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Biogeosciences Discussions

Interactive comment on "Key Arctic pelagic mollusc (*Limacina helicina*) threatened by ocean acidification" *by* S. Comeau et al.

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We thank the anonymous referees as well as Donna Roberts and E.V. Thuesen for their very useful comments. We are pleased to note that referees #1, #2 and D. Roberts recommend publication after minor revision, referee #4 being more critical. We list below all the issues raised by the referees and describe which steps have been taken to address them in the revised version of the manuscript.

1- Reply to referee #1

1.1- "Although Calcein staining, based on the author description of a darker portion of the shell, is apparent in Figure 2c, it is not obvious in Figure 2b."

As explained in the manuscript the darker portion of the shell represent the 5 days

linear growth following the calcein staining (green part of the shell). The linear growth is illustrated by the area comprised between the white arrows. The aim of this figure was to show the linear growth at the same scale in both conditions. We agree that the upper edge of this area is fainter, in the printed version, Fig. 2B than in Fig. 2C, especially on the right side of the photograph. The original photographs are of much better quality; we will try to improve the quality of the figure in order to get a better quality after printing.

1.2- "What was the condition of the animals after 5 days of incubation? What was their survival rate?"

The rate of survival was 100% and only 30% of the individuals were actively swimming, the others were at the bottom of the beakers and exhibited limited activity. This information will be provided in the revised version of the manuscript.

1.3- "Were observations of faster growth rates at high pH consistent for animals in the calcein experiment?"

The reproducibility of the calcein staining is an important point, unfortunately only 4 photographs were taken at each pH level. Additionally, measuring the linear extension rate from the photographs is rather subjective depending were the measurement is made. For these reasons, we believe that statistical testing is not possible on these data and prefer to keep the information gathered in the calcein experiments as qualitative. Data collected in the 45Ca experiments are much more solid, quantitative, and conclusive.

1.4- " In addition, please provide plots and slopes of calcification rates for the individual pteropods measured at time 0, 2, 4, and 6 hours, rather than just descriptions."

The plot and slopes of calcification rates will be provided in the revised version of the manuscript.

2- Reply to referee #2

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2.1- “Please give a size-range of the experimental pteropods in order to give readers an idea of the developmental stage you have been working with (might be valuable information for future related work)”

The size of the individuals at the end of the calcein and 45Ca experiments ranged from 5 to 10 mm. This information is provided in the revised version of the manuscript

2.2- “Was the linear shell extension in calcein staining for all individuals in the same range as shown in Fig 2? I agree with Ref #1 that you should be a bit more precise here and include a plot on shell growth.”

See point 3 of the reply to referee #1.

2.3- “ It is not clear to me how often you sampled sea-water in the field (surface?): in 2.2 it seems to me you collected regularly but in the first paragraph of the results section, L14 you report on only one fjord water sample.” and “Discussion”

Only one field sample was collected, on the 1st of June, for measurement of pH and total alkalinity. This will be clarified in the revised manuscript. As mentioned in the manuscript, this sample does not provide any information on the diurnal or geographic variations of the total alkalinity in the fjord. We also agree with the referee’s comment that large changes in the carbonate chemistry occur in the Kongsfjorden as a result of ice-melting and changes in the pattern and magnitude of the Atlantic inflow. However, the seawater used in the experiments was pumped at 80 m depth and likely was Atlantic water. This proved very useful as it permitted to maintain stable chemical characteristics during the experiments. This information will be provided in the revised version of the manuscript.

2.4- All the technical changes suggested were done.

3- Reply to referee D. Roberts

3.1 - “I would recommend including full details of the sample sites, individual experimental pteropods, carbonate chemistry and laboratory experiments in a supplemen-

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tary section, if not inside the main manuscript itself. For example, it would be good for other pteropod researchers to know how many pteropods from the original 50 were 'un-healthy', their size, sample depths, sample location details, etc"

As mentioned above in the replies to referees #1 and #2, information on pteropods will be added. That will include survival rates and conditions after the 5 days of incubations and also the size range. As suggested, full details on the carbonate chemistry and other experimental parameters will be given as supplementary information.

3.2- "I agree with referee #1 regarding clarification of the Calcein staining results, particularly concerning Figure 2b."

Figure 2b will be modified as described above.

3.3- "I also agree that the manuscript would benefit from figures showing shell growth under the two conditions, with measurements from all animals and a figure showing the slopes of calcification rates for the individual pteropods measured at time 0, 2, 4 and 6 hours."

The plot and slopes of calcification rates will be provided in the revised version of the manuscript.

3.4 - "I agree with referee #2 regarding clarification of where/what type of water the experiments used."

See point 3 of the reply to referee #2.

3.5- " I note that the fjord temperature was 2.2âŮę C but experiments were done at 5âŮę C. Could you mention why you chose 5âŮę C for the experimental temperature over the natural field temperature experienced by your Limacina helicina individuals? "

The difference of temperature between the fjord and the laboratory experiments was only due to the limitation of the experimental set-up which could not maintain in situ temperature. This point will be discussed in the revised version of the manuscript.

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3.6- “I would recommend adding a citation for the recent Moy et al 2009 Nature Geoscience paper to this manuscript, particularly as it documents a major polar calcifiers calcification response to changing carbonate chemistry.”

The recent reference of Moy et al. (2009), published after the submission of our manuscript, will be included in the revised version of the manuscript.

4- Reply to referee 4:

4.1- “A major concern of the experiments is the lack of replication.”

Only one beaker was investigated at each pH level. However, regressing calcium uptake versus time is statistically valid and comparing the slopes with a t-test is also valid as the beakers are independent. We agree that replication (that is more beakers set at each pH level) would provide stronger inferential capabilities. Unfortunately, it was not possible due to the technical difficulty of controlling pH in multiple beakers and the short access time to polar individuals. Numerous publications on ocean acidification and presentations at conferences expressed concern that polar pteropods could be among the organisms most sensitive to ocean acidification. The very fact that we report the first data on the impact of ocean acidification on pteropods calcification demonstrate how technically and logistically challenging such experiments are.

4.2 - “Regarding the 45Ca experiments, I was not certain what “tissue-dried” means; is this wet weight or were tissues dried in a drying oven?”

Wet weight was used. Tissue-dried means that the pteropods were gently dried with a tissue.

4.3 - “ Subtraction of the soft tissue weight from the shell + tissue + sea salt wet weight would over-estimate of the actual shell weight, however the significance would depend on the size of the animals. I recommend including the size range of the individual pteropods used in all experiments.”

The overestimation of the shell weight resulting from sea salt was negligible as seawa-

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ter was removed with a tissue. This is clarified in the revised version of the manuscript. As mentioned in the reply to referees #1 and #2, complementary information, including size-range will be added.

4.4- “In the calculation of the calcification rate, I was not sure what P referred to: the ratio of radioactive calcium to stable calcium in the shell or the incubation water? In either case, how was concentration of the stable calcium analyzed?”

P refers to the ratio of radioactive calcium to stable calcium in the incubation seawater. The concentration of stable calcium was estimated using its relationship with salinity (Dickson et al., 2007). This information will be added in the revised version of the manuscript.

4.5 - “I also note that the non-biological adsorption of 45Ca onto the shell was high, approximately 50% of the calcification rate of the low pH treatment. The pteropods used to measure the adsorption of 45Ca were killed with mercuric chloride. Did use of mercuric chloride possibly dissolve the shell or change the shell surface chemistry such that additional 45Ca would be adsorbed? I also suggest that the mean and SD of the non-biological adsorption of 45 Ca be stated.“

A high non-biological adsorption was already reported by Fabry (1990) on pteropods not killed by mercuric chloride. In our experiment, pteropods were placed in a solution of seawater containing mercuric chloride for a few seconds. Mercuric chloride was at low concentration (0.05%) and did not alter the pH values of the seawater. It is therefore highly unlikely that the shell was not affected by mercuric chloride. Furthermore, there is no significant difference in the passive adsorption of Mediterranean pteropods killed by mercuric chloride or by freezing. The mean, standard deviation and sample number of the non-biological adsorption of 45Ca will be included in the manuscript.

4.6 - “With regard to the carbonate chemistry, I suggest including a graph showing the variability of the pH and alkalinity measurements during the 5-day, calcein experiments the mean and SD of the experimental containers should be shown. Similarly, the mean

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and SD of pH and alkalinity should also be shown for each experimental container in the 45Ca experiments."

As mentioned above in the reply to referee #3, information on the carbonate chemistry will be given as supplementary information.

4.7 - "I was curious why the experiments were conducted at 5C when the fjord temperature was 2C?"

As mentioned before in the reply to referee #4, the difference of temperature between the fjord and the laboratory experiments was only due to a limitation of the experimental set-up.

4.8 - "As other referee comments have noted, the relationship between the experimental seawater conditions and those in the fjord environment needs to be better defined. Without this, the title of the ms, as well as some of the conclusions, may not be warranted."

See point 3 of the reply to referee #2.

4.9 - "In the Discussion, paragraph 2, the authors state that this ms is the first to provide both qualitative and quantitative evidence that ocean acidification affects calcification rates in pteropods. Later in the Discussion, however, the work of Orr et al 2005 is acknowledged as providing evidence that net dissolution exceeded net calcification when live pteropods were exposed to high CO2. I would suggest that the present work is the first to present quantitative evidence, while Orr et al presented qualitative evidence only."

As mentioned in the first version of the manuscript, Orr et al. (2005) have reported the important observation that the shell of live pteropod dissolves at low pH. It should be noted that the carbonate chemistry of this experiment was unfortunately not reported and that the rate of calcification was not measured. We disagree with the referee that these data provide "evidence that net dissolution exceeded net calcification" because,

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although they do document dissolution at low pH, they provide no evidence with respect to net calcification. Net calcification being the balance between dissolution and gross calcification, two processes that take place simultaneously, qualitative information on dissolution only does not enable to draw any conclusion on net calcification. Hence, it seems fair to say that our study is the first to provide both qualitative and quantitative evidence that increased ocean acidification affects calcification rates in pteropods.

5- Reply to E. V. Thuesen

5.1- Means $\pm$ SD, the sample sizes as well as the p-value will be added for the non-biological rates.

5.2- A t-test was applied on the slopes, which correspond to the calcification rates of the pteropods. This test was used to test the significance of the mean calcification rates.

5.3- An ANOVA is not useful for this data set as the experiment design only comprised two pH levels. In such a case an ANOVA is identical to a t-test.

Interactive comment on Biogeosciences Discuss., 6, 2523, 2009.