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Interactive comment on “Impact of seawater $p\text{CO}_2$ changes on calcification and on mG/cA and sR/cA in benthic foraminifera calcite (*Ammonia tepida*): results from culturing experiments” by D. Dissard et al.

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Anonymous Referee #3

Comment: These both represent ‘extreme’ seawater carbonate system conditions, (as pointed out by one reviewer already) with the experiments at the nominal $p\text{CO}_2$ of 2000ppmv seawater being highly undersaturated at 10°C and only slightly oversaturated at 15°C. One might hypothesise in the lack of evidence to the contrary that these conditions are likely beyond the boundaries that this particular species might encounter

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in any modern environment and that this could impose a significant associated stress on the organism. Control experiments are vital to drawing any significant conclusions from these experiments; given the reported experiments provide only a comparison of opposing extreme conditions. The lack of any reported control experiments is particularly puzzling given these same authors have conducted and reported relevant control experiments (performed at a nominal pCO₂ of 380 ppmv and salinities of 24 and 33) in a 'sister' manuscript that has been submitted recently (early 2009) to *Geochimica et Cosmochimica Acta*. These experiments appear to have been conducted at the same as those reported in the current submission, and are highly relevant to the interpretation of any results. Indeed the results reported for the pCO₂ = 380 ppmv experiments go counter to the several of the 'significant' trends that have been based on the extreme pCO₂ experiments reported in this study. It is my strong view that the authors be required to incorporate results from the 'ambient' pCO₂ experiments into the current manuscript and resubmit it for review. Answer: As explained in our answer to reviewer 1, a wide range of conditions is necessary in order to get a clear trend that is not blurred by the natural variability. However, as mentioned by the reviewer, we cannot exclude the possibility that the organisms are stressed under these extremely high and low pCO₂ conditions. Therefore, the results of a control experiment at 15°C, salinity 33 and a CO₂ concentration of 380ppmv were added to the manuscript. Initially we did not include these control data because the organisms were not from the same batch, and the experiments were run during a different period of the year. We have now included those experiments in the current manuscript.

Comment: In undertaking this I suggest that that authors need to further explain why the measured seawater carbonate system properties return much higher than the nominal experimental pCO₂ values for the 120 and 380 ppmv experiments. Is this a problem with achieving equilibrium in these 'gas bubbling' experiments or with the measurement of one or more carbonate system properties? Answer: The system was set using predetermined pH values. Using the CO₂sys program, the pH was calculated for systems of which pCO₂ corresponds to 120 and 2000ppmv. The actual pCO₂, however,

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was determined based on measured DIC and ALK, and those results are presented in table 1. To accommodate the reviewer comment we have now renamed the “120ppmv” experiment to “230ppmv” and the “2000ppmv” experiment to “1900ppmv” based on the average values of the experiments, as these values are more representatives of the “true” experimental conditions.

Comment: The current manuscript harbours a number of additional significant shortcomings that require attention in the event of revision and resubmission. These include: The assessment of calcification changes during culture is compromised by the impossibility of weighing the amount of foram calcite prior to culture and by the addition of new calcite layers in culture over pre-existing chambers. Answer: We agree with the reviewer that the impossibility of weighing the amount of foraminiferal calcite prior to culture compromises the estimation of the impact of [CO₃²⁻] on shell weight. For that reason, the relationship between shell weight and [CO₃²⁻] is not presented as a calibration for *Ammonia tepida*, but as a general (underestimated) impact of [CO₃²⁻] on foraminiferal shell weight. This is clearly stated in the text of section 3.2.: “Only the newly grown chambers are responsible for the observed differences, since the initial parts of the tests were grown under natural conditions. The observed differences between the different experiments will, therefore, underestimate the impact of the different variables.”

Comment: Importantly, to what extent are new chambers identified by the presence of the fluorescent marker, instead the occurrence of pre-existing chambers with new calcite layers. How could you distinguish these two cases. Answer: The referee is right that during *Rotaliid* calcification new calcite is also added over pre-existing chambers. However, in case of fluorescence of multiple chambers, the last chamber always consist of new calcite and in this paper we only analysed final (F) chambers of specimens that grew at least two new chambers. Indeed, figure 2 was misleading as it suggested that we analysed all newly grown chambers. We have replaced figure 2.

Comment: One possibility for distinguishing new versus merely thickened chambers

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during culture might be the laser ablation ICPMS profiles. However, based on the appearance of the ablation ‘pits’ in the provided SEM image I would be surprised if analyses undertaken as part of this study have the necessary depth resolution. The laser ablation ‘pits’ shown in the SEM are surprisingly poorly formed, with evidence of mechanical fragmentation of the shell to the extent that it is not clear what has been sampled and analysed during the ablation process. This is also important for confidently interpreting any Mg/Ca analysis given the strong concentration of Mg in any residual cytoplasm that may not have been removed from within or on the test prior to analysis. Answer: With regard to the comment on mechanical fragmentation, we would like to add that ablation was stopped when the laser fragmented the test. The signal did not show abnormal values at the end of the acquisition. The possibility of contamination with residual cytoplasm is excluded also because cytoplasm was effectively removed with NaOCl.

Comment: Lesser technical and other comments the data tables are difficult to read in some instances due to the use of too many significant figures, the inconsistent use of orders of magnitude and uncertainties (Table 4 specifically), and unclear statement of uncertainties (1 or 2 stdev?) in tables and figures. Answer: Tables were corrected, figure 5 was removed and the orders of magnitude as well as the uncertainties were made consistent. For clarity the following sentence was added to the legend of table 4: "Uncertainties (standard deviation (one sigma) calculated per experimental condition) are presented in brackets."

Comment: References for Bentov and Erez, and also Nehrke et al are missing. Answer: The references: Bentov and Erez, (2006) and Nehrke et al., 2007; were added to the reference list

Comment: Bernhard and Benthov are misspelled. Answer: The names were corrected

Comment: Beyond an introductory sentence to the impacts of ocean acidification driven by rising pCO₂, the first three sentences of the abstract are generic and un-

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informative. I suggest their removal. Answer: The first three sentences of the abstract are meant to introduce ocean acidification, and to highlight the importance of understanding the impact of ocean acidification on marine calcifiers. We did not remove them but rephrased it a bit.

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