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Biogeosciences Discussions

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

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We would like to thank the referee for his/her numerous comments and suggestions on our manuscript. We agree with most comments and will modify/update the manuscript accordingly. Details and answers to the referee follows.

1. New analyses

1.1. Presenting and analyzing growth rates instead of shell length data.

This suggestion by the referee is absolutely appropriate and we will follow it by adding a third plot to Fig. 2 (c) showing the variation of growth rates during the experimental period. We agree that it is not possible to know from our study if the observed decrease in growth rates would translate in an increase of the time spent in the water column

(allowing larvae to reach a minimal size before settlement) or if this will translate in a miniaturization of the shells. Both alternatives have been included in the discussion section, and we now discuss these potential effects on larval mortality in the field:

"Although no effect on hatching and mortality rates have been observed after 2d and after 15d of development, the consequences, in the field with the presence of predators, of a potential decrease of shell resistance and/or an augmentation of the time spent in the water column (delay in settlement) due to a reduction in growth as observed for a 0.25-0.34 pH unit decrease, are still unknown. Since the experimental period did not extend to the settlement and metamorphosis of the organisms, it is impossible to know if the observed decrease in growth rates would translate in a miniaturization of the spats and/or an increase of the time spent in the planktonic compartment. Nevertheless, both effects could have major consequences for the survival of the populations. Suspension-feeding benthic invertebrates can be important predators of pelagic larvae. In the Oosterschelde estuary, it has been shown that larviphagy from adult bivalves is a major source of mortality for bivalve larvae (Troost et al., 2009). However, several studies showed that, thanks to their shell, larvae could be rejected unharmed with the feces (Mackenzie, 1981). A reduction of the shell both in terms of length and thickness has therefore the potential to increase mortality rates during the planktonic larval stage. Finally, decreases in size during the early developmental stages of marine organisms have been shown to effect juvenile fitness by reducing competitive ability and increasing postsettlement mortality (Anil et al., 2001). "

1.2. Over-interpretation of observed results

The referee is correct. As we did not test the effect of OA on the settlement success in the hatchery and that the experiment was conducted under conditions not necessarily relevant for field conditions (temperature and food conditions, see below), we cannot conclude on the effect of decreasing growth rates observed in our study on the species fitness in the field. We have therefore nuanced our conclusion in that sense, it now reads as:

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"Although these results show that blue mussel larvae are still able to develop a shell in seawater undersaturated with respect to aragonite, the observed decreases of hatching rates and shell growth could lead to significant decreases of the settlement success. In order to investigate the potential ecological and economical losses of a decrease of this species fitness in the field, future studies will need to consider the whole larval cycle (from fertilization to settlement) under environmentally relevant conditions."

2. New information

The referee suggests to add more information on the variations (pH, Alkalinity etc..) to which adults and larvae of Mytilus are exposed to in their natural environment, and to discuss the role of these natural variations in terms of resistance to pH decrease.

Carbonate chemistry

The conditions with respect to the carbonate chemistry in the Oosterschelde from where the adult oysters were sampled were already mentioned in the manuscript: P2935L13 "In the Oosterschelde tidal inlet (5 stations, monthly measurements), surface pHNBS varies annually between 8.00 and 8.24, while TA varies between 2.334 and 2.567 meq kg-1 (data not shown). In the fall (that is the time of the experimental period), pHNBS and TA in the tidal inlet are, on average, 8.04 and 2.436 meq kg-1, respectively." Prior to the experiment, bottom-cultured adults were kept in acclimation tanks receiving water from the Oosterschelde, cooled at a temperature of 10°C. In order to clarify this, we added at L353 of Material and Methods:

"A group of approximately 150 ripe, bottom-cultured mussels from the Oosterschelde, a tidal inlet, were kept at a constant temperature (10ËŽC) for about 4 months. These animals originated from a same age-class and were fished in the tidal inlet and cultivated for about 2 years on commercial production plots."

Moreover, we now mention the pH levels at which larvae are naturally exposed in the field in the discussion section and mention that our control levels are significantly below

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the ones in situ at the time of spawning due to CO2 consumption by the phytoplankton (see below).

Food conditions

As the experiment was carried out in a commercial hatchery, food conditions are considered as optimal as this has been the object of intensive research in this hatchery for several years and has been published in international journals (e.g. Pronker, A. E., Nevejan, N. M., Peene, F., Geijsen, P., and Sorgeloos, P.: Hatchery broodstock conditioning of the blue mussel Mytilus edulis (Linnaeus 1758). Part I. Impact of different micro-algae mixtures on broodstock performance, Aquaculture International, 16, 297-307, 10.1007/s10499-007-9143-9, 2008.) Food availability was obviously higher in our experiment than in the field and we cannot exclude that this had an effect on the resistance of these larvae as compared to natural conditions, this will be acknowledged in the Discussion section.

Spawning period

The experimental period does not correspond to a natural spawning period. In the Oosterschelde, mussels naturally spawn in April when the water temperature exceeds approximately 10°C, sometimes followed by a second peak in autumn, depending on food availability. Again, this experiment was carried out under optimal and artificial growth conditions, adults were fed in an optimal way and maintained at low temperature (10°C), spawning was induced after maturation by an artificial temperature increase.

Temperature

We agree that temperature is a very important parameter. This parameter, in our experiment, in contrast with pH and total alkalinity (see above) is significantly different from the temperature level experienced by larvae in situ at the time of spawning. This will be acknowledged and the potential bias with respect to the relative decrease in shell growth will be discussed with a reference to the paper from Parker et al. 2009.

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However, we do not believe that the difference in temperature between Exp#1 and #2 is such of a problem as we do not directly compare these 2 experiments. A temperature difference between the 2 sub-experiments of Exp#1 that we directly compare to each other would have been more critical, but this parameter was quite constant.

Genetic variability

We do not have any idea on the genetic variability of the population from which the adults were taken from. The animals used in this study have been fished in mussel beds of the Oosterschelde estuary and bottom-cultured (and not rope-cultured as mentioned in the Discussion paper) for about 2 years in the Oosterschelde before they were taken to the hatchery and kept at low temperature. This issue of genetic variability vs. adaptation is, however, a very important issue pointed out by the referee. This potential adaptive capacity of mussels would deserve much more attention in future experiments (by comparing different populations of the same species for instance, wild vs. aquaculture populations). This is mentioned in the discussion section: "As mentioned previously, in the Oosterschelde estuary, adults are exposed to a relatively narrow range of pH with winter pH levels never falling below $\sim$7.9 and high pH levels in springtime ($\sim$8.3) when spawning and larval development occur. There is, therefore, a great need to evaluate the adaptive capacity of this species to low pH conditions. This could be achieved by comparing the responses, to a decrease in seawater pH, of populations originating from areas with contrasting conditions with respect to the carbonate chemistry and/or by performing such experiments over several generations."

Summary

Our experiment was conducted in a commercial hatchery, and both food and temperature conditions were set to reach optimal growth rates that do not necessarily reflect the conditions experienced by the larvae in situ. A paragraph on this issue has been added in the discussion section:

"The conditions at which the larvae were exposed in our experiment must be regarded

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as optimal. In the field, mussels usually spawn in spring when the water temperature is ∼8-18 °C and chlorophyll a concentrations vary between 0.5 and 19 μg l-1 (April-June, 5 stations, monthly measurements; see Table 1 for experimental levels). Therefore, as both experimental parameters were significantly higher than the ones encountered in situ at the time of spawning, the extrapolation, to the field, of our laboratory-based observations on the effects of decreasing pH on the blue mussel larval development, must be performed with caution. Indeed, Parker et al. (2009) have shown that the effects of ocean acidification on the growth of Sydney rock oyster larvae were greater at sub-optimal temperatures. Moreover, as food availability is a very critical parameter in limiting larval development, the fact that, in the present experiment, food concentrations were optimal could have led to a high resistance of mussel larvae to decreasing pH levels. The experimental pH level used for the control incubations in this study also does not reflect the conditions experienced by larvae in situ. Indeed, at the time of spawning, the spring bloom occurring in the Oosterschelde estuary, drives seawater pCO2 to values below atmospheric equilibrium corresponding to an average pH level of 8.27 $\pm$ 0.09 (April-June, 5 stations, monthly measurements), a value much higher than the one used as a control during the incubations. In order to evaluate the potential effect of ocean acidification on this species fitness, there is a great need to conduct future experiments under conditions similar to the ones experienced by the organisms in the field."

3. Literature review

The referee suggests to add more information on 1) the biology/ecology of this species, 2) the potential consequence of a decrease in growth and/or a delay in development on the fitness of this species in situ and 3) the capacity of these organisms to calcify under non optimal conditions.

We have followed the referee suggestions by:

1) adding a paragraph on the life-cycle of this species in the Introduction section:

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"The bivalve Mytilus edulis is a benthic invertebrate typical of the North Atlantic coast of North America, Europe, and in other temperate and polar waters around the world. They live in intertidal areas attached to rocks and other hard substrates. This species reproduces by means of a planktonic larval stage (meroplanktonic species). Eggs are fertilized in the water column and, thanks to their internal energetic resources (lecithotrophic phase), develop to the ciliated trochophore stage and to the D-shaped veliger (shelled) stage within few days depending on the temperature conditions (Pechenik et al., 1990). These veliger larvae start to feed in the water column and gain weight until they reach the pediveliger phase (after few weeks) during which they try to find a place to settle. Larvae become competent to settle at a shell length of $\sim$260 μm but can delay metamorphosis and remain in the planktonic compartment until they reach $\sim$350 μm (Sprung, 1984). Once the settling conditions are favourable, metamorphosis occurs, plantigrade larvae attach to the substrate thanks to the secretion of the byssus and start to secrete the adult (dissoconch) shell."

2) discussing the role of Mytilus edulis in the ecosystem the organisms were taken from (The Oosterschelde) in the introduction section:

"Blue mussel aquaculture is very important in The Netherlands and consists almost entirely of bottom-culture, carried out on leased sites in the Wadden Sea and in the Oosterschelde estuary (Smaal, 2002). In the Oosterschelde estuary, mussel beds (both wild and from aquaculture) play a major role in the cycling of nutrients and are able to filter the entire volume of the basin in 4-5 days (Prins and Smaal, 1994). In the last two decades, there has been an overall decline in available mussel seed due to intense fishing strategies that has forced local farmers to initiate the production of spats through hatchery techniques (Pronker et al., 2008)."

3) adding more information on the effect of delayed or decreased growth on the recruitment success and on the fitness of the population in the discussion section: "See above"

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4) extending the paragraph dealing with the regulation of the calcification process by mollusks in the discussion section:

"It is important to notice that even under aragonite undersaturated conditions, mussel larvae were able to produce a shell, highlighting that molluscs exert a control over calcification (McConnaughey and Gillikin, 2008) and are therefore not completely dependent on environmental conditions. This does not appear as a surprise since most freshwater molluscs are clearly well adapted to such conditions and bivalve growth has been showed by Tunnicliffe et al. (2009) under the extremely undersaturated conditions of deep hydrothermal sites. Most calcifying species, including molluscs, are able to concentrate Ca2+ and CO32- ions at the site of calcification. Adult molluscs appear to use conventional calcification physiology by pumping protons from the calcification site (extrapallial fluid: EPF), largely through Ca2+/2H+ exchange catalyzed by Ca2+ ATPase (McConnaughey and Gillikin, 2008). The elevation of pH in the EPF (Misogianes and Chasteen, 1979) allows an elevation of the concentration of CO32- that favours calcification. However, as this mechanism requires energy, this can lead to substantial energy shifts from other processes and to important costs for the growth of the organism as observed by Wood et al. (2008) for the brittlestar Amphiura filiformis. Although the regulation of calcification by this mechanism is well documented for adults, few studies have focused on the mechanisms of larval calcification and on the capacity of bivalve larvae to regulate calcification rates by controlling the carbonate chemistry at the site of calcification. There is, however, some indication that biomineralization of Mytilus edulis larvae is physiologically controlled, as the activity of the carbonic anhydrase, an enzyme that catalyses the reversible hydration of CO2 to HCO3- and H+, reaches a maximum at the end of each developmental stage connected with biomineralization (Medakovic, 2000). This study also reported that these larvae, as showed for other molluscs and echinoderms larvae (Weiss et al., 2002), produce mainly amorphous calcium carbonate (ACC) during the first 2-3 days of development and aragonite in the following days. As the solubility of ACC is 30 greater than that of aragonite (Brecevic and Nielsen, 1989), early larval stages should be much more vulnerable than

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older larval stages and adults that precipitate aragonite and/or calcite. Again, the fact that 2-days old larvae were able to produce a shell under aragonite undersaturation highlights the strong regulation capacity of these organisms under sub-optimal growth conditions."

We do not agree with the referee on the fact that we do not show a decrease in growth rates. Since we started the control and low pH incubations at the same time and sampled the organisms at the same time, the fact that shells were smaller at low pH means that there was a decrease in growth rates (we actually now show growth rates as a function of initial shell length as suggested by the referee). However, we agree that we cannot exclude the possibility that larvae would finally reach the same size before they settle and that this decrease in growth rates will translate in an increase in the time spent in the water column. We therefore extended the paragraph on this matter in the discussion section (see above)

4. Other questions/remarks

How long did the eggs and sperms were kept together before the fertilized eggs were filtered?

It is difficult to say how long they exactly stayed together. Mussels spawned in a flow-trough-tank so upon release the eggs flushed out of the tank and were retained on a 30 μm mesh sieve. This sieve was placed in a shallow bowl so the eggs were constantly submersed in water. Every 10 minutes or so we removed the eggs from the sieve, flushed them to get rid of sperm cells and placed them in our embryo-tanks.

How many males and females did actually spawn?

We spawned a group of approximately 150 mussels (as stated in the Material and Methods section). Since it was a mass-spawning and we did not remove any individuals from the tank upon spawning, it is impossible to know how many individuals spawned exactly.

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What about egg size?

Egg size was 57.7 $\pm$ 4.9 μm of diameter. This was added in the revised manuscript in the Material and Methods section. P2932L19: “After fertilization (see above), embryos (57.7 $\pm$ 4.9 μm of diameter) were counted, divided into 6 groups and transferred to the enclosures”

Adding pictures showing “normal” and “abnormal” larvae.

We do not believe a picture showing normal and abnormal larvae will bring a lot of information to the reader. Interested readers are invited to have a look at His et al. (1997) paper.

Many recently published papers are dealing with early life history stages.

The referee is absolutely right and this was corrected in the text.

P2929L2: It now reads as “Several experiments have shown a decrease of growth and calcification of organisms at decreased pH levels. There is a growing concern to focus on early life stages that are believed to be more sensitive to environmental disturbances such as hypercapnia.” P2931L11: “Most studies have investigated adult stages whereas the” has been removed

A recent paper from Watson et al. 2009 was actually added in the reference list (Watson, S.-A., Southgate, P. C., Tyler, P. A., and Peck, L. S.: Early larval development of the Sydney Rock oyster Saccostrea glomerata under near-future predictions of CO2-driven ocean acidification, Journal of Shellfish Research, 28, 431-437, 10.2983/035.028.0302, 2009.)

Replace “tosligh” by “to light”

The text was corrected. “a decrease of calcification due to slight undersaturation of seawater with respect to aragonite”

Fig. 2: final shell length is significantly smaller a low pH. Does that mean that the other

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days are not significant?

Shell lengths among treatments are not significantly different at day 6, 8 and 10. Shell lengths are significantly smaller at low pH after day 13. This was added in the text. Result section: "This relative decrease of shell length was statistically significant after day-13 of development. Growth rates, calculated as the difference in shell length between 2 sampling times divided by the time elapsed (d), decreased with increasing shell length (Fig. 2c) under both control and low-pH conditions. Statistically significant linear relationships between growth rates and initial shell length showed a shift to lower growth rates under low-pH conditions which was maintained throughout the experimental period."

Avoid using "alteration" and description such as "processes were diminished". P2937L1

We have tried our best to be more specific on the effects that were observed. This paragraphs now reads as:

"In the past few years, several papers have reported on the impacts of seawater acidification on the growth and development of shellfish early life stages. Kurihara et al. (2007; 2008) have demonstrated that a pHNBS decrease to $\sim$7.4 (-0.7 as compared to control values) caused a significant alteration of Crassostrea gigas and Mytilus galloprovincialis early (up to 6d) larval development, with significant decreases in hatching rates and shell growth. It has to be noted that at this pH level, which is much lower than the levels projected for the end of the century, the seawater was clearly undersaturated with respect to aragonite ($\sim$0.68). Parker et al. (2009) studied the synergistic effects of ocean acidification and temperature on the fertilization and early (up to 48h) embryonic development of the Sydney rock oyster (Saccostrea glomerata). These authors found that both fertilization and embryonic development success were diminished at lowered pH values in the range of projected levels for 2100 (pCO2 levels of 600, 750 and 1000 μatm) while temperature revealed an optimal level (26°C) below and above (i.e. 18,

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22 and 30°C) which embryonic developmental rates decreased. Talmage and Gobler (2009) performed a multi-species comparison of the effects of ocean acidification on growth and metamorphosis (from 4 to 18 days of development). They actually showed that, although the growth of the 3 studied species (Mercenaria mercenaria, Argopecten irradians, and Crassostrea virginica) was negatively affected, they did not exhibit the same sensitivity to a decrease of up to 0.6 pH unit. This species-specific sensitivity to ocean acidification has also been observed by Miller et al. (2009) who showed that the development (from 96h to ∼30d) and growth of the Eastern oyster (Crassostrea virginica) was significantly reduced at lowered pH levels (up to a 0.4 pHNBS unit decrease), while the Suminoe oyster (Crassostrea ariakensis) did not appear to be sensitive to the same acidified conditions."

On presenting the results of the literature in terms of pH decrease

The relative pH decreases and/or pCO2 levels have been included in the corresponding section (see above).

Presenting a summary table.

Our manuscript is not a review paper, we are confident that, following the large number of studies in that field of research that have been published or that will be published in the coming months, there will be an initiative to review the existing knowledge on the effect of OA on mollusks larval stages in the near future.

Replacing "environmental condition" in Appendix 1 and P2936 L10.

We have replaced "environmental parameters" by experimental abiotic parameters in these sections.

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