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Biogeosciences Discussions

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

Anonymous Referee #4

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This manuscript describes a very interesting study to investigate the effects of high CO2 on the Arctic pteropod *Limacina helicina*. I congratulate the authors on undertaking this important work. I do have some questions and concerns regarding the methods and data analyses, however, which I discuss below.

A major concern of the experiments is the lack of replication. In the calcein staining experiment, 50 pteropods were put in one 5L beaker with seawater maintained at pH 8.1 for 5 days and 50 pteropods were put in a second 5-L beaker with seawater maintained at pH 7.8 for 5 days. Each pH treatment has a sample size of one, as the sample unit is the beaker and pteropods are subsampled from this. Similarly, in the 45Ca uptake experiments, there are two beakers at each pH level: one beaker contained pteropods that were analyzed at time zero and the second beaker was subsampled at

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2, 4, and 6 hours; again, however, the sample unit is the beaker. Subsampling from the same beaker – even at different times – is pseudoreplication. Thus, any inferential statistics, that is, hypothesis tests based on p-values, are not valid. While other referees call for additional statistical analyses of the calcification data, this cannot be done with these data. And even the t-test reported at the end of the Results Section is not valid. I strongly encourage the authors to repeat the study using a different experimental design which includes adequate replication. In my view, the data in the present manuscript are not sufficient to support robust conclusions, and publication at this point is premature.

Regarding the 45Ca experiments, I was not certain what "tissue-dried" means; is this wet weight or were tissues dried in a drying oven? If this is wet-weight, I assume this would include sea salts. When the shell is dissolved and the soft tissues removed, the sea salts would likely remain in the acid-solution. 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.

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? 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 45Ca be stated.

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 and

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

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

Minor point:

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.

In summary, the authors have developed a very intriguing project. I strongly urge them to repeat the experiments with increased sample replication. Then, the appropriate statistical analyses can be done and the data interpretation will be much stronger.

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