

GEOMAR | Düsternbrooker Weg 20 | 24105 Kiel | Germany

**Handling associate editor of
Biogeosciences
Prof. Dr. C.P.D. Brussaard**

Dr. Silke Lischka

Tel +49 431 600-4029
Fax +49 431 600-4446
slischka@geomar.de

Revision of our manuscript bg-2015-618

September 7, 2016

Dear Corina,

please find enclosed our revised 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“*.

You may notice, that we have changed the title slightly according to a suggestion of one of the reviewers who asked us to use the term „ciliates“ instead of microzooplankton.

The point-by-point-response includes our original response to all referee’s comments („**Author response**“ in black letters), and directly below each response, detailed comments of the changes that we performed in the revised manuscript (in **blue letters**, equivalent to a detailed list of changes).

The most substantial changes that we performed according to the reviewers suggestions, are with respect to some of the figures (stacked bar plots instead of line plots), we considered most important predator/ prey interactions and included a respective paragraph in the discussion, and shortened the discussion on *Myrionecta rubra* and *Bosmina* sp. For evaluating predator/ prey relations, we have used, amongst others, data from Kate Crawford and yourself (as you know already). Seeing

GEOMAR
Helmholtz-Zentrum für
Ozeanforschung Kiel

Düsternbrooker Weg 20
24105 Kiel | Germany

Tel +49 431 600-0
Fax +49 431 600-2805
www.geomar.de

Deutsche Bank AG Kiel
BLZ 210 700 24
Kto. 144 800 000

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IBAN DE 69210700240144800000

Steuernummer 1929401912
VAT DE281295378

the revised manuscript now, if you should feel this addition would deserve co-authorship, please, don't hesitate to contact me.

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

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 Tvärminne, Sweden, using natural plankton communities during a summer situation. The focus of the present study was on micro- and mesozooplankton communities and their vulnerability to changes in ocean pH. In addition, ambient temperature and chlorophyll a (as a proxy for phytoplankton biomass) were considered as additional factors in order to relate these to changes in micro- and mesozooplankton abundances.

While the overall aim of the present study as well as the experimental approaches addressed are of great relevance, the manuscript has some considerable shortcomings. The ms is written in a very descriptive manner presenting many details on specific taxonomic groups/species/genera while a thorough elaboration of the main results and conclusions is missing. The way the data is presented should be re-considered in order to concentrate on the main important results instead of including too many details (e.g. showing both abundance data of each specific group and the percent contribution of major taxonomic groups each in a separate graph).

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

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

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

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

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

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

- 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).

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

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

- See our response just above. Different phytoplankton taxonomic groups were included in the pairwise correlations addressing predator/prey relationships (Table 2, supplemental material).

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

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

- 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.

Specific comments Introduction

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

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

- We have included a paragraph with some more details on the food web structure in the Tvärminne region (L73–82).

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 response: Other microzooplankton groups such as heterotrophic dinoflagellates were present but not part of this analysis. Data on heterotrophic dinoflagellates are shown in Spilling et al. 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'.

- In the revised manuscript, we have changed the category 'microzooplankton' to 'ciliates'.

Material & methods

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

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

- 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 response: 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.

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

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

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

Results General Comment:

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

Author response: Please see our response above.

Ref #1: Figure1: It would be helpful if the 3 different phases of the experiment would be mentioned within Figure 1. Further, adding temperature and total chlorophyll a as additional y-axes will help to improve the interpretation of the results.

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

additional y-axes.

- 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 response: Principally, these data are available and were mostly included in the overview paper to this study (Paul et al. 2015) and some others are shown in Spilling et al (2016) and Crawford 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.

- 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 response: Bacteria data are presented in the manuscript by Crawford 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.

- We have considered bacteria data in the Pearson correlations (Table 2, Supplemental material).

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

Author response: We will change Figure 3 accordingly.

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

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

- The reason why temperature was chosen to characterize phases was explained earlier in response to a previous comment.

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

subplots (s.a.) and show them if reasonable.

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

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

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

- 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:

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

- 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).

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

- 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).

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 were overall rather low and the results seem to be over-interpreted. The argument that increased CO₂ will strongly stimulate growth in *M. rubra* needs to be re-considered.

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

- 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*.

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 III (L. 477). As a suggestion, the authors could consider to correlate cryptophyte abundances with the different size classes of *M. rubra* in order to analyse predator-prey relationship in this species in more detail.

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

- 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.

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

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

- Section was condensed and argumentation consolidated through correlations with cryptophyte abundances, s. above.

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

- We have inserted a new paragraph "Predator/ prey relationships" (4.2.4) and moved much of the former conclusion to this section. As the focus 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 extend 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.).

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

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

discussion accordingly.

- 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.

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

Author response: Will be considered in a revised version.

- **Conclusions have been customized accordingly.**

References:

Crawford, 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., Crawford, 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., Stühr, 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.

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. 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 CO₂ 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 response: 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.

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 response: The abstract will be modified in consideration of all revisions applied to the manuscript.

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

1. Introduction

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

Where is “Storfjärden” and “Tvärminne”? (page 20029 / line 2, line 6)

Author response: 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 manuscript.

- We have included this information in the introduction (L66/67).

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 response: 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 response: 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.

- We have condensed the results section and removed text passages that were not of major importance and sometimes a bit repetitive.

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 response: 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 CO₂ 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.

- We have included the CO₂ development in Fig. 1 now.

Ref #2: 3.1.4: I would also like to see the temporal development in the Shannon index H.

Author response: 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.

- 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 response: We will do that.

- We have extended section 3.1.5 and described the most important statistical findings in more detail.

4. Discussion

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

Author response: To make it clearer, we will rephrase this sentence towards: “We found no significant relation between microzooplankton total abundance and fCO₂ 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 fCO₂, respectively.”

- Sentence was changed accordingly, L455/456.

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 response: We will consider this comment carefully in a revised version and better detail the most significant and meaningful findings.

- 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 response: 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.

- This section was condensed, s. response to referee #1. Also, we changed the heading to “*Myrionecta rubra*”.

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 response: 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).

- In the revised version, we have shortened section 4.2.1 by about the half and removed section 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 response: 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 CO₂ 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 CO₂ 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.

- 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.

Ref #2: 4.2.3 The finding of correlation between empty-filled brood chamber ratio and CO₂ 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 CO₂ will have any effect. There are simply too many open issues between the relationship between CO₂ increase and *Bosmina* food conditions in the Baltic Sea.

Author response: 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.

- 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.

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 response: 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.

- We have inserted a new paragraph "Predator/ prey relationships" (4.2.4) and moved much of the former conclusion to this section. As the focus 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).

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.

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

Silke Lischka¹, Lennart T. Bach¹, Kai-Georg Schulz^{1,2}, Ulf Riebesell¹

¹GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany
²present address: Centre for Coastal Biogeochemistry, Southern Cross University, Military Rd, East Lismore, NSW, 2480, Australia

Correspondence to: Silke Lischka (slischka@geomar.de)

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 CO₂ enrichment experiment (55 m³) 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 CO₂ elevations expected for this century. Besides the response to fCO₂, we also considered temperature and chlorophyll a variations in our analyses. Shannon diversity of ciliates significantly decreased with fCO₂ and temperature with a greater dominance of smaller species. The mixotrophic *Myrionecta rubra* seemed to indirectly and directly benefit from higher CO₂ concentrations in the post-bloom phase through increased occurrence of cryptophytes at higher CO₂ levels. With respect to mesozooplankton, 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 CO₂-enriched mesocosms except for the highest CO₂ level. The ratio of *Bosmina sp.* with empty to embryo/ resting egg bearing brood chambers, however, was significantly affected by CO₂, temperature and chlorophyll *a*. An indirect CO₂ effect via increased food availability (cyanobacteria) stimulating *Bosmina sp.* can not be ruled out. 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 CO₂ emissions have increased at an unprecedented rate and cause a concomitant increase of CO₂ concentration in the oceans. Thereby, ocean carbonate chemistry is altered with the main changes being reduced carbonate ion concentrations [CO₃²⁻] 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

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5 | already and projections suggest a further decrease of 0.14–0.43 units by the end of the century IPCC2013. The Baltic Sea, one of the largest brackish water systems, is sensitive to CO₂ 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 fCO₂ 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; Almen et al., 2014).

10 | 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 CO₂ 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 fCO₂/ pH changes expected for this century and direct negative effects usually do not occur unless exposed to much higher fCO₂ levels projected only much later (Kurihara et al., 2004; IPCC2013). 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 CO₂ effects may be more indirectly mediated to the zooplankton level through CO₂ induced changes in the biochemical and/ or stoichiometric composition of their food (Urabe et al., 2003; Rossoll et al., 2012).

25 | Holistic approaches studying CO₂ 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.

30 | The Tvärminne/ Storfjärden 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/ Storfjärden, among mesozooplankton rotifers, copepods and cladocera (Kivi 1986; Viitasalo 1992; Koski et al., 1999). In the Tvärminne/Storfjärden area during late summer and autumn, the microbial food web (MFW) is of particular importance

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5 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 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 on the mesozooplankton species composition (Hansen et al., 1994). Elevated CO₂ 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 CO₂ ranges projected to occur within this century (Aberle et al., 2013; Niehoff et al., 2013; Schulz et al., 2013).

10 As part of the KOSMOS Tvärminne mesocosm experiment, we examined CO₂ effects on the enclosed micro- 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 fCO₂ gradient was set up in six approximately 55 m³ mesocosms covering fCO₂ projections for this century or beyond (IPCC2013). 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. Within the category 'microzooplankton' (MiZP) we focus on ciliates only and use the term 'ciliates' when referring to our study. 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).

15 Temperature can have a general effect on 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 CO₂ driven chlorophyll *a* differences (Schulz et al. 2013), we also included temperature and chlorophyll *a* as explanatory variables in our statistical analyses.

30 2 Methods

To study the effect of elevated fCO₂ on a natural plankton community in the Baltic Sea, nine KOSMOS offshore pelagic mesocosms (Kiel Off-Shore Mesocosms for future Ocean Simulation) 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

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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.

5 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 CO₂ 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 m³ (Paul et al., 2015). The nine mesocosms were enriched with different amounts of CO₂ saturated seawater to set up an initial

10 gradient of $f\text{CO}_2$ 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. $f\text{CO}_2$ 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

15 more technical details about the experimental set-up, the CO₂ manipulations, and sampling procedures for various analyses see (Paul et al., 2015). Sampling days were enumerated consecutively with t_3 indicating three days before CO₂ manipulation, t_0 as the day of the first CO₂ manipulation, and t_{1+x} as the days following the first CO₂ manipulation.

2.1 Microzooplankton sampling

20 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

25 100 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. (2009), 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

30 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 CO₂ addition (t_{-3} , t_{-2} ,

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t_1), at the day of the first CO₂ addition (t_0), and after the first CO₂ addition ($t_3, t_{10}, t_{17}, t_{24}, t_{31}, t_{38}, t_{45}$). After collection, the samples were brought back to the lab 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 microzooplankton 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, $f\text{CO}_2$, 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 $f\text{CO}_2$ 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 (R Developmental Core 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-/ mesozooplankton community over time and in response to different abiotic parameters such as the $f\text{CO}_2$ 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* (≤ 10 μm), *M. rubra* (11–20 μm), *M. rubra* (> 20 μm), *Rimostrombidium* sp., *Spathidium*

sp., *Strobilidium* spp. ($\leq 20 \mu\text{m}$), *Strobilidium* sp. ($> 20 \mu\text{m}$), *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. ($\leq 20 \mu\text{m}$). Most of the Strobilids found, however, were probably *Lohmaniella* sp.

5 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).

10 2.3.1 Microzooplankton

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

15 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.

20 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} and 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 CO_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

25 data, GLM models (generalized mixed effects) were applied on a Gaussian distribution using $f\text{CO}_2$ (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

30 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).

Silke Lischka 8/9/2016 17:00

Gelöscht: : phase I, II, II.

Silke Lischka 8/9/2016 17:01

Gelöscht: . (Paul et al.2015)

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 $f\text{CO}_2$ 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).

3 Results

3.1 Ciliates

3.1.1 Ciliate total abundance

Total abundance of microzooplankton 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 ($\leq 10 \mu\text{m}$, $11\text{--}20 \mu\text{m}$, $> 20 \mu\text{m}$) 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* $> 20 \mu\text{m}$ occurred only exceptionally.

Silke Lischka 8/9/2016 20:32

Gelöscht: Microzooplankton

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Gelöscht: Microzooplankton

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*, *Strombidium* 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 CO₂ enriched mesocosm (mean: 1,250 cells l⁻¹ (SD 180) and mean: 2,205 cells l⁻¹ (SD 851), respectively). *Balanion comatum*, *Rimostrombidium* sp., *Strombidium* 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 CO₂ concentration with higher mean cell numbers in the control and lowest enriched mesocosm compared to the three high CO₂ mesocosms (mean: 1680 cells l⁻¹ (SD 139) and mean: 880 cells l⁻¹ (SD 223), respectively). Likewise, small *Strombidium* sp. developed some CO₂ 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 CO₂ enriched mesocosms, respectively. *Mesodinium* sp., *Strombidium* 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, *Strombidium* 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 ~ 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 microzooplankton

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 ($p_{\text{temperature}} = 0.022$; $p_{fCO_2} = 0.03$), total abundance of *Strombidium* cf. *epidemum* by chlorophyll a ($p = 0.002$), that of *Strombidium* sp. Showed synergistic responses to the combination of the factors fCO_2 *temperature and

- Silke Lischka 8/9/2016 21:05
Gelösch: microzooplankton
- Silke Lischka 8/9/2016 21:05
Gelösch: Mesodinium sp.,
- Silke Lischka 8/9/2016 21:05
Gelösch: Strombidium sp. (< 20 µm and > 20 µm),
- Silke Lischka 8/9/2016 21:07
Gelösch: Peak values ranged between 1,160 cells l⁻¹ and 4,000 cells l⁻¹ o
- Silke Lischka 8/9/2016 21:07
Gelösch: and showed
- Silke Lischka 8/9/2016 21:07
Gelösch: On t_{23} peak values ranged between 2,300 cells l⁻¹ and 3,840 cells l⁻¹, and between 1,980 cells l⁻¹ and 6,740 cells l⁻¹ on t_{37} .
- Silke Lischka 8/9/2016 21:08
Gelösch: of 1,760 cells l⁻¹ on t_7 , 1,680 cells l⁻¹ on t_0 , 3,640 cells l⁻¹ on t_{11} , 1,760 cells l⁻¹ on t_0 , ar... [6]
- Silke Lischka 8/9/2016 21:09
Gelösch: on
- Silke Lischka 8/9/2016 21:09
Gelösch: , respectively.
- Silke Lischka 8/9/2016 21:09
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- Silke Lischka 8/9/2016 21:11
Gelösch: Later in the experiment, these sp... [7]
- Silke Lischka 8/9/2016 21:11
Gelösch: and
- Silke Lischka 8/9/2016 21:13
Gelösch: < 550 cells l⁻¹ and < 700 cells l⁻¹... [8]
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Gelösch: . From t_0 onwards,
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Gelösch: cysts and unidentifiable ciliates... [9]
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Gelösch: and 3b
- Silke Lischka 8/9/2016 21:22
Gelösch: microzooplankton
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Gelösch: . It started with relatively low... [10]
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Gelösch: ied
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Gelösch: 4a, 4
- Silke Lischka 8/9/2016 21:26
Gelösch: . Overall, *H* showed a non-mon... [11]

$f\text{CO}_2$ *chlorophyll a, respectively ($p = 0.0005$ and $p = 0.0002$, respectively), and the Shannon index H a synergistic effect between $f\text{CO}_2$ *temperature was determined ($p = 0.0008$). Depiction of the statistical results of H showed a non-monotonic relationship with slightly increasing trend at lower $f\text{CO}_2$ and a decreasing trend the more the $f\text{CO}_2$ 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 90975 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 (29,325–44824 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., including 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 usually 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_{11} and t_{31} . 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_{22} and t_{30} . 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_{11} 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.

Silke Lischka 8/9/2016 21:38

Gelöscht: in response to one or more of the included explanatory variables.

Silke Lischka 8/9/2016 21:38

Gelöscht: Detailed s

Silke Lischka 8/9/2016 21:52

Gelöscht: caught in the net samples on t_{22} varied between 4841 ind. m^{-3} in M1 and 31471 ind. m^{-3} in M8

Silke Lischka 8/9/2016 21:53

Gelöscht: During the course of the experiment abundances increased in all mesocosms continuously until peak abundances were reached between t_{24} and t_{31} .

Silke Lischka 8/9/2016 22:04

Gelöscht: Low abundances of cladocerans occurred only at the beginning (t_{22} , t_{11}) and on t_{17} when they only had a share of between 8% and 28% of the whole community.

Silke Lischka 8/9/2016 22:04

Gelöscht: a relatively low share early in the experiment and at the end (~10–20%), but half way through the experiment copepods constituted

Silke Lischka 8/9/2016 22:08

Gelöscht: ,

Silke Lischka 8/9/2016 22:08

Gelöscht: later on they almost disappeared in all mesocosms (< 1%) except for M8 where on t_{35} 2% of the whole community were rotifera.

Silke Lischka 8/9/2016 22:08

Gelöscht: , only in a few cases (M1 and M5 on t_{10} and t_{24}) gastropods had a share of 5% and at the last day in M8 of 14% of the mollusca group.

Silke Lischka 8/9/2016 22:09

Gelöscht: By day t_{10} their abundances decreased to 7–0.6% of the total community.

Silke Lischka 8/9/2016 22:10

Gelöscht: with highest numbers at the beginning of the experiment.

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

5 *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 ind. m^{-3} and 2,496 ind. m^{-3}). Peak abundances were reached around day t_{17} and t_{24} (19,192 ind. m^{-3} and 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.

Silke Lischka 8/9/2016 22:20

Gelöscht: until day t_{10} ranging between ... [12]

Silke Lischka 8/9/2016 22:27

Gelöscht: , except on t_3 and t_{10} they had a lower share in all mesocosms (~ 50%)... Adult fe... [13]

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} and 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* 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 CO₂ mesocosms, M7 (497 μ atm), M6 (821 μ atm), and M3 (1007 μ atm) (138,394 ind. m^{-3} , 114,169 ind. m^{-3} , 127,080 ind. m^{-3}) compared to the two controls M1, M5 and the highest CO₂ mesocosm (M8, 1231 μ atm) (72,020 ind. m^{-3} , 58,107 ind. m^{-3} , 63,182 ind. m^{-3} respectively). (Fig. 8a, only *Bosmina* sp. shown).

Silke Lischka 8/9/2016 22:39

Gelöscht: ranging between ...84 ind. m^{-3} ... [14]

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 CO₂ 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 f CO₂ 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. 10³ 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 f CO₂ 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 significant interaction term between f CO₂ 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 ± 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 f CO₂ 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.

Silke Lischka 8/9/2016 22:54

Gelöscht: (Fig. 9a, 9b)

Silke Lischka 8/9/2016 22:55

Gelöscht: During the remaining days values fall between 0.15 and 8.44, except for M1 on t^3 when no organisms with filled brood chambers were counted.

Silke Lischka 8/9/2016 22:56

Gelöscht: occurred mainly during the first part of the experiment until t_{17} . During that time,

Silke Lischka 8/9/2016 22:56

Gelöscht: s

Silke Lischka 8/9/2016 22:57

Gelöscht: Later, the importance of *Podon* sp. was negligible, but among the few organisms that still occurred were both animals with empty and with full brood chambers.

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 *Strombidium* spp. (≤ 20 µm) due to difficulties with clear identification. However, most of the Strombidiids ≤ 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 ciliate 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 CO₂) 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 CO₂ 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 CO₂ concentrations were found for dinoflagellates and respectively prasinophytes and haptophytes but these effects did not translate to the MiZP level (Schulz et al., 2013).

Silke Lischka 9/9/2016 09:44

Gelöscht: Microzooplankton

Silke Lischka 9/9/2016 08:59

Gelöscht: MiZP

Silke Lischka 9/9/2016 09:02

Gelöscht: MiZP

Silke Lischka 9/9/2016 09:03

Gelöscht: MiZP

Silke Lischka 9/9/2016 09:44

Gelöscht: 1

Silke Lischka 9/9/2016 09:10

Gelöscht: microzooplankton

We found no significant relation between ciliate total abundance and $f\text{CO}_2$ 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 $f\text{CO}_2$, respectively. 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. ($< 20\mu\text{m}$) show some distinct differences in abundance between the three higher and lower $f\text{CO}_2$ 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 $f\text{CO}_2$ concentrations. 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.

4.1.3 *Myrionecta rubra*

Increased abundances of the mixotrophic ciliate *Myrionecta rubra* ($< 10 \mu\text{m}$) 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 ($< 2 \mu\text{m}$). 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 picophytoplankton of approx. 2.9 μm cell diameter most likely representing cryptophytes had highest abundances during phases II and III, but showed a distinct negative correlation with $f\text{CO}_2$ (Crawford et al., 2016). Cryptophyte biomass decreased from t_3 to t_{17} as did the total abundance of *M. rubra*, but the small size-class cells remained and 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

Silke Lischka 9/9/2016 09:12

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Gelösch: microzooplankton

Silke Lischka 9/9/2016 09:13

Gelösch: and the microzooplankton community seemed to change

Silke Lischka 9/9/2016 09:43

Gelösch: 2

Silke Lischka 9/9/2016 09:43

Gelösch: May

Silke Lischka 9/9/2016 09:43

Gelösch: benefit from OA?

Silke Lischka 9/9/2016 09:22

Gelösch: For the present study, a positive CO_2 effect on community chlorophyll a is described with significantly higher concentrations between day t_{21} and t_{29} (phase II and III) attributed for up to 90% to picophytoplankton ($\sim 2 \mu\text{m}$).

Silke Lischka 9/9/2016 09:22

Gelösch: This period of increased chlorophyll a concentrations under high CO_2 also coincides well with increased abundances of the probably predominantly photoautotrophic ciliate *Myrionecta rubra* ($\sim 10 \mu\text{m}$) in the high CO_2 mesocosms.

Silke Lischka 9/9/2016 09:23

Gelösch: can cause red tides and are characterized by high uptake rates of inorganic nutrients and they

Silke Lischka 9/9/2016 09:23

Gelösch: . In the absence of cryptophytes, they sustain a larger cell volume but exposure to cryptophytes stimulates incorporation and cell division of *M. rubra* resulting in a decreased average cell but increased population size (hence biomass)

Silke Lischka 9/9/2016 09:28

Gelösch: and showed a significant negative effect of CO_2 during the first phase of the experiment

Silke Lischka 9/9/2016 09:29

Gelösch:

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Gelösch: and

Silke Lischka 9/9/2016 09:29

Gelösch: whereas the 11–20 μm size class of *M. rubra* almost disappeared by that time or even earlier for the $\sim 20 \mu\text{m}$ size class,

performance of *M. rubra* may have been stimulated by elevated CO₂ concentrations and thus this ciliate may be 'co-responsible' for the CO₂ driven total chlorophyll *a* differences observed during phases II and III. Consequently, higher cell numbers of small sized *M. rubra* at elevated CO₂ may be a combination of indirect and direct CO₂ effects through 1) availability of cryptophytes in particular during phase I, and 2) through a CO₂-mediated higher photosynthetic rate of *M. rubra* supporting its own growth. Strong positive Pearson correlations between *M. rubra* and cryptophytes suggest a high grazing pressure of *M. rubra* on cryptophytes supporting our assumption. Overall, a CO₂ 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 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 (*Synchaeta* 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 ~22,000 – ~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 10,000 ind. m⁻³ and ~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,

Silke Lischka 9/9/2016 09:33

Gelöscht: II and III maybe being partly responsible for the negative CO₂ effect on cryptophyte biomass and abundances reported by Crawford et al. (2015) and Paul et al. (2015),

Silke Lischka 9/9/2016 09:35

Gelöscht: We have no (strong) support, however, that the negative CO₂ effect on cryptophyte abundance during phase I is related to higher grazing pressure of *M. rubra* resulting in an abundance increase. A certain affinity of *Mesodinium rubrum* (synonymous *Myrionecta rubra*) to low pH is supported by a microcosm study with a coastal plankton community that revealed clearly higher abundance at the lowest pH level (6.0) (Nielsen et al., 2010). During this phase, *M. rubra* abundances showed much variability between sampling days. Some higher abundances in the small and medium size class *M. rubra* especially in M6 and M8 between *t*₂ and *t*₁₁, however, may point in this direction. During phase II and III, however, [text] [*M. rubra*] may have benefitted from CO₂ stimulated photosynthetic activities and controlled cryptophyte abundances, and hence, decreased cryptophyte numbers would represent an indirect CO₂ effect through *M. rubra* (Crawford et al., 2015).

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Gelöscht:

Silke Lischka 9/9/2016 09:50

Gelöscht: , respectively,

Silke Lischka 9/9/2016 09:50

Gelöscht: respectively

1987). The MZP community in the surrounding water did not entirely correspond with the mesocosms 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 were 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 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; Riebesell et al., 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 CO₂ 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 pCO₂ 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., 2015), 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 CO₂ 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 CO₂ on reproductive success of *Eurytemora affinis* are in agreement with the observations from the mesocosms (Almén et al., 2015, this issue). Incubated *Acartia bifilosa* showed fCO₂ unaffected egg production, but slight negative effects on egg hatching and development were found and adult females were smaller in the two highest CO₂ mesocosms (Vehmaa et al., 2015, this issue). Our results are also in line with (Niehoff et al., 2013) who do not describe any apparent CO₂ effect on an Arctic MZP community including copepods. Copepods in the study region naturally experience fCO₂, 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 conspicuous differences found in mesozooplankton abundance are due to the cladoceran *Bosmina* sp. Between t_{24} and t_{31} . In three of the four CO₂ enriched mesocosms (497 µatm, 821 µatm, 1007 µatm) peak numbers were twice or even more

Silke Lischka 9/9/2016 09:52

Gelöscht: 1

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Gelöscht: Up to date, there are only very few large CO₂ enrichment mesocosm studies with as far as possible holistic plankton community approach

Silke Lischka 9/9/2016 09:53

[1] nach unten verschoben: (Riebesell et al., 2008; Riebesell et al., 2013a)

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Gelöscht: 13a). ...herefore, t...is study is ... [15]

Silke Lischka 9/9/2016 09:53

[1] verschoben (Einfügung)

Silke Lischka 9/9/2016 09:55

Gelöscht: a

Silke Lischka 9/9/2016 09:55

[2] verschoben (Einfügung)

Silke Lischka 9/9/2016 09:56

Gelöscht: This Arctic MZP community was dominated by meroplanktonic larvae and copepods played a minor role, and thus differed in species type composition compared to the Baltic community enclosed in our mesocosms (Niehoff et al., 2013; Riebesell et al., 2013). In general, on the mesozooplankton level, calcifiers seem to be more sensitive to CO₂ increases than crustaceans (Kurihara, 2008; Kroeker et al., 2013), i.e. copepods which dominate zooplankton communities in boreal and higher latitude regions. While copepods are thought to be rather robust against ocean acidification with negative effects occurring usually not until pCO₂ 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., 2015), 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. Mortality of the nauplii stage *Acartia tonsa* for example increased threefold already at CO₂ concentrations expected for the end of this century (Cripps et al., 2014). These authors highlight ... [16]

Silke Lischka 9/9/2016 09:55

[2] nach oben verschoben: (Niehoff et al., 2013; Riebesell et al., 2013) ... [17]

Silke Lischka 9/9/2016 09:57

Gelöscht:

Silke Lischka 9/9/2016 09:57

[3] verschoben (Einfügung)

Silke Lischka 9/9/2016 09:58

Gelöscht: e... et al., 2015), more recent st... [18]

Silke Lischka 9/9/2016 10:03

Gelöscht: 4.2.2 Mollusks ... [19]

than twice as high compared to the control and the highest CO₂ mesocosms, though a significant relation with *f*CO₂ could not be proved. Nevertheless, this striking difference may possibly point to an indirect CO₂ effect through higher food availability under high CO₂.

Cladocerans are highly reproductive at times of favourable environmental conditions. The lifespan 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 CO₂ 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 CO₂ mesocosms (Paul et al., 2015). The highly positive correlation between cyanobacteria and *Bosmina* sp. supports this assumption. Only M8, the mesocosm with the highest CO₂ 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 CO₂-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., 2013). 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 CO₂ mesocosms had higher reproductive activities during the time of actual peak abundance. In particular, *Bosmina* in M8 and M3 (two highest CO₂ 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 missed to sample the abundance peak of *Bosmina* in M8 possibly obstructing to prove a significant indirect *f*CO₂ effect on *Bosmina* abundance through increased food availability.

Silke Lischka 9/9/2016 10:05

Gelöscht: In the inner parts of the Baltic proper, the endemic *Bosmina longispina maritima* is most abundant among the genus *Bosmina*. Its life cycle includes many parthenogenetic generations in the summer, sexual reproduction occurs only during late summer and autumn from which fertilized resting eggs result that overwinter in the sediment and develop in spring (Kankaala and Wulff, 1981, and references therein).

Silke Lischka 9/9/2016 10:06

Gelöscht: , they can bear several developmental stages in the brood pouch of the mother and at favourable conditions,

Silke Lischka 9/9/2016 10:06

Gelöscht: --

Silke Lischka 9/9/2016 10:08

Gelöscht: . For example at high food concentration more broods occur and more eggs are produced

Silke Lischka 9/9/2016 10:08

Gelöscht: ,

Silke Lischka 9/9/2016 10:08

Gelöscht: whereat not only food quantity but also quality influences life time and together were shown to shorten it to up to 10 days (Hanazato and Yasuno, 1987). *Bosmina* is also very quick in taking advantage of favourable environmental conditions and has a high plasticity to customize its life cycle and growth patterns. For example, to avoid predation they can allocate energy to somatic growth instead of early reproduction to attain larger size and escape prey size spectrum of copepods (Kankaala, 1983; Jankowski, 2000).

Silke Lischka 9/9/2016 10:09

Gelöscht:) and has two modes of feeding: small-particle filtering and large-particle grasping (DeMott, 1982; DeMott and Kerfoot, 1982; Bleiwas and Stokes, 1985)

Silke Lischka 9/9/2016 10:10

Gelöscht: I

Silke Lischka 9/9/2016 10:10

Gelöscht: hlorophyll *a*, chlorophytes and particulate organic matter

Silke Lischka 9/9/2016 10:10

Gelöscht: probably

Silke Lischka 9/9/2016 10:12

Gelöscht: The stoichiometry of food organisms can affect growth in cladocerans, for example, growth of *Daphnia pulex* was reduced d (... [20])

Silke Lischka 9/9/2016 10:12

Gelöscht: Most likely

Silke Lischka 9/9/2016 10:14

Gelöscht:

4.2.4 Predator/ prey relationships

We have some evidence for $f\text{CO}_2$ 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 $f\text{CO}_2$ concentrations. With respect to *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strombidium* sp., the $f\text{CO}_2$ 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. 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). Filter-feeding cladocerans directly feed on bacteria and flagellates and effectively 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). 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, state 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 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. 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.

5 Conclusions

This study describes for the first time $f\text{CO}_2$ 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 $f\text{CO}_2$ levels. On the mesozooplankton level, significant $f\text{CO}_2$ 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 mixotrophic *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 $f\text{CO}_2$ 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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Silke Lischka 9/9/2016 10:30

Gelöscht: Our study gives evidence for direct and/or indirect CO_2 effects on the micro- and mesozooplankton community. For the MiZP community composition we determined significant changes with a shift towards smaller species/ genus with increasing CO_2 levels. The phototrophic ciliate *Myrionecta rubra*, as well as *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp. and the short-lived cladocera *Bosmina* seemed to benefit from increased CO_2 concentrations, the first one probably directly, the others rather indirectly. Although our results show no direct significant relation with abundance, we assume *Bosmina* growth and reproduction was stimulated from increased food availability at elevated CO_2 mostly during phases II and III (higher post-bloom chlorophyll *a* and particulate organic matter). This may 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. Filter-feeding cladocerans directly feed on bacteria and flagellates and effectively transfer carbon from the microbial loop to higher trophic levels. 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). Therefore, we conclude, under increasing ocean acidification in cladoceran dominated MZP communities, the importance of trophic transfer from the microbial loop to higher trophic levels becomes more efficient.

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of 1,760 cells l ⁻¹ on t ₇ , 1,680 cells l ⁻¹ on t ₀ , 3,640 cells l ⁻¹ on t ₁₁ , 1,760 cells l ⁻¹ on t ₀ , and 1,080 cells l ⁻¹		
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Later in the experiment, these species/ groups were not of importance anymore.		
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< 550 cells l ⁻¹ and < 700 cells l ⁻¹ , respectively		
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cysts and unidentifiable ciliates never accounted for more than 700 cells l ⁻¹ and 850 cells l ⁻¹ , respectively.		
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. It started with relatively low contributions during the first days of the experiment and increased depending on the mesocosm from t ₁₉ / t ₂₁ on to proportions of 17–36%. Maximum contributions		
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. Overall, <i>H</i> showed a non-monotonic relationship with a slightly increasing trend at lower <i>f</i> CO ₂ and a decreasing trend the more the <i>f</i> CO ₂ increased, and as well as a decreasing trend with temperature.		
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Seite 15: [15] Gelöscht **Silke Lischka** **09/09/2016 09:54**

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Seite 15: [16] Gelöscht **Silke Lischka** **09/09/2016 09:56**

This Arctic MZP community was dominated by meroplanktonic larvae and copepods played a minor role, and thus differed in species type composition compared to the Baltic community enclosed in our mesocosms (Niehoff et al., 2013; Riebesell et al., 2013). In general, on the mesozooplankton level, calcifiers seem to be more sensitive to CO₂ increases than crustaceans (Kurihara, 2008; Kroeker et al., 2013), i.e. copepods which dominate zooplankton communities in boreal and higher latitude regions. While copepods are thought to be rather robust against ocean acidification with negative effects occurring usually not until pCO₂ 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; Almen et al., 2015), 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. Mortality of the nauplii stage *Acartia tonsa* for example increased threefold already at CO₂ concentrations expected for the end of this century (Cripps et al., 2014). These authors highlight the importance of a holistic life-stage approach in order to provide meaningful data for climate change projections.

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(Niehoff et al., 2013; Riebesell et al., 2013)

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Seite 15: [18] Gelöscht	Silke Lischka	09/09/2016 09:58
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Seite 15: [19] Gelöscht	Silke Lischka	09/09/2016 10:03
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4.2.2 Mollusks

Mollusks enclosed in the mesocosms comprised for more than 90% of juvenile bivalves of the species *Macoma balthica* and occurred during the first ten days of the experiment. Calcifiers are among the most vulnerable organisms to ocean acidification, and within this investigation bivalve larvae were therefore subjected to a more detailed study on their occurrence and length distribution over time. The main findings from this study suggest reduced settling rates and a developmental delay with increasing *f*CO₂. For more details see (Jansson et al., 2015, this issue).

Seite 15: [19] Gelöscht	Silke Lischka	09/09/2016 10:03
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Seite 16: [20] Gelöscht **Silke Lischka** **09/09/2016 10:12**

The stoichiometry of food organisms can affect growth in cladocerans, for example, growth of *Daphnia pulicaria* was reduced due to reduced C:P ratios of high CO_2 cultivated food algae (Urabe et al., 2003). In the present study the average C:P ratio of particulate organic matter did not vary with $f\text{CO}_2$, however this can only be seen as an estimate for the food that was effectively ingested by *Bosmina* (Paul et al., 2015).

Seite 19: [21] Gelöscht **Silke Lischka** **09/09/2016 10:59**

DeMott, W. R.: Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*, *Limnology and Oceanography*, 27, 518–527, 1982a.

DeMott, W. R.: Competition Among Cladocerans: Nature of the Interaction Between *Bosmina* and *Daphnia*, *Ecology*, 63, 1949–1966, 1982b.

Seite 20: [22] Gelöscht **Silke Lischka** **09/09/2016 11:03**

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J. P.: Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming, *Global Change Biology*, 19, 1884–1896, doi:10.1111/gcb.12179, 2013.

Seite 20: [23] Gelöscht **Silke Lischka** **09/09/2016 11:03**

Kurihara, H.: Effects of CO_2 -driven ocean acidification on the early developmental stages of invertebrates, *Marine Ecology Progress Series*, 373, 275–284, doi:10.3354/meps07802, 2008.

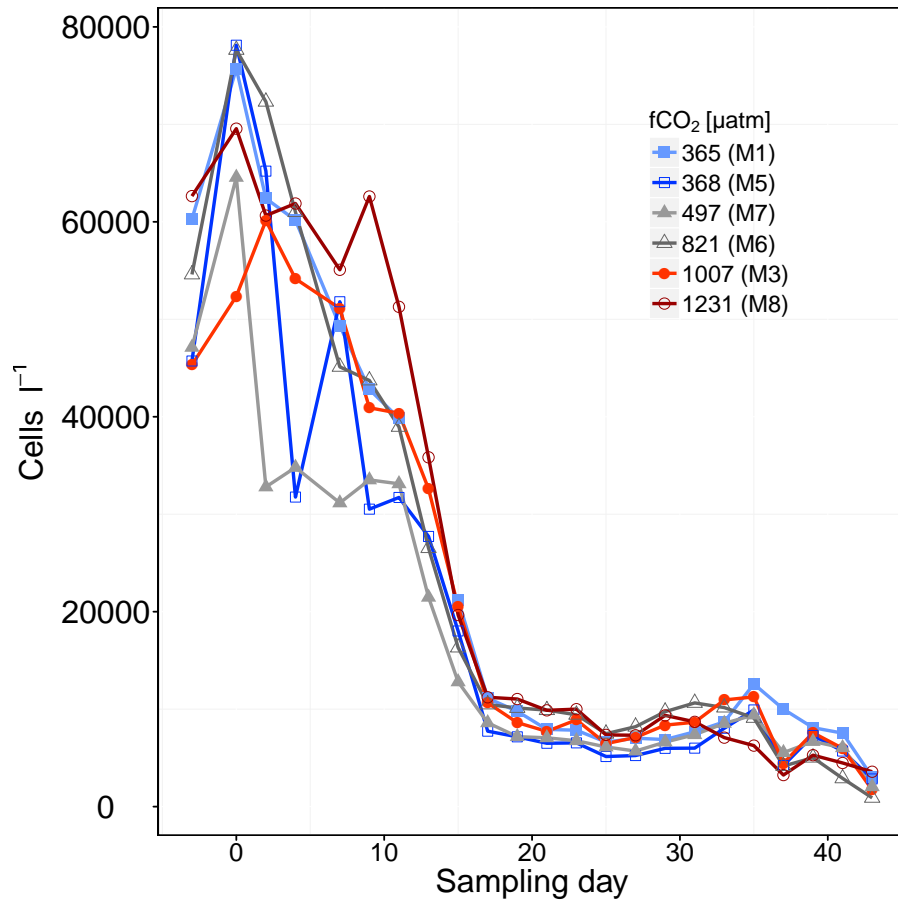


Figure 1. Total abundance of microzooplankton during the course of the experiment. Note there is one missing value in M1 on t_{13} .

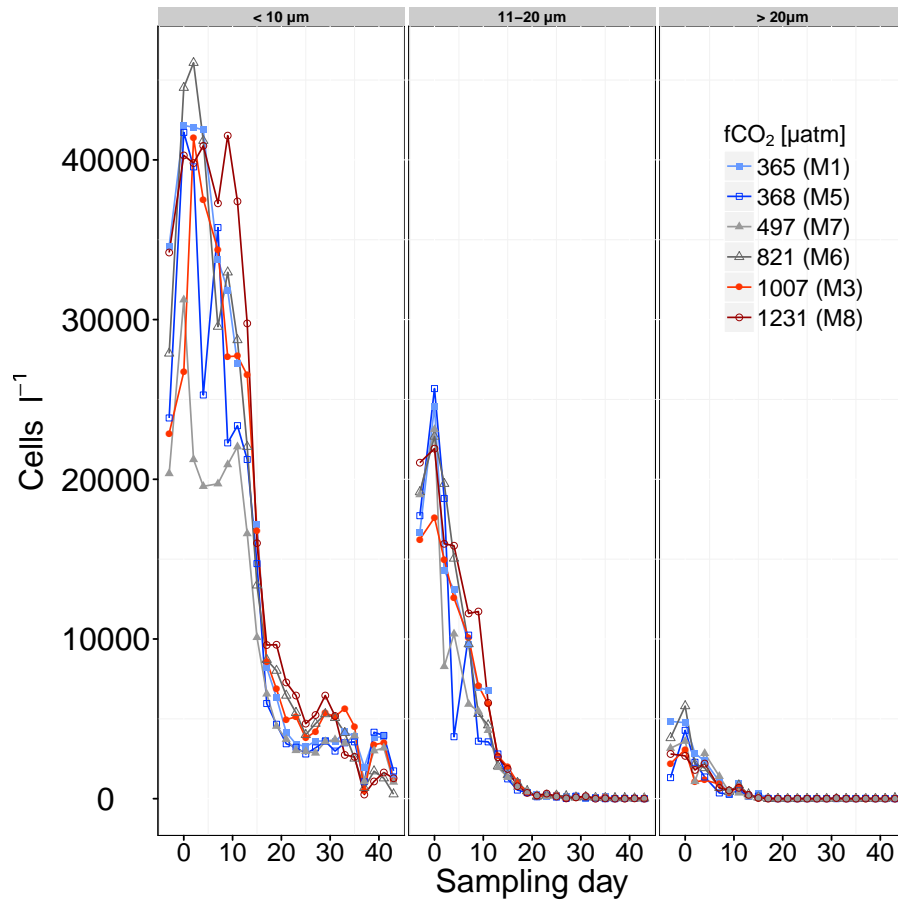


Figure 2a. Abundance of different size classes of *Myrionecta rubra*. Note there is one missing value in M1 on t_{13} .

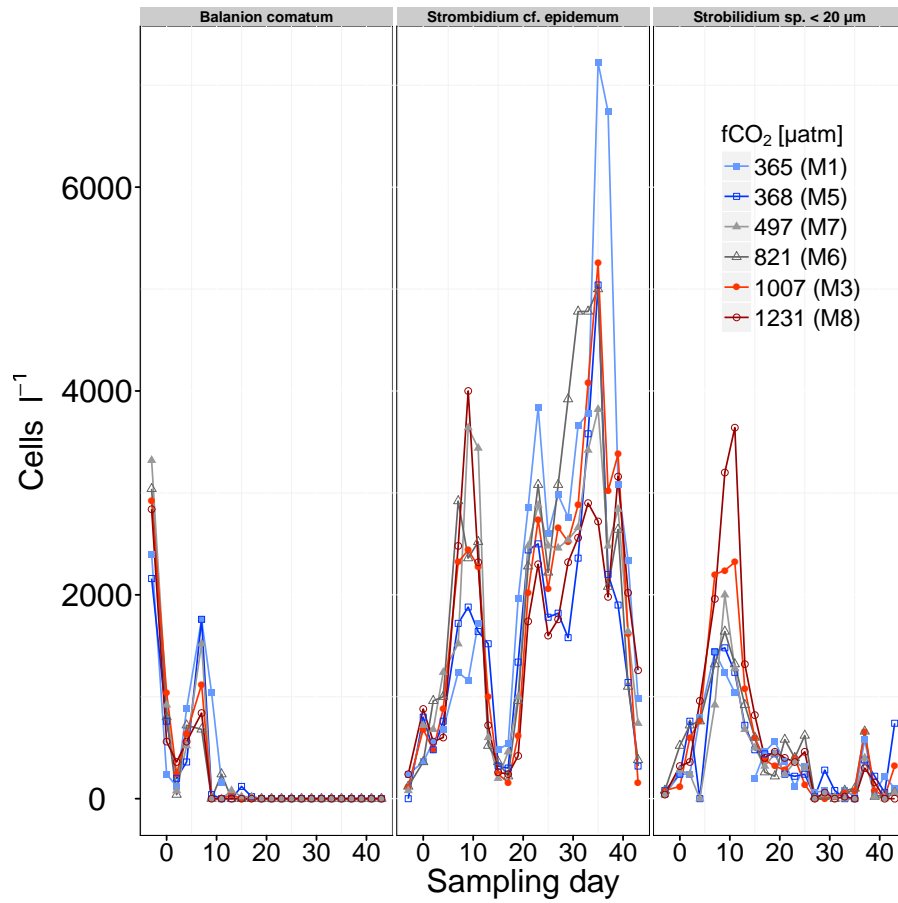


Figure 2b. Abundance of other microzooplankton species/ genera/ groups. Note there is one missing value in M1 on t_{13} .

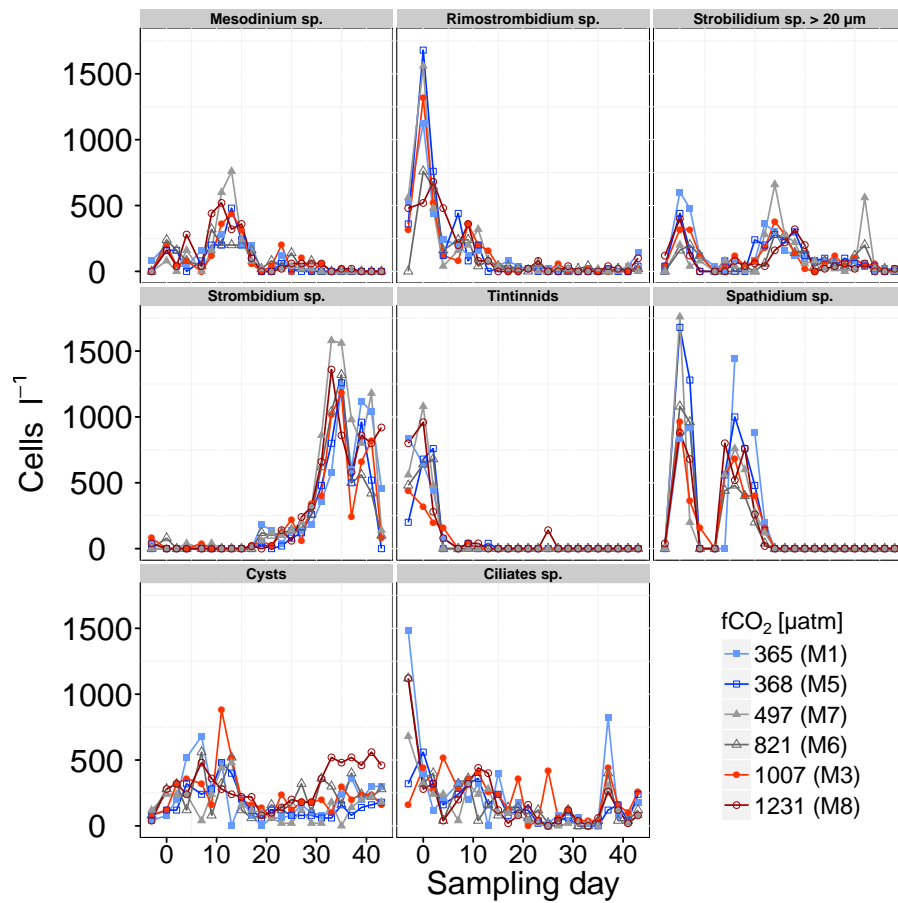


Figure 2c. Abundance of other microzooplankton species/ genera/ groups. Note there is one missing value in M1 on t_{13} .

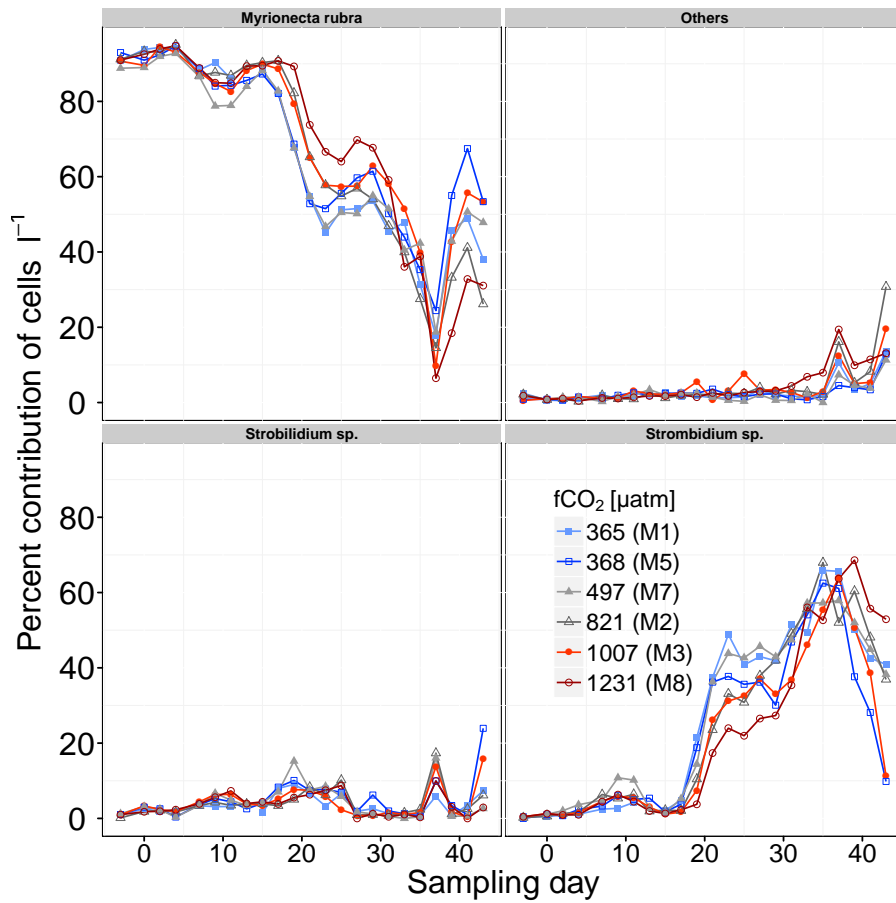


Figure 3a. Percent contribution of major taxonomic species/ genera/ groups to the microzooplankton community. Note there is one missing value in M1 on t_{13} .

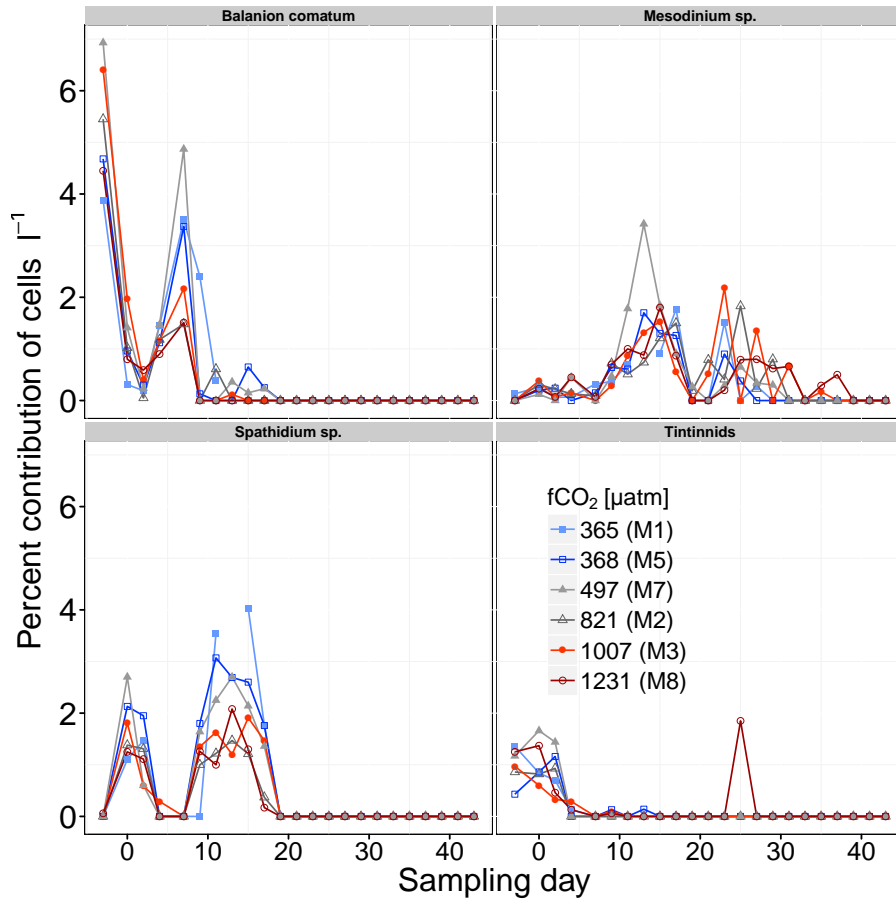


Figure 3b. Percent contribution of major taxonomic species/ genera/ groups to the microzooplankton community. Note there is one missing value in M1 on t_{13} .

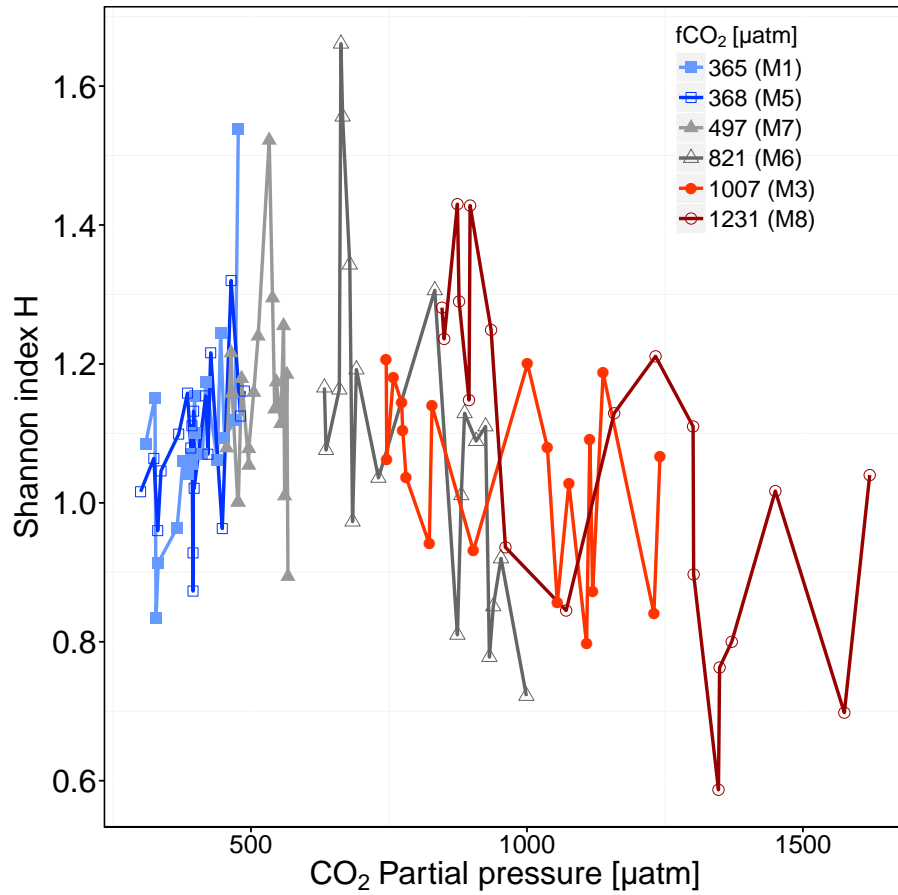


Figure 4a. Microzooplankton, Shannon diversity index H in relation to the daily change of $f\text{CO}_2$. Symbols and colours identify the mean $f\text{CO}_2$ for each mesocosm.

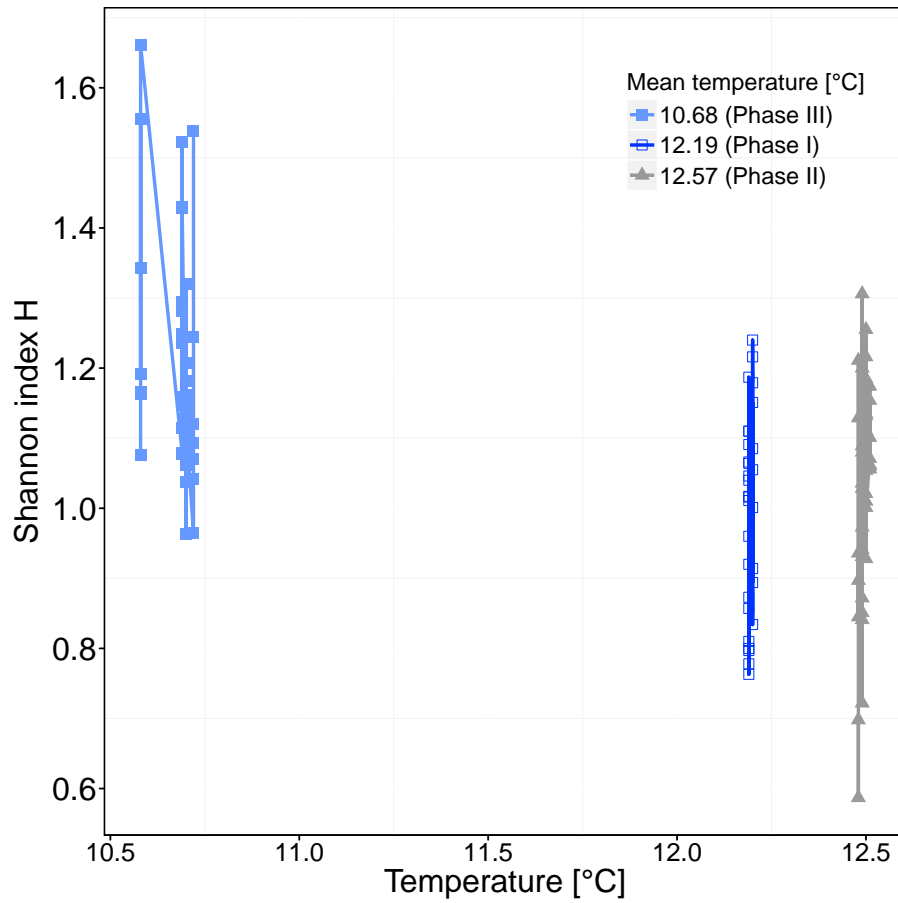


Figure 4b. Microzooplankton, Shannon diversity index H . For better visibility, H is plotted against the mean phase (I, II, III) temperature of each mesocosm. Symbols and colours identify mean phase temperature across all mesocosms.

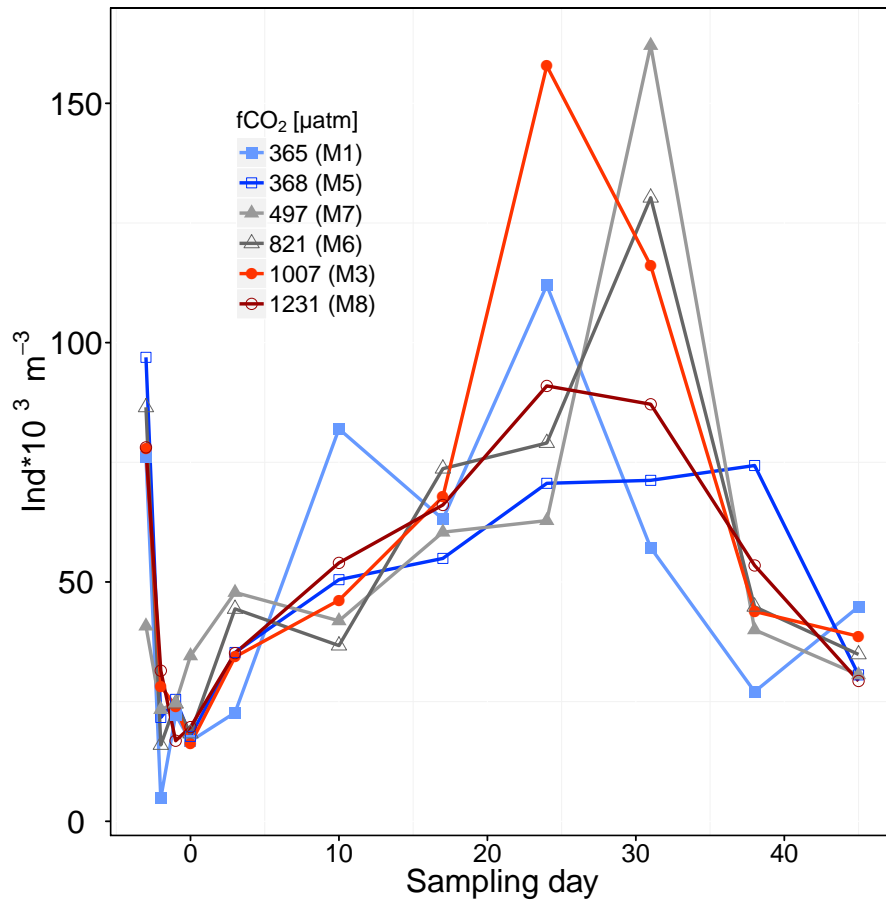


Figure 5. Mesozooplankton total abundance.

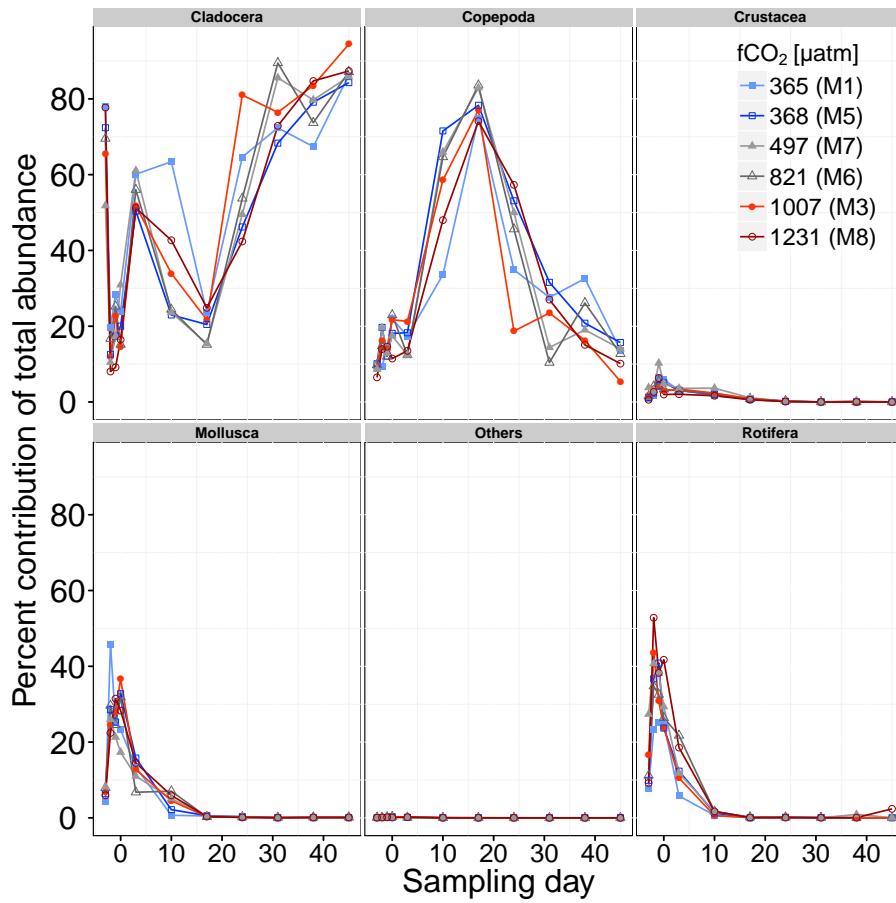


Figure 6. Percent contribution of mesozooplankton main taxonomic groups.

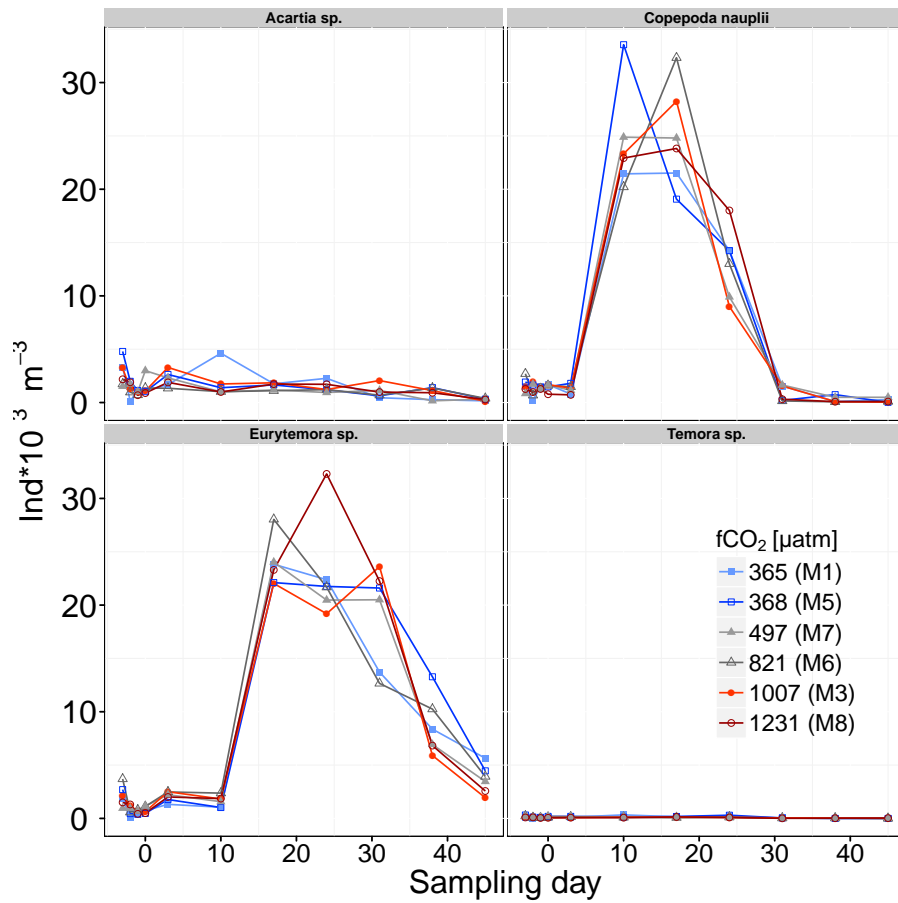


Figure 7a. Abundance of the dominant copepods species *Acartia* sp., *Eurytemora* sp., *Temora* sp., and copepod nauplii.

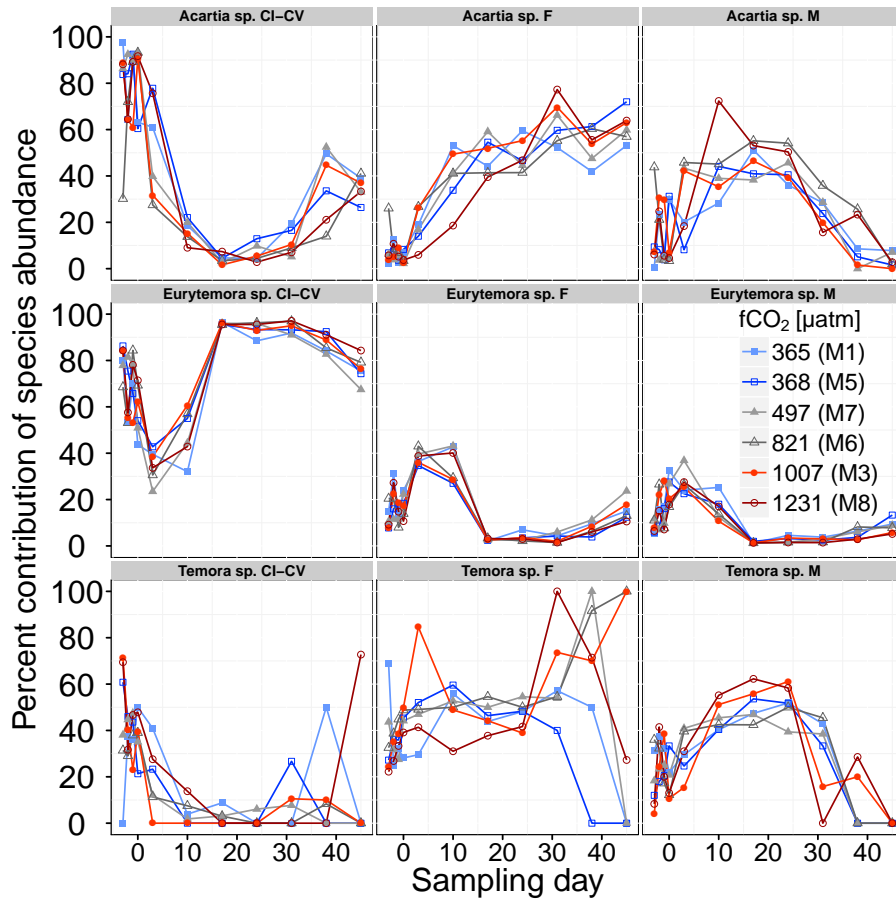


Figure 7b. Percent contribution of different stages of dominant copepods. CI-V: copepodite stages, F: females, M: males.

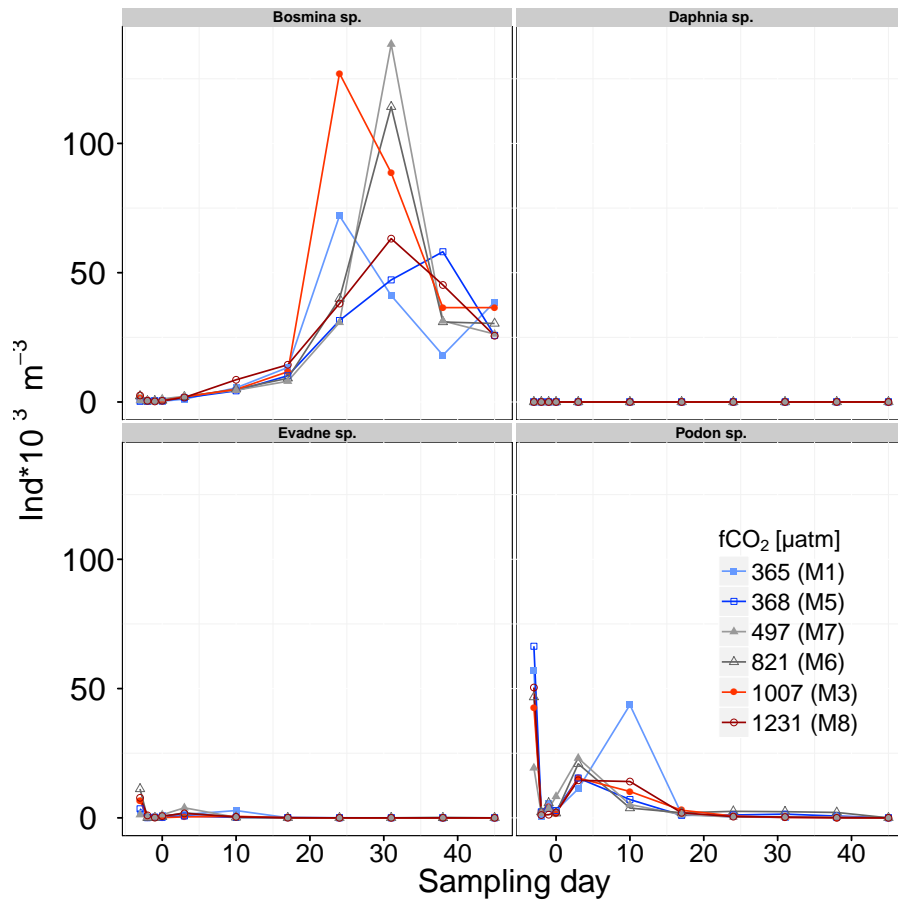


Figure 8a. Abundance of cladoceran species.

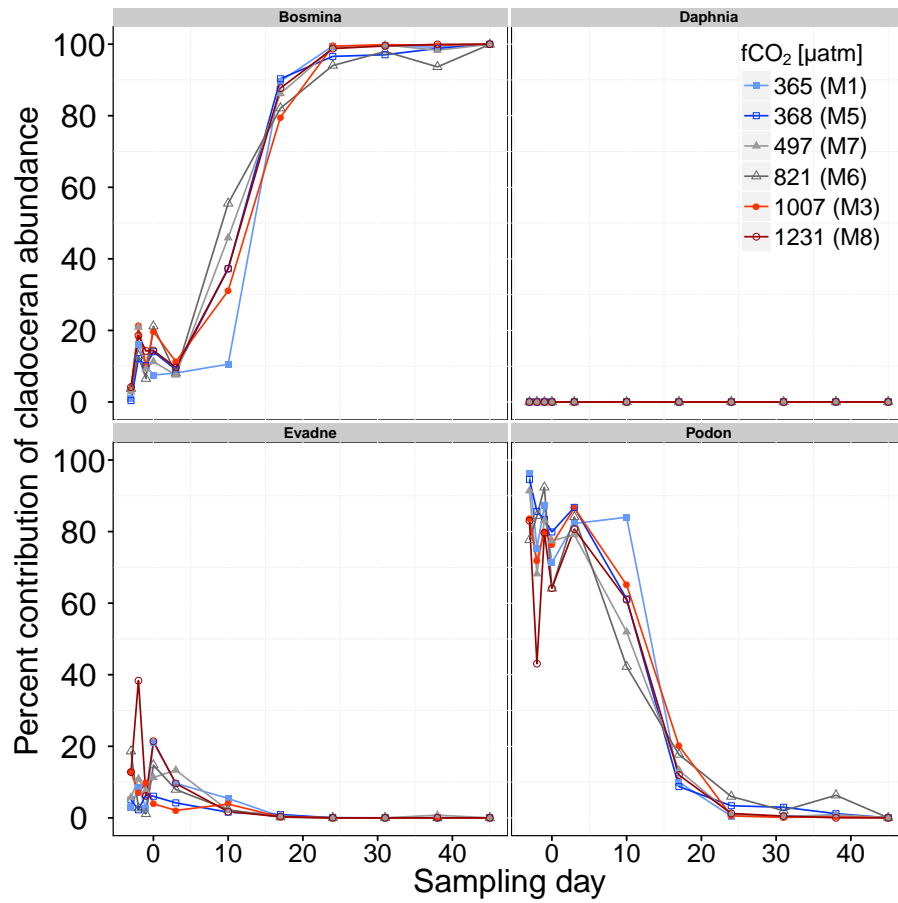


Figure 8b. Percent contribution of different cladoceran species to the total abundance of cladocera.

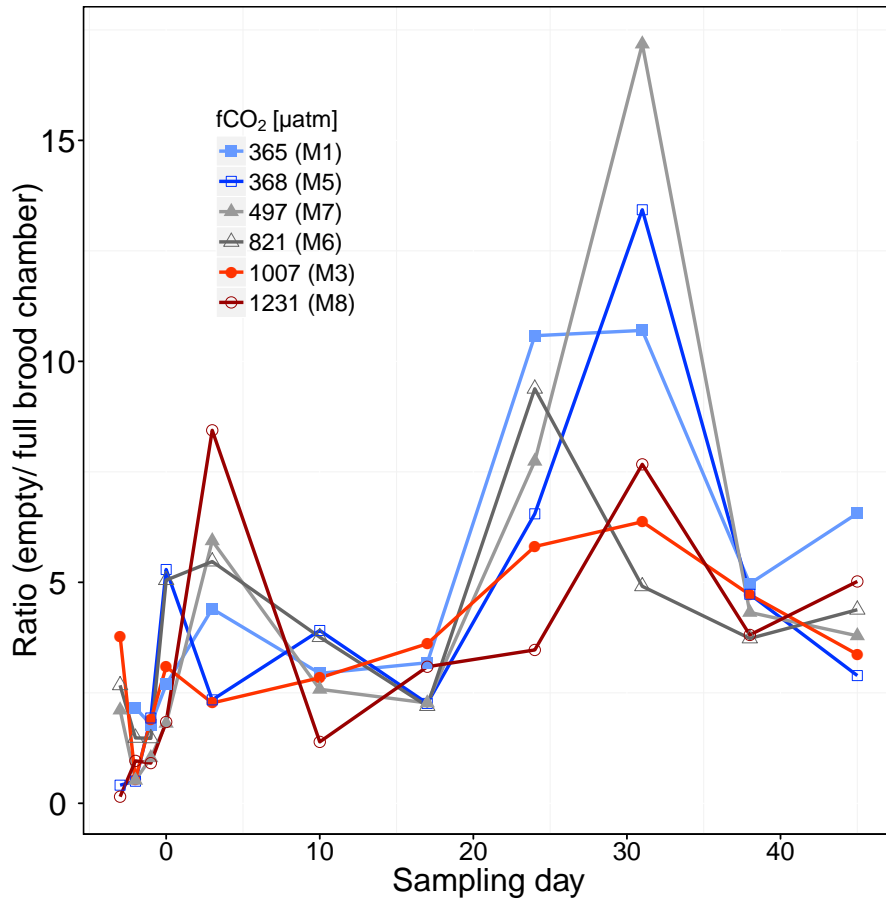


Figure 9a. Ratio of *Bosmina* with empty to full brood chambers. Note: Figure shows all data, but statistics were done on data from t_3-t_{45} only to assure equally spaced data.

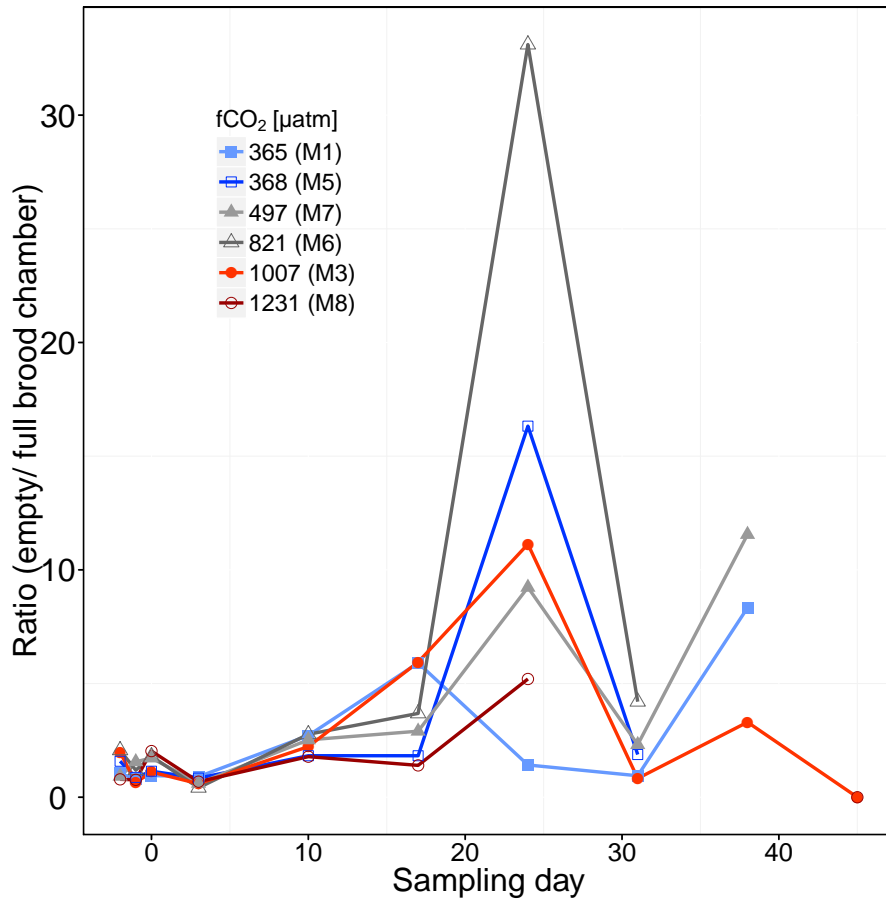


Figure 9b. Ratio of *Podon* with empty to full brood chamber. Note 1: Ratio on $t-3$ was huge and therefore values not shown here to obtain reasonable scaled y-axis. Note 2: occurrence of missing values means no individuals with full brood chambers were present, hence, no ratio could be calculated.

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
Microzooplankton					
MiZP total abundance	Temp	1	-3.506	0.0007	GAMM
<i>Myrionecta rubra</i> , $\leq 10 \mu\text{m}$	Temp	1	2.376	0.019	GAMM
<i>Myrionecta rubra</i> , $\leq 10 \mu\text{m}$	$f\text{CO}_2$ * Temp	1	-2.298	0.024	GAMM
<i>Myrionecta rubra</i> , $\leq 10 \mu\text{m}$	$f\text{CO}_2$ * Chl <i>a</i>	1	2.936	0.004	GAMM
<i>Balanion comatum</i>	Temp	1	2.320	0.022	GAMM
<i>Balanion comatum</i>	$f\text{CO}_2$	1	-2.210	0.030	GAMM
<i>Strobilidium</i> cf. <i>epidemum</i>	Chl <i>a</i>	1	-3.229	0.002	GAMM
<i>Strobilidium</i> sp., $< 20 \mu\text{m}$	Temp	1	2.811	0.006	GAMM
<i>Strobilidium</i> sp., $< 20 \mu\text{m}$	Chl <i>a</i>	1	-4.603	< 0.00001	GAMM
<i>Strobilidium</i> sp., $< 20 \mu\text{m}$	$f\text{CO}_2$ * Temp	1	-3.600	0.0005	GAMM
<i>Strobilidium</i> sp., $< 20 \mu\text{m}$	$f\text{CO}_2$ * Chl <i>a</i>	1	3.926	0.0002	GAMM
Shannon index <i>H</i>	Temp	1	3.652	0.0004	GAMM
Shannon index <i>H</i>	$f\text{CO}_2$	1	2.824	0.006	GAMM
Shannon index <i>H</i>	$f\text{CO}_2$ * Temp	1	-3.454	0.0008	GAMM
Mesozooplankton					
MZP total abundance	Temp	31	-1.155	0.257	GLM
MZP total abundance	$f\text{CO}_2$	31	-0.025	0.980	GLM
MZP total abundance	Chl <i>a</i>	31	0.550	0.586	GLM
MZP total abundance	$f\text{CO}_2$ * Temp	31	0.947	0.351	GLM
MZP total abundance	$f\text{CO}_2$ * Chl <i>a</i>	31	-1.081	0.288	GLM
<i>Bosmina</i> sp.	Chlor <i>a</i>	1	0.76	0.453	GAMM
<i>Bosmina</i> sp. ratio empty/ full brood chambers	Temp	1	-3.572	0.001	GAMM
<i>Bosmina</i> sp. ratio empty/ full brood chambers	$f\text{CO}_2$	1	-2.684	0.011	GAMM
<i>Bosmina</i> sp. ratio empty/ full brood chambers	Chl <i>a</i>	1	-3.980	0.0004	GAMM
<i>Bosmina</i> sp. ratio empty/ full brood chambers	$f\text{CO}_2$ * Chl <i>a</i>	1	2.738	0.01	GAMM
Shannon index <i>H</i>	Chl <i>a</i>	1	-0.555	0.582	GAMM

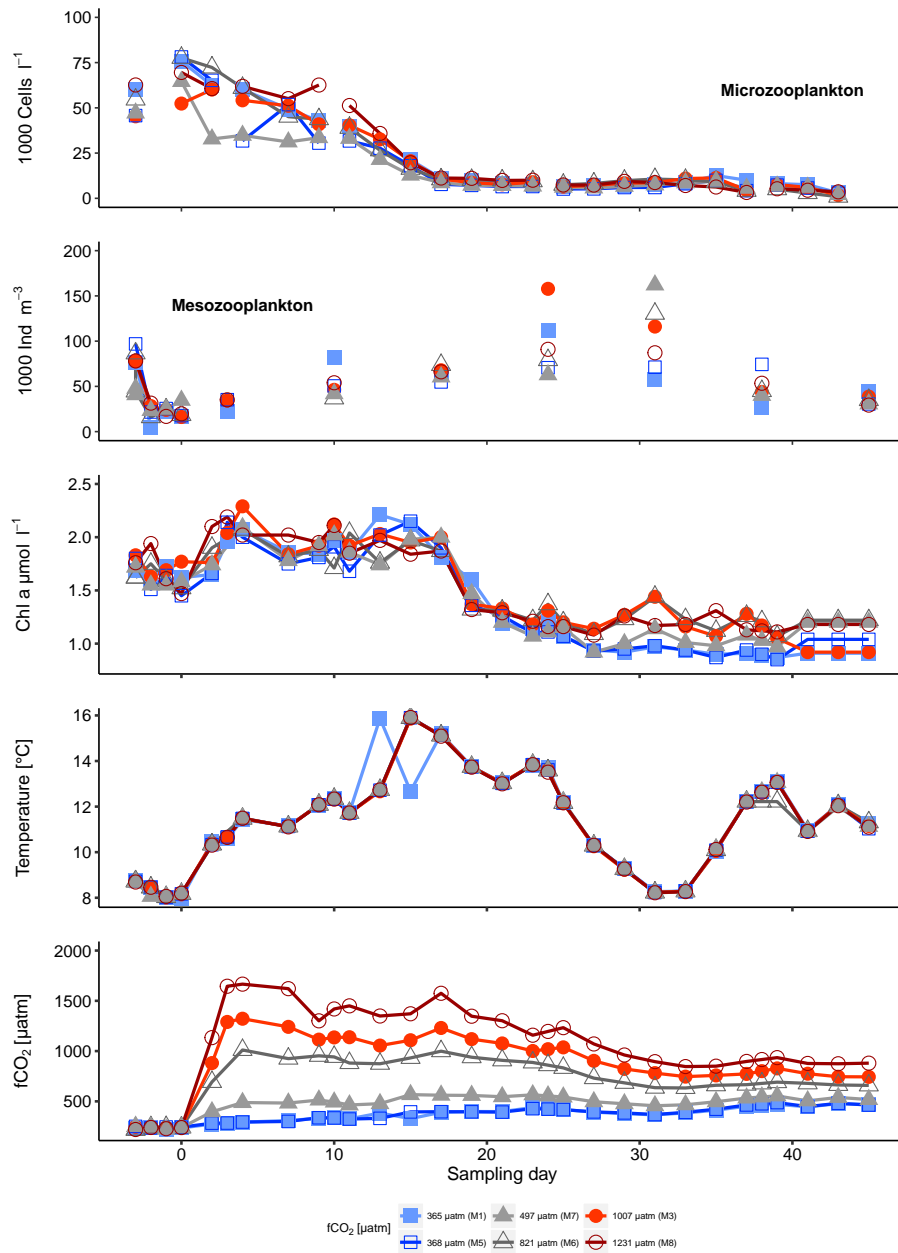


Figure 1. Total cell numbers of ciliates and total abundance of mesozooplankton during the course of the experiment as well as chlorophyll *a* succession, temperature and fCO_2 development. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: 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} .

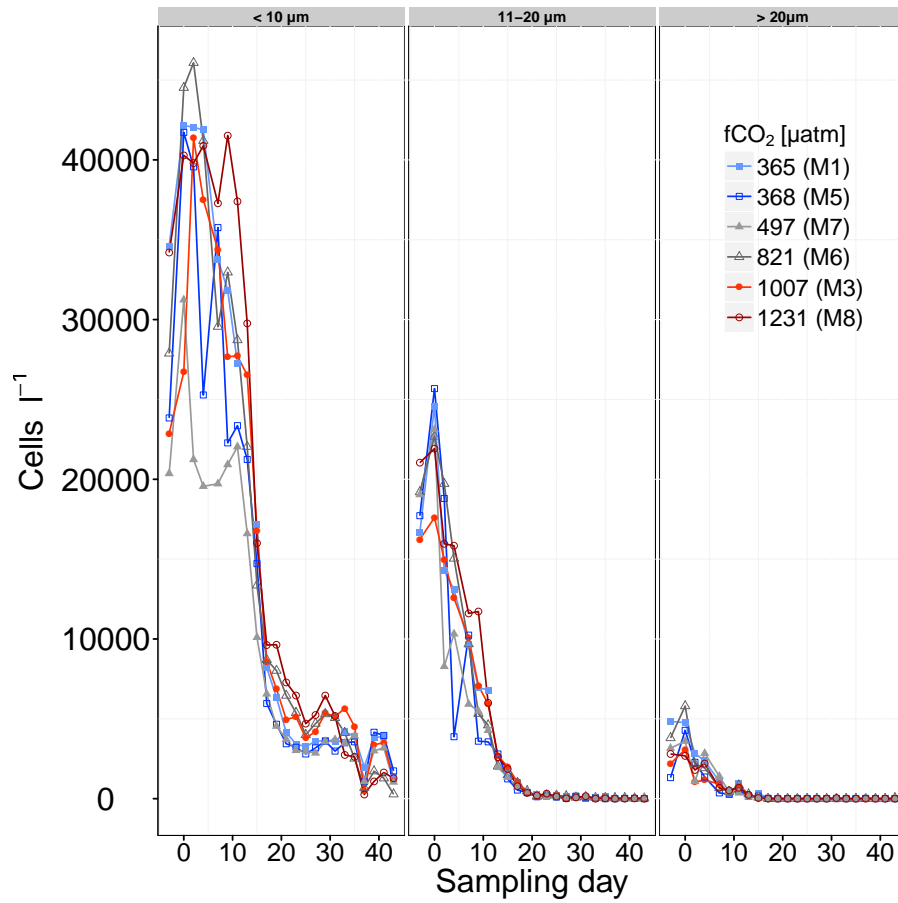


Figure 2a. Abundance of different size classes of *Myrionecta rubra*. Note there is one missing value in M1 on t_{13} .

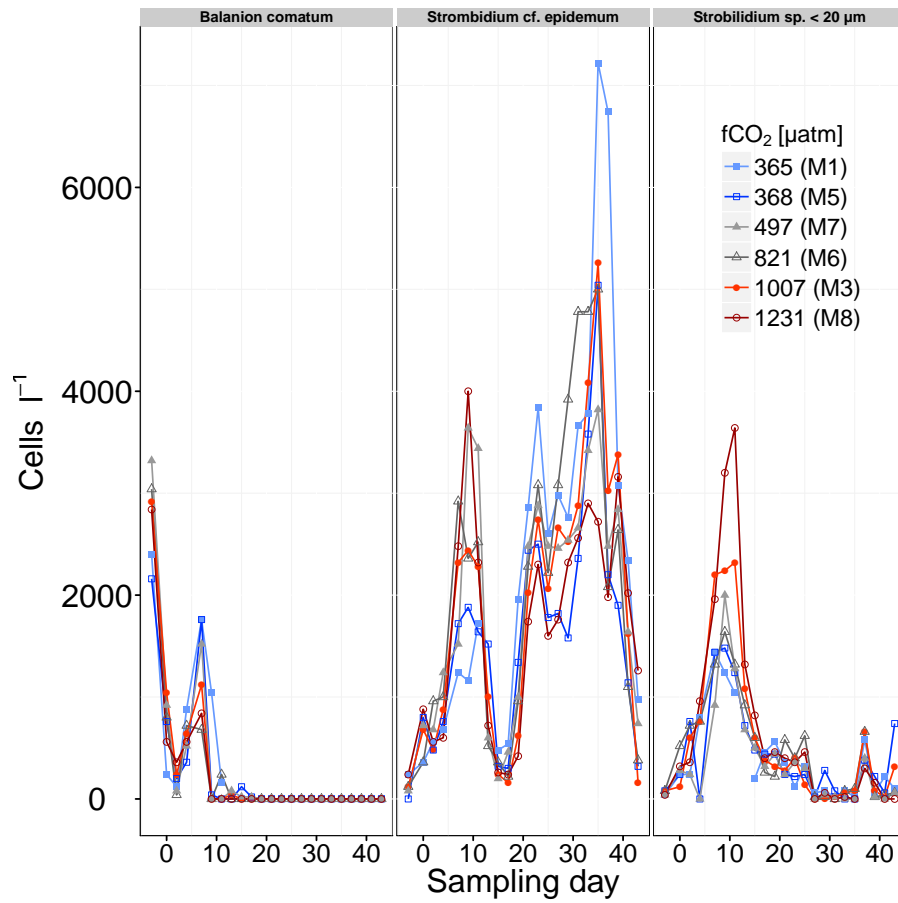


Figure 2b. Abundance of other ciliate species/ genera/ groups. Note there is one missing value in M1 on t_{13} .

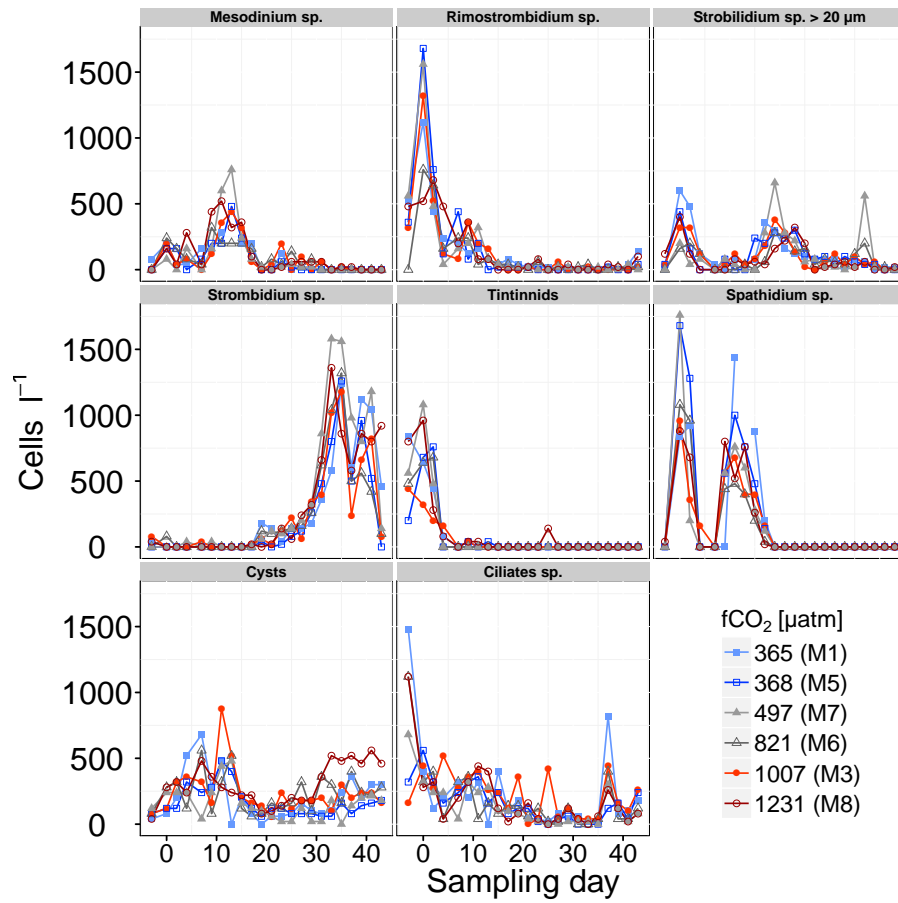


Figure 2c. Abundance of other ciliate species/ genera/ groups. Note there is one missing value in M1 on t_{13} .

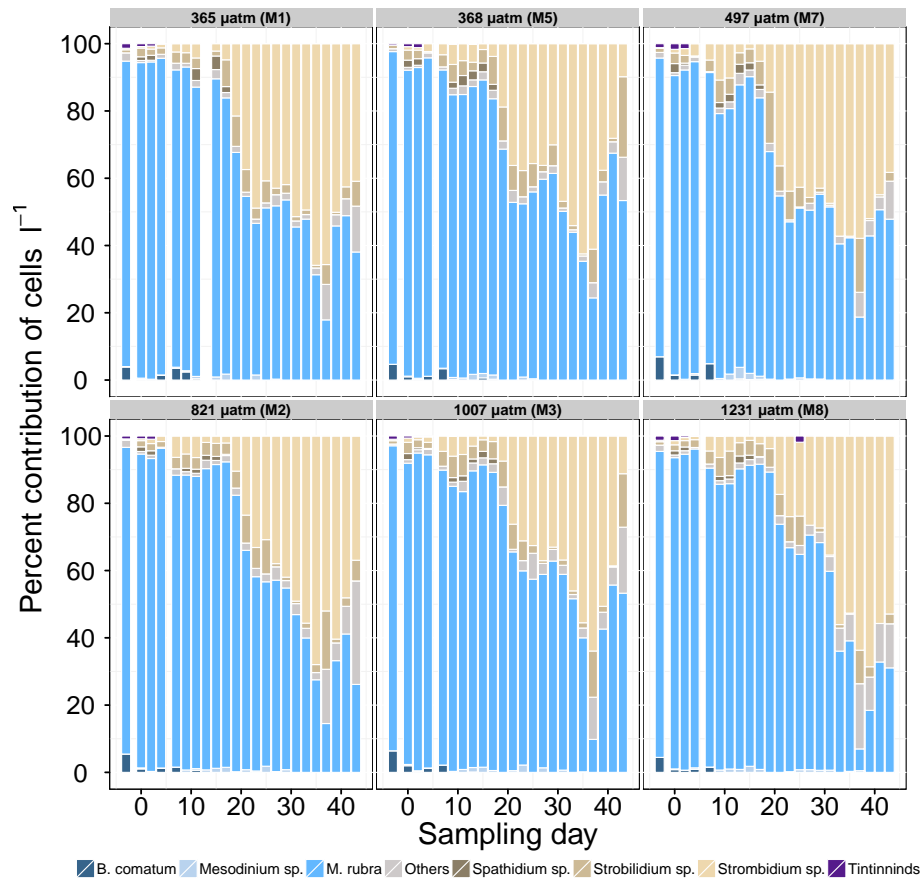


Figure 3a. Percent contribution of abundance of major taxonomic species/ genera/ groups to the ciliate community. Note there is one missing value in M1 on t_{13} .

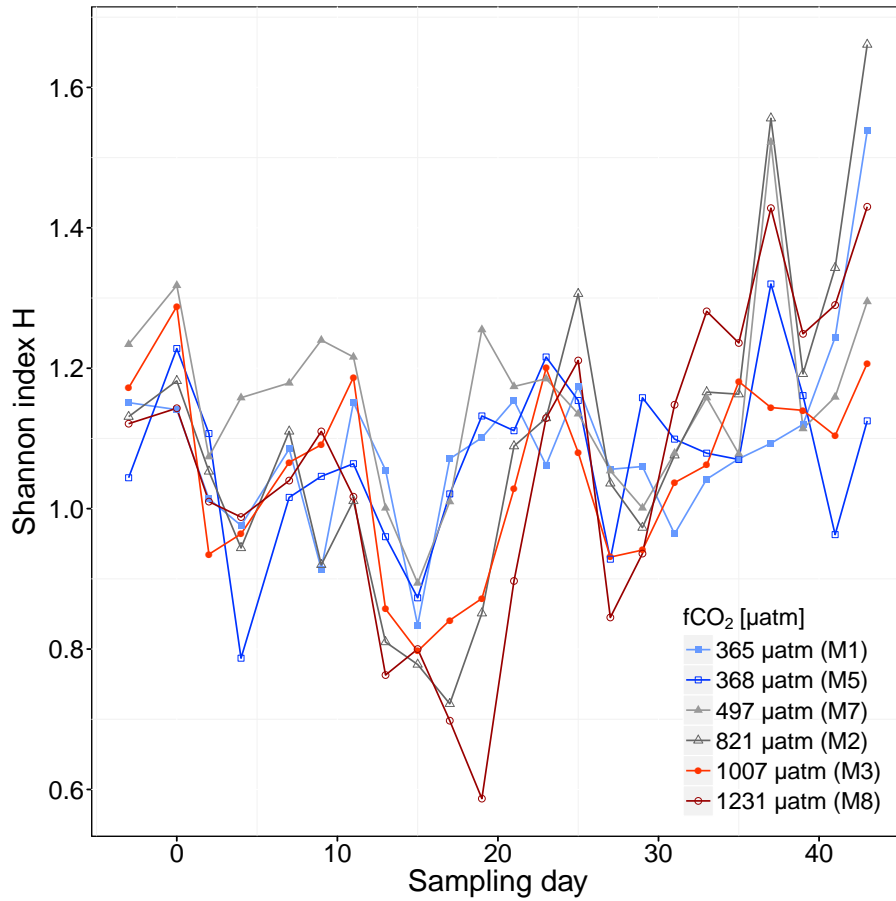


Figure 3b. Ciliates, daily change of the Shannon diversity index H at the different $f\text{CO}_2$ levels in the mesocosms.

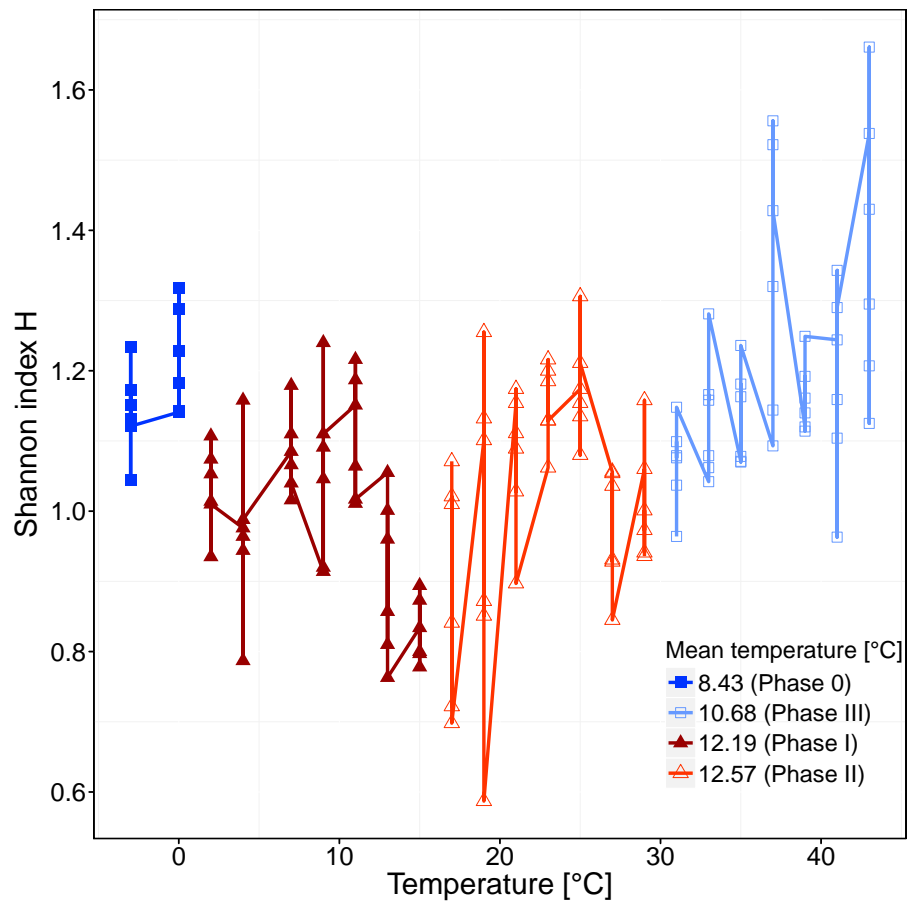


Figure 3c. Ciliates, daily change of the Shannon diversity index H during the 4 different temperature phases defined. Colour legend gives mean temperature during Phase 0 (12.57 °C), Phase I (8.43 °C), Phase II (10.68 °C), and Phase III (12.19 °C).

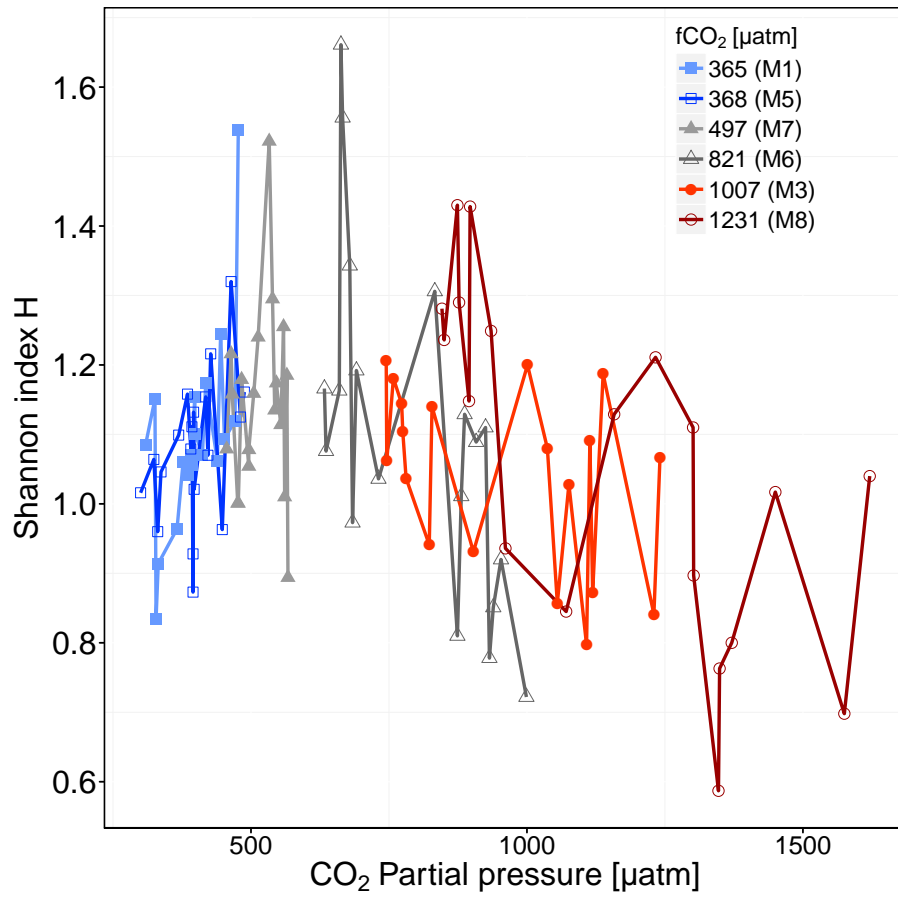


Figure 4a. Ciliates, graphical depiction of statistical results for Shannon diversity index H as a function of $f\text{CO}_2$: H is shown in relation to the daily change of $f\text{CO}_2$. Symbols and colours identify the mean $f\text{CO}_2$ for each mesocosm.

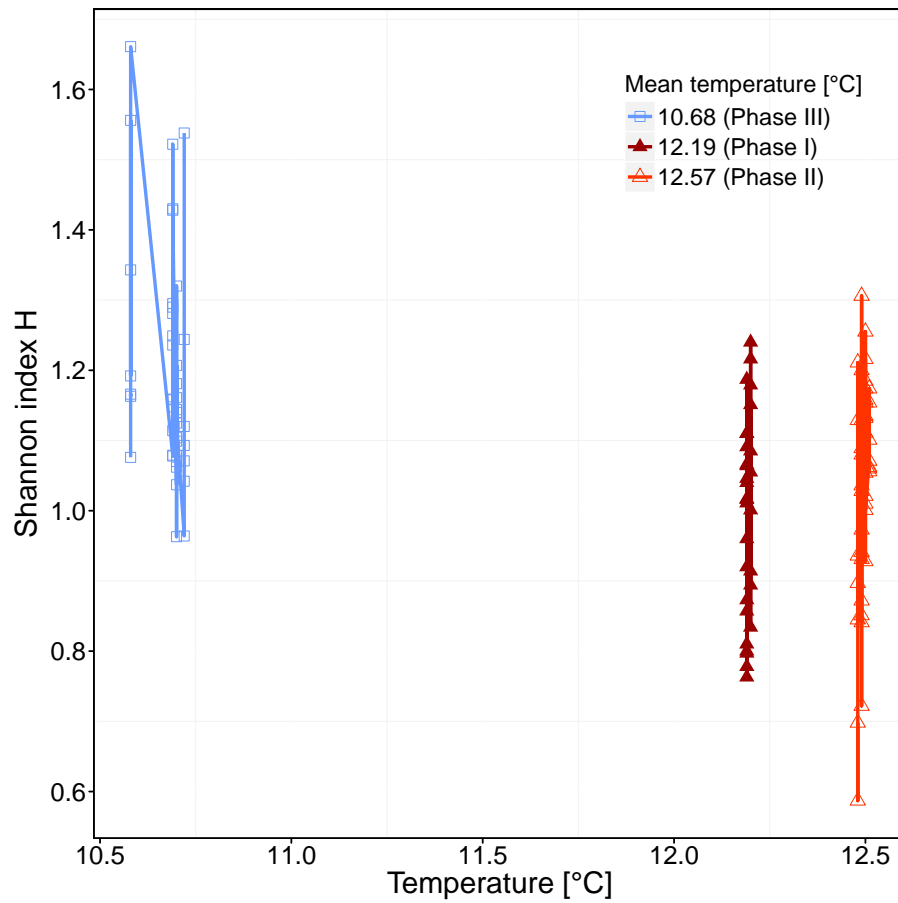


Figure 4b. Ciliates, graphical depiction of statistical results for Shannon diversity index H as a function of temperature. For better visibility, H is plotted against the mean phase (I, II, III) temperature of each mesocosm. Symbols and colours identify mean phase temperature across all mesocosms.

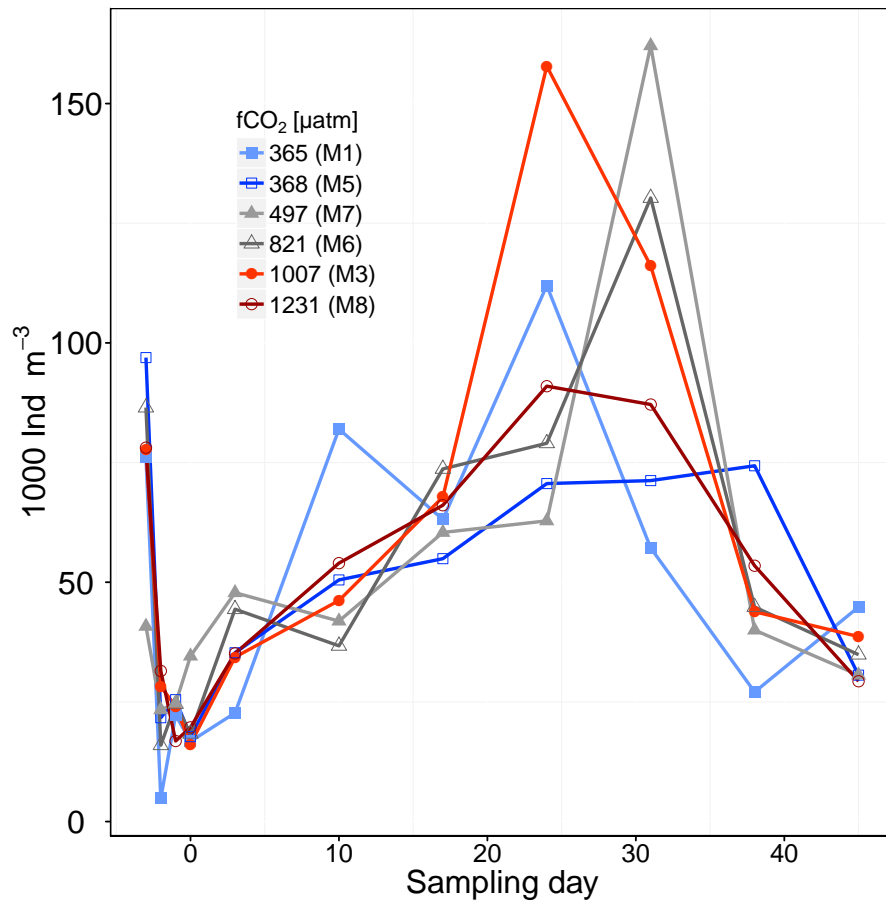


Figure 5. Mesozooplankton total abundance. According to temperature variations and the first CO₂ manipulation, different experimental phases were defined: Phase 0 = t₅ to t₀, Phase I = t₁ to t₁₆, Phase II = t₁₇ to t₃₀, Phase III = t₃₁ to t₄₃.

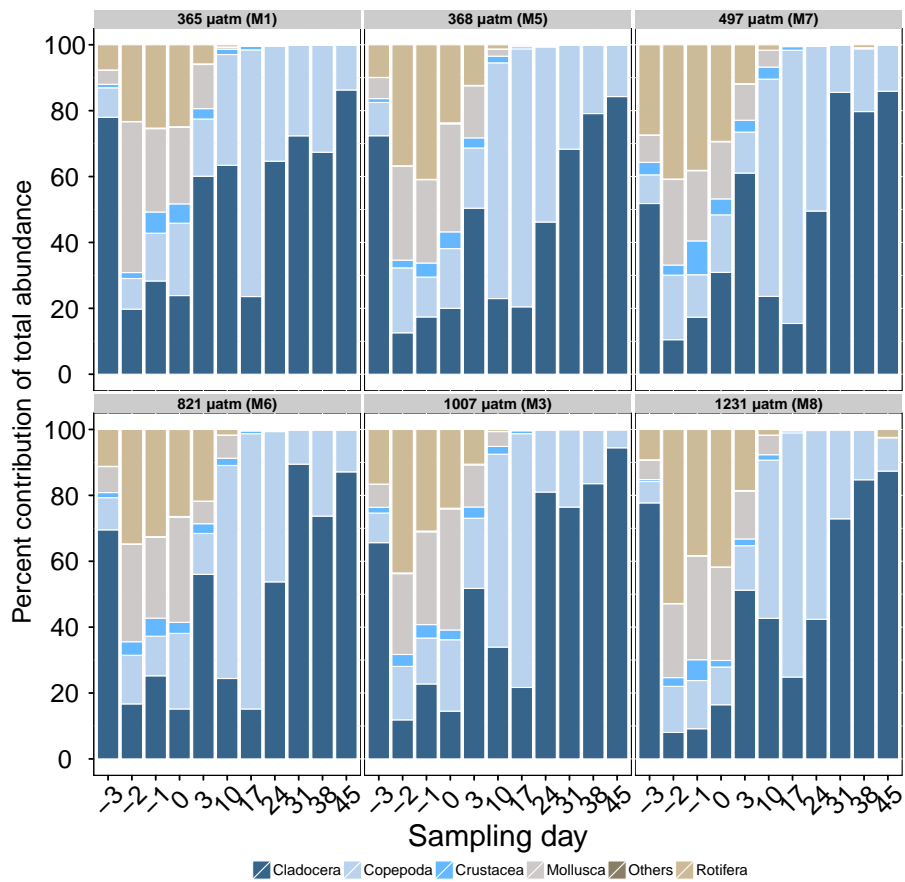


Figure 6. Percent contribution of mesozooplankton main taxonomic groups.

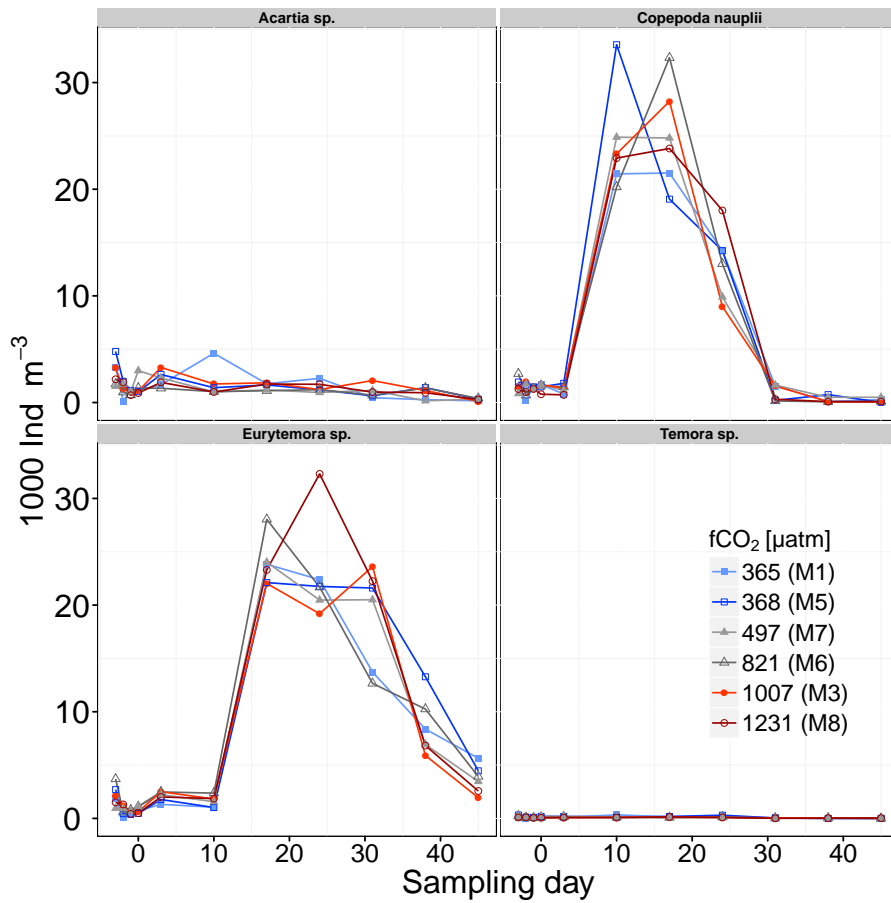


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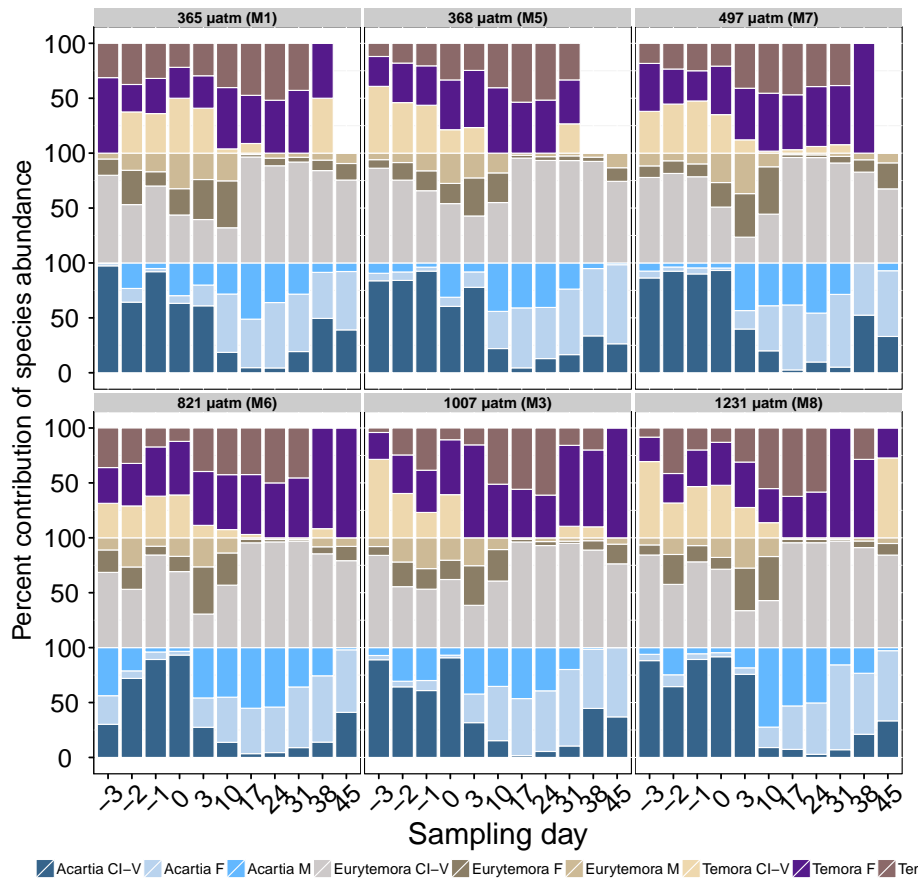


Figure 7b. Percent contribution of different stages of dominant copepods (*Acartia* sp., *Eurytemora* sp., *Temora* sp.). CI-V: copepodite stages, F: females, M: males.

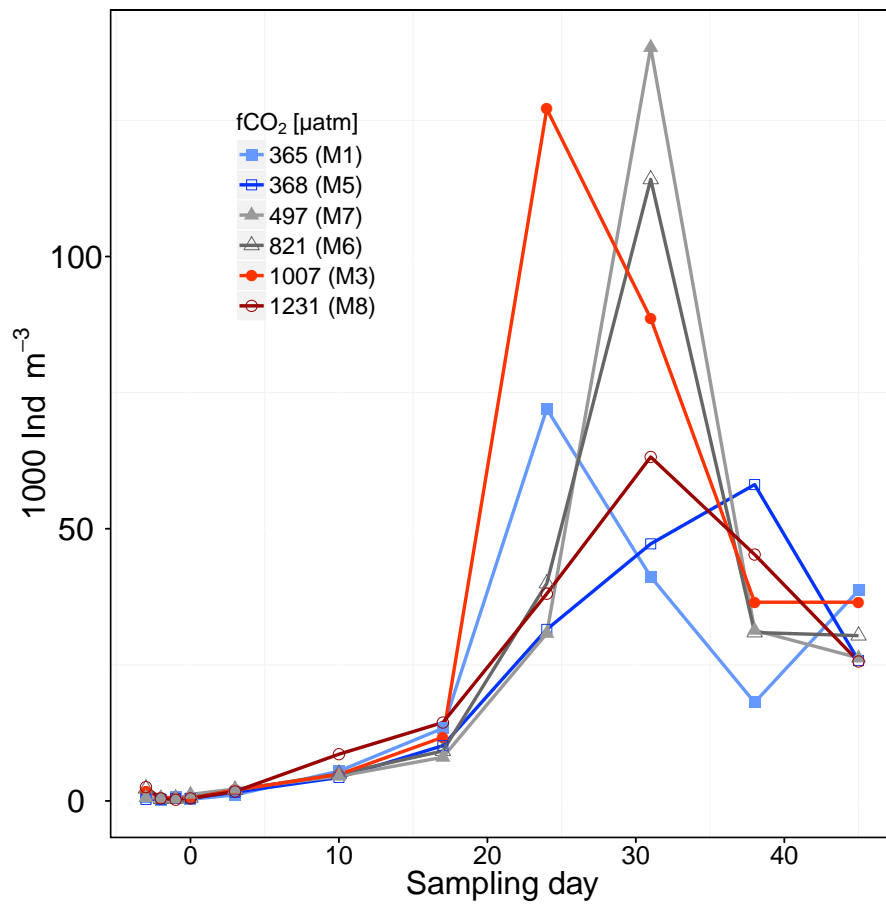


Figure 8a. Total abundance of the most dominant cladoceran species *Bosmina* sp..

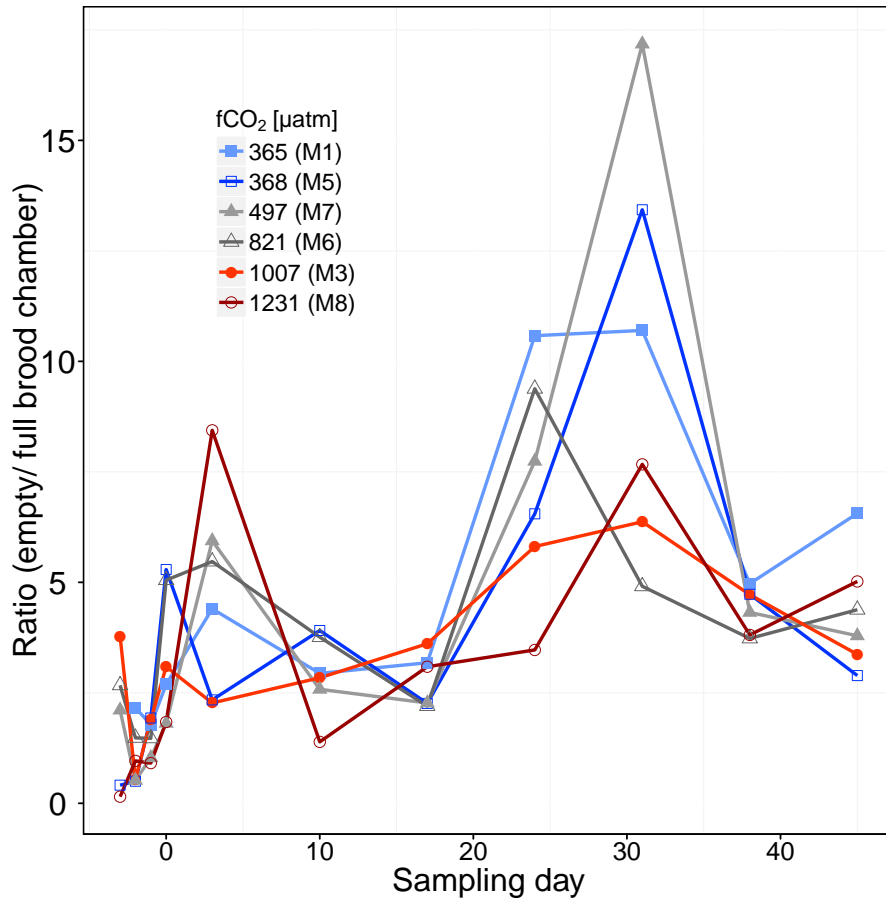


Figure 8b. Ratio of *Bosmina* with empty to full brood chambers. Note: Figure shows all data, but statistics were done on data from t_3-t_{45} only to assure equally spaced data.

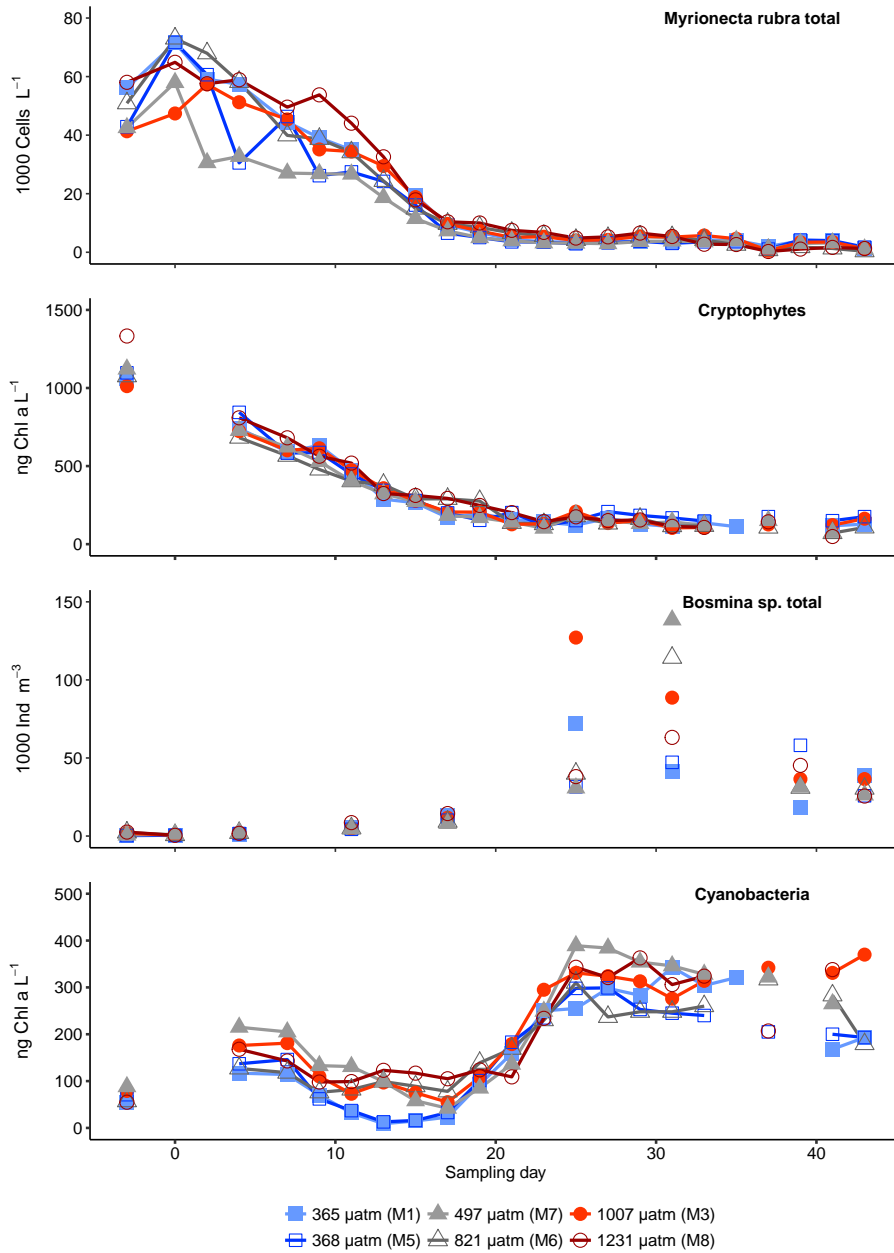


Figure 9. Succession of total cell numbers of *Myrionecta rubra*, total biomass of Cryptophytes, total abundance of *Bosmina* sp. and total biomass of Cyanobacteria during the course of the experiment. According to temperature variations and the first CO_2 manipulation, different experimental phases 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} .

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<i>Bosmina</i> sp. ratio empty/ full brood chambers	$f\text{CO}_2$	1	-2.684	0.011	GAMM
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<i>Bosmina</i> sp. ratio empty/ full brood chambers	$f\text{CO}_2$ * Chl <i>a</i>	1	2.738	0.01	GAMM
Shannon index <i>H</i>	Chl <i>a</i>	1	-0.555	0.582	GAMM

Table 2. Pearson correlation for various predator/ prey relationships. Listed are only correlations ≥ 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 $f\text{CO}_2$ levels and also separate for low (365 μatm , 368 μatm , 497 μatm) and high (821 μatm , 1007 μatm , 1231 μatm) $f\text{CO}_2$ levels. ¹data from Paul et al. (2015), ²Crawford et al. (2016), ³data from A. Stuhr (unpublished), ⁴this study.

Predator/ Prey	Pearson correlation	$f\text{CO}_2$ levels	Method
Ciliates/ Bacteria, Phytoplankton groups			
<i>Myrionecta rubra</i> < 10 μm / Cyanobacteria	-0.7	high	CHEMTAX ¹
<i>Myrionecta rubra</i> < 10 μm / low DNA bacteria	-0.7/ -0.7/ -0.7	all/ low/ high	Flowcytometry ²
<i>Myrionecta rubra</i> < 10 μm / Picoflagellates III	-0.7/ -0.7	low/ high	Flowcytometry ²
<i>Myrionecta rubra</i> < 10 μm / Synechococcus	-0.7	high	Flowcytometry ²
<i>Myrionecta rubra</i> < 10 μm / Cryptophytes	0.8/ 0.8/ 0.8	all/ low/ high	CHEMTAX ¹
<i>Myrionecta rubra</i> 10–20 μm / Cryptophytes	1.0/ 0.9/ 1.0	all/ low/ high	CHEMTAX ¹
<i>Myrionecta rubra</i> > 20 μm / Cryptophytes	0.9/ 0.8/ 0.9	all/ low/ high	CHEMTAX ¹
<i>Myrionecta rubra</i> < 10 μm / het. Dino.	0.8	all	Microscopy ³
<i>Myrionecta rubra</i> 10–20 μm / het. Dino.	0.7	all	Microscopy ³
<i>Myrionecta rubra</i> < 10 μm / het. Dino. (<i>Ebria</i> sp. excl.)	0.8	all	Microscopy ³
<i>Myrionecta rubra</i> 10–20 μm / het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	Microscopy ³
<i>Myrionecta rubra</i> > 20 μm / het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	Microscopy ³
<i>Balanion comatum</i> / Cryptophytes	0.8	all	CHEMTAX ¹
<i>Mesodinium</i> sp./ Euglenophytes	0.7	all	CHEMTAX ¹
<i>Rimostrombidium</i> sp./ Cryptophytes	0.8	all	CHEMTAX ¹
Tintinnids sp./ Cryptophytes	0.7	all	CHEMTAX ¹
<i>Spathidium</i> sp./ Euglenophytes	0.7	all	CHEMTAX ¹
Mesozooplankton/ Bacteria, Phytoplankton groups, Ciliates			
<i>Podon</i> sp./ Cryptophytes	0.9	all	CHEMTAX ¹
<i>Bosmina</i> sp./ Cyanobacteria	0.7	all	CHEMTAX ¹
<i>Podon</i> sp./ het. Dino.	0.7	all	CHEMTAX ¹
<i>Podon</i> sp./ het. Dino. (<i>Ebria</i> sp. excl.)	0.7	all	CHEMTAX ¹
<i>Eurytemora</i> sp./ Picoflagellates II	0.7	all	Flowcytometry ²
<i>Eurytemora</i> sp./ Cryptophytes	-0.7	all	CHEMTAX ¹
Copepod nauplii/ Euglenophytes	0.7	all	CHEMTAX ¹
Copepod nauplii/ Nanoflagellates II	0.8	all	Flowcytometry ²
<i>Podon</i> sp./ <i>Balanion comatum</i>	0.8	all	Microscopy ⁴