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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.***

**A.-L. Agnalt et al.**

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## **Response to Anonymous Referee #1**

The authors thank Anonymous Referee #1 for useful comments. Our response to the suggested changes is as follows:

### **Major comments:**

Regional variability and knowledge of the local system are now understood to be important when running OA experiments, rather than following specific atmospheric CO<sub>2</sub> concentrations outlined in, for example, the Guide to Best Practices. Presumably the

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raw water was monitored for pH, temperature, salinity, alkalinity, etc. as this was used as the control? Therefore these data should be presented alongside the mid- and low-pH conditions in the table.

Our reply: This information will be added to the table.

It does not surprise me that at 90 m, over the autumn-winter period in a fjord, the authors found the pH to be quite low in their ambient water (see for example, Blackford et al. 2007; Salisbury et al. 2008; Green et al. 2009, and other regional conditions for the Norwegian Sea by Bellerby et al.). And in fact you briefly discuss this in the last paragraph of the conclusion. . . If this is indeed the “real” conditions at 90 m in the fjord then these are indeed the “control”. However, this does make the assumption that that the organisms are used to living in these ambient conditions? This is then the critical question for later interpretation – would the lobsters normally inhabit the 90 m, fjord environmental conditions to which they are being subjected? For the planktonic larval stages, my guess would be not necessarily, but for the juveniles, it could be. If there is information about the distribution of larvae, juveniles and adults in the fjord or regional environment, it would be useful to include that here to justify that these ambient fjord conditions are what the lobsters experience. If this is the case then, the “mid-pH” conditions actually are a stable version of the “control”, being kept at constant conditions by the CO<sub>2</sub>-pH feedback system, while the “control” fluctuates according to the fjord water.

Our reply: To our knowledge there are little or no information about distribution of larvae and juveniles in Norwegian coastal environments. More information exists on adult lobster and we will look into this to see if this information can elucidate this question.

7584: Line 6: “believed to be” – if this wasn’t measured then remove this. I suggest just stating it is ambient water in the methods is enough. In the results, I suggest a short section on the environmental conditions: Please add the real data – pH conditions, alkalinity? Calculated pCO<sub>2</sub> conditions, etc. Here is where you can show the ambient

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water conditions.

Our reply: Water chemistry data will be added to the table. We will also consider adding a short section on the environmental conditions.

Then in the discussion, there is the opportunity to discuss the fact that the ambient fjord conditions were already low in pH, high pCO<sub>2</sub> and were similar to the mid-pH conditions. However this fluctuated over time.

Our reply: We will adjust the discussion as suggested.

With respect to the freezer breaking – an unfortunate incident many of us have dealt with in the past – I suggest additional information is given for how many individual were used for each stage. At the moment the information is (7587 Line 1-4) giving on average 20 larvae for each treatment. Was this equally spread over the stages? i.e. 5 individuals per stage, or was it more in one stage? This would make the interpretation of the results easier – for instance in Fig 4, “note that lacking bars are due to a freezer broke and the samples decayed”, in which case the only bars in 18 \_C stage 4, are from ambient conditions. However there are juveniles in fig 5 (and further results) from the other treatment too?

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.

Minor comments: 7581; Line 17: “cold waters of Atlantic origin” Atlantic waters are not the cold part of the Norwegian Sea. . . please amend.

Our reply: This sentence will be rephrased.

7581; Line 17-18: “low pH levels will most likely decrease with depth” What do the authors mean? pH levels will decrease with depth? Or that the low pH level will deepen with time? Please clarify.

Our reply: This will be clarified in the updated ms.

BGD

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7582; Other references for Lobster and CO<sub>2</sub> could include Keppel et al. 2012.

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Our reply: Thank you. This will be considered.

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7584; Line 10-15: Was CO<sub>2</sub> gas mixing used (as implied on line 13) or was a pH controlled CO<sub>2</sub> feedback system used (as implied on line 14)? The method for reaching the desired values (of pH?) is unclear, please amend. Was pure CO<sub>2</sub> bubbled in, was there a flow meter, was ambient air also bubbled in?

Our reply: This will be clarified.

Was the pH (and other parameters) measured in each exposure container, as opposed to the “bubbled enclosures”? I.e. do you know if there was a change in pH between the enclosure and the container that housed the individual larvae or juveniles?

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

7584; Line 27–: This section is really results – see above comment about rearranging the information regarding the control levels.

Our reply: Information about the drop in pH will be moved to the Results

7588; Statistics: did you analyse the six treatments (two temperature, three pH treatments) using a two-way ANOVA? Because that would be an unbalanced design.

Our reply: Sorry about this. We run a one-way ANOVA at each stage and temperature. We will look further into if this is sufficient.

Also, were tests for normality and homogenous variance tested, I suspected the numbers were quite unbalanced because of the issues with the loss of samples. Please explain more specifically what tests were carried out.

Our reply: You are quite right in that the numbers were unbalanced. That is also why sizes have not been given so much emphasize in the results. However, we will clarify what tests were carried out.

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7588; Line 10: what is normal pH, where does this information come from?

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Our reply: We are referring to ambient pH. This will be changed.

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7591; Line 10: This section about the ambient conditions can now discuss information suggested in the major comment above.

Our reply: We will adjust the discussion as suggested.

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Table 1: please include the control treatment.

Our reply: The table will be updated

Figure 4: are the values in the graphs means? What are the error bars representing—standard deviation? Indicating the number of samples in each graph (instead of just missing bars) above each variable would be useful.

Our reply: The values in the graph are mean  $\pm$  one standard deviation. We will add this information to the figure captions and also add the number of samples in each graph as suggested.

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

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