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***Interactive comment on “Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth” by S. Lischka et al.***

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We appreciate the comments of Anonymous Referee #2 very much, they are of great help to improve our manuscript specifically with regard to a deepened interpretation of our results. In the following we answer all comments consecutively.

General comments:

This manuscript describes an interesting study on the effects of elevated CO<sub>2</sub> and temperature on juveniles of the Arctic pteropod *Limacina helicina*. Pteropods are key

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components of many marine ecosystems which, due to their aragonitic shell, are vulnerable to ocean acidification. This is a particularly timely manuscript in light of the need to acquire data and knowledge on the response of pteropod early life stages to global change. So far, only adult Arctic pteropod's response to global change has been investigated. The study of overwintering Arctic juveniles is particularly interesting as it is a critical period with lower aragonite saturation state and lower food supply. Impacts of a 29 days incubation, under 4 pCO<sub>2</sub> conditions (180, 380, 750 and 1150  $\mu$ atm) and 3 temperatures (3, 5.5 and 8°C), on the mortality, shell preservation state and shell increment/extension are presented in this manuscript. The authors demonstrate that the main parameter affecting the mortality is the temperature whereas the parameter affecting the shell is the pCO<sub>2</sub>. There are few points (described below) that need to be clarified. One of the points of the experimental approach open to criticism is that the experiments were performed on starving organisms (see specific comments: discussion). The results presented are generally interesting, well presented, and are based on a relatively robust experimental approach. The discussion of the results provides some new ideas, but a deepened interpretation of the results would be welcome to reinforce the manuscript.

- The points brought up by Referee #2 are comprehensible and we clarify these in the following. A deepened interpretation of the results in the discussion specifically with respect to our shell degradation data is in accordance with suggestions of Referee #1, and will be provided in a revised manuscript.

Abstract, L3: The authors should indicate that the undersaturation is expected locally and less than 1 month per year.

- We will change this sentence accordingly.

Introduction

P8181, L3: A reference would be useful.

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This point was also brought up by Referee #1. As suggested by him, we will remove this sentence.

P8181-, L21: Comeau et al. 2010a, also report on shell dissolution of live pteropods.

- We will include this reference here.

#### Material and Methods

P8182, L10: What were the proportion of organisms damaged by the collection at 300 m? What was the density of pteropods (organisms per trawl)?

- About 200 individuals were caught per haul, less than 5% were damaged due to collection. We will include this information in a revised manuscript.

P8182, L14: What was the minimum acclimation time of the organisms?

- We are not quite sure, if we understand this question correctly. Minimum time pteropods were kept under in situ conditions until experiments started was one day. With respect to acclimation to experimental conditions, pteropods are not easy to cultivate for a longer period, and a classical acclimation period prior to experiments would mean additional stress to the organisms due to an additional transfer step from acclimation to experimental jars. During the relatively long incubation time of 29 days in our experiments, pteropods had the possibility to acclimatize to experimental conditions. This approach is similar to previous experimental studies with pteropods that lasted between 6 hours and 13 days (Comeau et al. 2009, 2010a, 2010b), however, incubation time in the present investigation was about 2 weeks longer. Furthermore, the control treatment allows separation of cultivation effects from temperature and CO<sub>2</sub> effects.

P8183, L9: Why did you use these temperatures?

- Temperatures were chosen according to a projected 1–2°C temperature increase for the upper 100–200 m of the Arctic ocean (Steinacher et al. 2009). The natural

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temperature range of *Limacina helicina* goes from  $-0.4^{\circ}\text{C}$  to  $+4^{\circ}\text{C}$ , infrequently up to  $7^{\circ}\text{C}$  (van der Spoel 1967). Hence, experimental temperatures are within its natural range ( $3$  and  $5.5^{\circ}\text{C}$ ) and according to a projected  $2^{\circ}\text{C}$  increase slightly above the upper temperature limit ( $8^{\circ}\text{C}$ ).

We will include this information in the text.

P8183: Were the incubations performed in the light or dark?

- Incubations were performed in darkness, which is according to in situ conditions at depth of occurrence. We will include this information.

P8187, L1–3: To my point of view, this classification is not clear. For example, the shell in stage III-4 seems more damaged than the shell in stage IV-1. In contrary in your 19 levels scale, the shell in the stage III-4 are classified “less damaged” than the shell in stage IV-1. How do you think it could impact the results presented?

- I think this is a misunderstanding. It is not stage I–V (in the sense of degradation), it is category I–V, meaning five different and “independent” patterns of degradation that were visible on the shell surface. These categories simply describe a variety of “things” that happened on the shell surface. Every shell was checked for each of these five categories and its stage (according to a scale from 1–4). In the referee’s example the shell in category III (scale 4) is very strongly degraded with respect to scarred structures. With respect to category IV (corrosion) this same shell was scaled 3 (strong), because all these scarred structures were deep but not very deep (as compared to others). So, this figure is only an example to illustrate scaling of each of the five categories.

We will amend the figure caption accordingly to make it easier understandable for the reader.

P8187, L19: Why didn’t you measure all the organisms? The results exposed would be much stronger.

- Our experimental design comprised of six replicates, which is more than the usually

applied three replicates and assures statistical validity with respect to the applied pCO<sub>2</sub> and temperature steps. We measured one individual out of each of the six replicates as a compromise between expenditure of time and statistical validity.

P8189, L7: Was the mortality higher at pCO<sub>2</sub> 350  $\mu$ atm at each temperature or only at one temperature?

- Mortality at 350  $\mu$ atm was significantly higher at 5.5°C and 8°C as compared to 3°C.

P8189 (part 3.3 and 3.4): Did you find a relation between one of your measured parameters and the aragonite saturation state (with or without taking into account the temperature)?

- We only related our data to pCO<sub>2</sub> so far, but since our results negatively correlate with pCO<sub>2</sub> they will also negatively correlate with the aragonite saturation state. Furthermore, we think the influence from increased aragonite saturation at higher temperature will not be distinguishable from the direct temperature influence on the physiological rates of the organisms. However, we will prove this and in case of significant effects include respective results in a revised version.

#### Discussion

P8190, L9–11: This is a major concern as I do not think that pteropods are totally starving at this time of the year. Indeed even if the phytoplankton concentration is reduced, pteropods are general suspensivores which can also feed for example on organic particles. Hence, the conclusion on the effect of temperature on the mortality of pteropods might be misled by this parameter. It is probable that pteropods which are fed (even a little) would exhibit much lower mortality rates than the one reported in your manuscript. It would also be interesting to discuss what would be the impact of a higher temperature on the food availability (higher or lower phytoplankton population. . .) in the part 4.2.

- We agree that feeding on POM cannot be totally excluded and that (if feeding on

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POM) mortality rates would probably be lower in organisms that were fed. However, we don't think, that our main result "rising temperature caused higher mortality" would be altered, since organisms in all (temperature) treatments were kept under the same conditions with respect to food. However, we will consider this point in our discussion.

Concerning temperature effect on phytoplankton availability, we don't think higher temperature would have any effect on phytoplankton food availability during this time of the year, because at this latitude light is the limiting factor. Light intensity ceases drastically from mid-September on which is why phytoplankton biomass decreases in Kongsfjord beginning already in late summer (Hop et al. 2002). Moreover, pteropods in our study had already migrated down to overwintering depth (below 100 m), where light intensity is negligible or even not existent.

P8191, L5: Did you measure the oxygen concentration at the end of the experiment? Low oxygen concentration would explain a part of the mortality.

- We did not measure oxygen concentration in these specific experiments, but we did some oxygen measurements in a separate approach. As calculated from these separate measurements, oxygen saturation (% air saturation) in the jars at experiment termination was still above 80–90%.

P8194, L7: The pteropods used by Orr et al. 2005 are from the Subarctic Pacific.

- We will correct this accordingly.

P8194, L12–22: Did you check if this difference is not only due to the fact that for similar CO<sub>2</sub> levels, the aragonite saturation state is higher at higher temperature?

- This point is certainly worth considering, so far we did not check it (s.a.). However, we think, it will not be possible to separate an effect caused by higher aragonite saturation at higher temperature from an effect caused by higher metabolic rates due to higher temperature since we have no according metabolic rates measurements. Therefore, for a revised version we suggest to include these points into this part of our discussion

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after we have re-analyzed our data with non-metric statistics as suggested by Referee #1.

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