

# ***Interactive comment on “Response of benthic foraminifera to ocean acidification in their natural sediment environment: a long-term culturing experiment” by K. Haynert et al.***

**K. Haynert et al.**

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## REPLY TO REVIEWER'S COMMENTS:

We thank all four reviewers and the short comment by Vasilis Kitidis for taking time and for their helpful, thoughtful and constructive comments which helped to improve the manuscript. The reviewer's comments are addressed in detail as follows.

REVIEWER 1

MAJOR COMMENTS

REVIEWER 1:

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The salinity at which the culturing experiment was conducted, was 16. This makes it difficult to see how the results of this paper are applicable to foraminifera in general, since most live at higher salinities where saturation states are higher and undersaturation is less of a problem. In fact, Haynert et al., have conducted an experiment at which the effect of both low salinity and elevated pCO<sub>2</sub> is investigated. This should be stressed in the title, abstract and discussion. The resulting low saturation states furthermore make it very difficult to compare their results with previously published results and the authors should refrain from that (see Discussion). As often with OA studies, no condition was included with a lower than current-day atmospheric pCO<sub>2</sub> (i.e. <380 ppm). This would have allowed testing whether any observed trends also hold for low pCO<sub>2</sub> conditions and whether the observed changes are not simply the result of an adaptation to natural conditions (i.e. whether decreased chamber addition rates would also have occurred at low pCO<sub>2</sub>'s). This is also a shortcoming in the experimental design of the authors and the resulting potential bias should be mentioned in the discussion.

#### REPLY:

The relatively low salinity value of 16 applied in the experiment corresponds to the natural conditions in the southwestern Baltic Sea which is a brackish water habitat. (Wefer, 1976, Haynert et al., 2012). Observations from field and laboratory studies revealed that seawater salinity had no significant effect on change of diameter of *A. aomoriensis*. In the natural environment of Flensburg Fjord, salinity ranged from 16.8 to 26.3 in the bottom water during the seasonal cycle (Haynert et al., 2012). An earlier experiment revealed also no significant changes in test diameter to the closely related species *Ammonia tepida* at salinities ranging from 13 to 27 (Bradshaw, 1957). Studies of the recent distribution of *Ammonia beccarii* and related species, as for instance *A. aomoriensis*, designated them as euryhaline species, inhabiting hypo- to hyper saline waters (Wefer, 1976). Accordingly, the salinity levels between 16 and 17 applied in our experiment were within the tolerance range, where no effect on life cycle of *A. aomoriensis* has been observed. Therefore, we have just investigated the effect of elevated pCO<sub>2</sub> dur-

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ing the experimental time. We added a related statement in the revised manuscript: 2.1 Field sampling and preparation. Furthermore, we simulated pCO<sub>2</sub> levels in order to match the specific environmental conditions in the southwestern Baltic Sea. During the course of the year, pCO<sub>2</sub> levels >3000  $\mu\text{atm}$  can be observed (Haynert et al., 2012). In future, even higher levels will occur as present day CO<sub>2</sub> variability (Melzner et al., 2012). Therefore, the treatment levels are adjusted to the present variability in order to test future scenarios. As seawater and pore water, respectively, conditions differ significantly from atmospheric conditions, a lower pCO<sub>2</sub> treatment would have had only limited explanatory power. A statement has been added in materials and methods.

## REVIEWER 1:

Abundances of living foraminifera are highly variable. Even right after homogenization, population densities are different between culture vessels. The problem with this variability is that it makes it difficult to interpret (small) increases or decreases in density, since they may be the result of a 'natural' variability. In my opinion, this prevents interpretation of decreases in population density (Figure 3) as reproduction events. Densities at 907  $\mu\text{atm}$ , for example, are all within the variability observed at  $t=0$ , so that occurrence of reproduction is not justified by this figure. Additional 'proof' comes from the next figure (4) by occasional increase in small-sized specimens. However, this is a relative increase, and may well be caused by decreased abundances of large-sized specimens. The periods with no claimed reproduction events may well have seen reproduction, albeit at a much lower rate. If the authors could convincingly show that there has been reproduction at certain months, it should be expressed as increased reproduction rates to avoid the suggestion that at other months there was reproduction.

## REPLY:

We only determined parameters which are measured on test diameter and population density/abundance. Therefore, we can't convincingly show reproduction events. The different growth cohorts made it impossible to determine exactly either the possible

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reproduction intervals or the number of juveniles per parent. We revised the results and refer to changes in the measured test diameter and population density/abundance.

REVIEWER 1:

The background variability in densities is in my opinion also clear from the occasional decrease in densities of dead specimens, even at relatively low pCO<sub>2</sub>'s, where undersaturation cannot account for the decrease in densities. Since it is to be expected that abundances of dead specimens only increase, the decrease in densities is likely 'caused' by variability in densities that was present when the experiment started.

REPLY:

The reviewer is right that some of the observed variation might be due to the variances of the initial compositions. However, the initial densities were comparable between the treatments and did not lead to systematic deviation and the applied homogenization technique appeared to be an appropriate method to ensure similar initial densities. Therefore, the changing densities observed during the experimental period cannot result entirely from varying initial densities but need necessarily be related to environmental conditions. As this was the first laboratory study on a natural foraminiferal community, we cannot explain these fluctuations completely, but reproduction events seem to play an important role as catastrophic events and predation can be ruled out.

REVIEWER 1:

The EDS maps are not very informative. The obtained Mg-concentrations and distributions are at best qualitative, and any explanation based on Mg/Ca of these foraminifera may better be taken from the literature. Surprisingly, there authors do not refer to known Mg/Ca for Ammonia's (e.g. Toyofuku et al., 2011; Marine Micropaleontology, Diz et al., 2012; Biogeosciences) and only rely on their qualitative maps. These results and the associated discussion should be removed altogether from the manuscript.

REPLY:

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Due to the fact that results and the associated discussion of the EDS maps are not informative, we removed these figures from the manuscript.

REVIEWER 1:

The discussion lacks focus and does not take full advantage of the carefully recorded and provided parameters. Instead, it contains numerous sections (see below) that should be omitted since they are irrelevant here, or represent overinterpretation of the data. The discussion should therefore be restructured altogether.

REPLY:

We agree, the discussion has been restructured.

MINOR COMMENTS

REVIEWER 1:

Affiliations: Is there a difference between 1 and 4?

REPLY:

The GEOMAR is shared of the three places in Kiel, therefore the name of street varied between 1 and 4.

REVIEWER 1:

Abstract: Line 2: ', However,' should be ', however,'.

REPLY:

Correction has been made.

REVIEWER 1:

Introduction: Page 9525, line 8: What does 'negatively affected' mean?

REPLY:

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We changed 'negatively affected' to 'hampered calcification'.

REVIEWER 1:

Introduction: Page 9525, line 10-11: What does 'indistinct sensitivity' mean? Does it mean that growth rates or chamber addition rates were not impacted by elevated pCO<sub>2</sub>?

REPLY:

'indistinct sensitivity' mean that some of the cultured species showed no significant change of calcification under simulated future pCO<sub>2</sub>, whereas another species benefit from elevated pCO<sub>2</sub> and showed an increase of growth. This has been clarified.

REVIEWER 1:

Introduction: Page 9525, line 13: I guess 'dry weights' mean weights of the tests. Please change, since 'dry weights' often refer to organic matter from which the water has been extracted.

REPLY:

This has been changed.

REVIEWER 1:

Introduction: Page 9525, line 21-22: What exactly was not affected? Species composition? Standing stocks?

REPLY:

The composition and population density of the benthic foraminiferal community were not affected. The information has been added in the sentence.

REVIEWER 1:

Introduction: Page 9526, line 9: 'population' should be 'community' or 'population and

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community’.

REPLY:

This has been changed to ‘population and community’.

REVIEWER 1:

Methods: Page 9526, line 16: ‘is’ should be ‘consist of’.

REPLY:

Change has been made.

REVIEWER 1:

Methods: Page 9526, line 22: What does ‘graduated’ mean here?

REPLY:

‘graduated’ mean that we used a plastic ring marked with a cm-scale to slice the uppermost one centimeter of the sediment core. This has been clarified.

REVIEWER 1:

Methods: Page 9527, line 3: Remove ‘room temperature’.

REPLY:

Has been removed.

REVIEWER 1:

Methods: Page 9527, line 7: Replace ‘sucked off’ with ‘removed’.

REPLY:

We have added ‘removed’.

REVIEWER 1:

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Methods: Page 9527, line 7-12: It is not clear to me, whether the culture vessels were filled with homogenized sediment from one sampled core (and therefore likely introducing differences between culture vessels since the cores may have different foraminiferal community compositions/population densities) or that material from different cores were combined before homogenization.

REPLY:

After four weeks of acclimation, the sediment material in the different Kautex<sup>TM</sup> wide-neck containers was combined in one plastic container. After homogenization in the plastic container, new culture vessels were used. This information has been added.

REVIEWER 1:

Methods: Page 9527, line 10: Was the sediment not sieved over a 1 mm screen to remove macrofauna?

REPLY:

On board, the surface sediment was first passed through a 2000- $\mu$ m screen in order to remove molluscs shells and pebbles. The samples were gently washed with seawater from Kiel Fjord. This has been clarified.

REVIEWER 1:

Methods: Page 9529, lines 13-14: 'in dependence of' should be 'independent of'.

REPLY:

The measurement precision of silicate varied in dependence of the silicate concentrations from 2.5 to 6 %. In this context, 'dependence' is the correct word.

REVIEWER 1:

Methods: Page 9529, line 1-page 9530, line 16: Please include the frequency during the 6 months incubation at which samples for DIC, TA, etc were taken. Also for the

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pore waters!

REPLY:

The sampling frequency of the water chemistry parameters during the incubation time has been included in the section.

REVIEWER 1:

Methods: Page 9529, lines 26-27: Was there any sign of anoxia? Particularly with the decaying macrofauna, I would expect so. This would require sampling the pore waters also under anoxia in order to accurately determine phosphate concentrations. Or was PO<sub>4</sub><sup>2-</sup> only measured on the overlying water?

REPLY:

Our experimental set-up included no macrofauna. However, we observed the formation of an organic detritus layer at the sediment-water interface (mentioned in the discussion part, at the beginning of chapter 4.1 Sediment chemistry). On the bottom of the culture vessels, we observed a dark grey sediment layer. Therefore, we assume that O<sub>2</sub> consumption at the sediment-water interface exceeded the delivery of O<sub>2</sub> via diffusion, which could induce anaerobic conditions in the sediment pore water. This would be in line, with the observed elevated AT values in the pore water which may result from anaerobic AT generation. The assumption was mentioned in the discussion part at the end of chapter 4.1 Sediment chemistry. PO<sub>4</sub><sup>3-</sup> was only measured on the overlying water.

REVIEWER 1:

Methods: Page 9530, line 18: Were the contents of complete vessels processed in this way?

REPLY:

The complete content of benthic foraminiferal sediment samples were transferred from

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the culture vessels in 100 ml Kautex™ wide-neck containers. The clarification has been added.

REVIEWER 1:

Methods: Page 9530, line 28: Staining with rose (no capital 'r') Bengal does not allow accurate quantification of living vs. dead stocks. In these samples, I think staining with rB is likely to approximate the portion of truly living foraminifera. However, I would like to see that the authors at least mention the possibility that they have overestimated population densities (e.g. Bernhard et al., 2006; Paleoceanography).

REPLY:

Correction to 'rose Bengal' has been made. We agree, rose Bengal staining is not the optimal method to check if foraminifera still alive or presumably dead. It could be possible that the faunas are already dead, while stainable cytoplasm inside the tests could be conserved for a long time. However, due to the populations densities and the high amount of work, we decided to use this established and favorable method to discern between empty and living specimens. We added the information in the section and mentioned the possibility that we observed overestimated population densities. The reference of Bernhard et al., 2006 has been included.

REVIEWER 1:

Methods: Page 9532, line 21: From which treatment and at what time were the 100 specimens of *A. aomoriensis* taken to determine organic C content?

REPLY:

From the control pCO<sub>2</sub>-line of 430  $\mu$ atm, 100 living *A. aomoriensis* from size fraction 200 to 300  $\mu$ m were picked to calculate the total organic content at the beginning of the experiment (t=0). The information has been added in the section.

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Results: Page 9533, line 14: Change 'achieved' into 'returned to'

REPLY:

Word has been changed.

REVIEWER 1:

Results: Page 9533, line 15: Replace 'In dependency to' by something like 'As a consequence of the elevated pCO<sub>2</sub> treatments,'.

REPLY:

Replace has been made.

REVIEWER 1:

Results: Page 9534, line 13: E. exclavatum exclavatum and E. exclavatum clavatum are not different species, but two subspecies/ morphotypes. Therefore, the authors have found 4 instead of 5 foraminiferal species.

REPLY:

Corrections have been made.

REVIEWER 1:

Results: Page 9535, lines 1-29: The authors repeatedly refer to reproduction events while there is no direct evidence for this. The only parameters that are measured are test diameter and abundance. Therefore, the results should be confined to reports on changes in test diameter/abundance over time/at certain pCO<sub>2</sub>'s. Interpretation of these data in terms of growth cohorts and reproduction (which I doubt can be made) should be reserved for the Discussion.

REPLY:

We agree and revised the results chapter. Now, the results report the measured

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changes in population density/abundance and test diameter of living and dead *A. aomoriensis* during six month incubation time. Interpretations in terms of growth cohorts and reproduction have been added in the discussion.

REVIEWER 1:

Results: Page 9536, line 4: from which condition/ month do the densities range between 24 and 61 tests/cm<sup>3</sup>?

REPLY:

At the beginning of the experiment densities ranged from 24 to 61 tests 10cm<sup>-3</sup>. This information has been added.

REVIEWER 1:

Results: Page 9536, line 16: 'frequent' should be 'increased'.

REPLY:

Word has been changed.

REVIEWER 1:

Results: Page 9536, line 21: 'dry weight' should be 'CaCO<sub>3</sub> weight', or combined with the measured sizes, 'size-normalized weights'.

REPLY:

According to you next suggestion, section has been removed.

REVIEWER 1:

Results: Page 9537, lines 1-22: this whole section can be removed. I don't see the added value of dry weight vs. CaCO<sub>3</sub> weight if the difference between the two is constant and only a few % of the total weight.

REPLY:

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Section has been removed.

REVIEWER 1:

Results: Page 9537, line 23-page 9538, line 12: the lower CaCO<sub>3</sub> 'production' rates may well be the result of increased dissolution at two highest pCO<sub>2</sub>'s. This is likely because saturation states in these conditions are lower than, or close to 1. The term 'production' is therefore misleading and these observations may instead be rather trivial. Moreover, to avoid the suggestion that the 'production' rates are a direct consequence of the applied pCO<sub>2</sub>, it may be better to refer to the treatments by their  $\Delta\text{pCO}_2$ . This is related to one of my main comments, since the low salinity amplifies the effect on  $\Delta\text{pCO}_2$  caused by increased atmospheric CO<sub>2</sub> concentrations. Once more, the authors have investigated not just the effect of pCO<sub>2</sub>, but the combined effect of low salinity and elevated CO<sub>2</sub>, both reducing the saturation state with respect to calcite.

REPLY:

We agree that lowered saturation state is probably the most important parameter for foraminifera. However, the only factor manipulated in this experiment was seawater pCO<sub>2</sub> which influences all other carbonate system parameters. In general, salinity and seawater alkalinity values are lowered compared to other studies but were similar in all treatments. Therefore, the treatments were characterized by the applied pCO<sub>2</sub>.

REVIEWER 1:

Results: Page 9538, line 19: It is unlikely that there is only one layer of calcite (see e.g. Erez, 2003 or Sadekov et al., 2008) due to bilamellar calcification in Elphidium and Ammonia. It may be that the different layers have a similar Mg/Ca, and are therefore difficult to distinguish by EDS.

REPLY:

This section has been deleted.

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REVIEWER 1:

Results: Page 9538, line 20: What is 'high Ca and low Mg calcite'? I think calcite always contains much Ca.

REPLY:

According to your suggestion that the EDS maps are not very informative, results have been removed from the manuscript.

REVIEWER 1:

Results: Page 9538, lines 22-23: Is the smooth surface of *E. incertum* visible from figure 7? It is true that this species has smaller pores that are commonly present at relatively low densities compared to those of *Ammonia aomoriensis*, but that is already known and much better visible on SEM pictures. Why include a scan on *E. incertum* in the first place? By far most of the results and discussion deals with *A. aomoriensis* anyway.

REPLY:

See comment above: EDS map have been removed.

REVIEWER 1:

Discussion: Page 9539, lines 1-9: The second part of the paragraph is wrong and awkwardly formulated. There is also no need for repeating the outline of this experiment. This paragraph can therefore be removed.

REPLY:

Paragraph has been removed.

REVIEWER 1:

Discussion: Page 9539, lines 12-13: Should be mentioned in the Results.

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REPLY:

We mentioned in the results that a layer of organic detritus formed at the sediment-water interface.

REVIEWER 1:

Discussion: Page 9540, lines 4-6: Should have been mentioned in the Results.

REPLY:

Has been mentioned in section: 3.1 Carbonate chemistry, last paragraph.

REVIEWER 1:

Discussion: Page 9542, lines 1-3: This explanation is, at best, incomplete. If dominance of Ammonia and Elphidium are both explained by availability of diatoms, what can explain their dominance in intertidal vs. deeper stations?

REPLY:

Our explanation refers to the environmental conditions in the southwestern Baltic Sea, where phytoplankton-blooms play an important role for degradation processes at the sediment-water interface. Especially Ammonia, as well Elphidium, are opportunistic species and tolerate high nutrient, trace metal levels (Nikulina et al., 2008), and salinity variations (Polovodova and Schönfeld, 2008). Intertidal and deeper regions are also characterized by high food supply (algae biomass, nutrients and remineralization processes), which explain the dominance in that region. A relationship between diatom spring blooms and reproduction events was reported for *E. excavatum clavatum* at deeper waters off Boknis Eck, southwestern Baltic Sea (Schönfeld and Numberger, 2007).

REVIEWER 1:

Discussion: Page 9542, lines 6-13: I don't see the added value of explaining the occur-

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rence of a very rare species in the experimental set up. I also don't understand why it has to be brought into the experiment as propagules and not as an erratically occurring juvenile or adult.

REPLY:

The information is an additional explanation, because the species was recorded by Brodniewicz (1965) in the southern Baltic Sea the last time. Therefore, the appearance of this species was rare! We included your thought that an erratically occurring of juveniles or adults could explain the sporadic occurrence.

REVIEWER 1:

Discussion: Page 9543, lines 20-21: The authors have kept foraminifera in sediment (although not necessarily under natural conditions), but have not experimented with different types of sediment, organic matter content, etc. A general comparison to previous culturing studies (particularly those using *Ammonia*) that have not kept specimens in sediment would suffice here. The possibility that pore water chemistry changed (severely) during the experimental period should make the authors cautious in generalizing their conclusions (lines 10-12).

REPLY:

We agree that investigations on different sediment types which most probably differ significantly would be an interesting research topic. The pore water chemistry, however, seem to be similar over the entire experimental period as no signs of dissolution were visible for living foraminifera throughout the experiment which would have been the case if lowered saturation states were present (see Haynert et al. 2011). However, as we have not measured the carbonate chemistry more often, we reformulated our conclusion in the revised manuscript.

REVIEWER 1:

Discussion: Page 9543, line 22-page 9544, line 5: This paragraph can be deleted since

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it contains very little information.

REPLY:

Paragraph has been removed.

REVIEWER 1:

Discussion: Page 9544, line 6: 'Reveals' should be 'may be explained by (a combination of)'.

REPLY:

Change has been made.

REVIEWER 1:

Discussion: Page 9545, lines 6-23: Although interesting, this information bears little relevance to the here measured parameters. If the organic vs. calcite production would have been determined for each of the treatments, this paragraph would have been more relevant.

REPLY:

We agree, the determination of carbonate production and organic content for each treatment would present very interesting results. Nevertheless, this paragraph is relevant, also for the measured parameters in the current study.

REVIEWER 1:

Discussion: Page 9546, lines 19-24: Please note that these results are in line with some sedimentfree experiment showing no or very little impact of pCO<sub>2</sub> on foraminiferal growth and size-normalized weights (e.g. Keul et al., BGD).

REPLY:

In the revised version, we discuss the results of Keul et al. in the discussion.

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## REVIEWER 1:

Discussion: Page 9547, lines 8-9: Erez (2003) does not show that tests of Ammonia, nor Elphidium consists of a single layer (on contrary for Rotallids in general), characterized by a low Mg/Ca. Surprisingly, the authors do not refer to any papers that published Mg/Ca ratios of Ammonia. This genus, however, produces calcite with a low Mg/Ca (Dissard et al., 2010; Duenas-Bohorquez et al., 2011; Toyofuku et al., 2011; Diz et al., 2012). Elphidium produces calcite with a similar Mg/Ca, and the authors should avoid the suggestion that their EDS pictures (Figure 7) would provide a reliable alternative to published Mg/Ca for these genera. Solubility due to 1-2 mmol/mol higher or lower Mg/Ca is negligible, btw. Therefore, the sections on the EDS maps can be discarded from this manuscript.

## REPLY:

According to your suggestion this section has been removed from the manuscript.

## REVIEWER 2

### MAJOR SHORTCOMINGS

## REVIEWER 2:

Page 9557 – 9564, Table 2 and 3: Beside the ‘%’ indication, it is not clear what the presented numbers actually stand for? Labels/explanation in table title, legend or the like are completely missing. I assume these tables are meant to show abundance data of the individual species for the different months of the experimental period, as explained in 3.2.1, right? (the following is stated under this assumption)

## REPLY:

We agree, explanations for the presented numbers are completely missing, both in table and legend. We revised the Table 2 and 3 and their legends and added the missing information.

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## REVIEWER 2:

Page 9557 – 9564, Table 2: The abundance data is incorrect and non conclusive. Example: Page 9557 Table 2, row '430\_A': the table implies a presence of 68 individuals of *A. aomoriensis* per '15. cm<sup>-3</sup>' in June 'total number of living species'. Problems: These numbers are extremely low. What does '15.' mean? This abundance would imply 45 individuals instead of 449.0 per 10 cm<sup>-3</sup>!

## REPLY:

We checked the submitted manuscript with the published BGD version. We agree, the data of number of living specimens are wrong, as well as the sediment volume data (cm<sup>3</sup>). pCO<sub>2</sub>-treatment of '430\_A' should presented 680 living *A. aomoriensis*, which equates a population density of 449.0 ind. 10 cm<sup>-3</sup> and the sediment volume was 15.2 cm<sup>3</sup>. We assume a formatting error occurred during the translation from MS-Word-format to BGD-format, which had cut off the last number or decimal point. We overlooked the flaw during proofreading and are grateful indeed that you found the shortcoming. The revised version includes the correct dataset.

## REVIEWER 2:

Page 9557 – 9564, Table 2: Looking at July we see 64 individuals of *A. aomoriensis* and 33 of *E. incertum* = a total of living/calcareous individuals 97 (not 67). Additional problems: How can 0.3 % and 0.1 % of *Elphidium excavatum* exist, if there is not a single individual present? Severe rounding errors of the stated percentages.

## REPLY:

In July we collected 640 individuals of *A. aomoriensis* and 33 of *E. incertum*. In the correct dataset, we recorded 2 individuals of *E. excavatum excavatum* and 1 of *E. excavatum clavatum*, which represent the calculated percentage of 0.3 and 0.1 %. On this base, the calculated total number of calcareous individuals/living specimens equates 676. The revised version included the correct dataset (see above).

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## REVIEWER 2:

Page 9557 – 9564, Table 2: These inconsistencies exist through all abundance data of Table 2 and led me to believe in a ‘systematic/order of magnitude error’. After consulting primary literature of ‘Haynert 2013 – Dissertation’, available at [http://eldiss.uni-kiel.de/macaus/receive/dissertation\\_diss\\_00012460](http://eldiss.uni-kiel.de/macaus/receive/dissertation_diss_00012460). See page 145-152, the above suspicion turned true. Species and total abundance (not population density) data in this Table 2 are by an order of magnitude larger here! (I now assume all last digits are cut off in Table 2 → single digits vanished completely)

## REPLY:

We agree, the online version of the dataset in the dissertation of Haynert 2013 is correct. Through the translation from MS-Word-format to BGD-format, the last digits are cut off and single digits vanished completely. The revised version included the correct dataset.

## REVIEWER 2:

Page 9561 – 9564, Table 3: Unfortunately the comparison turned out additional complications: ‘dead foraminiferal species’: Starting from Page 9562 Treatment 907\_B to page 9563 Treatment 1865\_B: abundance data stated in ‘Dissertation Haynert 2013’ and the submitted manuscript are quite different. Abundance values changed in some instances by >45 fold! These differences cannot be explained by the above mentioned ‘digit mistake’, but must have a different source. Please elaborate in a resubmission on this point. (I expect this stems from a severe copy and paste mistake of the data strip between Table 2 ‘living’ and Table 3 ‘dead’ tests in ‘Haynert 2013 – Dissertation’?) Starting from page 9563 treatment 1865\_C till the end of the table: data is in both versions of Table 3 are in unison again. These errors/uncertainties are logically carried over to all presented data in Figure 3 and 4 and Table S2. The abundance data of Table 2, 3, Table S2, Figure 3, 4, however, form a major/large part (and a very important one) of the findings of this manuscript. Due to the large uncertainties rising from the above

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problems, a reasonable evaluation of the here presented data is therefore not possible.

REPLY:

We thank the reviewer for the information. Unfortunately, the dataset in the mentioned section from page 9562, pCO<sub>2</sub>-treatment of 907\_B to page 9563, pCO<sub>2</sub>-treatment of 1865\_B stated in 'Dissertation Haynert 2013' is wrong. Probably, it has been due to a copy and paste mistake between Table 2 and 3. However, the correct dataset of the abundance of dead tests is presented in the manuscript. Consequently, the presented data in Fig. 3 and 4 and Table S2 are correct. The important data which form a large part of the findings in this manuscript were carefully revised and is now acceptable for publication in BG.

REVIEWER 3

SPECIFIC COMMENTS

REVIEWER 3:

Material and methods: Page 9527, line 11: Are the culture vessels the same Kautex wide-neck containers like mentioned before?

REPLY:

The culture vessels are not the same Kautex wide-neck containers like mentioned before. After four weeks of acclimation, the sediment material in the different Kautex<sup>TM</sup> wide-neck containers was combined in one plastic container. After homogenization in the plastic container, new culture vessels were used. This information has been added.

REVIEWER 3:

Material and methods: Page 9529, line 1-3: I suggest adding the following: "... the sediment of three culture vessel replicates for each pCO<sub>2</sub>-line was analyzed...."

REPLY:

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We added the missing phrase “for each pCO<sub>2</sub>-line”.

REVIEWER 3:

Material and methods: Page 9530, line 15-16: In table 1, not all mentioned chemical parameters were shown. I miss the data for the extracted pore waters of selected culture vessels that are mentioned in the text.

REPLY:

We agree, in Table 1 are not all chemical parameters are mentioned. But the measured sediment pore water parameters for 4 pCO<sub>2</sub>-levels after six month incubation are presented in the supplemental Table S1. We revised the section and added two sentences for a better understanding, where the particular data sets are reported.

REVIEWER 3:

Material and methods: Page 9530 and the following pages: From this page on, species names lost their formation and are not italicized any more.

REPLY:

We do not observe the same problem.

REVIEWER 3:

Material and methods: Page 9532, line 21-23: These 100 individuals of a specific size (200-300  $\mu\text{m}$ ), where did they come from? From pCO<sub>2</sub>-line 430  $\mu\text{atm}$ , t=0?

REPLY:

The 100 individuals of *A. aomoriensis* were picked from the control pCO<sub>2</sub>-treatment of 430  $\mu\text{atm}$  at the beginning of the experiment (t=0). We included the missing information in the revised version.

REVIEWER 3:

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Material and methods: Page 9533, line 3: The order of tables mentioned in the text is confusing, Table 4 follows Table 1, Tables 2 and 3 are mentioned later. This order should be changed by renumbering the tables.

REPLY:

We order and renumbered tables in the manuscript and supplement.

REVIEWER 3:

Results: Page 9534, Fig. 2: I suggest cancelling figure 2 because it shows a species that is very rare in these sediments and I find no reason to show it here.

REPLY:

We deleted Fig. 2 in the manuscript and added the figure to the supplement (Fig. S1).

REVIEWER 3:

Results: Page 9535, line 21-23: It is not clear for me, how reproduction events can be recognized in the datasets and in Fig. 4. Is there a certain threshold of individuals  $<100 \mu\text{m}$  to define a reproduction event (15%?; 20%?, references?)? High numbers of individuals  $<100 \mu\text{m}$  can also be found in lines 430  $\mu\text{atm}$  and 907  $\mu\text{atm}$  at  $t=0$ .

REPLY:

During the experimental time, the size distribution revealed growth cohorts which were characterized by a fixed, simultaneously reproduction period. We found no references to define a reproduction event of foraminifera, but we define a clearly reproduction with a proportion of more than 9 % from the sum of juveniles from size class  $<50 \mu\text{m}$  and  $50-100 \mu\text{m}$  in the current study. We included the statement in the text. The initial number of individuals  $<100 \mu\text{m}$  might result from earlier reproduction event as well. However, as these samples represent the initial distribution we were not able to characterize this event.

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REVIEWER 3:

Results: Page 9536, line 23-25: The order of figures mentioned in the text is confusing, figure 6 follows figure 4, and figure 5 is mentioned later. This order should be changed by renumbering the figures.

REPLY:

We order and renumbered figures mentioned in the text.

REVIEWER 3:

Results: Page 9537, line 24: The statement “total organic content was found to be 4.3%” should be worded more carefully here. This is an average value measured from 100 individuals of a specific size (200-300  $\mu\text{m}$ ). Juveniles or bigger individuals may have different TOC contents. This value can be used for estimations, but maybe include possible over- or underestimations.

REPLY:

We included the missing information into a carefully worded statement.

REVIEWER 3:

Results: Page 9538, line 14-16: A figure with pictures from the mentioned destroyed tests may be interesting here.

REPLY:

We added a picture from the mentioned destroyed tests in Fig. 2, B at 3247  $\mu\text{atm}$ . The destroyed tests were just providing additional information, because it is common for an empty test to dissolve at seawater undersaturation of  $\Delta\text{pCO}_2$ .

REVIEWER 3:

Results: Page 9538, line 19-20: “: : a single calcium carbonate layer..” Is this in accordance with the bilamellar character of many foraminiferal species?

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REPLY:

According to the suggestion of Reviewer 1 that the EMP maps are not informative, we removed the results from the manuscript. However, the bilamellar test of foraminifera consists of primary and secondary calcite. The primary calcite consists of high Mg-calcite, whereas the secondary calcite consists of low Mg-calcite. High Mg-calcite is the most soluble form, therefore both calcite types are likely to be affected at such extremely low omega.

REVIEWER 3:

Discussion: Page 9543, line 10-21: Are there any reports of ocean acidification experiments with other organisms tested in natural sediments that can be compared with the foraminiferal results?

REPLY:

We are not aware of any ocean acidification studies which have used natural sediment in laboratory experiment. Therefore, a comparison is not possible to date. We included the information at the beginning of the section. However, the study of Kitidis et al. (2011) found that benthic ammonia oxidation remained unaffected by ocean acidification, in sharp contrast to water column NH<sub>3</sub> oxidation. They suggested also that buffering in sediments through AT-generation may explain the absence of response to ocean acidification. We added the results of their study, which are in agreement with our findings to the revised manuscript.

REVIEWER 3:

Table 2 and 3: What about gaps in the tables, e.g. 430\_C, Sep?

REPLY:

The gaps in the table are due to the fact that some samples were lost during treatment. According to the suggestion of Reviewer 4, we summarize the results of 3 replicates.

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We hope that the tables are clearer and comprehensible now.

REVIEWER 3:

Table 4: I cannot see any bold results?

REPLY:

In our version the significant results are presented in bold letters.

REVIEWER 3:

Figure 5: Legend: “: : : living specimens including: : :”

REPLY:

We included the missing blank between specimens and including.

REVIEWER 4

SPECIFIC COMMENTS

REVIEWER 4:

A.) The saturation state in the pore water was much higher and differed significantly from the “manipulated” values in the water column by pCO<sub>2</sub> aeration. Pore water pH was not monitored continuously during the study, and only measured at the end. So we do not know if changes due to microbial activity (natural, feeding) on pore water pH occurred. In my opinion the authors cannot conclude that the sediment chemistry did create a microhabitat which supported growth independently from highly elevated pCO<sub>2</sub> conditions over the course of the study. Another replicate just for sampling continuously pore water would have been needed to justify that manipulated pCO<sub>2</sub> aeration did receive the aimed results at the sediment water interface over the entire period of manipulation.

REPLY:

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In our previous studies, foraminifera isolated from the sediment and exposed to elevated pCO<sub>2</sub> dissolved within a two months experimental period (Haynert et al., 2011). The experimental conditions (applied pCO<sub>2</sub> levels, salinity, temperature, food supply) were almost identical to the conditions of the present study, with the exception that the specimens were kept in natural sediment. In contrast to the previous studies, no dissolution and elevated mortality was observed in the present study. Therefore the sediment provides a sheltered microhabitat which enables successful development of a foraminiferal community and prevents test dissolution and mortality. Our measurements on carbonate chemistry were performed to understand why the results of the present study differed from the previous studies and revealed completely different conditions, such as saturation state, in the water column and the sediment pore water. As the reviewer stated in his comment, we are not certain of the development of this microhabitat but obviously it provided adequate conditions throughout the experimental period as we did not observe any dissolution of living foraminifera at any phase of the incubation.

#### REVIEWER 4:

B.) During the experiment the cultures have been additionally fed weekly with living microalgae which influences pH due to respiration and production especially on a daily cycle. So diurnal variations might have been large potentially because of accumulated algae in the cultures and no cleaning. Microalgae might have even been fertilized in the elevated pCO<sub>2</sub> treatments enhancing photosynthetic production.

#### REPLY:

Diurnal variations are quite common in the eutrophicated Baltic Sea, therefore benthic foraminifera are used to it. However, as the applied light levels were low, we do not expect that the algae had high photosynthetic rates and thereby the effects on the carbonate chemistry are expected to be low. As our measurement revealed, the applied pCO<sub>2</sub> level was clearly distinguishable therefore food supply did not significantly disturb our experimental conditions.

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REVIEWER 4:

C.) Salinity values are very low at 15-16 and make comparison to other studies difficult. No explanation is provided why those have been chosen.

REPLY:

Similar to Reviewer 1: the relatively low salinity values from 15 to 16 in the experiment correspond to the natural conditions in the southwestern Baltic Sea. In comparison to the open oceans, this coastal region is characterized by low salinity (Wefer, 1976, Haynert et al., 2012). A similar salinity value was applied in the present study. We added a related statement in the revised manuscript: 2.1 Field sampling and preparation.

SPECIFIC COMMENTS IN ORDER

REVIEWER 4:

Abstract: Page 9524, Line 12: Please add that there is no significant effect between the different pCO<sub>2</sub> treatments and add according statistics in Table 4.

REPLY:

We added the information in the abstract and revised the statistical test (see the following comments).

REVIEWER 4:

Abstract: Page 9524, Line 22: Please indicate in which treatment and approximately how many specimen did show dissolution features.

REPLY:

We noted that approximately 30 % of the empty tests of *Ammonia aomoriensis* showed dissolution at a high pCO<sub>2</sub> of 3247  $\mu$ atm during the last two month of incubation. According to Reviewer 3, we added a picture from the destroyed tests mentioned in Fig.

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2, B at 3247  $\mu\text{atm}$ .

REVIEWER 4:

Abstract: Page 9524, Line 25: Please indicate how large the species specific effect is and give % to compare between species, high Mg-Calcite is the most soluble form but both calcite types are likely to be affected at such extremely low omega reported here.

REPLY:

According to the suggestion of Reviewer 1 that the results of the EMP maps are not informative, we removed the results from the manuscript. Thereby, we abstained to indicate, how large the species specific effect in percentage is to compare between low and high Mg-calcite species. As stated above, the relatively high carbonate saturation in the sediment pore water caused only minor dissolution.

REVIEWER 4:

Introduction: Page 9525, Line 9-11: The introduction is relatively short, it could benefit by shortly reviewing the content of studies listed here and introducing work from Japanese co-workers on pCO<sub>2</sub> and foraminifera which are cited later in the discussions part.

REPLY:

The introduction is relatively short, but comprised all relevant information for the study. In our opinion, the introduction presents an overview about previous ocean acidification experiments. Due to the fact that all these studies used isolated cultured living benthic foraminifera without any natural sediment it is difficult to compare these studies with our approach. Therefore, we are reviewing these studies in more detail in the discussion part. According to the reference suggestion of Kitidis et al. (2011), who performed a similar experimental design, we included their results in more detail in the introduction and discussion part.

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#### REVIEWER 4:

Material and methods: Page 9533, Line 1-3: Statistics part is not well presented, I doubt that raw data was sufficient to meet the assumptions of ANOVA (normality, equal group variances)? Because density data (living and dead specimen) are given in percentages those data usually needs to be transformed, it is inconsistent that Table 4 (Page 9565) does not show all raw data collected but it is stated here that statistics were performed on all raw data. The results of the statistics in Table 4 should be referred to in the results section and then the numbering of the Tables adapted. Statistics on test diameter and population densities are difficult because you report various reproduction events in the cultures. Did you try to see if results are different if you exclude cultures where reproduction had occurred? Within one to two month offspring can reach sizes close to adults.

#### REPLY:

We thank the reviewer or her/his comment. The statistic has been changed to Kruskal-Wallis test. Due to the strong changes of test diameter by the reproduction events we only tested the number of living and dead foraminifera in order to test or pCO<sub>2</sub> effects on this parameter. We refer to production events while there is no direct evidence for this. According to the comment of Reviewer 1 and 3, we refer our results in the revised manuscript to changes in populations density/abundance and test diameter of living and dead *A. aomoriensis* during six month incubation time. The different growth cohorts made it impossible to determine exactly the possible reproduction intervals or the number of juveniles per parent. More detailed informations are given in the discussion part 4.3 Response of *A. aomoriensis* life cycle. We revised the statistics part and Table 4, now it should be better comprehensible. All data of the 3 replicates are stated in the tables of the manuscript or in the supplements.

#### REVIEWER 4:

Results: Page 9533-9538; Results section lacks clarity and does not address the data

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presented in the results well enough, I suggest to re-structure and to start on carbonate chemistry, and then statistical results followed by percent changes and general observations in the measured parameters.

REPLY:

A re-structuring of the results has been made in the revised version.

REVIEWER 4:

Conclusions: Page 9547, Line 24: The statistical tests have only been applied on one parameter (test diameter). The conclusion cannot be made that "Growth, reproduction and mortality of *A. aomoriensis* were unaffected by elevated pCO<sub>2</sub> from the presented dataset.

REPLY:

We revised the statistical test (see comment above). We conclude that survival of *A. aomoriensis* was unaffected by elevated pCO<sub>2</sub>." in the revised manuscript.

REVIEWER 4:

Table 1: Page 9556: Can be improved by summarizing incubation time, give incubation time as 0-2 month, 3-4 month and 5-6 month so the results are presented in a clearer way.

REPLY:

We do not agree with the reviewer. The carbonate chemistry measurements presented the trend of each parameter in a clear way. The trends of phosphate (PO<sub>4</sub><sup>3-</sup>) and silicate (Si) play an important role in the discussion chapter. Therefore it is essential to present the data for each month during the experimental time.

REVIEWER 4:

Table 2 and 3: Page 9557-9564: Please give mean % values of data by pCO<sub>2</sub> treatment

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and write that the Table summarizes results of 3 replicates, this will make the Table clearer and your results will stand out better.

REPLY:

Table 2 and 3 were revised. We summarized the results of 3 replicates, now the tables should be clearer.

REVIEWER 4:

Figure 2: Page 9567: Does not related to the hypothesis and shows a rare species in the cultures, it should be omitted or put in the supplement.

REPLY:

We deleted Fig. 2 in the manuscript and added the figure in the supplement (Fig. S1).

INTERACTIVE COMMENT BY VASILIS KITIDIS

COMMENT BY KITIDIS:

This is an interesting paper. We also found that benthic ammonia oxidation remained unaffected by ocean acidification, in sharp contrast to water column NH<sub>3</sub> oxidation. In our paper we also suggested that buffering in sediments through TA generation may explain the absence of response to OA (Kitidis, et al., 2011. Impact of ocean acidification on benthic and water column ammonia oxidation. Geophysical Research Letters 38, doi: 10.1029/2011GL049095).

REPLY:

Thank you for letting us know your reference. Your study provided very interesting results! We added your reference to the revised manuscript.

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Interactive comment on Biogeosciences Discuss., 10, 9523, 2013.

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