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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: This paper deals with the effect of ocean acidification on larval development of the European lobster, Homarus gammarus. It is an interesting and important subject, which appeals to a wide audience of ecologists and marine scientists. The ms follows the patterns of growth and calcification of the carapace during larval development by measuring length, dry mass and Mg and Ca contents of the carapace. All these parameters are proxies of the larvae to grow (discussion page 10, line 208) and should be stated in the objectives of the introduction. Unfortunately, the objectives remain quite unclear, due to insufficient definition of the terms growth and development ("aspects

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of growth and development“ page 5, line 94) until reading the discussion. The quantification of development is unclear (Fig. 1b) and the presentation of the experimental design in the methods section is confusing. The sequence in which the parameters are examined in the results section differs from that in the methods section. The text would flow better if the methods and results were organized in the same way, and should be consistent with the order in which the objectives are presented in the introduction. A consistent order would also help to follow the flow of ideas in the discussion.

AC: These issues have been addressed in the manuscript and are explained in further detail below in response to the referees specific comments/questions.

RC: Some aspects of the experimental design are difficult to visualize. It is unclear how many females and larvae were used in each experiment and data analysis, whether data for the 4 larval stages were obtained from a same set of larvae that were reared through all the stages or from different batches of larvae (one batch for each stage), and whether the same females and larvae were used in different experiments or an entirely new set of females and larvae were used for each. The sample size for “development” seems to be 1. If this interpretation is correct, then do the authors feel this experiment is sufficient to reach reliable conclusions about developmental time between treatments?

AC: Adjusted in manuscript - Page 6, line 123-127: 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), with all flasks containing larvae from all females. Page 7, line 149: Measurements of the calcium and magnesium content of the carapace from the same individuals measured above.

RC: Page 6, line 110: specify “newly-hatched, free-living larvae”: Were all larvae of the same age? What does “free-living” mean? It is unclear what is meant with “when required”. Were the experiments with 5 controls and 5 CO2 incubated flasks not started at the same day?

AC: See comment above. Adjusted in manuscript - Page 6, line 127: Both treatments

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commenced simultaneously and were incubated for 28 days.

RC: Page 6, line 113-115: How many different females were taken to receive the newly hatched Zoea I larvae? Did all flasks maintain larvae from all females?

AC: See comment above.

RC: Page 6, line 118-119: Were the various stages separated or were all larvae left in the same bottle during the complete experiment?

AC: All larvae were in the same flask throughout the experiment.

RC: Page 6, line 117: “flasks were left to acclimate for 2h”: Do you mean “equilibrate CO2 levels” instead of “acclimate”?

AC: Adjusted in manuscript - Page 6, 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 6, line 120-133: Were the flasks left open? The production of seawater with high CO2 levels is unclear.

AC: Adjusted in manuscript – Page 6, line 111: Sea water was placed in ten open conical flasks (vol. = 1 l).

RC: Page 6, line 128-130: This sentence should be removed because it is already mentioned in the introduction

AC: We agree with this comment and have removed this in the manuscript.

RC: Sampling of the 4 larval stages is unclear. Were they all in the same flask or were they separated? Were measurements of larval growth and survival made in different flasks? How many flasks from how many females per treatment were used to get all the samples?

AC: Survival of larvae was only noted and recorded from the same flasks where in-

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dividuals were being removed for sampling. Larvae were removed at a concentration of more than 10% of the original population, which is statistically proven to affect survival rates (Castex et al., 2008). We have therefore only commented on survival in the results to avoid confusion.

RC: Page 7, line 136: Explain how “Carapace area” was measured

AC: Adjusted in manuscript – Page 7, line 142: 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 7, line 138: “Morphological differences” should be more specific.

AC: Adjusted in manuscript - Page 7, line 144: Larval moult stage was recorded daily, as a measure of development

RC: It should be briefly explained in the materials and methods why it is important that the mineral concentration of Mg and Ca were expressed as percentage of total mass of animal carapace and as per unit of total carapace area.

AC: The observed changes in Ca and Mg are not absolute; they are relative between treatments and samples and therefore require standardising. There are two ways of doing this, as a percentage of the total carapace mass, or as a unit of total carapace area. The percentage of carapace mass gives an indication of the relative proportion of each mineral taking into account carapace thickness as well as size, this explanation has been added to the materials and methods (Page 7, Line 153-158).

RC: Data on survival and morphological differences are not presented but mentioned in the material and methods part.

AC: Survival and zoeal progression were both only monitored during experimental exposures. Effects on survival and development through each progressive zoea have therefore been added to the results only as observations.

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RC: Figure 1: The standard deviation is missing in Figure 1b. The x-axis is not clear: The days 7, 14, 21 and 28 represent the day of moulting to the next stage or the middle of each stage? How was development monitored? Are the developmental stages I-IV (Figure 3 and 4) the same as the days 7, 14, 21 and 28 in Figure 1 and 2? Sampling time should be explained in more detail in the material and methods as the ca and mg can vary substantially within the stages, depending on the day within the moulting cycle.

AC: Figure 1: The graph for development (Figure 1b) 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. 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. The sampling days represent the mid-point of development through each of the four larval stages (i.e. Zoea I, II, III, and IV).

RC: The ms emphasizes that growth not differed significantly among treatments. I would argue the opposite. The dry mass, which is also a parameter to measure growth, decreased with progressive developmental stages. Therefore, the thickness of the carapace might decrease with development when exposed to high CO2 levels as discussed by the authors. The authors should be more precise with their terms, which make it easier to read the discussion, e.g.:

RC: Page 10, line 206: “Certain morphological parameters” should be replaced by “carapace length and mass”

AC: Morphological parameters has been removed from this section

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RC: Page 10, line 212: “growth” should be replaced by “carapace length”

AC: Adjusted in manuscript – Page 10, line 210: The fact that carapace length was not affected by culture in CO2-acidified sea water. . .

RC: Page 10, line 209: “Survival” is not displayed in the results sections and should be added. The zoeal progression should be displayed. Figure 1b is not very informative and should be explained in more detail in the “materials and methods” and the “results section”.

AC: See comment above about survival. Figure 1b removed also explained above.

RC: The authors state that CO2 induced acidification affected the calcified exoskeleton in late zoea larval stages. They argue that it is the most critical period for production of viable post-larvae (page 11, line 249-250). According to the data high CO2 levels show a progressive effect of decreasing % Ca as well as % Mg with developmental stage. This could simply be an effect of incubation time, as Zoea I in comparison with Zoea IV are less time exposed to the experimental high CO2 levels. Therefore, I suggest that we see a long term CO2 effect in Zoea IV that cannot be measured in Zoea I.

AC: Adjusted in manuscript – Page 12, line 264: CO2-induced acidification displayed a progressive long-term CO2 effect on the calcified exoskeleton in Zoea IV.

FC: Figure legends: Figure 1. 1000pp

AC: Mistype, adjusted in manuscript

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