Biogeosciences Discussions

Interactive comment on "Mesozooplankton community development at elevated CO$_2$ concentrations: results from a mesocosm experiment in an Arctic fjord" *by* B. Niehoff et al.

B. Niehoff et al.

barbara.niehoff@awi.de

Received and published: 5 December 2012

Response to Anonymous Referee #2

Dear Referee #2, thank you very much for your considerate and thorough review of our paper. Below we have addressed all your comments and we correct the manuscript following your suggestions. We believe that this will considerably improve our manuscript.

- General comments: The manuscript reports the study of the effects of ocean acidiïňĄcation on zooplankton community composition in a mesocosm set-up. The study is one of the ïňĄrst of this kind and therefore novel and interesting. The manuscript is well structured and also well written. From their results, the authors conclude that ocean

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

acidiïňĄcation could have some future impact on zooplankton composition by nega-
tive affecting particular groups such as cirripedia or bivalves. Mesocosm studies like
the present often suffer from restricted sampling by the limited volume. The authors
overcame this problem by relatively infrequent sampling (six times) and by averaging
data over the whole sampling period for subsequent linear regression analysis. I have
some problems with this method as it assumes that responses to increasing CO2 will
be linear and that zooplankton development is independent of time and not different in
the various mesocosms.

Answer: We fully agree that for studying zooplankton a replicate approach would have
been desirable. However, the present paper is embedded in a large study within the
European Project on Ocean Acidification and 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."

Following the suggestion of referee 1, who also made us aware that our statistical anal-
ysis was not correct, we have now 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 t11representative 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. Compuations 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 water column data; p=0.46 for sediment data).

Referee: This is not necessarily the case, particularly in such groups, which have several development stages, e.g. copepods. For instance, mortality is not independent of development rate as it is stage dependent, which has not been analyzed here. Answer: We did indeed determine developmental stages in Calanus, including nauplii and copepodites. We had chosen not to present these data in this paper to focus on the species composition only. However, as abundance is of course influenced by mortality and this may change with developmental stage, we have now added the stage distribution data. These data indicate that the development of Calanus did not differ among the mesocosms.

Referee: Differences in timing, caused by various environmental factors, as also discussed by the authors, can therefore inïňĆuence the results of the analysis. Moreover, conclusions drawn for cirripedia and bivalves are based on selected data (either from the sediment trap or the water column) and it is not clear for me why this selection is justiïňĄed. Answer: These 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 structure. We have now included a comment on that in the "Methods" section.

Modified text: Data from the water column present zooplankton concentrations (n m-3) at the particular day, while the sediment trap data present how many organisms have

been lost to the upper 12 m of the water column during 48h. Thus, owing to their different quality characteristics, the analyses of the water column and the sediment trap samples are presented separately.

Apart from these critical issues, the methods lack some detailed information on procedures and some wording could be corrected.

Introduction p. 11481, Line 6: Before CO2 dissolves it is absorbed by the sea. The carbon system is described too short (only by one sentence) for a general introduction. Improving this would increase understandability of the following for a non-specialist and shorten text, e.g., line 31 when 'carbonate ion' is used and when 'decreasing pH-increasing pCO2' can be exchanged by OA. Answer: We have now included a short summary of the carbon system and we hope that this will improve the understanding for a non-specialist.

New text: When CO2 in the gas phase dissolves in seawater, it equilibrates with carbonic acid (H2CO3). Carbonic acid dissociates immediately to bicarbonate ([HCO3-] und hydrogen ions ([H+]). In a second reaction on pH, bicarbonate ions dissociate to carbonate ([CO3-2] and [H+]; this reaction is dependent on pH. Thus, with increasing pCO2 the seawater pH decreases and free carbonate ions protonate and form bicarbonate.

p. 11481, Line 17: Citations are missing for the observed changes; e.g. are there many examples for a changed stoichiometry? I thought changes occur largely when carbon availability is limited. Answer : We have now included recent examples for a changed stoichiometry.

New text: At elevated CO2 levels, pelagic primary production may increase due to lower costs of carbon fixation, the stoichiometry and the biochemical composition of some algal species may change (Emiliana huxlei, Leonardos and Geider 2005, Borchard et al. 2011, Thalasisosira pseudonana, Rosoll et al. 2012) and carbon overconsumption may lead to increased exudation of transparent extracellular particles (Engel 2002).

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

p. 11482, Line 5 ff: long sentence Answer: This sentence has been split.

New text: In shelf seas and coastal areas, meroplanktonic larvae at times occur in high abundances (e.g. Fransz et al., 1991; Fetzer et al., 2002; Walkusz et al., 2009). Among these, non-calcifying larvae of some benthic species were also shown to be sensitive to pCO2, e.g. barnacle nauplii by Findlay et al. (2009, 2010).

p. 11482, Line 14: Bergen experiments: any citations? Answer : The results have been summarized by Riebesell et al. 2008, which is referred to later in the text. For clarification, we have now added this reference to the respective sentence.

New text: Up to date, there has been a mesocosm experiment studying the impact of pCO2 in the outdoor facilities at Espegrend, Bergen, Norway (summarized in Riebesell et al., 2008).

p. 11482, Line 20: Any important results from Caretenuto? Answer : We have now briefly summarized the results of the study by Carotenuto et al. In doing so, we have also corrected the year of publication, which was wrong in our manuscript.

New text: Only Carotenuto et al. (2007) studied the effect of CO2 on a mesozooplankton species, i.e. Calanus finmarchicus, during the mesocosm experiment in Bergen and they suggest that the algal food quality was altered by elevated pCO2, which in turn affected nauplii recruitment.

p. 11482, Line 24: Any hypotheses? One wonders if there no strong effects, why is this studied? 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

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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.

To the same comment (One wonders if there no strong effects, why is this studied?) Answer: In this respect, we do not fully agree with Ref 2. The information we have to date on the response of zooplankton species is based on laboratory studies only as outlined above. We believe that it is crucial for understanding the effects of OA on pelagic communities to study the communities in near-natural environments. We also strongly believe that negative results (i.e. no changes associated with elevated pCO_2) should be made available to the scientific community in order not to bias the findings on the effect of OA towards positive results.

Methods p. 11483, Line 14: I guess 15 m 'depth' not 'length'. Answer: Length has now been changed to depth.

p. 11483, Line 19: explain: t-7; what deïňĄnes day 0 or 1? Answer: The labelling of the days has been used by all groups, which participated in the experiment. We have now clarified this in our manuscript.

p. 11484, Line 8: Just out of curiosity: how much water was added to each of the mesocosms? Did the amount of water (dilution) differ substantially between different CO_2 treatments? Answer: Between 70 and 320 L corresponding to 0.15-0.7% of the total volume, not-manipulated mesocosms (3 and 7, 180 μatm) were treated with filtered seawater (251L); this information has now been added to our manuscript.

p. 11484, Line 14: Please give the time span for the decline in CO_2. From which day are the ïňĄnal measurements? Answer: The CO_2 decreased continuously over the entire experiment and the date of final pCO_2 measurements was t30. We have now modified the text accordingly.

Modified text: Due to gas exchange and biological processes, the CO_2 concentration decreased continuously over the entire experimental period in all mesocosms (pCO_2

at t30 was 165 μatm in M3, 160 μatm in M7, 220 μatm in M2, μatm 290 in M4, μatm 365 in M8, μatm 500 in M1, μatm 555 in M6, μatm 715 in M5 and μatm 855 in M9) (Czerny et al., 2012a; Bellerby et al.. 2012).

p. 11485, Line 12: At which time of the day the samples were taken? During the day, zooplankton might have been missed due to vertical migration. Sub-sampling from the fjord would have helped to resolve the insecurity about the abundance estimates. Answer: Samples were always taken during the morning hours until early afternoon; the different sampling days are thus comparable. Zooplankton, which migrated to deeper layers, were sampled by the sediment traps, which integrates over 48hrs.

Diel migration itself, however, cannot be evaluated based on the data of our study but we believe that this aspect has been covered by sediment trap sampling. We will add this aspect to our discussion. With regard to additional sampling in the fjord, we are not sure whether this would have improved the insecurity about the abundance estimates as the community in the fjord was so very different from that in the mesocosms.

With regard to the sampling variability, we believe that our estimates of species abundance in the mesocosms are reliable: In total, we have identified ten taxa, which contributed >5% to the community in the mesocosms and the fjord. In the water column, cirripedia, bivalve and polychaete larva, copepod nauplii, Calanus, Acartia, Oithona and Microsetella were all present in >80-100% of the samples (total of 59). Euphausid larvae were found in 66% of the samples and gastropod larvae were present in 39%. Accordingly, usually either eight (in 16 samples) or nine (in 27 samples) groups were found in a sample. This consistency among the data sets indicates, in our opinion, that our data reflect the dominant groups in the community. Species/taxa, which were only rarely found, were not included in our analysis owing to the limitations in sampling. We will comment on that in the revised version of the manuscript.

p. 11485, Line 13: The order of days is confusing. Why is d-2 twice mentioned? D-11 is before the iňĄlling (and if it is d11, then this is close to biweekly, not weekly). Answer:

Interactive Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Sorry, second sampling took place on t2 and not t-2, and it has to be t11 not t-11; we have corrected the text accordingly.

p. 11485, Line 19: What were the criteria for splitting the samples, how many individuals were counted? What about the treatment of rare vs. abundant species regarding the splitting? Answer: We have now added the information as requested by all referees to our manuscript.

New 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 μm, e.g. early trochophora larvae, were not sampled quantitatively with the Apstein net and are thus not further considered.

p.11486, Line 5: What is the underlying hypothesis for assuming linear responses to pH/CO2? Answer: see also comment above on the experimental design. We have removed the linear regressions from the manuscript and to test whether there is a relation between CO2 and abundances we have now used the (two-tailed) Spearman-rank-test as this test does not depend on normally distributed data and on linear responses.

p.11486, Line 16-17: Citations are missing in the references. Answer: Sorry and thanks! As we have now removed the MDS plots from our manuscript, these citations are, however, not to necessary anymore.

Results p.11486, Line 20: While I can understand that sampling in the mesocosms was restricted by the available volume, this was not the case in the fjord. Here one would have expected that replicated sampling would have been done. Furthermore, one wonders why the last day of mesocosm run was not used to establish estimates of sampling variability. Answer: Unfortunately, as the experiment cannot be repeated, we cannot mitigate that we did not estimate sampling variability and in future experiments, we will certainly follow the suggestion of the referee(s).

p.11487, Line 7: 'The number of organisms changed with time. . .' contrasts with ' the total abundance changed only slightly. . .' on page 11486, line 22. It is doubtful to give numbers here (averaged over all mesocosms? What justiïňĄes this?), as there are no estimates of sampling variability and trends can result from compositional differences. Answer: The text on page 11486 describes the development in the water column whereas the text on page 11487 describes the development in the sediment trap samples. To be more precise, we have now added "in the water column" to the first paragraph. Modified text: The initial total zooplankton abundance in the water column of the mesocosms ranged from 9,286 ind. m-3 in M8 and 27,858 ind. m-3 in M1 (Fig. 1A).

p.11487, Line 26: AS described in the legend to Fig 3, M7 is not visible, but is one of the 185 μatm CO2 mesocosms. Answer: We do not fully understand this comment but we realized that there was no information in the legend that data from t30 are missing for M7. Therefore we have now included the following information and we hope that this clarifies the referee's question. The same sentence has been added to the legend to Fig. 7 as this information was lacking here, too. New text: At t30, no samples were taken in M7 (185 μatm) and no data are available.

Fig 2C: Does the copepod composition include nauplii? Otherwise it should be mentioned that this describes the copepodite composition. Answer : The copepod composition includes only copepodites and adults; this is now mentioned in the legend.

Interactive Comment

Fig 1C: The zooplankton carbon and total carbon can be separated in this ïņ̃gure, as zooplankton were mostly swimmers (according to line 4, p 11487) while diatoms are probably 'real' export. Why were they summarized here? 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), however, calculate the contribution of zooplankton to the total carbon. However, your comment (and the comment of Prof. Kurihara) made us aware that these data are not necessary for our manuscript. In the revised version we will therefore remove Fig. 1C.

p.11488, Line 5: The differences between fjord and Mesocosms are no surprise considering the mesh used to exclude large zooplankton. The manuscript lacks information on the composition of the zooplankton left out: was this Calanus or other species? Answer: We agree that the differences between fjord and mesocosms communities are no surprise, due to the lack of larger predators and also due to advective processes in the fjord. Moreover, including the data from the fjord does not add to the question whether mesozooplankton communities develop differently in relation to pCO2. We do, however, feel that presentation of the data from the fjords completes the manuscript and should not be left out.

To sample Calanus females and CV, I usually use a 500μm net, smaller stages are of course sampled with smaller meshes. With 3000μm mesh size (see Methods, page 11483, line 20), only larger zooplankton was excluded and we do not believe that Calanus was among those. Moreover, the catches from the fjord give some (limited) information on which species were excluded although the Apstein net certainly was not ideal for evaluating the abundance of larger species. To estimate the abundance of chaetognathes, amphipods etc., WP2/3 or Bongo nets would have been appropriate. However, sampling had to be done by hand from rubber boats. Using larger nets - preferably towed to sample a larger volume in order to account for lower densities -

was unfortunately not feasible during our study.

p.11489, Line 2: Please specify: what is meant by 'development was influenced by CO_2'. Do you suggest that the development rate was reduced, so that nauplii instead of cypris settled? How much is this trend driven by the two 'outliers' at day 16? If this trend is related to the CO_2, should it not be visible in the whole mesocosms, and not only in the sediment traps? Answer: Following the suggestion of Ref. 2, we have now removed the linear regression analysis as we realized that this statistical test is not appropriate for our data; the Spearman-Rank-Test does not give indication that there is a relationship between CO_2 and the ratio of cypris to nauplii. We modify the revised version of our manuscript accordingly.

p.11489, Line 7: Throughout the text the labeling of mesocosms according to number (M1, M2. . .) is not very satisfying as no additional information is provided and one always has to go back to the Mat& Meth to look up which mesocosm these have been. Can these exchanged to the CO_2 labeling (185 μatm. . .). This is also consistent with the labeling in figures. Answer : We have modified the text following the suggestions of the referee.

p.11489, Line 11: This is very unclear. Polychaetes apparently settled as they were found in the sediment traps, and were removed. What kind of larvae was then found at day 11? The large size argues against that these have been trochophora larvae. So where did these larvae come from? The mesh size of the net used should have allowed collecting trochophora larvae, but these should have been present earlier then. Answer: Actually, the only explanation we have is that the larvae (these were early polychaete larvae, not trochophora) developed from eggs or very early trochophora larvae – they appeared in all mesocosms and are thus not an isolated phaenomenon. As stated in the Methods section, we did not count eggs and trochophora larvae and thus we can unfortunately not relate the polychaete larvae to any previous developmental stage.

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

p.11490, Line 15: Correlation between what? Answer: In agreement with the referee's comment (see our comment above) we removed the linear regression analyses.

p.11491, Line 20: Which data went into the linear regression of copepods? Stages? Why is – as in all the other cases – a linear response to CO2 expected? Dose-effect responses often follow a sigmoid response. Furthermore, it is not clear for me how the data has been treated: was the abundance at the end used, was the data averaged over time – and if yes – is regression analysis correct statistic here? Abundance of copepods is a function of stage dependent mortality and development rate – and thus the treatments cannot necessarily be compared by averaging or using end samples when the different mesocosms develop differently over time – so time and timing cannot be ignored. Answer : Following the suggestion of the referee (see our comment above) we removed the linear regression analyses. We did, however, now include a paragraph describing the stage composition of Calanus spp. to illustrate that it did not differ among the mesocosms.

Discussion: Apart from the discussion of the set-up (outgasing/uptake of CO2), I miss a critical evaluation of the lacking estimate of sampling variability on potential conclusions. The sample volume of an Apstein net is small (in the case roughly 0.2 m3), but small differences occurring in the analysis are potentially up-scaled (by a factor of 5). This might be critical for estimating effects of OA on rare groups (e.g., bivalves). In addition, time periods in between single samples were long. Moreover, the ïňﬁrst samples revealed a strong variation in the initial abundance of zooplankton. Can this inïňﬂuence detection of any trends? There is also a layer of 3 m depth between sediment trap and the depth of net sampling. Answer: We have followed the suggestion of the referee and included a more thorough discussion on these topics (see also comments above).

p.11494, Line 18: I might have missed it, but were mortality rates estimated that would allow this conclusion? Answer: We did not calculate mortality rates. Our conclusion is based on Fig. 6, which shows that the number of nauplii decreases considerably in all

mesocosms without any apparent trend.

p.11494, Line 27: When OA is delaying the development of nauplii, one should see this as well in the mesocosms which contained by far the larger pool of cirripedia nauplii. Fig 6 A,B, however, do not indicate such a delay, and one wonders if such trend could have been detected with the low sampling frequency. Answer: The only ways of a reduction in numbers of nauplii would be (1) mortality and (2) molting to cypris larvae; therefore, we have used the cypris:nauplius ratio as an index for development. We did, however, not determine nauplius stages in the cirripedia and we will therefore remove this statement from the discussion.

p.11496, Line 25: Again, the conclusions seem to be based by using only one of the available data sets. The data from the sediment traps is ignored here, although they constituted a considerable pool for this group. In addition, please specify what is meant by negative inïňĆuence on 'development'. Answer: See our comment above on separating water column and sediment trap data and on our answer to your comment on p. 1194, line 27.

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

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper