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Biogeosciences Discussions

Interactive comment on "Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.)" *by* K. E. Arnold et al.

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RC: The authors studied the effect of CO2 on larval development of the European lobster, Homarus gammarus (L.) focusing on the effect on calcification structures. This is an interesting and timely aspect with respect to the impact of ocean acidification on crustaceans. The critical point of the manuscript is the methodology. The description of methods is very unclear diffuse. Without adequate classification of larval developmental stages, it will be not possible to be certain about the correct larval age. This can influence the results with respect to larval composition and growth.

AC: When larvae reached approximately mid-point of each development stage, the 9

randomly selected individuals were removed for analysis. As larval development can alter under varying conditions, a preliminary study was carried out using the same conditions set in the final experiment. This gave us the chance to ascertain the possible mid-point of development through each of the four larval stages. As stated in the manuscript larval moult stages were determined using the schemes of Aiken (1973) and Chang et al. (2001).

RC: Page 3090 line 10: the authors argue: "Early investigations suggest that early life stages of development may be slowed ... or even completely disrupted ... at CO2 levels ... " It will be not possible to make evidence about larval development times when moulted larvae were not separated per larval stage and age. See methods...

AC: This was an introduction to ocean acidification impacts on early life stages and not a specific point about what we were attempting to provide evidence for. We feel this is a good introduction to the subject of ocean acidification impacts on early life stages and highlights the limitations and gaps in knowledge.

RC: Page 3091 line 20: How many larvae were used from how many females? Was the start and timing of the incubation the same for all treatments?

AC: Adjusted in manuscript – Page 6, line 123: Newly-hatched Zoea I larvae, from 3 different mothers, were (carefully) distributed haphazardly between a number of aquaria (flasks vol. = 1 l; N = 50 zoea per flask; T = 17 ïĆś 1°C).

RC: Page 3091 line 21: 50 larvae in a 1-litre flask are a large biomass per volume of water. (The lobster zoea larvae stages have a size from 7 to 12mm from the rostrum to the end of the abdomen.) This density will lead to high mortalities and cannibalism among larvae.

AC: We understand that the density of larvae were high during these experiments, though as stated for lobster rearing, larvae were successfully reared at a density of 62 l-1 (MAFF, 1985). As larvae were being removed in high numbers during each moult

(for morphological and physiological analysis), we needed to ensure that we would have sufficient numbers of larvae in each flask surviving through to the final moult (Zoea IV). As stated in the methods we also removed any moults and mortalities at regular intervals, as well as provided the larvae with sufficient food to maintain low accumulation of material and to minimise cannibalism.

RC: Page 3091 line 22: The relatively large temperature variability from 18 to 20 _C can lead to differences between larval development times in the flasks.

AC: There is an inverse relationship between temperature and the time it takes for larval lobsters to reach Zoea IV (Templeman., 1936), however as all larvae reached each progressive zoea within a few days of each other, it can be seen that the temperature variability did not significantly affect development time between individual repeats.

RC: Page 3091-92: The treatments were explained in a very preliminary and diffuse way. Did you close the flasks of the different treatments? Which flask did you acclimate for 2 h?

AC: Adjusted in manuscript – Page 5, line 111: Sea water was placed in ten open conical flasks (vol. = 1 l). Page 5, line 128: The elevated CO2 treatment flasks were left to equilibrate for 2 h to the required CO2 levels before larvae were transferred to them.

RC: Page 3092 line 17-22: Which age within larval stages did the sampled larvae have?

AC: When larvae reached approximately mid-point of each development stage, the 9 randomly selected individuals were removed for analysis.

RC: Different ages within a zoea stage means different development times and, as a consequence, differences in the composition of larvae. How did you measure carapace length and carapace area?

AC: Larval development can vary depending on temperature; therefore in order to as-

certain larval development times under the control conditions, preliminary studies were first carried out. The sampling days represent the mid-point of development through each of the four larval stages (i.e. Zoea I, II, III, and IV). The measurement of carapace length remains constant during the inter-moult period until the next moult (Oliver and MacDiarmid, 2001). CL was calculated as shown in Figure 1, CA was calculated by taking measurements of the removed and flattened carapace again using digital photography under lower power magnification (x 10) and ImageJ software.

RC: Page 3092 line 24-26: Did you use the same individuals for larval growth and measurements of mineral content? This is not clearly explained in this part.

AC: Adjusted in manuscript – Page 7, line 149: Measurements of the calcium and magnesium content of the carapace from the same individuals measured above, for each of the four developmental stages (Zoea I, II, III, and IV).

RC: Page 3093 line 18: Which age did larvae have within each stage at the specific sample dates?

AC: The sampling days represent the mid-point of development through each of the four larval stages (i.e. Zoea I, II, III, and IV). When larvae reached approximately mid-point of each development stage, the 9 randomly selected individuals were removed for analysis.

RC: Fig.1b: It is better to depict the stages on the x-axis and the development time (in days) on the y-axis. For the different larval development times during one zoea stage it is important to show standard deviation.

AC: Figure removed - The graph for development was only added as a guide to show the developmental stage at each particular day of sampling. This should have been explained in the results. We have therefore removed this graph, as it is misleading, and have instead included a paragraph in the methods as to why these particular sampling days where used.

RC: 1200ppm not 1000ppm CO2 page 3093

AC: Mistype, adjusted in manuscript

RC: Line 20: What do you mean by “circa 28 days”? Specify your incubation and sampling in the material and methods part.

AC: Adjusted in manuscript – Page 6, line 127: Both treatments commenced simultaneously and were incubated for 28 days.

RC: Table 1: Why did you use 17 _C in your calculations and not 19_C? According to the text the control treatment occurs under 365ppm CO2 (page 3092 line 8) but in the table under 315 _ 18,83ppm CO2?

AC: Mistype in table, water temperature was maintained at 17°C, this has been adjusted in manuscript. Control treatment was 315 ppm, this has also been corrected in manuscript - Page 6, line 116: Control flasks were aspirated (10 l.min-1) with an air mixture containing 380 ppm of CO2, however the pressure was not high enough to completely equilibrate these flasks, which had high alkalinity, and hence the measured sea water CO2 value was slightly lower (mean 315 ppm).

Interactive comment on Biogeosciences Discuss., 6, 3087, 2009.