

***Interactive comment on “Mesozooplankton
community development at elevated CO₂
concentrations: results from a mesocosm
experiment in an Arctic fjord” by B. Niehoff et al.***

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Dear Prof. Kurihara,

Thank you very much for your helpful comments on our manuscript. Below we have addressed all of them. We corrected our manuscript accordingly and we believe that our manuscript certainly benefits from your suggestions.

General comments

This paper had evaluated the effect of high CO₂ on the zooplanktonic community for 6 weeks in the mesocosm established in Arctic fjord. The main found of this study

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was that the zooplankton plankton community structure is not affected by CO₂ while some correlation between CO₂ was observed in some individuals such as the ratio of cirripedia nauplii/cypris and number of bivalve significantly decrease with CO₂. Though I think that evaluation of CO₂ on zooplankton is extremely important issue and mesocosmic studies could be a strong experimental approach, I have several criticism for this paper and at least from the present data analysis, I am not convinced for the authors conclusion.

Specific comments

Introduction

P. 11481 Line 10 Describe in more detail, such as till when is expected that pH will decrease by 0.5 units according to which scenario etc.

Following the suggestion of the referee, we have now included more information on this study.

New text: A modelling study by Caldeira and Wicket (2003) who used the atmospheric pCO₂ as observed from 1975 to 2000 and CO₂ emissions from the Intergovernmental Panel on Climate Change's IS92a scenario, suggests that the pH may drop by approximately 0.5 units the end of this century and reach a maximum decrease of 0.77 at around the year 2300 (see their Fig. 1).

P11481 L14 References should be added

Answer: We have added the repetitive reference (Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N.; Ishida, A. ; Joos, F.; Key, R.M.; Lindsay, K.; Maier-Reimer, E.; Matear, R.; Monfray, P.; Mouchet, A; Najjar, R.G.; Plattner, G.P.; Rodgers, K.B.; Sabine, C.L.; Sarmiento, J.L.; Schlitzer, R.; Slater, R.D.; Totterdell, J.D.; Weirig, M.F.; Yamanaka, Y. and Yool A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, *Nature*, 437: 681–686, 2005.)

P. 11481 L 17 References should be added

Answer: The results from the experiment in Bergen are all summarized in the review by Riebesell et al. 2008, which is cited in the text. Since we do not refer to particular studies but to the entire experiment we feel that this is the correct citation.

11481 L. 20 Please add some words such as larvae or meroplankton etc for Echinodermata and Bivalve

Answer: We have now included "larvae".

L. 11482 Line 24 What is the hypothesis or question of this study? Why authors studied the impact of CO₂ on zooplankton community and what they expected to find? I think the authors should take more time to explain the basis of the meaning for evaluating the effect of CO₂ on zooplankton community, provide some hypothesis and explain the basis of these hypothesis. Answer: The underlying hypothesis of our study is that negative effects of high CO₂ concentrations on single species and their food source, respectively, can provoke lower growth (e.g. Yu et al., 2011), recruitment (Carotenuto et al., 2007) and reproductive rates (Rosoll et al., 2011) as well as higher mortality (Findlay et al., 2009, 2010). This may ultimately change the community dynamics (Doney et al., 2009) with possibly severe consequences for the food web (Fabry et al., 2008). At present, however, it is not known whether species-specific effects found in laboratory experiments will also occur in natural environments and whether they are strong enough to change the community structure. We have now added this information to the Introduction.

Methods p. 11483 line 17 Several citation is the methods not in the reference list and several "in preparation" papers (e.g. Bellerby et al. 2012; Czerny et al 2012a, b; Riebesell et al. 2012) are cited which I do not recommend as readers and reviewers are not able to read and judge the accuracy of the methodology. Answer: Thank you for making us aware that some references were missing. We fully agree that citing not-published papers is usually not desirable. However, in this particular case it

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is unavoidable since the present study is part of an international EU-project and the outcome will be jointly published in the Special Issue in Biogeosciences.

p. 11483 line 19 What is the definition of $t=0$? Answer: The time-line was defined by the entire group participating in the experiment and all papers in this Special Issue use the same labelling. $T=0$ is the first sampling after the initiation of the CO₂ manipulation; thus $t>0$ indicate the number of days of exposure to the different CO₂ concentration. These technical details are explained in Riebesell et al. (2012). We have now specified this in our manuscript.

p.11484 line line 10 Please give a table showing the seawater chemistry of each mesocosm and also the changing CO₂ by time during the 6 week experiment Answer: The seawater chemistry is described in detail in the paper by Bellerby et al. 2012, which is part of the present Special Issue. We would therefore rather refer to the paper instead of repeating the information.

p.11484 line 10 I expect that the CO₂ concentration highly differ between day and night as the CO₂ seems to be highly influenced by the phytoplankton photosynthesis. When (what time of the day) seawater alkalinity and DIC was evaluated and what was the diurnal change?

Answer: The seawater alkalinity and DIC were always measured in the morning hours (see Bellerby et al. 2012, this issue), and there was no diurnal study. The readers of the papers on our study should, please, keep in mind that each complete sampling of the mesocosms and the fjord took several hours. We were thus limited with respect to temporal resolution and we do not know whether and how the pCO₂ had changed over 24hrs. We have, however, now added more information on the sampling time.

Modified text: Depth integrated water samples were taken between 9:00 and 11:00 to measure total alkalinity and total dissolved inorganic carbon among other parameters (Bellerby et al., 2012). CTD casts were taken daily between 14:00 and 16:00 am to monitor the development of temperature, salinity and pH in the mesocosms and the

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fjord (Schulz et al, 2012).

p. 11484 line 19 What is the reason that pteropod was added in the mesocosm? This seems to be possibly cause strong artifact as authors aim is to see the effect of CO₂ on natural zooplankton community structure.

Answer: Pteropods were added as this group is of great interest in studies addressing ocean acidification. In the fjord, they are frequent but their distribution is very patchy. Including different numbers in the mesocosm just by chance was likely to affect the results. Therefore, exactly 170 pteropods have been added to each mesocosm by hand. This information has now been inserted in our manuscript.

p. 11485 line 12 Sampling were done at day or night? Though sampling in the mesocosm were conducted by vertical tow, I might expect that zooplankton could swim deeper than 12m principally at day time.

Answer: We have now included a more detailed description of the sampling, particularly mentioning that we sampled during the day. Moreover, the sediment traps covered the entire mesocosm area and we thus believe that vertically migrating zooplankton has been captured in these traps. We have now added information on the size of the traps to our manuscript and we will discuss the effect of (possible) vertical migration on our sampling.

Modified text: Two m above the bottom, a sediment trap was installed inside each mesocosm, covering the entire mesocosm area (3.14m²) to minimise material losses.

Modified text: Zooplankton was sampled between 9:00 and 14:00 in approximately weekly intervals by vertical net tows with an Apstein net of 17 cm diameter and 55 μm mesh size in the mesocosms and in the fjord.

Results p. 11486 Figure 1. Why results are only shown for 30 days and not 42 days? The duration of the experiment was not 6 weeks (=42 days)? Answer: The entire experiment lasted almost six weeks from lowering the mesocosms into the fjord to

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the end of the experiment. However, as the Prof. Kurihara pointed out, our sampling covered the period from t-2 to t30, which is only about one month. We have now modified the respective sentences in the manuscript.

Figure 2. Though I understand that authors make big effort to evaluate the fjord sample, why they did not show the data for the day -7 to day-5 (the day which the mesocosm were closed) which seems to be most important data to interpret the initial zooplankton composition in the mesocosm.

Answer: Biological sampling started at day-2, which was just before the CO₂ manipulation started; therefore these data describe the pelagic community at the starting point of our experiment. Sampling prior to this, data would have only described the development of the community in the mesocosms but would not give any information on the effect of CO₂. We thus do not fully understand why the data from t-7 to t-5 would be the most important data to interpret the initial zooplankton composition in the mesocosm.

Also please add methods of how the fjord samples were taken: how (mesh size of the net, vertical tow or not? What depth etc..) and when (day or night?) the fjord samples were taken?

Answer: The samples from the fjord were taken at the same time as the samples from the mesocosm following the same procedure (Apstein net, 55 μ m, 12 m depth, vertical hauls). This has now been clarified in the manuscript.

p. 11487 line 18 If most zooplankton are lived and just trapped rather than sink after dead, this is not organic carbon export. For discussing about carbon export or \dot{C} , authors should also show the zooplankton biomass in carbon base and also show the carbon \dot{C} of zooplankton and phytoplankton on the sediment trap separately.

Answer: This seems to be a misunderstanding as the zooplankton carbon was not separated from the total carbon in the analysis of the sediment trap samples, which were deep frozen, grinded and analysed for C and N content AFTER the subsamples for zooplankton analysis were taken. Czerny et al. (2012, this issue) calculate the con-

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tribution of zooplankton to the total carbon. However, your comment (and the comment of Ref 2) made us aware that these data are not necessary for our manuscript. In the revised version we will therefore remove Fig. 1C.

p. 11488 Fig. 5 It is very hard to distinguish the labels. Additionally please add any statistical results. Authors concluded that “there is no change on zooplankton community structure” however this conclusion is just base on “trend” and there is no any statistical result that they can prove that the community have not really changed during the experiment. Though I completely understand that in mesocosm study is very hard to have replicates, and using natural community is very hard to see any significant change in the highly heterogeneous community, but even so, since authors aim is to evaluate the effect of CO₂ on zooplankton community structure this is a very critical point of this study.

Answer: Following the suggestion of Ref. 1, who also made us aware that our statistical analysis was not correct, we have removed the MDS plots and we have used fitted linear mixed effects models to determine the dependency of diversity (i.e. the Shanon index H) of time and of CO₂ combined with two nutrient conditions (t-2, t2 and t11 representative of phase 1 (Schulz et al., 2012 and t18, t 24, t30 representative of phase 2 and 3 (Schulz et al., 2012)). Random effects were modelled by CO₂, i.e., grouping the data by mesocosms. Computations were performed in the computer program R, using lmer (ML method) from package lme4; H was computed in the vegan package. This analysis reveals that a fixed effect of CO₂ is not significant for the time dependency of H (ANOVA, p=0.11 for watercolumn data; p=0.46 for sediment data).

We should, however, be aware that statistical analysis cannot prove that there have been NO significant changes – statistics can always only prove that there are significant effects.

Also, we are very aware that changes in communities are much more difficult to detect than in species-specific studies. However, in order to understand ecosystem responses

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we believe that it is crucial to extend our approaches to the community level. We will add this aspect to the discussion in the revised version of our manuscript.

p. 11489 line 1, Fig. 7 The data “over entire experimental period” included data from which day to which day? Data for -2 (day before add CO₂) or/and data between day -1 to 4 (day that CO₂ was adjusted) are excluded? Answer: It should have been “over all sampling days”. This has now been corrected.

I have not been convinced from regression analysis data of ration of nauplii : cypris averaged data over entire experimental period that the development of naupli to cypris stage was influenced by CO₂. First authors should also show if there are same trend in the water sample, they also should show that at day-2 (before injecting CO₂) there is no such trend (there is no trend that M_{3,7}>M₂>M₄>M₈,M₁>M₆>M₅>M₉) and they also should not integrate data for whole experimental period as the development is time dependent. Additionally the data seems to be highly influenced by the data of one day (day 16) which seems to be very “special” or the same trend of naupli:cypris is shown in all other days? Answer: Following the suggestion of Ref. 2, we have now removed the regression analysis as we realized that this statistical test is not appropriate for our data. To test whether there is a relation between CO₂ and cypris larvae as well as the ratio between cypris and nauplii, we have now used the (two-tailed) Spearman-rank-test as this test does not depend on normally distributed data and on linear responses. This test does not indicate a response of cirripedia to CO₂ and we will change the manuscript accordingly.

The reason why we separate the data from the water column and the sediment traps is that the data are of different quality characteristics, i.e. the data from the water column present concentrations (n m⁻³) while the data from the sediment traps present the number of animals collected in the traps and thus leave the system: i.e. A copepod nauplius in the water column will eventually develop to a copepodite but a copepodite found in the sediment trap is removed from the mesocosms. Therefore, in our opinion it is not correct to mingle the two data sets for describing the community composition.

We have now included a comment on that in the “Methods” section.

p. 11490 line 9 Figure 8 Similar to the comment for Figure 7, authors should first show that at time -2 (before injecting CO₂) there is no such trend (there is no trend that M_{3,7}>M₂>M₄>M₈,M₁>M₆>M₅>M₉), but the relation between bivalve abundance and CO₂ start to be observed and become clear after day 4 or later. To test whether there is a relation between CO₂ and bivalve abundance we have now used the (two-tailed) Spearman-rank-test. Significant relationships between pCO₂ and bivalve abundance were found at t₂ and t₁₈ and we will change the manuscript accordingly.

Discussion

Since the discussion is mainly based on the cirripedia nauplii/cypris and number of bivalve conclusion is hard to evaluate before revision. Additionally, I would like to evaluate the discussion after the hypothesis of the authors become clear. Answer: Based on the suggestions of all referees, we will rewrite large parts of the discussion.

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