) effects were responsible for observed changes. Mesocosm studies off the coast of Norway and in the Arctic revealed no effect of different CO_2 concentrations on the MiZP community neither with respect to abundance nor community composition (Suffrian et al., 2008; Nielsen et al., 2010; Aberle et al., 2013). In the latter study, positive effects on the autotrophic biomass with higher and lower CO_2 concentrations were found for dinoflagellates and respectively prasinophytes and haptophytes but these effects did not translate to the MiZP level (Schulz et al., 2013).

We found no significant relation between ciliate total abundance and fCO_2 concentration, but total abundance was significantly affected by temperature. Moreover, there seemed to be a trend with respect to species diversity H towards a higher dominance of single species with increasing temperature and fCO_2, respec-

tively. Most likely, small species/genus are responsible for this change in diversity. During the first days of the experiment (t_5, t_5–t_9, and t_7–t_{13}, respectively) small species such as *Balanion comatum*, *Strombidium* cf. *epidemum*, and *Strobilidium* sp. (< 20 μm) show some distinct differences in abundance between the three higher and lower fCO_2 mesocosms. While *B. comatum* occurs at higher abundance in the control mesocosms and the lowest CO_2 enrichment level (M7, 497 μatm), *S.* cf. *epidemum* and *Strobilidium* sp. have higher abundances in the three high CO_2 mesocosms. Later in the experiment, between t_{19} and t_{31}, the small size class *Myrionecta rubra* for example occurred in much higher numbers in the mesocosms with the three highest fCO_2 concentrations. ~~F~~for the mentioned species, significant relations were determined for all factors included in our analyses, except for *Balanion comatum* that showed no significant response to chlorophyll *a* and *Strombidium* cf. *epidemum* that only showed a significant relation with chlorophyll *a*. Rose et al. (2009) also report on increased dominance of smaller taxa (mostly *Lohmaniella* sp. among ciliates) during the course of their experiment, but dependent on a combination of different factors, i.e. temperature, CO_2 and changes in the top-down control. Finally, they conclude on a more general effect of temperature on MiZP abundance and community composition. A relationship between temperature and Shannon diversity *H* on ciliate communities and on heterotrophic ciliates, respectively, was also shown by Setälä and Kivi (2003) and Aberle et al. (2007). In contrast to our present study, Aberle et al. found *H* to increase with higher temperature and it was larger ciliates (mostly *Strobilidium* species) that caused the community shift. Like Rose et al. (2009), the temperature effect determined in the present study, is most likely of more general nature related to the natural succession of ciliates during the summer season.

Although some of the species mentioned above significantly correlated with chlorophyll *a* concentrations (*Strobilidium* sp., *Strombidium* sp.), chlorphyll *a* had no significant effect on species diversity H. Most likely this is due to the occurrence of species with different (heterotrophic/autotrophic) food preferences during the course of the experiment. Species diversity was lowest during phase I and II and this was due to the dominance of the mixotroph *Myrionecta rubra*. Later in the experiment when chlorophyll *a* concentrations had decreased, *M. rubra* still occurred with lower cell numbers but also other ciliates like the mixotrophic *Strombidium* sp. increased in abundance and as a consequence H increased. Members of the genus *Strombidium* feed on a variety of organisms including bacteria, nano- and dinoflagellates (Fenchel and Jonsson, 1988; Ichinotsuka et al., 2006; Stoecker et al., 2009). Furthermore, this experiment was conducted during the post-bloom phase. Possibly, if our experiment also covered the phytoplankton peak-bloom phase and H was determined over the whole duration from the peak- to the post-bloom phase, the relationship between H and chlorophyll *a* was more pronounced.

4.1.3 *Myrionecta rubra*

Increased abundances of the mixotrophic ciliate *Myrionecta rubra* ($\leqslant$ 10 μm) in the high CO_2 mesocosms coincided well with increased chlorophyll *a* concentrations at high CO_2 levels during phases II and III attributed for up to 90% to picophytoplankton ($\leqslant$ 2 μm). The relative contribution of the 2–20 μm size fraction to total chlorophyll *a* was estimated as about 20% (Paul et al., 2015). Blooms of *M. rubra* can contribute significantly

to chlorophyll *a* values and primary production in estuaries, fjords and upwelling areas. *M. rubra* robs plastids from Cryptophytes (Lindholm, 1985; Gustafson Jr et al., 2000, and references therein). Cryptophytes were among the main contributors to total chlorophyll *a* in particular during phase I (Paul et al., 2015). Moreover, small ~~nanophytoplankton~~picoeukaryotes (PICO III) of approx. 2.9 μm cell diameter most likely representing Cryptophytes had highest abundances during phases II and III ~~but~~and showed a distinct negative correlation with fCO_2 (Crawfurd et al., 2016). Cryptophyte biomass decreased from t_3 to t_{17} (Paul et al., 2015) as did the total abundance of *M. rubra*, but the small size-class cells remained and during phase II developed a distinct difference in abundance between the higher and lower CO_2 mesocosms. Growth and photosynthetic performance of *M. rubra* is ultimately dependent on the availability of Cryptophytes, but the ciliate can sustain long periods without feeding by functioning as a phototroph and has the ability to control cryptophyte plastids' division and synthesize chlorophyll (Johnson and Stoecker, 2005; Johnson et al., 2006). Photosynthetic performance of *M. rubra* may have been stimulated by elevated CO_2 concentrations and thus this ciliate may be 'co-responsible' for the CO_2 driven total chlorophyll *a* differences observed during phases II and III. Consequently, higher cell numbers of small sized *M. rubra* at elevated CO_2 may be a combination of indirect and direct CO_2 effects through 1) availability of Cryptophytes in particular during phase I, and 2) through a CO_2–mediated higher photosynthetic rate of *M. rubra* supporting its own growth. Losses of PICO III during phase II were largely due to microzooplankton grazing (Crawfurd et al., 2016). In further support of our assumption are the strong positive Pearson correlations between *M. rubra* and Cryptophytes and Dinophyta suggesting a high grazing pressure of *M. rubra* ~~on Cryptophytes supporting our assumption~~. During phase II, Dinophyta showed a significant decrease in relative biomass with increasing CO_2 consistently with the CO_2 stimulated increase of small *M. rubra* (Bermúdez et al., 2016). Overall, a CO_2 effect on *M. rubra* was only visible during the post-bloom phase, when cell numbers were rather low compared to initial numbers. However, possibly, differences were established already before but we were not able to see that because we only looked at abundances but not at processes.

4.2 Mesozooplankton

4.2.1 Mesozooplankton succession

The MZP community enclosed in the mesocosms reflected fairly well the natural succession of MZP in Tvärminne/Storfjärden where rotifers, cladocerans and calanoid copepods comprise the major zooplankton taxa (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). Usually rotifers numerically dominate in spring/early summer (*Synachaeta* sp.) and reach a second peak in mid-summer/autumn (*Keratella* sp.). The calanoid copepods *Acartia bifilosa* and *Eurytemora affinis* show two abundance peaks, in mid-June and mid-September, respectively, and *Temora longicornis* occurs only at low numbers year-round. Cladocerans peak in summer (August/September) with *Bosmina longispina maritima* clearly dominating among *Podon* spp. and *Evadne nordmanni*. Highest MZP biomass is build up in summer (August/September) (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999).

The species composition in the mesocosms resembled well natural conditions and were dominated by the most common and successful genus/species known for the Gulf of Finland and the Tvärminne region such as *Acartia bifilosa*, *Eurytemora affinis*, *Bosmina longispina maritima*. Due to the rather late start of our mesocosm experiment after the spring phytoplankton bloom, the usual peak of *Synchaeta* sp. in spring/early summer – also one of the most successful species (i.e. *Synchaeta baltica*, Viitasalo (1992)) – was barely visible during the first days, later rotifers still occurred until termination but were not of great importance anymore.

Total population densities known for mesozooplankton in the Tvärminne area more or less coincide with abundances found in the mesocosms and range from median values between $\sim$ 22,000– $\sim$ 40,000 ind m^{-3} with occasional peak abundance for *Acartia bifilosa* and *Bosmina* sp. of up to 45,000 and 82,000 ind. m^{-3}, respectively. Average peak abundance of *Acartia bifilosa* and *Bosmina* sp. during a period from 1967–1984 was $\sim$ 10,000 ind. m^{-3} and $\sim$ 20,000 ind. m^{-3}, respectively (Viitasalo et al., 1995; Viitasalo, 1992). Between t_{24} and t_{31}, however, some exceptional high numbers (> 150,000 ind. m^{-3}) occurred in the mesocosms mainly attributed to extremely high occurrence of *Bosmina* sp.. Even higher densities exceeding 1,000,000 ind. m^{-3} during bloms of blue-green algae are known for *B. fatalis* in an eutrophic lake in Japan (Hanazato and Yasuno, 1987). The MZP community in the surrounding water did not entirely correspond with the mescosms over the course of the experiment. Whereas the dominance of particular species corresponded quite well until t_3, it diverged progressively after t_{10} when in the surrounding water the occurrence of colonies of blue-green algae (*Aphanizomenon*) and rotifera where higher than in the mesocosms, and the abundance of copepods and cladocerans comparatively lower (S. Lischka, pers. obs.). Most likely, this is a result of isolation of the mesocosm bags from surrounding water mass exchange and incoming plankton communities and selective advantage of single species in the mesocosms.

4.2.2 Copepods

This study is one of the first to follow MZP community development subjected to ocean acidification scenarios projected for this century in a close-to natural holistic plankton community (IPCC, 2013; Riebesell et al., 2008, 2013b). Previous study using the same mesocosm set-up investigated effects on an Arctic MZP community and found no significant difference neither in total abundance or abundance of single taxa nor in species diversity (Niehoff et al., 2013; Riebesell et al., 2013a).

Copepods comprised one of the two dominant taxonomic groups in the present study and the mesocosm approach allowed to investigate CO_2 effects on the succession of all different life stages from eggs to reproducing adults. While copepods are thought to be rather robust against ocean acidification with negative effects occurring usually not until pCO_2 levels far beyond projections for end of this century (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012; McConville et al., 2013; Almén et al., 2016), more recent studies give evidence that copepods' sensitivity may be highly stage dependent and thus so far mostly underestimated due to the fact that most studies done to-date considered only adult stage copepods (Cripps et al., 2014). Over the CO_2 range projected for this century, we found no distinct abundance differences for neither

of the species. The permanent occurrence of adult males and females together with copepodite stages and nauplii suggest more or less continuous reproduction. Concurrent lab experiments investigating the effect of CO_2 on reproductive success of *Eurytemora affinis* are in agreement with the observations from the mesocosms (Almén et al., 2016, this issue). Incubated *Acartia bifilosa* showed fCO_2 unaffected egg production, but slight negative effects on egg hatching and development were found and adult females were smaller in the two highest CO_2 mesocosms (Vehmaa et al., 2015, this issue). Our results are also in line with Niehoff et al. (2013) who do not describe any apparent CO_2 effect on an Arctic MZP community including copepods. Copepods in the study region naturally experience fCO_2, pH and also temperature fluctuations of more than 0.5 pH units and 5°C temperature during daily vertical migrations which is more than the predicted climate change for the year 2100. I.e. these copepods are probably well adapted to short-term physico-chemical changes (Lewis et al., 2013; Almén et al., 2014).

4.2.3 Cladocera – OA effect on *Bosmina* spp. through increased food availability?

Most conspiciuous differences found in mesozooplankton abundance are due to the cladoceran *Bosmina* sp. between t_{24} and t_{31}. In three of the four CO_2 enriched mesocosms (497 μatm, 821 μatm, 1007 μatm) peak numbers were twice or even more than twice as high compared to the control and the highest CO_2 mesocosms, though a significant relation with fCO_2 could not be proved. Nevertheless, this striking difference may possibly point to an indirect CO_2 effect through higher food availability under high CO_2.

Cladocerans are highly reproductive at times of favourable environmental conditions. The life-span of *Bosmina* spp. varies between 20–25 days, age of first reproduction is between 4–7 days (food dependent) and populations can increase twofold within 5–10 days (Purasjoki, 1958; Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Biswas et al., 2014). Population dynamics of *Bosmina longirostris* are highly food-sensitive with food quantity and quality having a significant effect on growth, net reproductive rate and rate of population increase to shorten life time to up to 10 days (Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Urabe, 1991). Cladocerans are opportunistic feeders that graze on nano- and microplankton, bacteria (including Cyanobacteria), and detritus (Purasjoki, 1958; Nanazato and Yasuno, 1985; Work and Havens, 2003; Kluijver et al., 2012). *Bosmina* tolerates low pH in acidic lakes well (Uimonen-Simola and Tolonen, 1987).

The above mentioned population increase of *Bosmina* in the mesocosms coincides with significant CO_2 mediated differences during phase II in Cyanobacteria during the respective days and may have represented favourable food conditions for this species enhancing asexual reproduction in particular in the elevated CO_2 mesocosms (Paul et al., 2015). The highly positive correlation between Cyanobacteria and *Bosmina* sp. supports this assumption. Only M8, the mesocosm with the highest CO_2 concentration, diverged from this trend. Peak abundance in all mesocosms occurred only on one sampling day, i.e. did not stay high for a longer period but was low at the preceding sampling day and had dropped already at the following sampling day. Possibly, the drop in population size that occurred earlier than to be expected from *Bosmina*'s lifespan of around 20 days was due to high mortality and/or change to sexual reproduction producing resting eggs. Therefore, a

possible explanation why *Bosmina* in M8 did not follow the trend observed in the other CO_2-elevated mesocosms may be that due to the rather low possible sampling frequency (every seven days) the actual abundance peak was missed (Riebesell et al., 2013a). Reason for mortality could be in response to the overall drop in available food during phases II and III and/or stress response due to extreme densities or reproductive rates of *Bosmina* itself. It is known, that *Bosmina* sp. can die earlier when they have higher reproductive rates and switch to sexual reproduction producing resting eggs, respectively, at too high population densities (so called "crowding phenomenon") (Purasjoki, 1958; Acharya et al., 2005). In Kankaala (1983), *Bosmina* started sexual reproduction at around 4,500 ind. m^{-3} which is about 1–2 orders of magnitude less than observed peak numbers in the mesocosms.

The significant results we found for the ratio of *Bosmina* with empty and full brood chambers strongly suggest that organisms in the high CO_2 mesocosms had higher reproductive activities during the time of actual peak abundance. In particular, *Bosmina* in M8 and M3 (two highest CO_2 levels) had continuously low brood chamber ratios (i.e. large proportion of actively reproducing organisms in the population) from t_{10} onwards (with the ratio in M8 mostly even lower than in M3). This supports our assumption that we may have missed to sample the abundance peak of *Bosmina* in M8 possibly obstructing to prove a significant indirect fCO_2 effect on *Bosmina* abundance through increased food availability.

4.2.4 Predator/prey relationships

We have some evidence for fCO_2 stimulated predator/prey relationships between *Myrionecta rubra*/Cryptophytes and *Bosmina* sp./Cyanobacteria, though the mixotrophic ciliate *M. rubra* may also have benefitted directly from elevated fCO_2 concentrations (see above) . With respect to *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp., the fCO_2 related abundance differences during particular phases of the experiment can not be explained through enhanced predator/prey relationships.

Although our results show no direct significant CO_2 effect on *Bosmina* abundance, we can not rule out that growth and reproduction was stimulated from increased Cyanobacteria availability at elevated CO_2 mostly during phases II and III or from increased heterotrophic bacterial production (Hornick et al., 2016). This would point to an indirect CO_2 effect that was masked as a consequence of too low sampling frequency not allowing to adequately capture the population dynamics of this short-lived and highly adjustable genus. For the study region, microbial loop has been shown to be of particular importance during late summer and autumn when most of the secondary production including fish is fueled by carbon channeled from the microbial loop to crustacean zooplankton (Uitto et al., 1997; Koski et al., 1999). In the present study, heterotrophic bacterial production and biovolume was strongly linked to phytoplankton dynamics and suggested several indirect responses to fCO_2 (Hornick et al., 2016). Enhanced bacterial grazing, and thus stimulated microbial loop, was assumed in relation to higher fCO_2 (Crawfurd et al., 2016). This was mostly reflected in relatively high rates of cell-specific bacterial protein production of particle-associated heterotrophic bacteria throughout the entire experiment, though they only contributed a minor fraction to the overall heterotrophic bacterial biovolume (Hornick et al., 2016). Filter-feeding cladocerans directly feed on bacteria and flagellates and ef-

fectively transfer carbon from the microbial loop to higher trophic levels. In the eastern and western Gulf of Finland as well as in the southern Baltic Sea, *Bosmina longispina* can be the dominant prey for herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and three-spined stickleback (*Gasterosteus aculeatus*) (Casini et al., 2004; Peltonen et al., 2004). Larger herring feed more on Mysids during autumn that in turn can effectively prey on cladocerans including *Bosmina* sp. (Rudstam et al., 1992). Increased bacterial production (Hornick et al., 2016) may have provided optimal feeding conditions to favor reproduction of *Bosmina* sp. at elevated fCO_2, but, unfortunately our data can not provide a sufficient proof. ~~Contrary, in copepod dominated communities, the carbon transfer from microbial loop is comparatively low because an intermediate trophic level is needed (heterotrophic flagellates, ciliates) (Koski et al., 1999, and references therein.~~

A more recent publication by Wikner and Andersson (2012)~~, however,~~ states that increased microbial heterotrophy decreases trophic transfer efficiency of biomass to higher trophic levels. This work investigated the influence of increased river discharge through increased precipitation on phytoplankton biomass production and finds a shift in the carbon flow towards microbial heterotrophy. This shift was mainly due to an increase in freshwater and riverine organic carbon supply on phytoplankton growth despite a concomitant increase in nutrients. As already mentioned above, during phase III, increasing importance of production of the microbial food web related to higher fCO_2 was found in the present study (Paul et al., 2015; Crawfurd et al., 2016; Hornick et al., 2016) , concomitant with the abundance peak of the cladoceran *Bosmina* sp.. In plankton communities comprising species able to effectively graze on bacteria such as *Bosmina* sp., trophic transfer to higher trophic levels may not be necessarily decreased but could still be enhanced.~~Effects on higher trophic levels were not included in this analysis, though. Contrary, our results may indicate that, under increasing ocean acidification in cladoceran dominated MZP communities, the importance of trophic transfer from the microbial loop to higher trophic levels may become enhanced.~~

The results described for *M. rubra* and *Bosmina* should be robust also if biomass estimates were considered. The significant response of *M. rubra* to CO_2 was determined for cells of the same size class (all mostly 10 μm), i.e. biomass-based results would scale proportionally with cell numbers. In case of *Bosmina* the significant CO_2 relation was found for the ratio of embryo-bearing to non-embryo bearing organisms which is an abundance-/biomass-independent measure. As regards a possible indirect CO_2 effect that we suggested for *Bosmina* sp., abundance increased more than two-fold in the mentioned mesocosms and consisted of different-sized individuals. I.e. a respective increase in biomass would probably be smaller as compared to the abundance increase, but certainly still existent.

5 Conclusions

This study describes for the first time fCO_2 related effects on the zooplankton community level in a close to natural plankton community. Some ciliate species as well as the species diversity of ciliates responded to elevated fCO_2 levels. On the mesozooplankton level, significant fCO_2 effects were only found for the ratio of empty to full brood chambers of the cladocera *Bosmina* sp. but an indirect effect on *Bosmina* abundance

via food seems likely. Although for the ciliates, in particular the mixotroph *Myrionecta rubra*, the magnitude of change in abundance was rather minor as effects were observed only in the post-bloom phase, and for the cladoceran *Bosmina* sp. a fCO_2 effect could only be carefully assumed, our study has shown that ocean acidification effects can potentially translate up from the primary production level to higher trophic levels. Certainly, this is not a general consequence but is probably highly dependent on the species composition of a pelagic community, i.e. the presence of species that have the ability to quickly respond to changes in food availability and composition with increased reproduction or cell division, respectively, such as the highly flexible cladocerans or the mixotroph ciliate *Myrionecta rubra*.

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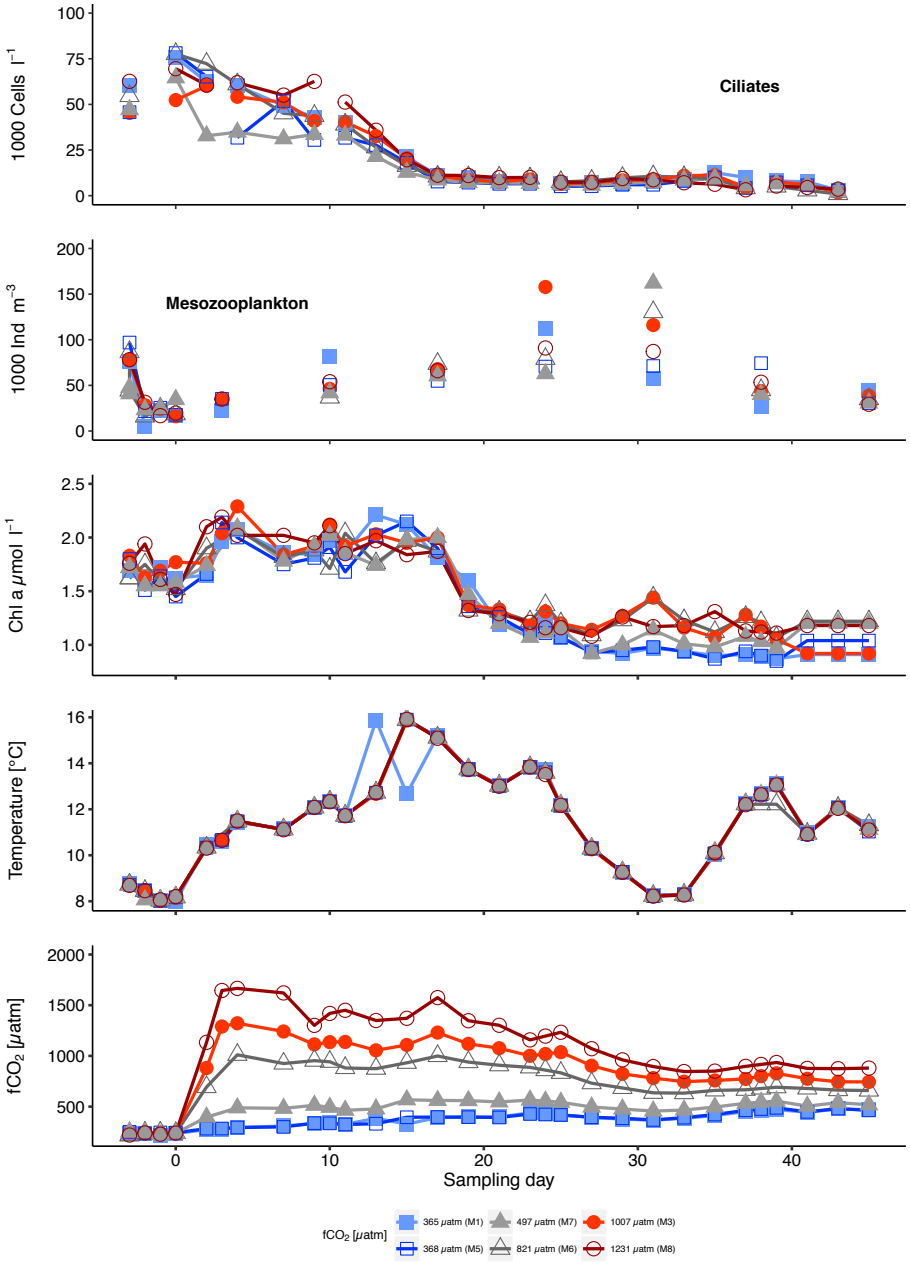

Figure 1. Total cell numbers of ciliates and total abundance of mesozooplankton during the course of the experiment as well as chlorophyll *a* succession, temperature and fCO_2 development. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}. Note there is one missing value in M1 on t_{13}.

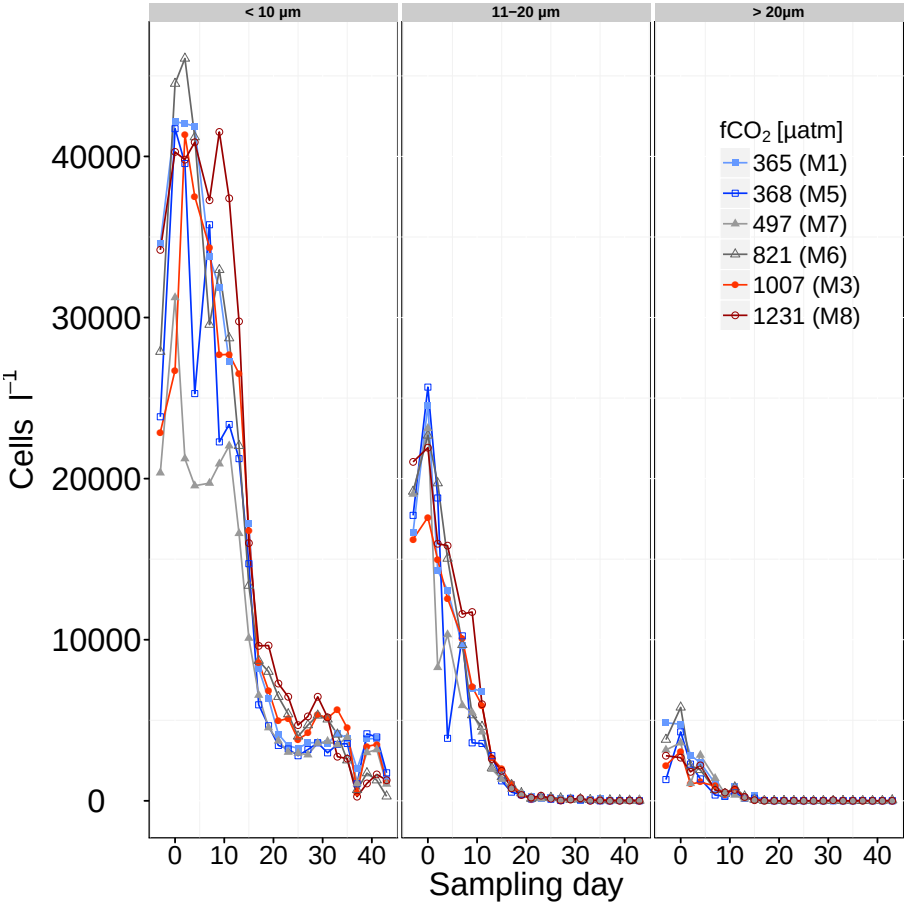

Figure 2a. Abundance of different size classes of *Myrionecta rubra*. Note there is one missing value in M1 on t_{13}.

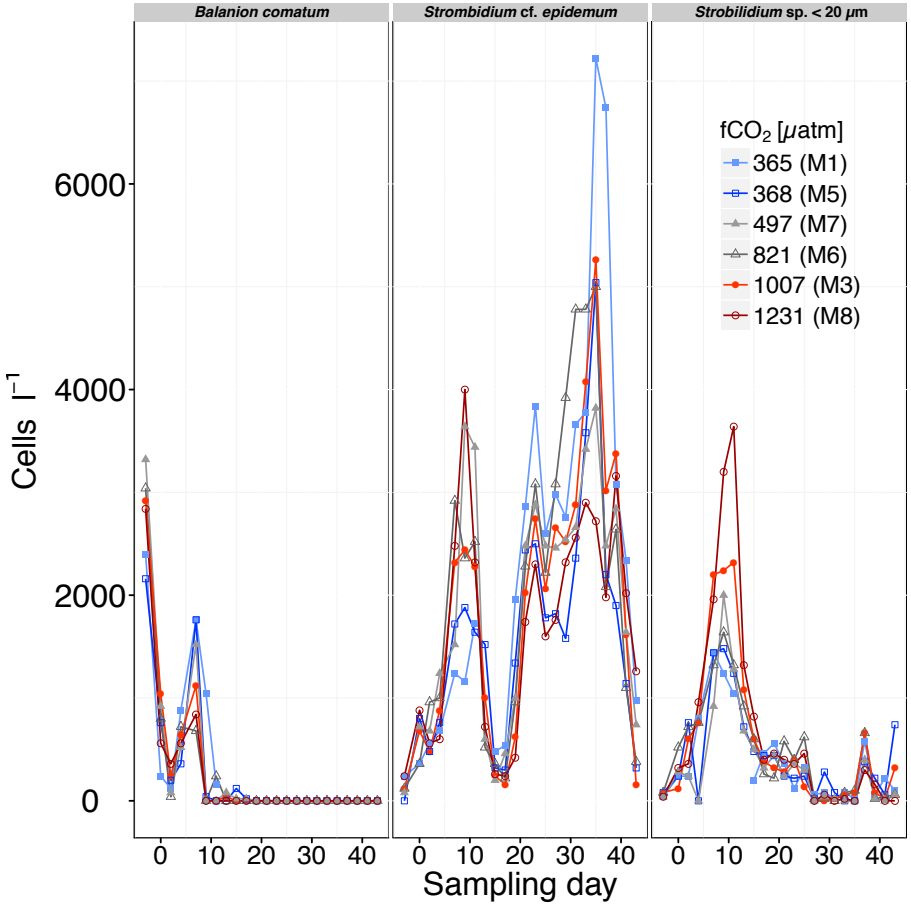

Figure 2b. Abundance of other ciliate species/genera/groups. Note there is one missing value in M1 on t_{13}.

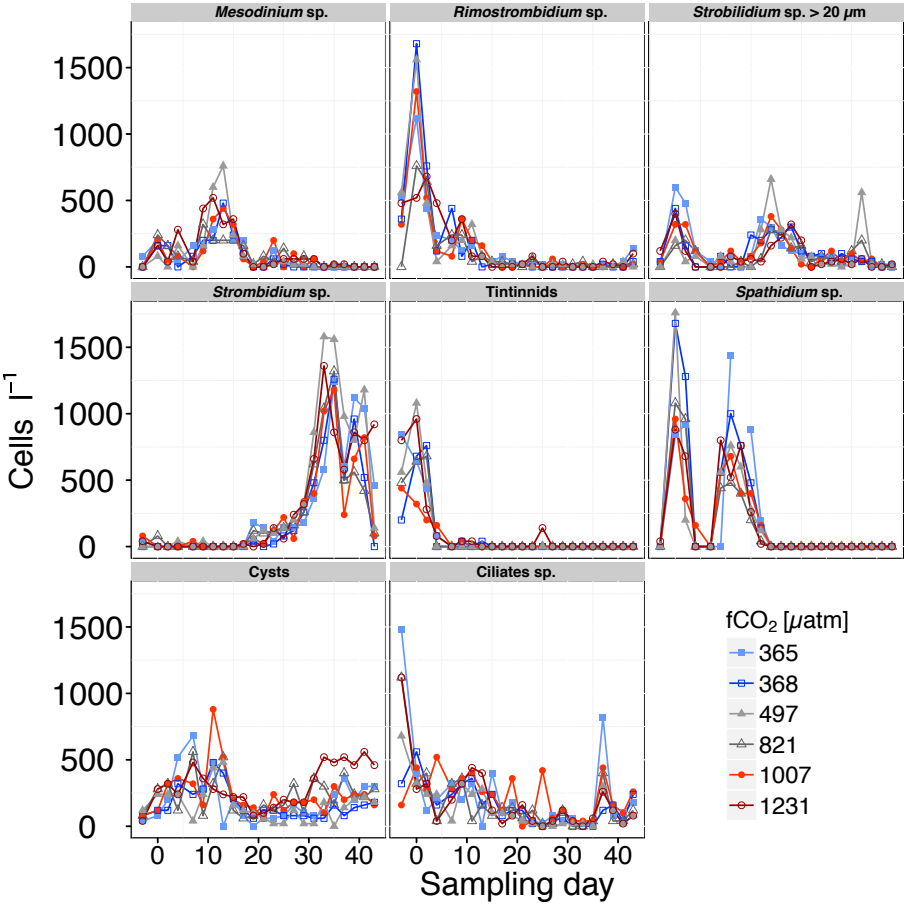

Figure 2c. Abundance of other ciliate species/genera/groups. Note there is one missing value in M1 on t_{13}.

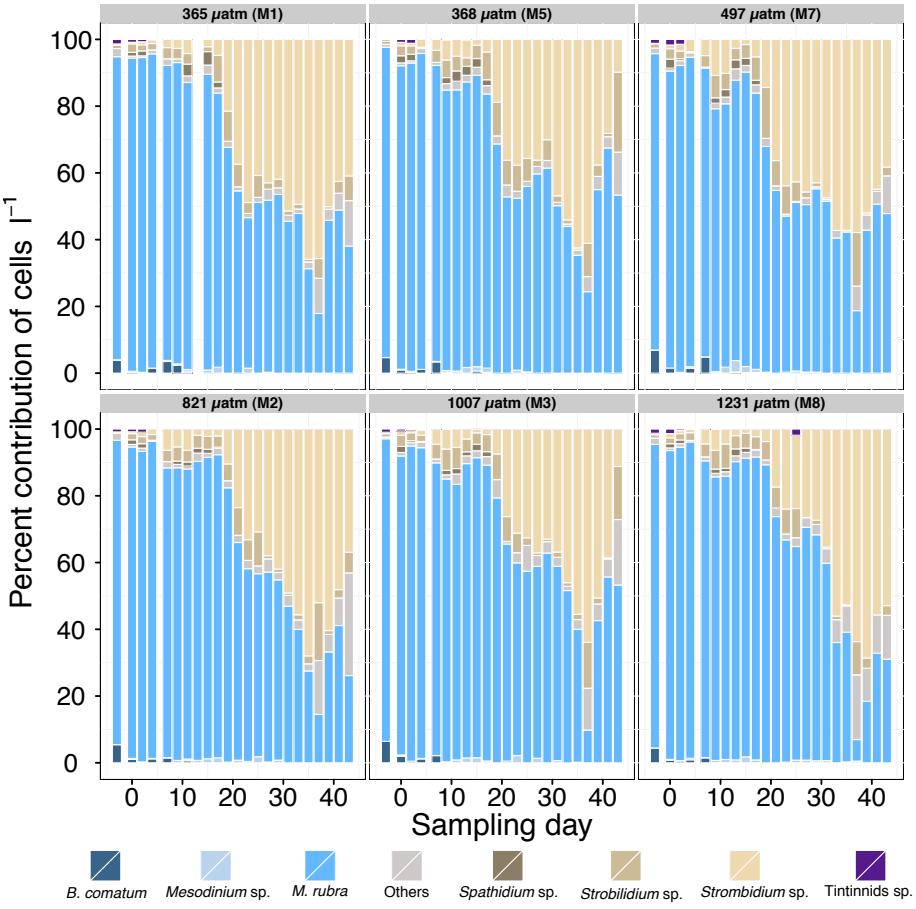

Figure 3a. Percent contribution of abundance of major taxonomic species/genera/groups to the ciliate community. *B. comatum* = *Balanion comatum*, *M. rubra* = *Myrionecta rubra*. Note there is one missing value in M1 on t_{13}.

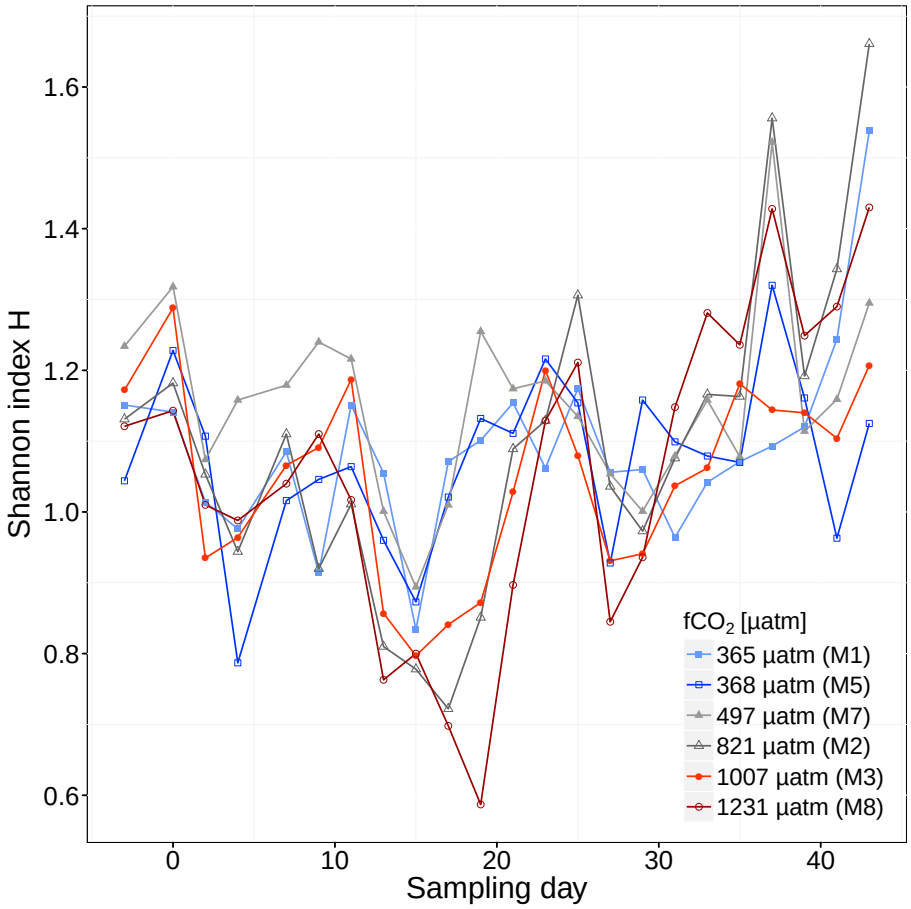

Figure 3b. Ciliates, daily change of the Shannon diversity index *H* at the different fCO_2 levels in the mesocosms.

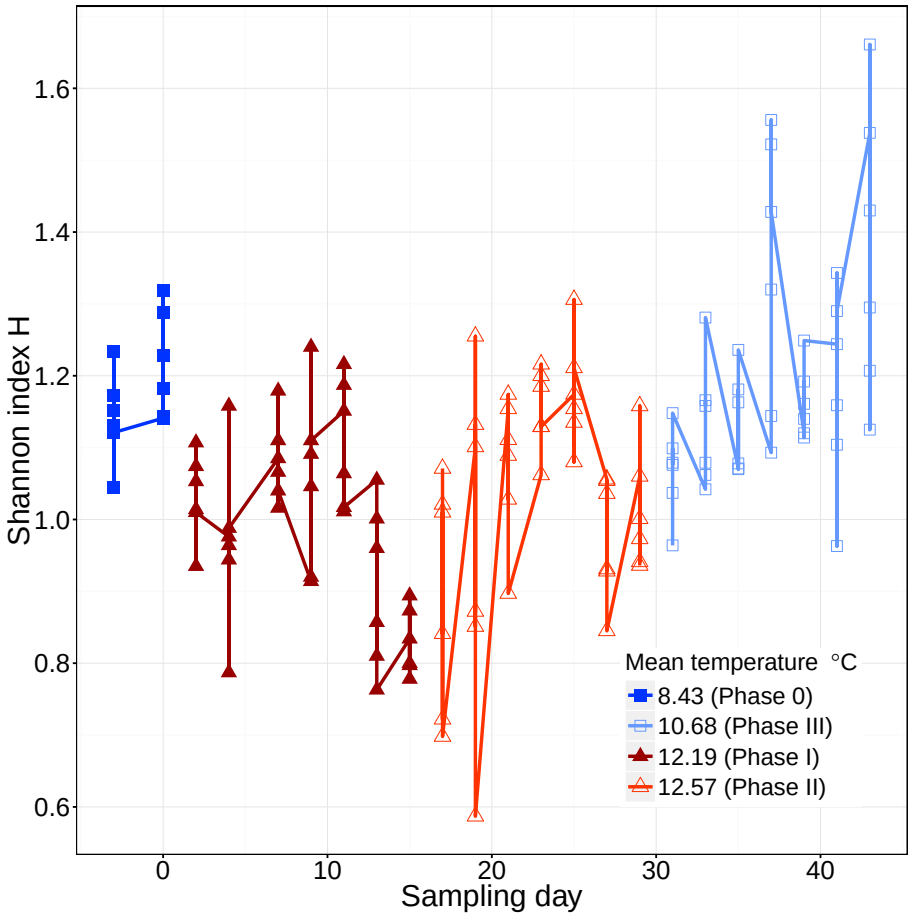

Figure 3c. Ciliates, daily change of the Shannon diversity index *H* during the 4 different temperature phases defined. Colour legend gives mean temperature during Phase 0 (12.57 °C), Phase I (8.43 °C), Phase II (10.68 °C), and Phase III (12.19 °C).

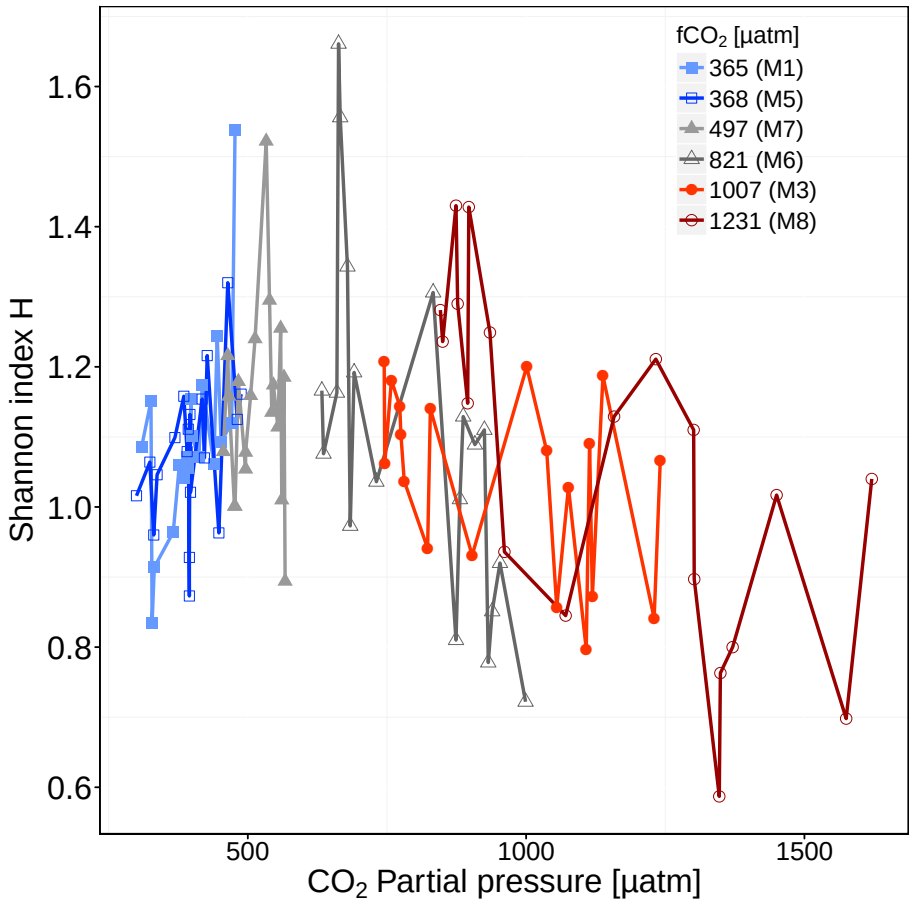

Figure 4a. Ciliates, graphical depiction of statistical results for Shannon diversity index *H* as a function of fCO_2: H is shown in relation to the daily change of fCO_2. Symbols and colours identify the mean fCO_2 for each mesocosm.

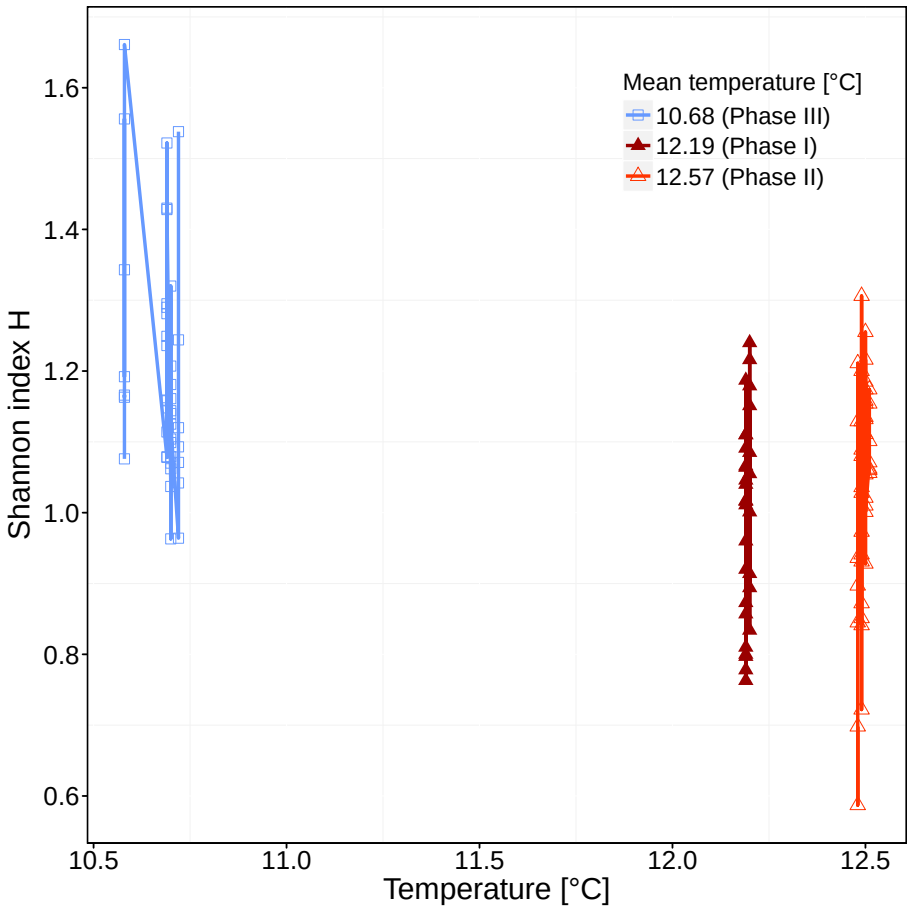

Figure 4b. Ciliates, graphical depiction of statistical results for Shannon diversity index *H* as a function of temperature. For better visibility, *H* is plotted against the mean phase (I, II, III) temperature of each mesocosm. Symbols and colours identify mean phase temperature across all mesocosms.

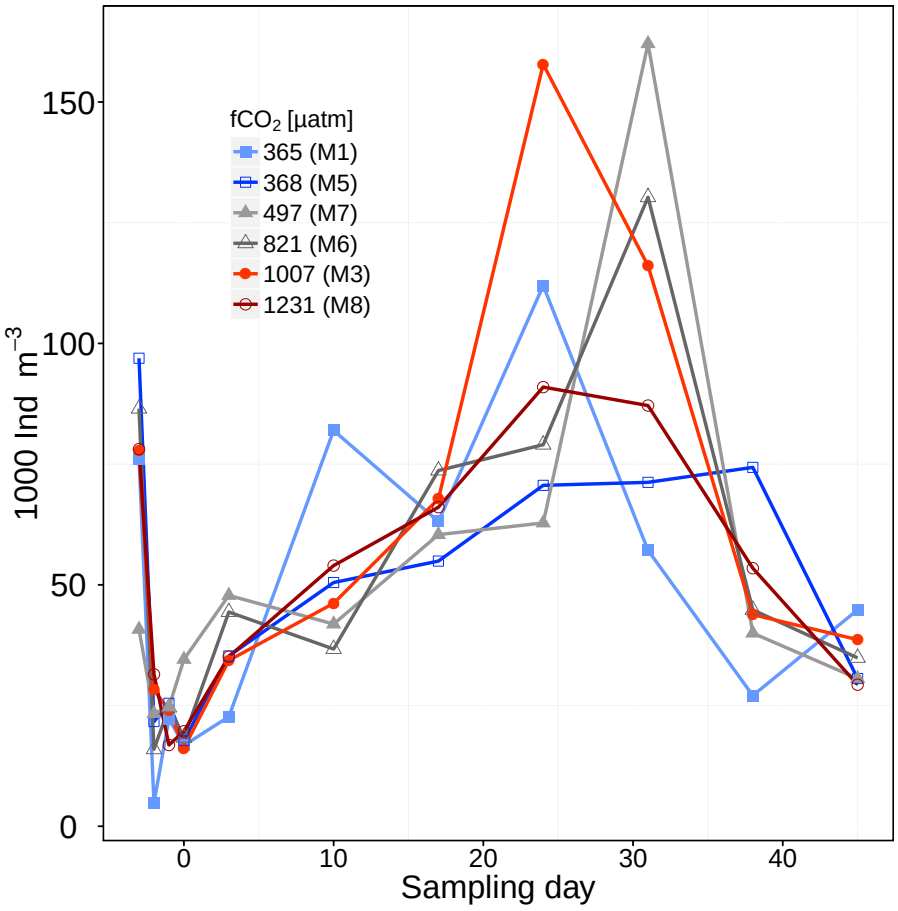

Figure 5. Mesozooplankton total abundance. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}.

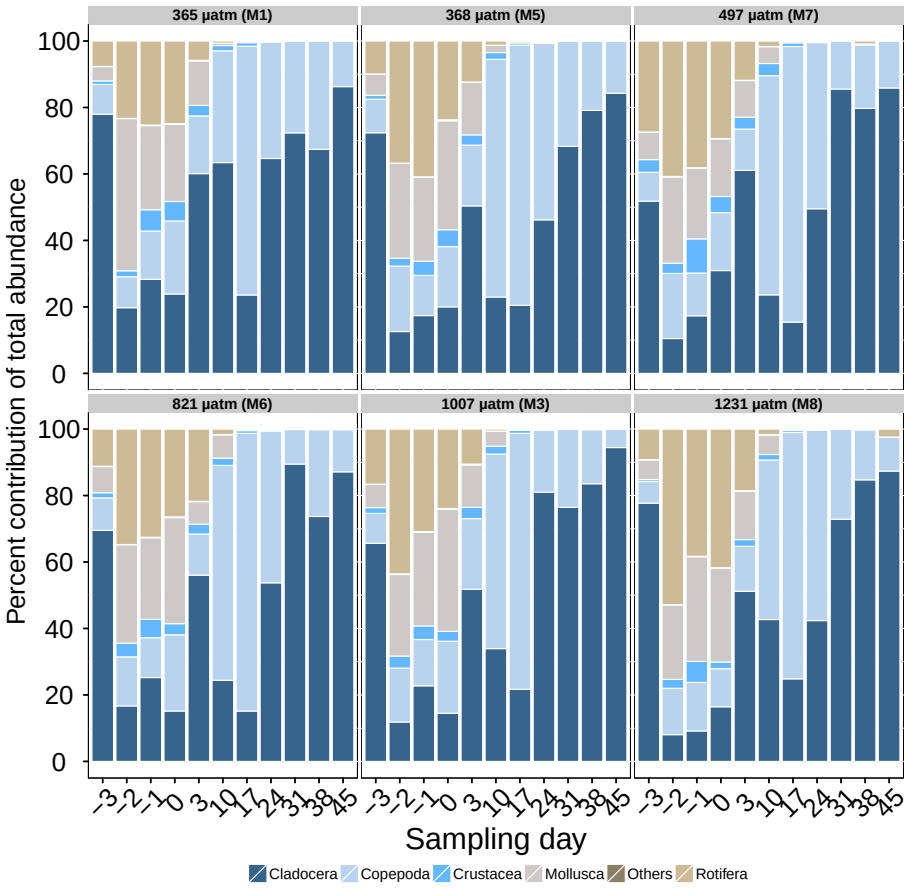

Figure 6. Percent contribution of mesozooplankton main taxonomic groups.

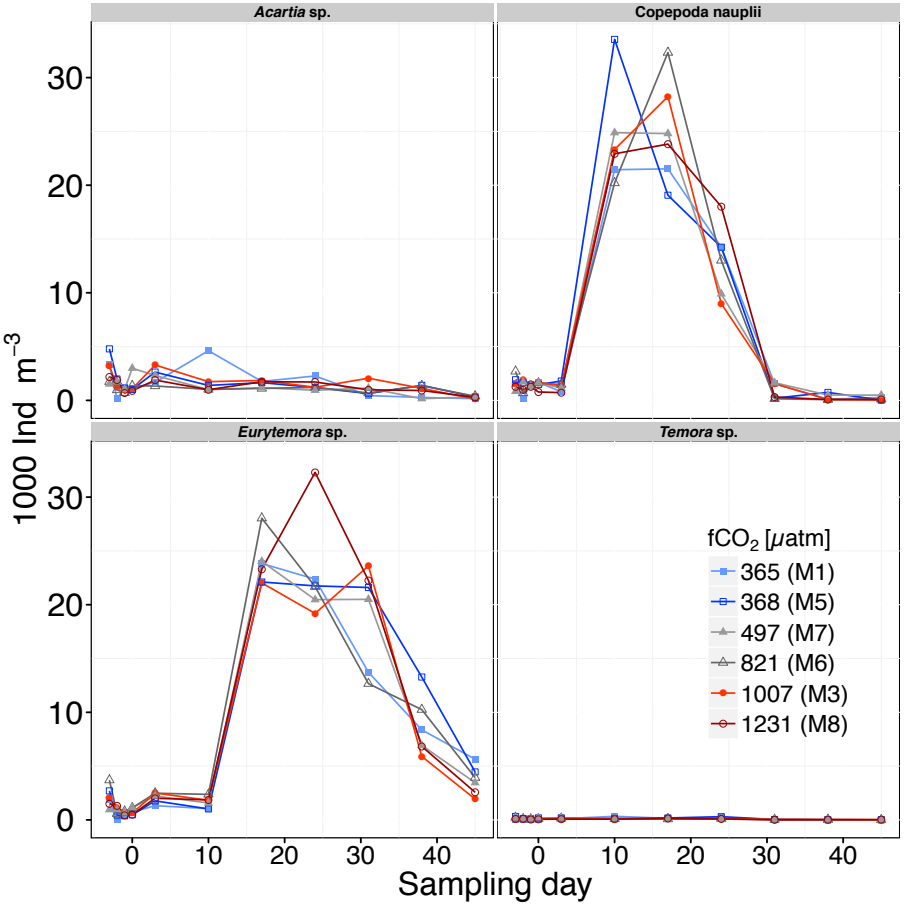

Figure 7a. Abundance of the dominant copepods species *Acartia* sp., *Eurytemora* sp., *Temora* sp., and copepod nauplii.

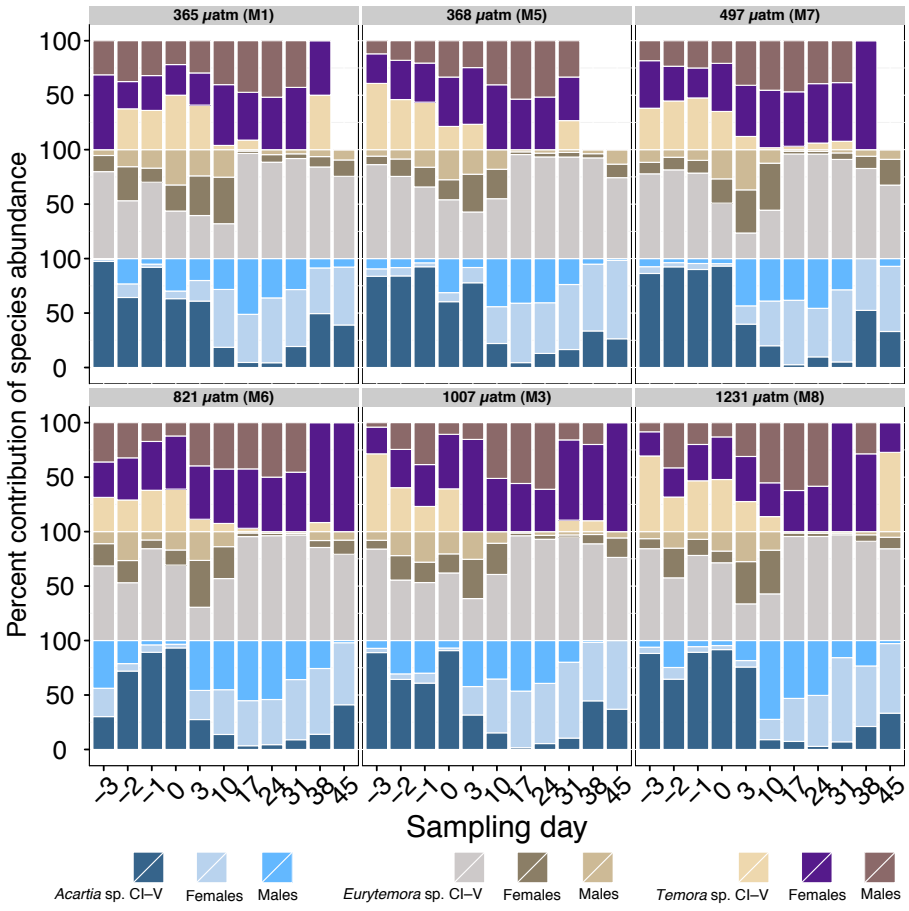

Figure 7b. Percent contribution of different stages of dominant copepods.

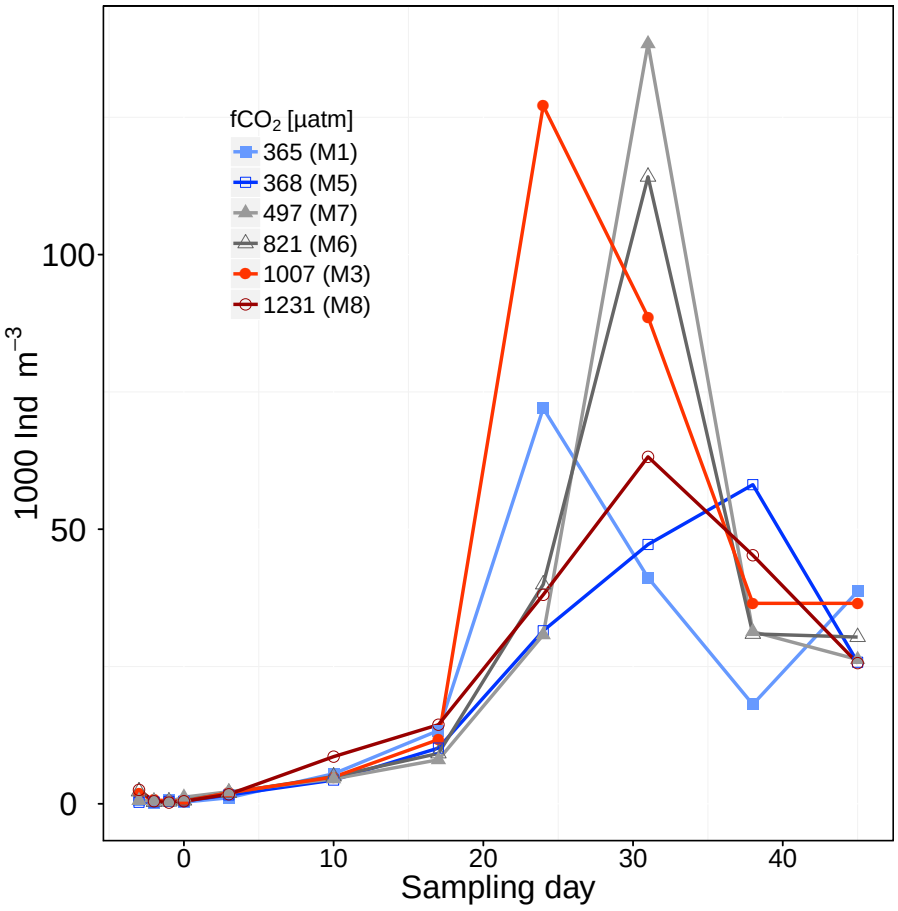

Figure 8a. Total abundance of the most dominant cladoceran species *Bosmina* sp..

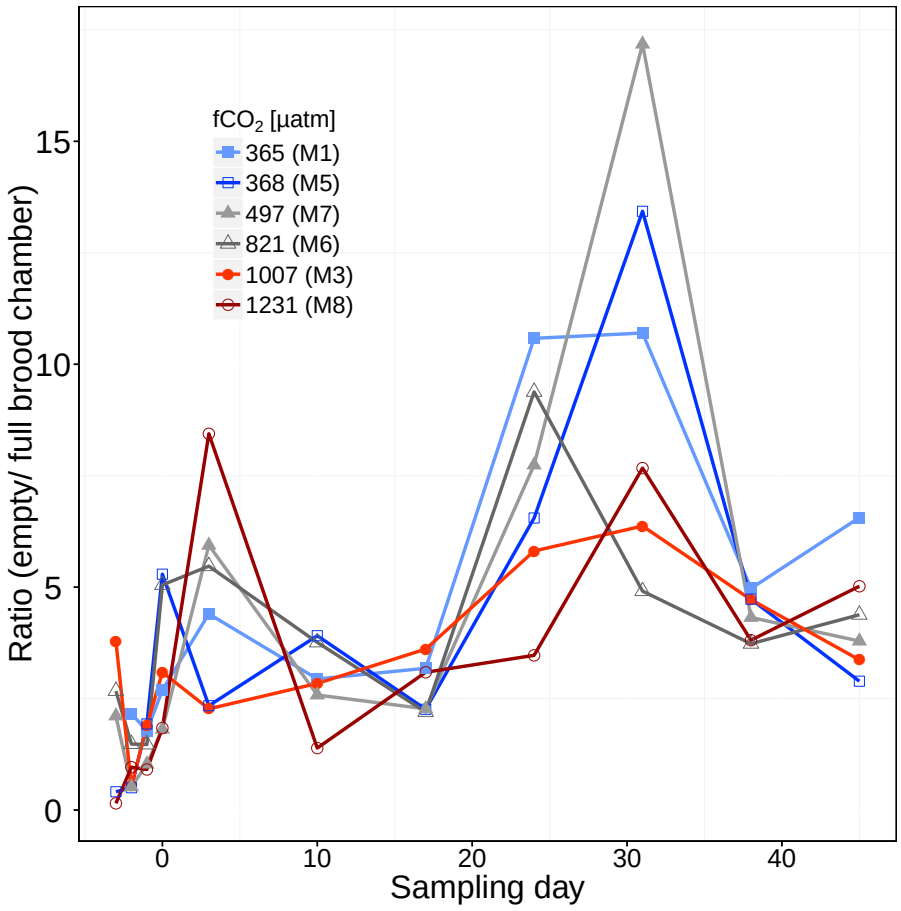

Figure 8b. Ratio of *Bosmina* with empty to full brood chambers. Note: Figure shows all data, but statistics were done on data from t_3–t_{45} only to assure equally spaced data.

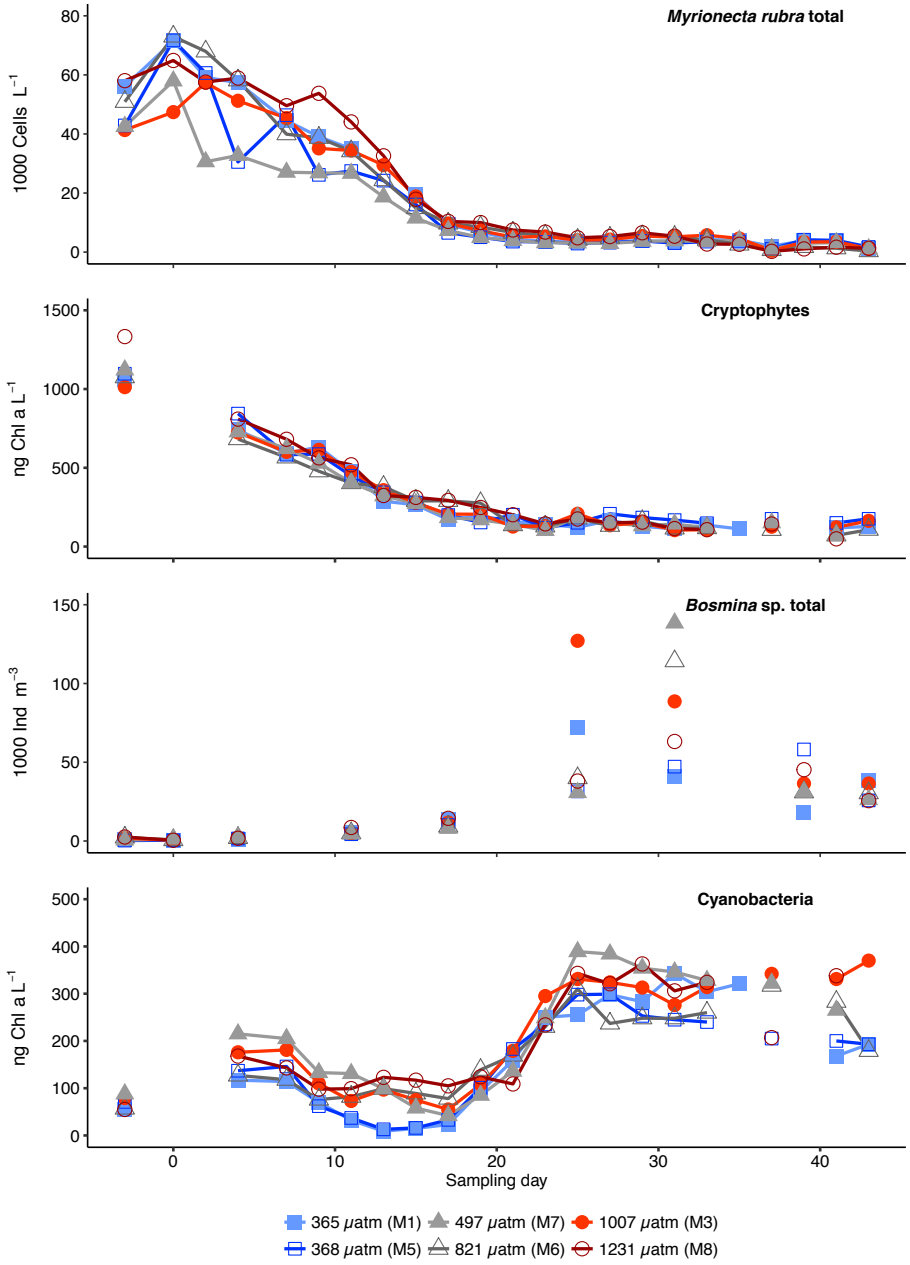

Figure 9. Succession of total cell numbers of *Myrionecta rubra*, total biomass of Cryptophytes, total abundance of *Bosmina* sp. and total biomass of Cyanobacteria during the course of the experiment. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}. Note there is one missing value in M1 on t_{13}.

Table 1. Statistics summary table of retained fixed effects of the GLM's and GAMM's. Significant p-values are indicated in bold (Temp: temperature).

	Explanatory variable	DF	t	p-value	Model
Ciliates					
Ciliates total abundance	Temp	1	-3.506	**0.0007**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	Temp	1	2.376	**0.019**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	fCO_2 * Temp	1	-2.298	**0.024**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	fCO_2 * Chl *a*	1	2.936	**0.004**	GAMM
Balanion comatum	Temp	1	2.320	**0.022**	GAMM
Balanion comatum	fCO_2	1	-2.210	**0.030**	GAMM
Strombidium cf. *epidemum*	Chl *a*	1	-3.229	**0.002**	GAMM
Strobilidium sp., < 20 μm	Temp	1	2.811	**0.006**	GAMM
Strobilidium sp., < 20 μm	Chl *a*	1	-4.603	**< 0.00001**	GAMM
Strobilidium sp., < 20 μm	fCO_2 * Temp	1	-3.600	**0.0005**	GAMM
Strobilidium sp., < 20 μm	fCO_2 * Chl *a*	1	3.926	**0.0002**	GAMM
Shannon index *H*	Temp	1	3.652	**0.0004**	GAMM
Shannon index *H*	fCO_2	1	2.824	**0.006**	GAMM
Shannon index *H*	fCO_2 * Temp	1	-3.454	**0.0008**	GAMM
Mesozooplankton					
MZP total abundance	Temp	31	-1.155	0.257	GLM
MZP total abundance	fCO_2	31	-0.025	0.980	GLM
MZP total abundance	Chl *a*	31	0.550	0.586	GLM
MZP total abundance	fCO_2 * Temp	31	0.947	0.351	GLM
MZP total abundance	fCO_2 * Chl *a*	31	-1.081	0.288	GLM
Bosmina sp.	Chlor *a*	1	0.76	0.453	GAMM
Bosmina sp. ratio empty/full brood chambers	Temp	1	-3.572	**0.001**	GAMM
Bosmina sp. ratio empty/full brood chambers	fCO_2	1	-2.684	**0.011**	GAMM
Bosmina sp. ratio empty/full brood chambers	Chl *a*	1	-3.980	**0.0004**	GAMM
Bosmina sp. ratio empty/full brood chambers	fCO_2 * Chl *a*	1	2.738	**0.01**	GAMM
Shannon index *H*	Chl *a*	1	-0.555	0.582	GAMM

Table 2. Pearson correlation for various predator/ prey relationships. Listed are only correlations $\geqslant 0.7$. The pairwise correlation plots for all group combinations and the Pearson correlation coefficients can be seen from supplemental material (Fig. S2−S1). het Dino.: heterotrophic dinoflagellates, excl.: excluded. For *Myrionecta rubra* Pearson correlation was determined combined for all fCO_2 levels and also separate for low (365 μatm, 368 μatm, 497 μatm) and high (821 μatm, 1007 μatm, 1231 μatm) fCO_2 levels. [1]data from Paul et al. (2015), [2]Crawfurd et al. (2016), [3]data from A. Stuhr (unpublished), [4]this study.

Predator/Prey	Pearson correlation	fCO_2 levels	Method
Ciliates/Bacteria, Phytoplankton groups			
Myrionecta rubra < 10 μm/Cyanobacteria	-0.7	high	CHEMTAX[1]
Myrionecta rubra < 10 μm/low DNA bacteria	-0.7/ -0.7/ -0.7	all/ low/ high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Pico~~flagellates~~eukaryotes III	-0.7/ -0.7	low/ high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Synechococcus	-0.7	high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Cryptophytes	0.8/ 0.8/ 0.8	all/ low/ high	CHEMTAX[1]
Myrionecta rubra 10–20 μm/Cryptophytes	1.0/ 0.9/ 1.0	all/ low/ high	CHEMTAX[1]
Myrionecta rubra > 20 μm/Cryptophytes	0.9/ 0.8/ 0.9	all/ low/ high	CHEMTAX[1]
Myrionecta rubra < 10 μm/het. Dino.	0.8	all	Microscopy[3]
Myrionecta rubra 10–20 μm/het. Dino.	0.7	all	Microscopy[3]
Myrionecta rubra < 10 μm/het. Dino. (*Ebria* sp. excl.)	0.8	all	Microscopy[3]
Myrionecta rubra 10–20 μm/het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Myrionecta rubra > 20 μm/het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Balanion comatum/Cryptophytes	0.8	all	CHEMTAX[1]
Mesodinium sp./Euglenophytes	0.7	all	CHEMTAX[1]
Rimostrombidium sp./Cryptophytes	0.8	all	CHEMTAX[1]
Tintinnids sp./Cryptophytes	0.7	all	CHEMTAX[1]
Spathidium sp./Euglenophytes	0.7	all	CHEMTAX[1]
Mesozooplankton/Bacteria, Phytoplankton groups, Ciliates			
Podon sp./Cryptophytes	0.9	all	CHEMTAX[1]
Bosmina sp./Cyanobacteria	0.7	all	CHEMTAX[1]
Podon sp./het. Dino.	0.7	all	Microscopy[3]
Podon sp./het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Eurytemora sp./Pico~~flagellates~~eukaryotes II	0.7	all	Flowcytometry[2]
Eurytemora sp./Cryptophytes	-0.7	all	CHEMTAX[1]
Copepod nauplii/Euglenophytes	0.7	all	CHEMTAX[1]
Copepod nauplii/Nano~~flagellates~~eukaryotes II	0.8	all	Flowcytometry[2]
Podon sp./*Balanion comatum*	0.8	all	Microscopy[4]