

Interactive comment on “Effect of CO₂-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.)”
by K. E. Arnold et al.

Anonymous Referee #1

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General Comments

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 of growth

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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.

Specific comments and questions

Materials and Methods

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?

2.1 Animal material

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?

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?

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

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

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

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

2.2 Larval growth and survival

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?

Page 7, line 136: Explain how "Carapace area" was measured

Page 7, line 138: "Morphological differences" should be more specific.

2.3 Measurements of mineral content

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.

Results

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

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

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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.

Discussion

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.:

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

Page 10, line 212: "growth" should be replaced by "carapace length"

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".

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.

Technical corrections

Figure legends: Figure 1. 1000pp

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