

#### REPLY TO REVIEWER'S COMMENTS:

We thank anonymous reviewers, as well as J.P Gattuso and S.Uthicke for taking the time to comment on our manuscript. Their reviews greatly helped to improve the manuscript. We addressed the comments and questions and will gladly submit a revised version of our manuscript.

Below, we have listed our replies to all comments.

#### Reviewer 1

##### GENERAL COMMENTS

**1-1) The manuscript by Keul and others presents an interesting approach to OA research on an important group of calcifying organisms. The authors need to be reminded, however, that results on one species (an undescribed / unnamed one at that!) can not be extended to the entire group, as they currently assert in the abstract and elsewhere.**

##### ANSWER:

We agree with the reviewer that caution should be taken when generalizing our results. For this reason, we have included a literature review compiling all known responses of foraminifera to OA. Not only does this put our results in perspective, but also allows for a preliminary assessment of the relation between taxonomy and biomineralization. We have also adjusted the text to avoid the suggestion that our results are representative for foraminifera *as such*.

The genus *Ammonia* forms a genetic and morphological continuum, and assigning species names within this genus is therefore prone to discussion. Previous field studies (e.g. Hayward et al., 2004) have identified *Ammonias* in the intertidal Wadden Sea as 'molecular type T6' and these foraminifera have been named *tepida* by a number of previous studies (e.g. De Nooijer et al., 2007; Munsel et al., Dissard et al., Dueñas-Bohórquez et al., Raitzsch et al.), but more recently it has been suggested that it might be more accurately described as *aomoriensis* (refs). Until consensus has been reached over this species nomenclature, we will refer to it as sp. We have changed the text accordingly:

We have conducted carbonate chemistry perturbation experiments with the benthic foraminifer "Ammonia molecular type T6", further referred to as *Ammonia sp.* (Hayward et al. 2004; please note that this species is also referred to as *Ammonia tepida* in other studies, e.g. Nooijer et al. 2007, Dissard et al. 2010, Duenas-Bohorquez et al. 2011, Raitzsch et al. 2010).

**1-2) The title does not reflect the content of the manuscript, which includes a lengthy review of earlier publications. If, as stated on page 1158 lines 7-9, "A thorough comparison of these studies is beyond the scope of this paper and should be addressed in a proper review paper" is true, then what is the point of reviewing all prior publications on foraminifera and OA, and including a multi-page table? Personally, I think a review paper and a new scientific contribution should be separate.**

ANSWER: We have discussed our data in perspective to the existing literature. Since the compiled literature is derived from 2 main fields using different approaches and species (i.e. **paleo-oceanography**, studying mostly planktonic species from downcore and coretop samples and **OA research**, focussing largely on cultured, benthic foraminifera), we have organized the available data into one table. This overview helps placing our results in a broader context.

**1-3) The statement on pg 1154 (line 8-10) that pCO<sub>2</sub> in the two manipulation methods were "slightly different" is a major understatement. In reality, Table 1 shows the lowest paired treatments were 61 and 217 uatm, which differ by more than a factor of three. That exceeds a "slight" difference. Plus, the 61 value differs from nominal values by a very large amount. The next higher values (479 and 396) both differed from the nominal target by quite a bit also.**

ANSWER: We agree with the reviewer insofar that the focus on co<sub>2</sub> is misleading. In fact co<sub>2</sub> is only one of the parameters of the carbonate system and for our purpose it is not necessary to obtain exactly matching values for any parameter. The reason for this is that our interpretation is based on trends over a range of values. The latter implies that the ranges of selected parameters should be similar in the two manipulation methods (which they are). The selected parameters here include CO<sub>2</sub>, CO<sub>3</sub><sup>2-</sup>, pH, and Omega. The setup also necessarily contains parameters whose ranges cannot match in the two manipulation methods, such as DIC and TA. This fact, however, does not invalidate this experimental approach (e.g. Keul et al. 2013, G3 and Langer and Bode, 2011, G3).

We have altered the Material and Method section of the manuscript accordingly to make this more clear and avoid the suggestion that dissolved CO<sub>2</sub> values were always very similar between replicates (see Results, 3.1. Carbonate Chemistry, second sentence, p1154, l. 8):

"Due to in- and outgassing, the respective pCO<sub>2</sub> of the culturing media were not exactly matching, in some treatments more than in others (esp. lowest pCO<sub>2</sub> values). However, in order to analyze the effect of individual c-chemistry parameters on foraminiferal calcification, obtaining similar ranges of selected parameters is crucial, which is given in our setup."

**1-4) Page 1158 states that "In order to address the effect OA might have on foraminifera it is crucial to develop a process understanding of the observed responses." This statement is awkward and requires clarification. Further, it is unclear why it is supposedly imperative to distinguish between the two processes. The authors are urged to better justify the study. Just because the two parameters can be varied independently (more or less), why do it?**

We agree and have changed the text, starting p 1158, line 11 until the end of the paragraph.

"Comparing these studies on a methodological level is beyond the scope of this paper and might better be done in a separate study. The variety in responses shows, however, that there is no uniform response of foraminifera to OA. This makes it difficult to predict the overall response of foraminifera to ongoing OA and the subsequent impacts on biogeochemical cycling and ecology. To facilitate estimations on future foraminiferal functioning despite inter-species variability in calcification responses, an understanding of the physiological basis of calcification (and variability between species) is necessary (see also Rost et al. 2008). At the same time, such a biomineralization model may also explain the apparent variability in calcification responses observed so far (Table 2). In addition, a process-based characterization of foraminiferal calcification will also help to understand the impact of different parameters of the inorganic carbon-system on element incorporation and stable isotope fractionation, thereby improving their paleoceanographic potential. On long timescales, changes in the individual parameters of the C-system (e.g. alkalinity and pH) may not have been tightly coupled. Together, these two objectives make it necessary to study impacts of the different C-system components individually. Therefore, we cultured specimens of the benthic foraminifer *Ammonia* sp. under two carbonate chemistry manipulations, allowing to quantify impacts of carbonate ion concentration as well as pH."

**1-5) It is quite surprising that the authors conclude (page 1163 line 20-21) that an increase in SNW and growth rate was caused by increasing CO<sub>3</sub><sup>2-</sup> especially given the paragraph labeled 3.2, where the authors state that "In general, we observed a high variability in final shell length and weight among specimens. Consequently linear regression of growth characteristic (final length and weight, and factors such as SNW and growth rates derived thereof) versus carbonate chemistry parameters yields low R<sup>2</sup> values in general." These are contradictory statements that require clarification.**

ANSWER: We fail to see why a relatively low  $r^2$  should be contradictory. We have, however, rephrased these sentences slightly to clarify that the relatively large variability is a natural feature among *Ammonia* sp. offspring, thus observed trends are still discernible.

#### SPECIFIC COMMENTS

**1-6) When relevant, the carbonate saturation state type should be noted. For example page 1149 lines 1-3 could be more specific (aragonite vs. calcite saturation states).**

changed

**1-7) Lines 24-26 page 1149 must specify that the observations are for one species only and may not apply to other foraminiferal species.**

changed

**1-8) Given cytoplasm of other foraminiferal species are also orange colored, how do the authors know all their specimens were this one particular species of *Ammonia*?**

We used the coloration of the cytoplasm to distinguish living from dead individuals, which we have made more clear in the text. At our sampling location, *Ammonia aomoriensis*, as defined by Hayward et al. 2004, is the only *Ammonia* species present (based on distinctive morphological features). Moreover, we have used asexually reproduced clones of a few adult *Ammonias*, allowing us to conclude that all our specimens used are from the same species.

**1-9) What do the authors intend by reporting length for a species that is round? Do the authors intend diameter?**

We replaced length by diameter throughout the text.

**1-10) Were tests weighed individually (line 21 page 1152)?**

Tests were weighed individually, which we have included in the respective passage.

**1-11) The rationale for “The factor 100 was added to enhance readability of SNW and growth rates” is unclear (line 18 page 1153).**

We have multiplied SNW and growth rates by the factor 100 in Figs 2 and 3 as to enhance readability of the figures (as to avoid an y-axis labeling with too many digits). We now mention this into the caption of the respective Figures, which should make the purpose more clear.

**1-12) It is not clear where the data are for section 3.2. Is the data discussed in section 3.2.2 shown in Figure 3?**

Table 1 contains this data, we have included references to this table in the respective section.

**1-13) Most readers would expect that discussion about Figure 2 would appear in the text before discussion on Figure 3. This is not the case (pages 1155-1156).**

We have changed the order of the figures accordingly.

**1-14) The discussion on page 1157 is a peculiar mix of data and literature review.**

We do not intent this section as a thorough and in-depth literature review, but this aids putting our results in perspective.

**1-15) Where is the data showing that Ca<sup>2+</sup> is constant in all treatments (page 1161 line 5)?**

We have added this to Table 1

**1-16) Figure 1 is misleading because only nominal pCO<sub>2</sub> is listed, not the actual values, which vary wildly from the nominal values (Table 1).**

We have discussed the mismatch in some of the target and actual pCO<sub>2</sub> values above and changed the manuscript accordingly. We depict the nominal values of pCO<sub>2</sub> in the Figure, since this is what the gas mixing system supplies and ergo also is present in the air in the boxes, which is depicted in the figure. We have changed the caption of the figure, as not to mislead the reader, which was not our intention. We also want to highlight that we have plotted the "actual" pCO<sub>2</sub> values in Figure 3, not the nominal ones.

**1-17) Regarding Figure 3, what happens if growth rate was assessed without dividing the populations into size classes? Is it valid to break this dataset into size classes? The divisions are completely arbitrary.**

Foraminiferal growth is not linear at all stages of its lifecycle. While growth in juveniles is exponential, it slows down as the individual gets older (expressed as chambers added per unit time). Thus a direct comparison of the growth rates of individuals is only possible, when all individuals display similar growth rates. One would expect that these larger individuals are characterized by smaller growth rates. We have updated Figure 3, to make this point more clear. Growth rates increase linearly with diameter. If larger individuals displayed slower growth the plot below would not be linear any more. Since this is not the case, it is safe to argue, that while growth rates vary, the growth rates between the individuals are comparable since larger individuals are characterized by larger growth rates, not smaller ones. We therefore exchange the respective figure with the following, clarifying our original point.

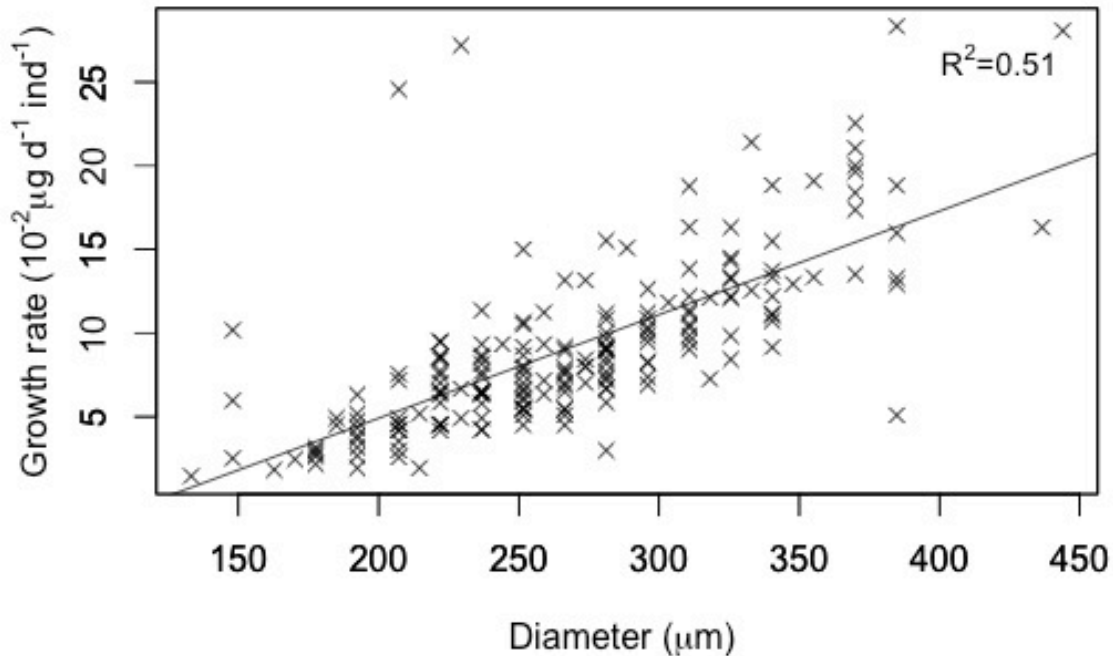


Figure: Growth rates ( $10^{-2} \mu\text{g d}^{-1} \text{ind}^{-1}$ ) of all treatments versus diameter ( $\mu\text{m}$ ).

#### TECHNICAL CORRECTIONS

Line 2 page 1148 is poor grammar (and makes little sense). It would be better to write “. . .by the oceans; such uptake causes surface ocean pH. . .”

changed

Line 6 page 1148 should read “compiled a state-of-the-art review of OA effects. . .”

changed into: "We have compiled the state of the art literature on OA effects on foraminifera,..."

Line 9 page 1148 should read “process-based” (same for line 14 same page)

changed

Line 11 of page 1148 should read “carbonate ions were varied ” (not “where”).

changed the whole sentence

The sentence spanning lines 12-13 on page 1148 is awkward nonsense (“the parameter of the parameter”).

changed

The sentence spanning lines 15-17 on page 1148 must be divided into at least two sentences, to read “We argue that [CO<sub>2</sub>] is the parameter affecting foraminiferal size-normalized test (shell) weights (SNW) and growth rates. Based on the presented data, we can confirm the strong potential of Ammonia sp. SNW as a . . .” You can NOT assert that this Ammonia species will respond like all other foraminifera!

changed

Line 20 page 1148: remove “the” to read “. . .start of industrialization. . .”

changed

Line 25 page 1148L “. . .by the end of this century. This decline is. . .”

changed

Line 1 page 1149 should read “saturation state, biogenic. . .”

changed

**Line 2 page 1149 should read “and foraminifera is expected. . .”**

changed

**Line 3 page 1149 should read “generally expected that coral. . .”**

changed

**Line 17 page 1149 should read “system that causes the effects.”**

changed

**Line 19 page 1150 notes 8 treatments, yet only 2 are listed in the following bulleted list.**

**Clarify.**

changed, to:

1.)

TA-manipulation: seawater with a range of pH's and [CO<sub>2</sub>- 3 ] (4 in total) while total inorganic carbon concentration remained constant.

**Line 21 page 1150: please define “TA”.**

defined, DIC as well.

**What is a “pHstable-manipulation”? First, grammatically, it should be “pH-stable manipulation”. Second, that phrase is an oxymoron (stable and manipulation).**

We have changed it to pH-stable-manipulation. We are aware that the name is not entirely correct, but we have chosen it as to underline, that this is the treatment, where C-chemistry was manipulated in a way, that pH remained stable, since this is the main characteristic of this treatment.

**The term “setup” is colloquial and should be avoided (appears at least twice on pg 1151).**

changed to experimental setup

**Dunaniella does not exist. Perhaps the authors intend Dunaliella.**

changed

**Line 3 page 1152 should read “Dickson’s CRMS”**

changed

**Line 4 page 1152 should read “water, Marine Physical Laboratory,. . .”**

changed

**Line 13 page 1152 needs an author name (“(1987)”).**

changed

**Line 15 page 1152: spell out concentrated.**

changed

**Line 14 page 1153 should refer to “wall thickness” not chamber thickness.**

changed

**When referring to the statistical tests employed, it is “post-hoc tests” not “post hoc tests”.**

changed

**What is meant by “means exactly differed” (line 26 page 1153)?**

changed to "means differed from each other"

**Is “quasi-constant” a word (page 1154 line 16)?**

changed to quasi constant

**Page 1157 line 1 should read “have been documented that range from. . .”**

changed

**Species specific effects should read “species-specific effects” (multiple places).**

changed throughout the document

**Define “normal” on page 1158 line 2.**

changed to "present day"

**Page 1158 line 27: please explain to which “the latter” refers.**

changed to "This unit might be applied...."

**Page 1159 line 8 should read “. . .of growth rates is . . . lower than. . .” (omit “with”).**

changed

**Species names need to be italicized in the references.**

changed

**Table 2 should be chronological and updated to include recent publications.**

Changed

#### **Reviewer 2:**

**2-1) This is an interesting manuscript aimed for investigating the effect of carbonate chemistry parameter on growth of the benthic foraminifera. In my opinion, the discussion par t 4.1 and table 2 should be in a separate paper.**

see answer to 1-14)

**2-2) The authors should discuss why the effect of carbonate ions might be modified by the presence or absence of the symbionts.**

We have added the following to the discussion(4.1.):

"Another factor that could result in different calcification responses under changing C-chemistry in different species, is the presence or absence of symbionts. By photosynthesis and respiration symbionts may alter the inorganic C-system in the direct surrounding of the foraminifer and thus modify ambient values for pH, TA, etc. (e.g. Rink et al.1998). "

**2-3)It should be noted in the discussion and conclusion that this proxy would apply to this species tested.**

changed

**2-4) The corrections of some typos and grammatical errors are needed.**

We have used the comments of reviewer #1 for this and have carefully checked our manuscript for grammatical errors and typos.

**2-5) The authors should note the number of samples used in each treatment or experiment.**

We have added sample number (n) to table 1

please find an updated version of Table 1 below, now including n and [Ca<sup>2+</sup>]

	Treatments							
	A1	A2	A3	A4	B1	B2	B3	B4
<i>seawater parameters</i>								
pCO <sub>2</sub> (µatm)	180	380	950	1400	180	380	950	1400
"nominal"								
pCO <sub>2</sub> (µatm)	217	479	850	1301	63	396	829	1252
CO <sub>3</sub> <sup>2-</sup> (µmol/kgSW)	401	224	136	88	21	152	405	563
HCO <sub>3</sub> <sup>-</sup> (µmol/kgSW)	1798	1999	2073	2063	223	1499	3536	5131
DIC (µmol/kgSW)	2205	2236	2232	2187	246	1662	3965	5729
TA (µmol/kgSW)	2747	2535	2400	2277	342	1884	4436	6343
pH total scale	8.32	8.02	7.79	7.60	7.95	7.98	8.03	8.01
Ω <sub>c</sub>	9.8	5.5	3.3	2.2	0.5	3.7	9.9	13.8
[Ca <sup>2+</sup> ] (mol/kgSW)	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.008
<i>physiological parameters</i>								
n (number of specimens)	15	14	37	39	36	21	19	24
final weight (µg)	7.3	6.1	5.1	6.3	3.4	5.3	7.3	5.3
2 SE	2.1	1.4	0.9	1.0	0.5	1.6	1.4	1.5
final diameter (µm)	288	255	252	280	247	288	280	229
2 SE	27	29	19	18	26	32	31	28
growth rate (*10 <sup>-2</sup> ) (µg/d/ind)	11.20	7.28	7.55	9.71	5.99	9.33	12.75	9.01
2 SE	3.17	2.34	1.17	1.43	0.89	2.72	2.53	2.56
SNW (*10 <sup>-2</sup> ) (µg/µm)	2.41	2.19	1.89	2.14	1.33	1.70	2.48	2.29
2 SE	0.48	0.37	0.23	0.24	0.14	0.33	0.38	0.67

**2-6) Page 1148, Line 11 should be "while pH and carbonate ions were varied in one, . . .".**

changed

**2-7)Page 1148, Line 13 should be "This allows the identification of the parameter of the carbonate system. . .".**

changed

**2-8)Page 1150, Line 23 should be "pH-stable manipulation".**

changed

**2-9) Page 1151, Line 18 should be "Dunaliella salina".**

changed

**2-10) Page 1152, Line 1 should be "repeated measurement".**

changed

**2-11) Page 1153, Line 26 should be "post-hoc tests".**

changed

**2-12) It would be clearer if the author use Ω<sub>c</sub> for the calcite saturation state.**

changed

**2-13) The authors should show the R2 value for the regression data.**

We have added the R2 values in the paper

**2-13b) Page 1155, Line 20: The authors should clarify this sentence “The linear regression model reveals that the carbonate ion concentration can explain the highest amount of variability (29%) in average shell weights and pCO<sub>2</sub> the lowest (6%)”. If it is only 29%, why the authors concluded that the carbonate ion concentration can be used as a proxy for the SNW.**

As explained above and in the manuscript, the variability in SNW's in *Ammonia* appear to a large degree to be "natural", in the sense that we observe regularly that offspring of *Ammonia* has a range of growth rates, despite similar environmental conditions. At the end of the experiments when foraminifera are harvested, individuals with a higher growth rate will naturally be larger and heavier than those with a lower growth rate. This reduces the certainty to quantify the effect of experimental treatments, since a huge part of the variability is "natural". Therefore, 29% compared to the high natural variability is still relatively large and much larger than that for other factors, such as pCO<sub>2</sub>. This means that carbonate ion concentration, despite the natural variability, significantly impacts SNW/ shell weights in our species studied.

**2-14) From page 1161, Line 5; the author should include the Ca<sup>2+</sup> concentration in the table 1.**

changed

**2-15) Page 1162, Line 2; Typo: “the inorganic carbon”.**

changed

**2-16) Figure 1 legend should be in more detail.**

changed

**2-17) Figure 2: x-axis of DIC, TA and bicarbonate should be in the same scale and inter val.**

changed

**2-18) Figure 3: “sizeclass” should be “size class”, “Growthrates” should be “Growth rates”, and “post hoc test” should be “post-hoc test”.**

Figure 3 (now Fig. 2) depicts now a new plot, which we hope is more clear (see comment of Reviewer 1)

#### **J.P. Gattuso (short comment):**

**In the abstract and text body, it is mentioned that foraminifera are "estimated to precipitate ca. 50% of biogenic calcium carbonate in the open oceans." I think that this is incorrect as Schiebel (2002) mentions different numbers and for calcite alone: "23–56% of the total global-marine “steady” calcite flux at 100 m depth".**

changed to correct numbers:

....precipitate 23-56% of the biogenic calcite flux in the open oceans

- p1552114: author name missing.

included now

**- I would suggest to avoid the use of acronyms, for example SNW, especially in section headings.**

We have changed this in the case of SNW in the headings, however, we could not realize this in the text, as the acronyms used by us occur relatively often and are commonly used in the field

**- R is not a statistics software but a software environment (see web site). R can be cited as follows: R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.**

changed

**- I suggest to reorder table 1 chronologically.**

changed

**- You should consider avoiding the use of grey tones in the figures because it is hard to see. Why don't you use color, which does not entail extra cost?**

Changed to colours, thanks for the tip

#### **Sven Uthicke (short comment)**

**The paper presented represents an interesting review of the literature and interesting new data. However, addressing the following comments could improve the present version**

**1) A couple of papers recently published need to be incorporated: a) Glas MS, Fabricius KE, de Beer D, Uthicke S (2012) The O<sub>2</sub>, pH and Ca<sup>2+</sup> microenvironment of benthic foraminifera in a high CO<sub>2</sub> World. PLoS ONE 7: e50010 b) McIntyre-Wressnig A, Bernhard JM, McCorkle DC, Hallock P (2013) Non-lethal effects of ocean acidification on the symbiont-bearing benthic foraminifer *Amphistegina gibbosa*. MEPS 472: 45-60c) Reymond CE, Lloyd A, Kline DI, Dove SG, Pandolfi JM (2013) Decline in growth of foraminifer *Marginopora rossi* under eutrophication and ocean acidification scenarios. Global Change**

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included, thanks for mentioning these studies

**2) Please take a closer look at Sinutok et al. there seem to be some issues with the methods used which cast some doubt on the results. For example, even the control foraminifera showed negative growth in this experiment, and the number of symbionts is exceptionally low. It appears experiments were conducted under very artificial conditions with weakened individuals. 3) Uthicke and Fabricius : Please note that we also conducted calcification experiments with pure CO<sub>2</sub>, not just with water from the vent. This was done to test if the effects observed may be due to an unknown factor. Results suggest this was not the case and we did observe an effect of CO<sub>2</sub>. 4) Vogel and Uthicke: You focus on our results for Marginopora, but there were also two further species investigated. 5) Page 1158, line 4: I think here you mean to cite Vogel and Uthicke, not Uthicke and Fabricius?**

we have updated this