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# *Interactive comment on* "Deformities in larvae and juvenile European lobster (*Homarus gammarus*) exposed to lower pH at two different temperatures" *by* A.-L. Agnalt et al.

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The authors thank Anonymous Referee #2 for useful comments. Our response to the suggested changes is as follows:

General comments:

This paper constitutes new information in a very timely topic of interest. The questions and scientific approach are clear, though the chemistry of the seawater should have been tested before proceeding with the experiments. This unfortunately led to the absence of a clear control for the study, leaving out valuable information. However, given the authors extensive background working with the study species, the absence

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of exoskeletal abnormalities from her long-standing experience allows some valuable conclusions to be made. The authors have made efforts to be upfront and clear about the lack of control pH, but may also benefit in greater clarity by including the pH information in the data tables.

Our reply: As the reader can see in figure 2, the pH in the ambient water was stable at the beginning of the experiment running at 10 degrees (first 3 weeks). Why this changed abruptly we do not know, but as everybody thought the conditions were stable this was not discovered until after the experiment was terminated. Thus, the entire 18 degree experiment was run without ambient water having a significantly different pH compared to the medium treatment group. However, if this change in pH had be discovered at an earlier point we would have had a dilemma; should we continue to use the ambient water reflecting the natural conditions or should we try to elevate the pH which means that we no longer used ambient water? For future experiments we need to consider how to deal with this challenge. Information about the pH will be included in the data tables.

Specific comments:

7584 - Temperature treatment choice – you mention the importance of warming temperature in combination with OA, yet I understand the high temperature treatment you chose is the optimum temperature for homarid crustaceans. I'm not sure this addresses the question of how future levels of temperature and OA affect lobster larvae at relevant temperature levels. Please describe how and why the study temperatures were chosen.

Our reply: We will elaborate our choice of temperatures in the revised manuscript.

7587 line 4 – It is unclear how many replicate larvae were measured per treatment per stage. This type of information is important to allow replication of your experiment for verification of findings, particularly when there is some question about the control missing. It is also necessary for understanding claims later in the paper attributing

some significant differences to low replication versus experimental treatment.

Our reply: The number of individuals in each stage for each treatment varied from 8 to 40. This will be clarified in the revised manuscript.

7588 – Regarding the statistical analysis, I understand that there were multiple (number unclear) replicate larvae sampled from each kreissel per treatment per stage. How did you treat replicates (kreissels and larvae within kreissels) within the ANOVA design in this study? Your replicates would be the 2 kreissels in each of the 6 treatments, with replicate larvae from each kreissel nested within each treatment combination. This would necessitate a nested ANOVA analysis unless there is no effect of kreissel within each of the treatments which could be checked with Cochran's test (see, for example,Underwood 1997).

Our reply: We will look into the statistics used, but the variation is small when comparing all data combined. It is strange that we do not find any decreased size (CL, TL nor weight) with increased $pCO_2$, as previously been reported in American lobster (Keppler et al. 2013, Hall & Bowden unpublished), although Arnold et al. 2009 did not find any such effect on European lobster. Species differences? This will be addressed.

7589 line 4 – You conclude that the lack of significant difference in CL between ambient and high $pCO_2$ treatments overrides that of the low-replicate significant difference between medium and high $pCO_2$. However, the ambient treatment provided higher pH conditions in the beginning of the experiment and more variability in pH overall. The effect of variable pH versus constant pH is emerging now in OA research. It may be worth considering that the difference in conditions (variable ambient pH versus stable medium pH) itself could be responsible for the difference between medium and ambient results, rather than the different sample sizes.

Our reply: We will look into how variable pH vs constant pH may explain the differences between medium and ambient results.

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7590 – How does the change in proportion of deformed larvae in 1 year juveniles compare with mortality? The decrease in proportion of deformed larvae in high pCO2 may be related to higher mortality in high pCO2 over the last 7 months, or higher mortality of already deformed larvae.

Our reply: We have looked into it, page 7590 line 13-21. However, we can consider simplifying if needed.

7591 line 11 – I do not feel it is appropriate to say that within the control group no larvae were deformed as there was no real control in this study. It is clear that the main author is very experienced with this species, there is no evidence from the present study on which to base this statement due to the ambient water pH variability. There is also a study by Keppel et al. (2012) not referenced in this paper which discusses effects of OA on larvae of the American lobster. There are few studies specific to this taxa and acidification, and there is much to be learned by comparing what is known about related species.

Our reply: The first 3 weeks of the larval phase run at 10C the pH was above 8.0, and is a true control in this part of the experiment. Within this period, all larvae developed normally i.e. none were deformed. Although we think this is clearly written, we will consider rephrasing to avoid misunderstandings.

Minor comments:

7584 - How frequently were water samples taken for chemical analysis?

Our reply: We will add this information to the text.

7584 line 10 – The actual pCO2 wasn't reported for the control here. While it is well discussed why this was different from expected elsewhere in the paper, it is important to include this information when reporting values for all treatments.

Our reply: We will include the pCO2 values in the revised manuscript.

7584 line 12 – It is unclear how the gas was controlled at experimental pCO2; did you bubble a premixed gas, or mix CO2 from a gas cylinder with ambient air? Or bubble CO2 and air separately?

Our reply: We realize that this needs to be explained better, and will be done.

7603 table 1 – please include a column for the control pCO2 treatment

Our reply: Will be done

Technical corrections:

7584 Line 25 - HgCl2 typo 7584 Line 26 – pH closed bracket 7587 line 20 'describen' should read 'described' 1593 line 19 – missing word: "The new exoskeleton..." 7604 Fig 4 – missing 's' at end of Homarus

Our reply: The technical corrections will be adjusted in the revised manuscript.

Interactive comment on Biogeosciences Discuss., 10, 7579, 2013.