

## ***Interactive comment on “Effect of ocean acidification on the early life stages of the blue mussel (*Mytilus edulis*)” by F. Gazeau et al.***

**Anonymous Referee #1**

Received and published: 3 May 2010

This paper by Gazeau et al. aims to investigate the impact of ocean acidification on the early stages of the ecologically and economically important mollusk species, *Mytilus edulis*. They have collected simple but important and interesting data on growth and survival under different scenarios of ocean acidification, comparing short (0-2d) and longer (2-13d) exposure.

This is an interesting paper leading testable hypotheses and I would recommend publication of this paper for Biogeoscience after several modifications including:

- New additional analyses: the dataset is interesting and more information can be extracted. Some results also need a new interpretation (e.g. impact of low pH on shell thickness).

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- New information: additional information is needed for a proper interpretation of the results.
- Broader literature review: this manuscript deals with a biological/ecological question and more biology/ecology is needed for a good understanding of the model used, the observed differences between the treatments and their consequences

All of this can easily be solved in a new version of the manuscript.

### 1. New analyses

1.1. For the average shell length, it would be possible to calculate the impact on growth rate rather than only present the raw data. I have made an attempt using the average data (see Figure, it would be necessary to do this using the raw data). As expected the growth rate is dependent on the initial size (see figure below): the smaller the larvae, the fastest the growth rate. This leads to a classic growth curve (Fig. 2 in the manuscript, a growth model can fit these data). Plotting initial size against growth rate for the two treatment conditions, you can see that the growth rate and size are negatively correlated. It is also clear that you have a very nice decrease in the growth rate under low pH conditions (this could be tested using statistical analysis, for example analysis of co-variance). It is interesting to notice that the two curves are shifted and that the impact on growth is similar for all the sizes. If we consider that the effect is rather a delay in development (suggested by new analysis of the data) rather than a larval miniaturization, we can then estimate (using a simple growth curve model and projecting values of the low pH on the control curve) that at day 15, the larvae at low pH are 2 days delayed compared to the control. If predation rate on larvae is similar to what is observed for urchins, a 2 day delay can induce 15 to 30% increase in mortality. It is then impossible to determine if the observed difference in shell thickness is due to a pH effect or just a delay in development. The only way to solve this would be to measure the relationship between shell thickness and shell length. Conclusions such as “shell thickness appeared to be affected as well with a relative decrease twice the relative

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decrease observed in shell length" (page 2939 line 13-14) is dangerous. There is no reason why shell length and shell thickness should grow at the same rate and then it is impossible to compare the effect on these two processes growing at different relative speeds.

1.2. Data interpretation. The authors should be careful to not over-interpret their results. If the collected data could be interpreted as a negative effect (but see below for some experimental limitations), they provide very little information on the consequence of ocean acidification on the species fitness (e.g. settlement success). It may be nice to tone down some dramatic conclusions such as "significant ecological and economical losses" (Abstract, p2929, line 20).

2. New information It is briefly mentioned in the Discussion that "sensitivity of the organisms might depend on the pH variability at which they are naturally exposed in the field." (page 2939, line 9). This should be developed and explained a little bit more (what kind of variation is related to what kind of resilience, there is a nice literature on these aspects). Recent and historical environmental variability experienced by the adult can have consequences on larval resilience to additional stressors as a consequence of both acclimation/carry-over effects and natural selection. Animal experiencing more natural environmental variations are potentially more resistant to additional stressors (e.g. Hofmann & Todgham 2010, *Annu. Rev. Physiol.*; Melzner et al. 2009, *biogeosciences*; Dupont et al. 2010). For example, individuals from an intertidal population may potentially be more resilient to stressors than subtidal ones. This highlights the importance to describe the natural environment (including variation at different temporal scales) of the population used. This is also complicated by the fact that *Mytilus* is an indirect developer and that the larvae experience a very different environment than the adults (and then a different level of variation). For a better interpretation of the observed effects and determine the ecological relevance of the observed differences (is it possible to translate the observed effects to natural environment), we need to know: - Conditions in which the adults used in the experiment

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were kept (environmental conditions and its variation), in the intertidal inlet but also in the acclimation tanks. What about the food conditions? - The information on the adults used in the experiment. Is there any idea on what is the genetic variability? Is this potentially reduced by the aquaculture culturing method? (see also 3.2. for the discussion on selection and adaptation potential and genetic variation). - To properly define the control (on an ecological perspective), it is important to provide information on the seawater parameters (pH, alkalinity, etc.) where *Mytilus* larvae naturally swim and at the time of spawning. At what depth the seawater used for this experiment was collected? Does this water present the same chemical characteristic than the water in which *Mytilus* larvae naturally swim? Is the natural period of spawning correct to the time of the experiment? This would be interesting to see the ecological relevance of the experiment. Some partial information is presented at the beginning of the Result and it may be good to present this earlier (to define what a proper control is in term of temperature, pH, etc.) For example, it is surprising that two different temperatures (and temperature is critical, see Parker et al. 2009 showing that impact of ocean acidification is higher at higher temperature) were used in experiments 1 and 2 and that control pH are not always the same. This is not a problem on the physiological point of view but it is critical for a proper ecological perspective. - More and more data tend to show that ocean acidification play a role on the energy balance (e.g. Wood et al. 2008). As a consequence, food availability is a critical parameter (you can expect higher impact when the food available is low and in high concentration you may under-estimate the real impact). Is the amount of food provided important (ad libitum)? And how is it compare to field condition? What is a normal food concentration in the field at spawning time?

### 3. Literature review

3.1. This manuscript deal a biological/ecological question: what will be the impact of near-future ocean acidification on *Mytilus* early developmental stages? Some more information on the species biology and ecology would be interesting (e.g. in the In-

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triduction, in the present version the balance between chemistry and biology does not seems right), including: - What is the role of *Mytilus* in the ecosystem and what would be the consequence if the species is seriously impacted? Not all species have the same importance in the ecosystem and "uniqueness" (some species can just be replaced by another one playing the same role). - What is the life-cycle of *Mytilus*? Different developmental stages, distribution, spawning time, etc. - "In the field, in presence of predator." (p2937, line 17). The interaction between the larvae and predator is probably a very important parameter (see point 1 for some info). This should be explained (what is the role of the shell for predation avoidance, if any?) more in details. The indirect effects should also be mentioned (e.g. impact of an increase of the developmental time). For example, it is said: "we show (...) decrease in growth rate" (p2937, line 13). This is not what is shown here (see 1). No growth rate was calculated and most observations are single time point. As a consequence, it is not possible to interpret the observed different as "delay in development" (real impact on growth rate) and "miniaturization" (plasticity, abnormality). Both non exclusive hypotheses should be discussed with potential consequences on indirect consequences on individual fitness. There is an extensive literature on this. For example, an increased time in the plankton due to a decreased growth rate can have drastic impact on larval survivor due to increased predation. This can also have impact on phenology. A decreased size will have other consequences (e.g. smaller size at settlement with potential carry-over effects on juveniles and then adults).

3.2. One of the very interesting finding of this paper is that some larvae of *Mytilus* are able to grow a shell in undersaturated water. It may sounds surprising under the current paradigm in the ocean acidification field claiming that calcifiers will be dramatically impacted by pH decrease. However, more and more data shows that things are more complicated than that and that an calcifying organism is much more than a piece of calcium carbonate. This result should be discussed a little bit further: - The authors could provide some figures/data on calcifying species (including molluscs) already living and growing shell in undersaturated marine waters. How is that possible? What are

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the mechanisms? - This result is also interesting on the "evolutionary" point of view. The fact that a significant portion of the larvae are able to calcify and survive in low pH water may be an indication of intraspecific genetic variation allowing selection of resistant genotypes.

3.3. Calcification is a central point in this manuscript and there is a need for more information on the calcification mechanism in *Mytilus* (it is just mentioned in the Discussion: "The mollusks exert a control over calcification" (p2397, line24)) and/or other mollusks but also buffering capacity. Another important point is the cost of calcification. Is there any information on the energetic cost of calcification (compared to other physiological processes). Is there any plasticity when exposed to different environmental conditions (e.g. food concentration) and how does this relate to individual fitness (there is an extensive literature on this subject). To be able to interpret the observed effects on the shell, it is also essential to understand the role of the skeleton in a *Mytilus* larvae. Is it used for feeding? Protection against predator? What would be the consequence in the field of a slightly thinner shell? Would it be a real problem for the larvae (only in the water for a short period)? Would it affect it feeding efficiency?

4. Other questions/remarks - p2932, line 8: How long did the eggs and sperms were kept together before the fertilized eggs were filtered. How many males and females did actually spawn? What about egg size? - It would be nice to have some pictures of what are "normal" and "abnormal" larvae rather than send the reader to His et al. 1997 publication (p2934). - "few studies have focused on early life stages" (p2929, line 3), "Most studies have investigated adult stages..." (p2931, line11-12). This is less and less true. Many recently published paper are dealing with early life history stages (oyster, barnacles, echinoderms, etc.) and in some phyla, there is more information available on early stages than on juveniles or adults (e.g. echinoderms, see Dupont et al. 2010 in Ecotoxicology for Review). - Page 2929, line 13: replace "tosligh" by "to light". - Fig 2: it is said in the text that final shell length is significantly smaller a low pH. Does that mean that the other days are not significant? - P2936 (lines20, 26). In

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the discussion, avoid using “alteration” and “altered” or description such as “processes were diminished” (p2937, line 1). This is too vague. Be more specific on what are the effects. For more clarity, it would nice to present the results in the literature not in term of pH but pH variations compared to the control (e.g. p2936, line 19). Actually, a summary table with the observed impacts and variations in environmental parameters would be helpful. - The use of “environmental condition” is confusing (e.g. Appendix 1, also p2936 line 10). I first thought it related to conditions in the field. What about “Experimental abiotic conditions”?

Interactive comment on Biogeosciences Discuss., 7, 2927, 2010.

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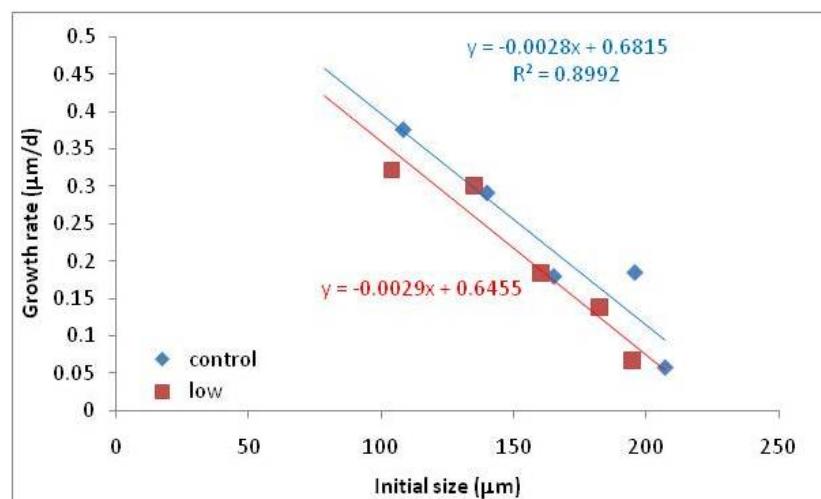


Fig. 1.

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