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## **Effect of ocean acidification on the early life stages of the blue mussel (*Mytilus edulis*)**

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## Abstract

Several experiments have shown a decrease of growth and calcification of organisms at decreased pH levels but relatively few studies have focused on early life stages which are believed to be more sensitive to environmental disturbances such as hypercapnia. Here, we present experimental data demonstrating that the growth of planktonic mussel (*Mytilus edulis*) larvae is significantly affected by a decrease of pH to a level expected for the end of the century. Even though there was no significant effect of a 0.25–0.34 pH unit decrease on hatching and mortality rates during the first 2 days of development nor during the following 13-day period prior to settlement, final shells were, respectively, 4.5±1.3 and 6.0±2.3% smaller at pH<sub>NBS</sub> ~7.8 than at a control pH<sub>NBS</sub> of ~8.1. Moreover, a decrease of 12.0±5.4% of shell thickness was observed. More severe impacts were found with a decrease of ~0.5 pH<sub>NBS</sub> unit during the first 2 days of development which could be attributed to a decrease of calcification due to slight undersaturation of seawater with respect to aragonite. Indeed, important effects on both hatching and D-veliger shell growth were found. Hatching rates were 24±4% lower while D-veliger shells were 12.7±0.9% smaller at pH<sub>NBS</sub> ~7.6 than at a control pH<sub>NBS</sub> of ~8.1. Although these results show that blue mussel larvae are still able to develop a shell in seawater undersaturated with respect to aragonite, decreases of hatching rates and shell growth suggest a negative impact of ocean acidification on the future survival of bivalve populations potentially leading to significant ecological and economical losses.

## 1 Introduction

The atmospheric partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) will continue to increase beyond the end of this century with projected values ranging from 500 to 1000 µatm, depending on the considered CO<sub>2</sub> emission scenario (IPCC, 2007). Because about one third of anthropogenic CO<sub>2</sub> emissions (from fossil fuel, cement production and land-use changes) has been stored in the oceans since the industrial revolution (Sabine et al.,

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2004), seawater pH has already declined by 0.1 unit compared with pre-industrial values (Orr et al., 2005) and it is projected to decrease by another 0.35 unit by the end of the century (Caldeira and Wickett, 2003). Ocean acidification may have profound impacts on marine biota. Beside the direct effect of decreasing pH on the physiology and metabolism of marine organisms through a disruption of inter-cellular transport mechanisms (see Pörtner et al., 2004, for a comprehensive review), calcareous organisms are particularly sensitive due to the decreasing availability of carbonate ions (CO<sub>3</sub><sup>2-</sup>) driven by increasing  $p\text{CO}_2$ . Indeed, this generates a decrease of the calcium carbonate saturation state ( $\Omega$ ):

$$\Omega = \frac{[\text{CO}_3^{2-}][\text{Ca}^{2+}]}{K'_{\text{sp}}}, \quad (1)$$

where  $K'_{\text{sp}}$  is the stoichiometric solubility product, which is a function of temperature, salinity, pressure and the mineral phase considered (calcite, aragonite or high-magnesian calcite). Cold waters will become undersaturated with respect to aragonite ( $\Omega_{\text{aragonite}} < 1$ ) in few decades (Orr et al., 2005). Since the seminal paper of Broecker and Takahashi (1966) reporting a dependency of calcification rates on CaCO<sub>3</sub> saturation state, several experimental studies have investigated the effect of a  $p\text{CO}_2$  increase on the growth of calcifying organisms. Most studies have investigated primary producers (corals, coralline algae and coccolithophores) and have shown a very large range of responses (Feely et al., 2004; Kleypas et al., 2006; Doney et al., 2009).

Among calcifying species, molluscs are very important both in ecological and economical terms. Shellfish are ecosystem engineers governing energy and nutrient flows in coastal ecosystems, providing habitats for many benthic organisms and constitute an important food source for birds (Gutiérrez et al., 2003; Norling and Kautsky, 2007). Moreover, with an average annual increase of 7.7% over the last 30 years, global shellfish aquaculture production reached 13.2 million tons in 2004, corresponding to a commercial value of US\$ 9.8 billion (FAO, 2007). The Pacific oyster (*Crassostrea gigas*) was the most cultivated species in 2004 with a volume of 4.6 million tons or 7.7% of

the total world aquaculture production while mussel production represented 1.9 million tons. A negative impact of ocean acidification on the growth of these species would, therefore, not only have also major consequences for coastal biodiversity and ecosystem functioning and services, but will also cause a significant economic loss (Gazeau et al., 2007; Cooley and Doney, 2009).

Several studies have focused on the effect of projected pH levels on the growth of benthic (e.g. Gazeau et al., 2007; Ries et al., 2009) and planktonic (e.g. Comeau et al., 2009) molluscs. Most of these studies have demonstrated a negative effect of ocean acidification on the growth of these organisms although recent experiments (Ries et al., 2009) have suggested a more complicated story with species-specific sensitivities to decreasing pH levels and positive effects on calcification rates in some cases. Most studies have investigated adult stages whereas the early life stages of calcifying organisms are generally considered to be more sensitive to environmental disturbances (Raven et al., 2005). Moreover, amorphous calcium carbonate (AAC) and aragonite have been identified as the main  $\text{CaCO}_3$  mineralization form in Molluscs larval stages (Medakovic, 2000). Therefore, as aragonite is 50% more soluble than calcite, these aragonitic larval stages are expected to be more sensitive to ocean acidification than calcitic organisms. Indeed, several recent studies have focused on the effect of ocean acidification on the early development of mollusc species (Kurihara et al., 2007, 2009; Ellis et al., 2009; Miller et al., 2009; Parker et al., 2009; Talmage and Gobler, 2009) and most of them have reported negative impacts of decreasing pH levels on the growth and development of these organisms. So far, there have been no studies on the effect of ocean acidification on the larval development of the blue mussel (*Mytilus edulis*), the second most cultivated bivalve species after *Crassostrea gigas*. This study aims to investigate the effects of future ocean pH levels on the development of these organisms.

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## 2 Material and methods

### 2.1 Test animals and experimental conditions

To investigate the effect of rising atmospheric  $\text{CO}_2$  on mussel (*Mytilus edulis*) larvae, experiments were carried out in mesocosms at the commercial hatchery *Roem van Yerseke* (Yerseke, The Netherlands) between 18 October and 27 November 2007. A group of approximately 150 ripe, rope-cultured mussels from the Oosterschelde, a tidal inlet, were kept at a constant temperature ( $10^\circ\text{C}$ ) for about 4 months. Before spawning, mussels (male and female) were cleaned with  $1\ \mu\text{m}$  filtered seawater and placed in a spawning tank. Mass spawning was initiated by rapidly raising water temperature from  $10^\circ\text{C}$  to  $19^\circ\text{C}$ . Fertilized eggs were retained on a submerged  $30\ \mu\text{m}$  sieve. During each experiment (see below), six enclosures were used, each of them containing 130 l of filtered ( $1\ \mu\text{m}$ ) seawater from the Oosterschelde. Three enclosures were continuously bubbled with ambient air ( $p\text{CO}_2 \sim 380\ \mu\text{atm}$ ) while the three others were bubbled with a mixture of ambient air and pure  $\text{CO}_2$ . The flow rates of  $\text{CO}_2$  were regulated by means of digital thermal mass flow controllers in order to reach the desired seawater pH.

### 2.2 Bioassay

In a first experiment (experiment #1), the effects of a pH decrease from  $\sim 8.1$  (control) to, successively,  $\sim 7.8$  and  $\sim 7.6$  were investigated during the first two days of development (from eggs to D-shape larvae). After fertilization (see above), embryos were counted, divided into 6 groups and transferred to the enclosures (3 controls, 3 low pH) at a density of approximately  $10\ \text{embryos ml}^{-1}$ . Embryos were maintained in batch conditions (no feeding, no water flowing) until the population reached the D-veliger stage (initial development of the shell, reached in about 2 days).

In a second experiment (experiment #2), larvae were exposed to pH values of  $\sim 8.1$  and  $\sim 7.8$  during the two weeks development period following the D-veliger stage. Embryos were grown at environmental pH ( $\sim 8.0\text{--}8.1$ ) during 2 days, then counted and

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evenly transferred to the 6 enclosures (3 controls, 3 low pH) at a density of approximately 10 embryos  $\text{ml}^{-1}$ . Cultivation period lasted for 13 days (day 2 to day 15 of development) until the population reaches the pediveliger stage. Larvae were fed in a continuous flow-through system with a mixture of *Isochrysis* sp. (T-Iso, CCAP 927/14) and *Chaetoceros muellerii* (CCMP 1332) (2:1, based on cell counts) at a concentration of approximately 80 000 cells  $\text{ml}^{-1}$ . From day 4 to the end of the experiment, larvae were fed with a mixture of *Isochrysis* sp., *Pavlova lutherii* (CCAP 931/1) and *Chaetoceros muellerii* (2:1:2, based on cell counts) at a concentration of approximately 150 000 cells  $\text{ml}^{-1}$ .

### 2.3 Sampling and analytical measurements

At the end of experiment #1 (day 2) and three times a week during experiment #2, the enclosures were emptied and cleaned with a mixture of diluted acetic acid and HCl. Water from the tanks was passed through a 90  $\mu\text{m}$  sieve and larvae were concentrated in 2-l jars. A sub-sample of 50 ml was fixed in a 5% neutralized-formalin seawater solution to determine the larval abundance, hatching rates (% of D-veliger larvae) and size. After sampling during experiment #2, we made sure that pH was constant and at the desired pH level before reintroducing the larvae in the enclosures.

Larval abundance was estimated based on triplicate counting of 500  $\mu\text{l}$  sub-samples, under a binocular microscope. Larvae shell length (measured on 100 individuals) was measured (anterior to posterior dimension of the shell parallel to the hinge) under a microscope (20 $\times$ ; 0.01  $\mu\text{m}$  precision in length measurement). Shell thickness was estimated from scanning electron micrograph (SEM) images acquired using the JEOL JSE 820 microscope at Cambridge University. Dried larval shells were mounted onto double sided carbon tape and sectioned using a flat edge needle. Loose organic matter and residual shell were removed with a dry paintbrush. Larval shells were removed using a wet paintbrush, reoriented and remounted onto fresh tape attached to aluminium stubs and gold coated. Shell thickness was determined on 20 individuals of each replicate treatment. Hatching rates were defined as the percentage of "normal"

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D-shape larvae following the criteria proposed by His et al. (1997), after observation of a minimum of 500 larvae.

During the two experiments, pH, temperature and oxygen concentrations were continuously monitored in each enclosure using Metrohm and Consort electrodes, which were calibrated daily on the N. B. S. scale for pH (pH 4 and 7). Salinity was measured at the beginning of each incubation periods (3) in each enclosure. Daily measurements of total alkalinity (TA) in the 6 enclosures were performed by Gran electro-titration on 50 ml samples filtered on GF/F membranes. Titrations of TA standard provided by A. G. Dickson (batch 82) were within 0.22  $\mu\text{eq kg}^{-1}$  of the nominal value ( $2334.8 \pm 3.3 \mu\text{eq kg}^{-1}$ ;  $n=5$ ).  $p\text{CO}_2$  was computed from  $\text{pH}_{\text{NBS}}$  and TA using the software package  $\text{CO}_2$  (M. Frankignoulle) and the thermodynamic constants of Mehrbach et al. (1973). The solubility products for calcite and aragonite were from Morse et al. (1980).

### 2.4 Statistical analysis

For final shell length (experiment #1 and #2) and shell thickness (only experiment #2), differences between replicates of each treatment as well as between control and low  $\text{pH}_{\text{NBS}}$  treatments were tested using one-way ANOVA after testing for normality (Kolmogorov-Smirnov test). No significant differences were found in any of these parameters between the replicate tanks within each experimental condition. For hatching rates and abundances, as only one value was estimated per replicate, this latter was used to obtain grand means and standard deviations (SD) values for each treatment. Since normality tests could not be applied due to the small sample size, differences of hatching rates between control and low  $\text{pH}_{\text{NBS}}$  conditions were tested by means of unpaired Student's t-tests using a Welch correction that does not assume equal variance between the two groups (Graphpad InStat software). For all tests, differences were considered significant at  $p < 0.05$ . In the following section, data are presented as means  $\pm$  SD.

### 3 Results

The environmental parameters as well as parameters of the carbonate chemistry are shown in Table 1. During experiment #1,  $\text{pH}_{\text{NBS}}$  was maintained, during the first set of incubations, at  $8.15 \pm 0.01$  (Control-1) and  $7.81 \pm 0.01$  (Low pH-1) corresponding to  $p\text{CO}_2$  values of 468 and 1124  $\mu\text{atm}$ , while during the second set of incubations, larvae were kept at  $\text{pH}_{\text{NBS}}$   $8.09 \pm 0.01$  (Control-2) and  $7.58 \pm 0.01$  (Low pH -2), corresponding to  $p\text{CO}_2$  values of 537 and 1929  $\mu\text{atm}$ . During experiment #2, seawater  $\text{pH}_{\text{NBS}}$  was maintained at  $8.03 \pm 0.03$  (Control,  $p\text{CO}_2 = 642 \mu\text{atm}$ ) and  $7.78 \pm 0.05$  (Low pH,  $p\text{CO}_2 = 1213 \mu\text{atm}$ ). Results obtained during these 2 experiments are presented in Figs. 1, 2 and 3. Data used for these figures as well as environmental parameters ( $\text{pH}_{\text{NBS}}$ , TA and temperature) measured at the time of sampling are presented in Appendix 1 (available as Supplementary Material at <http://www.biogeosciences-discuss.net/7/2927/2010/bgd-7-2927-2010-supplement.pdf>). In the Oosterschelde tidal inlet (5 stations, monthly measurements), surface  $\text{pH}_{\text{NBS}}$  varies annually between 8.00 and 8.24, while TA varies between 2.334 and 2.567  $\text{meq kg}^{-1}$  (data not shown). In the fall (that is the time of the experimental period),  $\text{pH}_{\text{NBS}}$  and TA in the tidal inlet are, on average, 8.04 and 2.436  $\text{meq kg}^{-1}$ , respectively. Significantly higher  $\text{pH}_{\text{NBS}}$  values have been obtained for the control tanks in our experiments due to the continuous bubbling with external air ( $\sim 380 \mu\text{atm}$ ), especially during experiment #1 which was performed in batch conditions. During experiment #2, the continuous flow-through system did not allow such an efficient equilibration with air, leading to significantly lower  $\text{pH}_{\text{NBS}}$  values ( $8.03 \pm 0.03$ ), closer to environmental levels. It must be stressed that the  $p\text{CO}_2$  levels for low- $\text{pH}_{\text{NBS}}$  treatments were slightly outside the range projected for the end of the century (500–1000  $\mu\text{atm}$ ) and must be considered as extreme conditions. The objective of the experiment was to test the effect of a  $\sim 0.3 \text{ pH}_{\text{NBS}}$  unit decrease of these organisms and, in that sense, experimental conditions were successfully set up and controlled.

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During experiment #1, a seawater  $\text{pH}_{\text{NBS}}$  decrease of 0.34 unit ( $\text{pH}_{\text{NBS}} = 7.81$ ;  $\Omega_{\text{aragonite}} = 1.38$ ) had a significant effect on mussel larvae development (Fig. 1). Although no significant effect was found on hatching rates (unpaired Student's t-test,  $p > 0.05$ ), the average shell lengths at the end of the 2-day incubation period at  $\text{pH}_{\text{NBS}}$  7.81 were significantly lower (ANOVA,  $n=100$ ,  $p < 0.001$ ) than at higher  $\text{pH}_{\text{NBS}}$ . The relative decrease in shell length after 2 days has been estimated to  $4.5 \pm 1.3\%$ . In contrast, a decrease of 0.51 unit ( $\text{pH}_{\text{NBS}} = 7.58$ ;  $\Omega_{\text{aragonite}} = 0.81$ ) had large effects on both the hatching and growth rates. The hatching rates decreased by  $24 \pm 4\%$  (unpaired Student's t-test,  $p < 0.001$ ), while D-veliger shells were  $12.7 \pm 0.9\%$  smaller (ANOVA,  $n=100$ ,  $p < 0.001$ ). From day 2 to day 15 (experiment #2, Figs. 2 and 3), a decrease of seawater  $\text{pH}_{\text{NBS}}$  by 0.25 ( $\text{pH}_{\text{NBS}} = 7.78$ ;  $\Omega_{\text{aragonite}} = 1.37$ ) also did not have significant effects on larvae survivorship (unpaired Student's t-test,  $n=3$ ,  $p > 0.05$ ) while a significant effect was found for final shell length (ANOVA,  $n=100$ ,  $p < 0.001$ ), corresponding to a relative decrease of  $6.0 \pm 2.3\%$ . Effects on final shell thickness were more important with a significant (ANOVA,  $n=20$ ,  $p < 0.001$ ) relative decrease of  $12.0 \pm 5.4\%$ .

### 4 Discussion

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  $\text{pH}_{\text{NBS}}$  decrease to  $\sim 7.4$  caused a significant alteration of *Crassostrea gigas* and *Mytilus galloprovincialis* early (up to 6 d) larval development. 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 48 h) embryonic development of the Sydney rock oyster (*Saccostrea glomerata*). These authors found that both fertilization and embryonic development were altered at lowered pH values in the range of projected levels for 2100 while temperature revealed an optimal level ( $26^\circ\text{C}$ )

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below and above which both of these processes were diminished. 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 96 h to ~30 d) and growth of the Eastern oyster (*Crassostrea virginica*) was significantly reduced at lowered pH levels (up to a 0.4 pH<sub>NBS</sub> unit decrease), while the Suminoe oyster (*Crassostrea ariakensis*) did not appear to be sensitive to the same acidified conditions.

In this study, we show that ocean acidification has a significant effect on the blue mussel larval development although the observed decrease in growth rates both in terms of length and thickness was not accompanied by a decrease of hatching rates and an increase in mortality rates as long as seawater remained oversaturated with respect to aragonite. Although no effect on hatching and mortality rates have been observed after 2 d and after 15 d of development, the consequences, in the field with the presence of predators, of a potential decrease of shell resistance due to a reduction in growth as observed for a 0.25–0.34 pH unit decrease, are still unknown. At pH<sub>NBS</sub> ~ 7.6, both hatching success and growth rates (as estimated by shell length decrease after 2 d of development) exhibited an important decrease coinciding with a slight undersaturation of seawater with respect to aragonite. 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 (Miller et al., 2009) 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 Ca<sup>2+</sup> and CO<sub>3</sub><sup>2-</sup>

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ions at the site of calcification. However, as this mechanism, obviously 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*.

At this point, it can only be speculated that the observed decrease in larval developmental success for a ~0.5 pH<sub>NBS</sub> unit decrease is due to the seawater undersaturation with respect to aragonite. It must be stressed that our data do not allow discriminating between the physiological impact of pH decrease alone via a disruption of inter-cellular transport mechanisms and the impact on calcification resulting from aragonite undersaturation, on the larval development of this species. More studies are needed to disentangle these different aspects of the potential effect of ocean acidification on marine organisms.

As the different pressures exerted by the environment and predators in the field result in a considerable mortality, approaching 99% (Bayne, 1976) during the free-swimming larval period, an additional 24% decrease in hatching rates as observed at a pH<sub>NBS</sub> of 7.6 can therefore compromise the survival of the population. Indeed, relatively small fluctuations in the abundance of these larval stages are known to regulate the size of the population (Green et al., 2004). Shellfish predominantly inhabit coastal regions, which usually exhibit lower pH values than the open ocean because of permanent or episodic low pH water inputs from rivers (Salisbury et al., 2008). While many estuaries already have high and variable pCO<sub>2</sub>, atmospheric CO<sub>2</sub> enrichment will shift the baseline toward even higher values (Miller et al., 2009) that could lead to extended periods of undersaturation with respect to aragonite. Therefore, these species will undoubtedly be exposed to suboptimal growth conditions in the coming years. In order to assess socio-economic and ecological effects of ocean acidification on shellfish, it is therefore crucial to predict accurately the evolution of pH as well as the saturation state of the ocean and its coastal waters with respect to aragonite in the near future.

Our observation of no significant effect of a ~0.3 pH<sub>NBS</sub> unit decrease on both hatching rates and survivorship stands in contrast with results obtained by Parker et

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al. (2009) on *Saccostrea glomerata* during the first 48h of development and by Talmage et al. (2009) on *Mercenaria Mercenaria* and *Argopecten irradians* between the veliger and metamorphosed stages (18–20 days). Our results are consistent with those from Talmage et al. (2009) on *Crassostrea virginica* who observed significant effects on growth rates but no significant effects on survivorship for a ~0.3 pH unit decrease. Altogether, these different studies reveal, as what is observed for adult stages, that the effects of ocean acidification of molluscs early life development are species-specific (Kurihara, 2008) and that the sensitivity of the organisms might depend on the pH variability at which they are naturally exposed in the field.

In the present study, we show that shell increase, by linear extension, which is the most commonly measured parameter in ocean acidification related studies for molluscs larvae, should not be the only measured parameter if one wants to investigate the effects of acidification on shell growth. Indeed, shell thickness appeared to be affected as well with a relative decrease twice the relative decrease observed in shell length. This is consistent with Miller et al. (2009) findings who estimated a much higher decrease of shell weight (estimated as the amount of calcium in the shells) than shell area (respectively –42 and –16% between pre-industrial and end of 21st century projected pH level for *C. virginica*). However, it must be stressed that, in our study, shell thickness measurements could not be performed on D-veliger (2 days old) larvae and appeared to be limited to large pediveliger larvae using our protocol. Calcium content in the shells, as an estimator of shell weight, is also an interesting parameter to follow and has been successfully applied by Miller et al. (2009). However, again, this technique has been applied to large metamorphosed larvae, and there is still a need to test its validity for smaller veliger larvae. Finally, more precise techniques such as <sup>45</sup>Ca labelling, recently used on pteropods (planktonic molluscs; Comeau et al., 2009) are promising and might be valuable tools to accurately evaluate the effect of ocean acidification within the range of expected levels for 2100 on calcification rates of mollusc early life stages.

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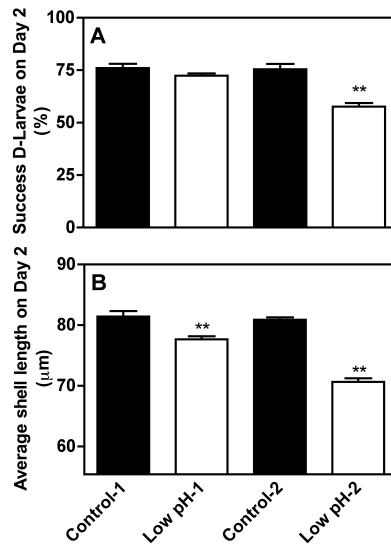
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**Table 1.** Environmental parameters and carbon chemistry of experimental seawater during the course of the different experiments.  $p\text{CO}_2$  and related carbonate parameters were computed from mean  $\text{pH}_{\text{NBS}}$  and Total Alkalinity during each incubation.

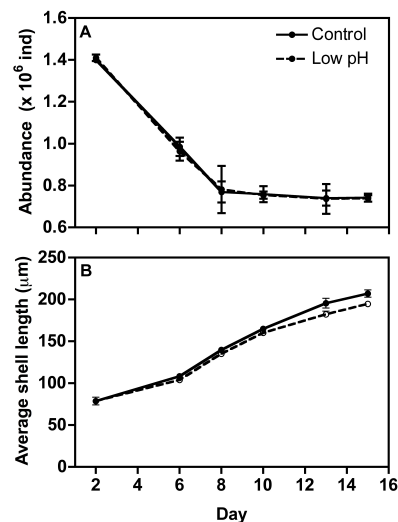
	Control-1	Experiment #1: day 0 to day 2		Experiment #2: day 2 to day 15		
		Low pH-1	Control-2	Low pH-2	Control	Low pH
Measured parameters						
Temperature (°C)	16.6±1	16.7±0.8	16.5±1	16.2±1.2	19.5±0.3	19.2±0.4
Salinity	32.1±0.1	31.9±0.2	32.0±0.1	32.1±0.1	31.4±0.3	31.5±0.1
Oxygen (% of saturation)	100±3	94.7±2.6	96.4±2.5	97.6±0.6	91.3±6.9	91.8±4.4
pH <sub>NBS</sub>	8.15±0.01	7.81±0.01	8.09±0.01	7.58±0.01	8.03±0.03	7.78±0.05
Chlorophyll- <i>a</i> (µg l <sup>-1</sup> )	—	—	—	—	29.12±8.87	31.12±12.55
Total alkalinity (meq kg <sup>-1</sup> )	2.486±0.006	2.483±0.006	2.436±0.006	2.437±0.004	2.402±0.02	2.403±0.02
Calculated parameters						
<i>p</i> CO <sub>2</sub> (µatm)	468	1124	537	1929	642	1213
DIC (mmol kg <sup>-1</sup> )	2.254	2.397	2.237	2.432	2.218	2.321
[HCO <sub>3</sub> <sup>-</sup> ] (mmol kg <sup>-1</sup> )	2.071	2.273	2.073	2.312	2.062	2.200
[CO <sub>3</sub> <sup>2-</sup> ] (mmol kg <sup>-1</sup> )	0.166	0.084	0.144	0.049	0.135	0.080
Ω <sub>aragonite</sub>	2.75	1.38	2.39	0.81	2.30	1.37
Ω <sub>calcite</sub>	5.12	2.58	4.45	1.52	4.27	2.54

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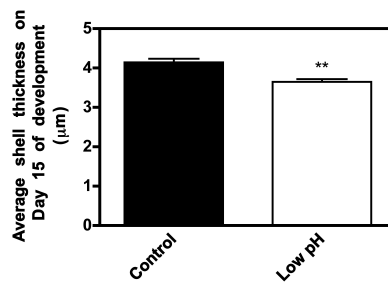
**Fig. 1.** Proportion of embryos that developed to D-shape larvae (**A**) and average length of D-shape shell (**B**) at the end of the two incubation periods during experiment #1, in control (black bars) and low pH (white bars) seawater. During the first incubation, seawater pH<sub>NBS</sub> was maintained at  $8.15 \pm 0.01$  (Control-1) and at  $7.81 \pm 0.01$  (Low pH-1). During the second incubation, pH<sub>NBS</sub> levels of  $8.09 \pm 0.01$  and  $7.58 \pm 0.01$  were used (Control-2 and Low pH-2, respectively). Errors bars represent standard deviations of the triplicate enclosures. \*\*Significant difference between control and low-pH groups.

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**Fig. 2.** Abundance (**A**) and average shell length (**B**), during experiment #2 (13 days, from D-shape to pediveliger larvae), in control (solid line, black dots) and CO<sub>2</sub> (dotted line, white dots) seawater. Seawater pH<sub>NBS</sub> was maintained at  $8.03 \pm 0.03$  and  $7.78 \pm 0.05$  in control and low pH enclosures, respectively. Errors bars represent standard deviations of the triplicate enclosures.

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**Fig. 3.** Average shell thickness at the end of experiment #2 for larvae on day 15 of development, in control ( $\text{pH}_{\text{NBS}}=8.03\pm0.03$ ; black bars) and low pH ( $\text{pH}_{\text{NBS}}=7.78\pm0.05$ ; white bars) seawater. Errors bars represent standard deviations of the triplicate enclosures. \*\*Significant difference between control and low-pH groups.