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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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Received and published: 5 December 2012

Answers to Ref.#1

Dear Referee #1, Thank you very much for your considerate and helpful review. We have addressed all your comments (see below) and we corrected our manuscript accordingly. We believe that this has considerably improved the quality of our manuscript. Particularly your comment on statistics has led to an intense and fruitful discussion and will inseminate future studies.

General comments This manuscript reports on the mesozooplankton community development in CO₂ enriched mesocosms in the Arctic Kongsfjord over a six-week period.

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The focus was on changes in abundances and taxonomic/species composition. Meso-zooplankton in the water column and in the sediment traps of the mesocosms were studied. The main finding of this work is a negative CO₂ effect on the development of cirripedia larvae and on the occurrence of bivalve larvae. As a whole, the zooplankton community composition was not affected by elevated pCO₂ concentrations. This is a well-structured and clearly written manuscript that is easy to follow for the reader. The experimental approach allows for the investigation of ocean acidification effects on whole planktonic communities on a large (ecological) scale, therefore this is a unique dataset. However, the authors' main conclusion "no significant change in community composition" is not justified by the way the data were analyzed and therefore, the manuscript should not be published without serious consideration of the comments below.

Specific comments – L245/246: My major criticism is on the MDS ordination and conclusions drawn from it. MDS ordinations do not give a significant result, i.e. no significance value. They simply map (multivariate) data in an n-dimensional space by distances based on a similarity (or dissimilarity) matrix among the samples. That means an MDS plot helps to see whether samples are similar and how close they are to each other. But it is NOT a statistical test!

What complicates the matter here is the repeated measures design with no replication. That means, in the analysis the factor "time" needs to be eliminated to be able to judge whether or not there is a CO₂ effect. If not, the time effect may mask a possible CO₂ effect. A simple MDS ordination technique cannot do this. Therefore, the authors have no justified reason for their conclusion "no significant change in community composition" any better than a subjective impression of their data. In fact, Fig. 5 nicely shows the separation of the samples by time, in that, I agree with the authors. But, as just pointed out, it is not the factor time that is of interest here (it is well known, that time has an effect on the plankton succession).

The question is, whether or not aside from a time effect there is also a CO₂ effect?

It needs more elaborate statistical techniques to determine whether or not there is a CO₂ effect. Mixed effects modeling would be an appropriate tool maybe using species richness or Shannon Wiener index as a measure. Also, it needs to be better specified which taxa/species were included to calculate the similarity matrix. I.e. how were the cirripedia entered, as “cirripedia” or as “cirripedia nauplii and cypris”? It is also not clear, whether copepods were entered as “copepods” or separated by “species and even stages”? The authors mention that they have staged copepods, but the stages counts do not show up anywhere in the ms. Included or not, this will change the similarity matrix calculated and thus the outcome of the analysis. This needs to be stated clearly in the ms in order to make the reader able to assess the data/results and the conclusions drawn.

Answer: The referee is absolutely right and we are very thankful that we were made aware of our mistake. Following the suggestion of the referee, we have now fitted linear mixed effects models to determine the dependency of diversity (i.e. the Shannon 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 phases 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 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 water column data; p=0.46 for sediment data).

As we omitted the MDS plots from the revised version of the manuscript, the following is just for your information: In the (previously presented) MDS plots, which targeted community composition, we had entered the different groups; copepod species were separated, but not the developmental stages. However, out of curiosity, we have re-computed the MDS plots including developmental stages and the outcome was actually not much different.

Also Referee 2 mentioned in his/her review that we determined copepod developmental

stages (Methods) but do not show the data. This was probably not well explained from our side: what we intended was to address that we had distinguished between cirripedia nauplii and cypris larvae and between copepod nauplii and copepodites I–VI, respectively - the latter were presented as one group. We did, however, indeed determine developmental stages in Calanus and we have now included a chapter on this topic. It shows that also the development of Calanus did not seem to be affected by the CO₂ treatment.

Referee 1 (as are Ref. 2 and Prof. Kurihara) is also right that the lack of repeated measurements was problem for study. However, this approach was used for the following reasons (cited from Riebesell et al. 2012, page 12994–12995): “1. Because of the low number of experimental units available and considering the risk of losing one or several mesocosms (e.g. due to damage by ice floats) a CO₂-gradient approach carries a lower risk of failure compared to a replicated approach (e.g. 3 CO₂ treatments with triplicates each) relying on ANOVA statistics. 2. If there is a threshold level for any of the CO₂/pH sensitive processes, a CO₂-gradient approach has a higher chance of detecting it. 3. With a CO₂-gradient approach the opportunity arises to include one or two CO₂ levels outside the range recommended for ocean acidification perturbation experiments (Barry et al., 2010), which would be more difficult to justify if such extreme levels were replicated. 4. Although CO₂ manipulation is relatively straight forward, it is challenging to precisely achieve the targeted CO₂ levels. While critical in a replicated approach, in a CO₂-gradient approach deviations from the targeted CO₂ levels can be tolerated.”

– L171–175: Sample processing needs to be clarified: What was the maximum split factor applied? Of the samples that were split, were only the very abundant taxa/species counted in the subsamples or were the abundances of the whole sample calculated from subsample counts? Usually, only the abundant taxa are counted from subsamples and the less abundant taxa/species are counted from the whole sample or the larger aliquots. What was the minimum number of individuals counted in each

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subsample? Please, clarify how samples were counted to make the abundance calculations reproducible. Answer: We have now added information on sampling and sorting procedures as requested by all referees to our manuscript.

Information now included in the text: Under a dissecting microscope, the organisms were sorted and determined to the lowest taxonomical level, if possible to species and/or developmental stage (*Calanus* spp.). In the fjord, zooplankton abundances were relatively low and thus all organisms were sorted and counted in a sample. In the mesocosms, the abundance of mesozooplankton organisms was considerably higher for most of the experimental period and therefore, the samples were subdivided with a Plankton Splitter (Hydrobios) usually to 1/8 (44 of the 59 samples) and at maximum to 1/32 (2 of the 59 samples). Abundant species ($n > 50$ in an aliquot) were sorted only from one subsample, while less abundant species were sorted from at least two subsamples. Comparing the subsamples indicates that the numbers of organisms, even of rare species, did not differ much among the subsamples. Abundances were calculated in terms of individuals m^{-3} . Eggs and larvae $< 55 \mu m$, e.g. early trochophora larvae, were not sampled quantitatively with the Apstein net and are thus not further considered.

– L96–98: Last sentence in the introduction is results, delete from the introduction
Answer: The respective sentence has been removed.

– L449–501: Is it possible that the author could not notice increased mortality of bivalve larvae because their shells dissolved already in the water column and the soft tissue was already degraded (or unidentifiable) before reaching the sediment trap? Answer: We found quite a few larvae during the first week in the sediment traps, also in the high CO₂ treatments. At that time, CO₂ concentrations were highest; later they decreased due to outgassing and biological processes (Bellerby et al., 2012, Czerny et al., 2012). Thus, the highest risk for not finding bivalve larvae in the sediment traps was during the first two weeks, and at that time, their contribution to the zooplankton in the sediment traps was high (see Fig. 4). Therefore, we do not believe that their shells dissolved in

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the water column within the two days before they reached the sediment traps.

– L525–529: The larger mesozooplankton that was not effectively collected by the 55 μm net, was it found in larger numbers in the sediment trap? If not, there was probably not too much larger plankton in the mesocosms. Answer: This part of the discussion will be completely changed as we include a more detailed discussion on the limitations of sampling the mesocosms.

Technical corrections – L111: Off-Shore: The “S” should be underlined, too, I guess? Answer: has been changed

– L123: Delete the dot after . . .2012b) Answer: has been changed

– L166: t-11 or t-1? Answer: has been changed, sorry for the confusion, it is t11

– L295 + L302 + L344: Fig 2? Should be Fig. 3. Answer: has been changed

– L312 + L348: Fig. 3 must be Fig. 4 Answer: has been changed

– L370: . . . up to several days. . . Answer: has been changed

– L393: grazing rates of *Calanus* spp. and cirripedia nauplii decreased with increasing or decreasing pCO₂? Answer: has been changed: we have included “increasing pCO₂”

– L457: Kongsfjorden lacks the “s” Answer: has been changed

– Fig. 2+6 (Figure caption): Please make the reader aware that the scales are different in the different graphs. Answer: has been changed; we have now included this information in the figure captions

– Axis labels of Figures: Please use consistent labels, some start with capital letters some don't. Answer: has been changed; we have now uniformed the labels (small letters)

– Fig. 2 lacks the x-axis label. Answer: changed

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– Fig. 7: shows the ratio nauplii : cypris of only 8 mesocosms, where is number 9? Answer: Two mesocosms were kept at ambient CO₂ conditions (M3 and M7), the averaged abundances of bivalves of these two mesocosm matched so closely that only one data point is visible. Due to the comments from the other referees, however, we have removed this graph from the manuscript.

Interactive comment on Biogeosciences Discuss., 9, 11479, 2012.

BGD

9, C6291–C6297, 2012

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