Biogeosciences
Discussions
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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.

Anonymous Referee #1

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This paper presents novel data on the effects of ocean acidification which fits within the scope of BG, the scientific methods and assumptions are clearly outlined, and the authors clearly emphasise problems that have arisen with the experiment and how they have dealt with them, which allows the reader to draw an informed conclusion, and not overly interpret the results. Relating to the issues that the authors outline and discuss, namely the "lack of a control" and the "incident with the freezer", could be made slightly more clear and transparent, and I have detailed below some information regarding this. Otherwise, the manuscript is clear, and concise. I believe the data is valuable and should be published subject to the major comments below:

Major comments: As the authors openly discuss, they had issues with their control

conditions and they say they do not have real control comparison as a result of low pH inflow water. However, they still see abnormalities which they attribute to pH changes, having personally the long-term experience of culturing lobster larvae. I agree that just because the "control" did not stay at high pH should not prevent this work from being published; however I believe a few revisions should be made, and have made some suggestions below which should make the work more transparent and interpretation of results more clear. Regional variability and knowledge of the local system are now understood to be important when running OA experiments, rather than following specific atmospheric CO_2 concentrations outlined in, for example, the Guide to Best Practises.

Presumably the 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. 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_2-pH feedback system, while the "control" fluctuates according to the fjord water.

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 pCO2 conditions, etc... Here is where you can show the ambient water conditions. Then in the discussion, there is the opportunity to discuss the fact that the ambient fjord conditions were already low in pH, high pCO2 and where similar to the mid-pH conditions. However this fluctuated over time...

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?

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

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.

7582; Other references for Lobster and CO2 could include Keppel et al. 2012

7584; Line 10-15: Was CO2 gas mixing used (as implied on line 13) or was a pH-controlled CO2 feedback system used (as implied on line 14)? The method for reaching the desired values (of pH?) is unclear, please amend. Was pure CO2 bubbled in, was there a flow meter, was ambient air also bubbled in?

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?

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

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... 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.

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

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

Table 1: please include the control treatment

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.

Suggested references: Blackford and Gilbert 2007 Journal of Marine Systems 64 (2007) 229–241 Green et al. Limnol. Oceanogr., 54(4), 2009, 1037–1047 Salisbury et al. 2008 Eos, Vol. 89, No. 50,513-528

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