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Effects of sea-water acidification on fertilization and larval development of the oyster *Crassostrea gigas*

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ABSTRACT

Oceans face a serious change to their natural biogeochemical cycle due to the rapid absorption of CO_2 generated by human activities. Ocean acidification is the common term used to describe the decrease of seawater pH caused by the absorption of atmospheric CO_2. To evaluate the effects of ocean acidification, we focused on the larval stage of bivalves, which produce a fragile calcareous skeletal structure, very sensitive to changes in seawater chemistry. In this context, we investigated sperm motility, fertilization rate and larval viability (survival, growth and abnormalities) of the Pacific oyster, *Crassostrea gigas*, a commercially important bivalve, in a controlled CO_2 perturbation experiment. The carbonate chemistry of seawater was manipulated by diffusing pure CO_2, to attain two reduced pH levels ($\Delta pH = -0.4$ and $\Delta pH = -0.7$) which were compared to unmanipulated seawater. The results show high sensitivity of *C. gigas* veliger larvae to low values of pH, as reflected by a decrease in survival and growth rates, as well as an increased frequency of prodissoconch abnormalities and protruding mantle. Moreover, results also show that sperm motility, fertilization rate, and hatching success, were negatively influenced by acidification. The exposure to $\Delta pH = -0.7$ had a higher impact on the fertilization and larval viability than $\Delta pH = -0.4$. The results suggest that the reproductive success and the biological mechanisms for calcification may be prematurely interrupted and disturbed when *C. gigas* veliger larvae are exposed to an acidified environment which may reduce their viability and compromise settlement and future abundances of this species.

1. Introduction

In the last 250 years, human activities have been producing greenhouse gas emissions and increasing the atmospheric concentration of carbon dioxide by a rate of approximately 0.5% year^{-1} from 180–300 ppmv (prior to industrial revolution, Siegenthaler et al., 2005) to 380 ppmv (Fabry et al., 2008; Forster et al., 2007). This acceleration of the natural CO_2 flux has created a surplus of CO_2 in the ocean surface, causing noticeable changes in global temperatures, climate and ocean carbon chemistry. The oceans absorb about 25–26% of global carbon emissions and without this ocean sink the rising trend in atmospheric CO_2 would have been even faster (*e.g.* Feely et al., 2009; Sabine et al., 2004) and the present concentration would be about 55% higher (Fabry et al., 2008).

Ocean acidification refers to the increase of hydrogen (H^+) and bicarbonate (HCO_3^-) ions, with the consequent reduction of pH and carbonate (CO_3^{2-}) ion concentration. Currently, the average surface seawater pH is 8.1 (already 0.1 units lower than in 1750, Bernstein et al., 2007; Raven et al., 2005). According to estimates based on the IPCC emission scenarios, pH will further decline between 0.3 and 0.5 units during the 21st century. The unrestricted burning of fossil fuels may cause a more extreme decrease, of 0.7 units from current values, by the year 2300 (Caldeira and Wickett, 2003). The reduction of 0.1 units on ocean pH corresponds to a 30% increase of $[H^+]$ in the seawater (Bindoff et al., 2007) and a pH downfall of 0.3 or 0.4 units is equivalent to a 150% increase of $[H^+]$ and 50% decrease of $[CO_3^{2-}]$ (Doney et al., 2009; Orr et al., 2009). Despite the fact that these reactions are thermodynamically reversible (Feely et al., 2004; Millero et al., 2002), Gattuso et al. (2009) consider that ocean acidification, as it is happening today, is irreversible on a human time scale.

Seawater carbonate chemistry is extremely important for the mineral formation of calcium carbonate ($CaCO_3$), which is a crucial structural element of shells and skeletons of marine calcifying organisms (Fabry et al., 2008). If ocean acidification continues to evolve at the present rate, the surface of the Arctic Ocean will be undersaturated with respect to aragonite (the more soluble crystalline form of $CaCO_3$) by the year 2050, with atmospheric CO_2 over 500 ppm and undersaturated with respect to calcite by the year 2100, with atmospheric CO_2 over 800 ppm (Gattuso et al., 2009). Feely et al. (2004) predicted that, by the year

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2300, the entire ocean surface would be undersaturated for aragonite. Experimental studies have shown that changes of the magnitude predicted by the IPCC scenarios can significantly reduce calcification rates, as well as other aspects of the physiology of planktonic and benthic marine organisms (see reviews by Fabry et al., 2008; Guinotte and Fabry, 2008; Hendricks et al., 2010; Kleypas et al., 2006; Widdicombe and Spicer, 2008).

Among the marine calcifiers predicted to be affected by ocean acidification, bivalves are of most concern due to their important role in marine and coastal ecosystems, their contribution to the inorganic carbon cycle as carbonate producers and mostly for being first level consumers. In many coastal areas, bivalves are also important economic resources for fisheries and aquaculture. The potential for significant ecological and economic impacts arising from the effects of ocean acidification on bivalves and the need for further research on commercially important species has been explicitly recognized (Cooley et al., 2012; Gazeau et al., 2007; Kleypas et al., 2006).

The composition of an adult bivalve shell consists of calcite, aragonite or both, however, all shells of bivalve larvae contain aragonite (Weiss et al., 2002), the most soluble form of $CaCO_3$. The segregation and mineralization of the first larvae shell is initiated during the trochophore larvae stage through a specialized group of ectodermic cells (Iwata, 1980; Kniprath, 1981; Weiss et al., 2002). These cells invaginate to form the shell gland and the mantle whilst the remaining surface cells start to produce the outermost organic shell layer, the periostracum (*i.e.* a thin layer that covers the shell permeable to Ca^{2+} and inorganic carbon, Weiss et al., 2002). The calcification of the shell layers occurs between the mantle and the larvae shell, in the extrapallial fluid (McConnaughey and Gillikin, 2008), evolving the larvae to the veliger stage featuring a distinct "D" shaped embryonic translucent shell named prodissoconch I (Zardus and Martel, 2002). The veliger larvae is a very common stage in marine bivalves and also extremely sensitive to the surrounding environment. The prodissoconch I is generally believed to be composed by amorphous calcium carbonate (ACC), a very unstable and soluble form of $CaCO_3$ that quickly transforms to aragonite (Weiss et al., 2002), although this widespread perception has been recently challenged (Kudo et al., 2010).

Studying the effect of ocean acidification on early life-stages on marine invertebrates is essential to understand and predict future consequences to the ecosystem. The present study aims to evaluate the effect of seawater acidification by CO_2 on fertilization and larval development of the oyster *Crassostrea gigas*.

2. Methods

2.1. Experimental conditions

The experimental set up was adapted from Range et al. (2011), incorporating some adjustments to accommodate the larval stages. It was installed in a bivalve hatchery in Tavira, Portugal, (37°7′ 17.73″N, 7°37′12.19″W), operated by IPMA, Instituto Português do Mar e da Atmosfera. The hatchery is supplied with seawater from the Ria Formosa lagoon, which is sand-filtered and stored in a 200,000 L concrete reservoir. In order to assure adequate oxygenation and stable pH during the experiment, seawater was aerated for 2–3 days in 2000 L fiber-glass tanks before being transferred to the 250 L header tanks (also fiber-glass).

Three levels of pH were considered: one control pH ($\Delta pH = 0.0$) and two manipulated pH levels ($\Delta pH = -0.4$ and $\Delta pH = -0.7$), with separate flow-through systems for each of these levels. Seawater in the 250 L header tanks was filtered through a 1 μm sieve and used to supply the 20 L conic larval tanks. The pH of seawater in the acidified treatments was adjusted to the predetermined set-points by diffusing pure gaseous CO_2 into closed reactors (Aqua Medic-reactor 1000), installed between the header tanks and the larval tanks. The flux of gas from the CO_2 tanks to the reactors was controlled using a pHstat system (Aqua Medic AT Control), by opening or closing a solenoid valve when the pH readings in the larval tanks deviated from the predetermined set-points by ±0.05 pH units. Seawater flow was regulated manually by PVC valves, at approximately 9 L h^{-1}, with renewal in the larval tanks exceeding 10 times per day. Temperature, salinity and pH of seawater were recorded twice per day in the larval tanks using dedicated electrodes and the data-logger function of the AT-Control unit. The automatic pH electrodes were routinely calibrated with NIST buffers (pH 7.0 and pH 9.0, Merck-CertiPUR®). These automatic readings were validated against manual determinations (in all the tanks) with an YSI Pro Plus multiprobe, standardized against Tris seawater buffers. The physical–chemical characteristics of seawater during the experiment are expressed in Table 1.

2.2. Bioassay

On April 8th, thirty wild adults of Pacific oyster, *C. gigas*, were collected from an intertidal clam rearing bank in the Ria Formosa lagoon (36°59′N; 7°55′W, South Portugal) and immediately transported to the hatchery. The oysters were at ripe stage and oocyte and sperm were obtained by stripping the gonads from 8 females and 11 males into separate beakers with filtered (5 μm) and sterilized (UV) seawater with natural pH. The sperm suspension was sieved with 100 μm mesh, to remove tissue debris, feces and shell waste subsequently kept at 4 °C to prevent degradation. The oocytes pooled from all females were separated into three beakers rinsed over a sieve of 100 μm mesh size and collected in a sieve of 20 μm. The three samples of oocytes were then suspended in three different levels of seawater pH: the control level ($\Delta pH = 0.0$) and two reduced levels ($\Delta pH = -0.4$ and $\Delta pH = -0.7$). The oocytes suspensions were kept at room temperature (20 °C) for 2 h to enhance maturation (germinal vesicle breakdown), with occasional stirring to prevent anoxia. The number of oocytes per treatment was counted and fertilization was done by adding the sperm mixture at a ratio of about ten spermatozoids to each oocyte.

The fertilized and non-fertilized oocytes were counted in three samples of 1 mL 2, 3 and 4 h after fertilization to evaluate the fertilization rate. The embryos were then transferred from each treatment beaker to 3 replicate larval rearing tanks, at a density of 60 embryos mL^{-1} and incubated for 48 h. After this time, the D-shape larvae were collected on a 20-μm mesh sieve. Hatching and abnormal veliger rates were calculated, relative to the initial number of embryos, based on three 0.1 mL samples. The remaining larvae were returned to the larval tanks where they were reared. The larvae were fed with *Isochrysis aff. galbana* (T-ISO) through a continuous-flow system at

Table 1
Seawater carbonate chemistry variables (mean ± SE, n = 3); pH (total scale), temperature (T) and salinity (S) values were automatically recorded twice per day; total alkalinity (TA) was not measure during the experiment, but is the average of 84 previous measurements on the seawater supply of the hatchery (Range et al., 2011, 2012); dissolved inorganic carbon (DIC); partial pressure of CO_2 in seawater (pCO_2) and saturation state for calcite (Ω_{cal}) and aragonite (Ω_{ara}) were calculated from *in situ* temperature and salinity, corrected pH and TA, using CO_2calc (Robbins et al., 2010).

pH level	T (°C)	S	pH_{TS}	TA (μmol kg^{-1})	DIC (μmol kg^{-1})	pCO_2 (μatm)	$\Omega_{cal.}$	$\Omega_{ara.}$
$\Delta pH = 0.0$	17.88 ± 0.16	30.56 ± 0.08	8.09 ± 0.01	3550 ± 2	3230 ± 5	580 ± 11	6.57 ± 0.09	4.20 ± 0.06
$\Delta pH = -0.4$			7.76 ± 0.01		3424 ± 5	1386 ± 32	3.37 ± 0.07	2.16 ± 0.04
$\Delta pH = -0.7$			7.37 ± 0.02		3600 ± 8	3573 ± 151	1.46 ± 0.06	0.93 ± 0.04

a concentration of 50 to 100 cells μL^{-1}. The sampling procedure for larvae was repeated at 96 and 148 h post fertilization.

At each time, samples were taken from each tank in order to estimate mortality and percentage of abnormal D-shape larvae. Fifty randomly selected larvae were also taken from each replicate and conserved in 4% formaldehyde solution to evaluate shell growth. Photomicrographs were taken to measure shell length (anterior to posterior length of the shell, parallel to the hinge line) and height (dorsal to ventral, perpendicular to the hinge) (Kurihara et al., 2008) and to evaluate the morphology of the larvae.The criteria used to differentiate between normal and abnormal veliger larvae were: shell degradation, presence of a convex hinge and mantle protuberances. The mean was calculated using the three replicates in each treatment except for mortality in the control and frequency of abnormalities in the control and $\Delta pH = -0.4$, where only two replicates were used. Larval growth rate was calculated through simple linear regression.

2.3. Statistical analyses

To analyze the consequences of pH decrease on the fertilization and larval development, differences in mortality and growth were tested by analyses of variance (ANOVA) or Kruskal–Wallis ANOVA on ranks, whenever the assumptions of ANOVA failed. Percentage data were arcsine transformed to normalize variance. Multiple pairwise comparisons among means were done on significant effects using the post-hoc parametric Tukey or Duncan's test or the non-parametric Dunn test. For all tests, differences were considered significant at $p<0.05$. Statistical analyses were done with Sigmastat© software.

3. Results

The percentage of fertilized eggs was substantially reduced for $\Delta pH = -0.7$ relative to the other treatments with just 41% of fertilized eggs present after 4 h. In contrast, the percentage of fertilized eggs exceeded 80 and 90% in the $\Delta pH = -0.4$ and the $\Delta pH = -0.0$ treatments during the same 4 h period. Although the sperm activity could not be quantified, a decrease in *C. gigas* sperm motility and velocity were observed under the microscope, showing slower sperm when exposed to acidified seawater. This phenomenon was accentuated in $\Delta pH = -0.7$. Results for hatching rate were very similar with 93% of hatching veligers in the control seawater, 87% for $\Delta pH = -0.4$ and 77% for $\Delta pH = -0.7$ (Fig. 1). However, statistical differences were found between the control ($\Delta pH = 0.0$) and $\Delta pH = -0.7$ treatments (ANOVA, $F = 10{,}332$, $df = 2$, $p = 0.011$; Duncan's method $- p<0.05$).

Concerning the mortality of larvae (Fig. 2), significant differences were found (K–W, $H = 15.70$, $df = 2$, $p \leq 0.001$; Dunn's test $- p<0.05$) between larvae reared in the control seawater and CO_2 manipulated seawater, with 83% and 98% mortality in $\Delta pH = -0.4$ and $\Delta pH = -0.7$, respectively, in contrast to 34% observed in the control at 144 h after fertilization (end of the experiment).

The percentage of abnormal D-shape larvae after the incubation was very low in the control treatment (<20%), compared to 54% in $\Delta pH = -0.4$ and nearly 70% in $\Delta pH = -0.7$ (Fig. 3). At 96 h after fertilization, the percentage of D-shape veliger slightly decreased in the control and $\Delta pH = -0.4$ treatments, however, there were significant differences among the three treatments (ANOVA, $F = 22.38$, $df = 2$, $p \leq 0.001$, Tukey test $- p<0.05$). The range of morphology of *C. gigas* seen in the treatments during the trial is shown in Table 2.

Larval growth (length) is shown in Fig. 4 and Table 3. Shell length of the D-shape veliger larvae was significantly smaller in the acidic seawater treatments (length: K–W, $H = 267.023$, $df = 2$, $p \leq 0.001$; height: K–W, $H = 233.199$, $df = 2$, $p \leq 0.001$), showing a distinct difference, 48 h after fertilization, in the three treatments. Despite the fact that the growth rate was similar in each treatment (1–2 $\mu m\ d^{-1}$), 144 h after fertilization, the length of the larvae in the control seawater was 10 μm larger (94.3 ± 1.1 μm) than in the CO_2 manipulated treatments which did not differ between them (84 ± 1.3 μm for $\Delta pH = -0.4$ and 83 ± 2.6 μm for $\Delta pH = -0.7$). Analogous results were found for larvae height.

4. Discussion

Our results put in evidence that the gametes of *C. gigas* react to acidification when exposed to realistic pH reductions ($\Delta pH = -0.4$ and $\Delta pH = -0.7$). Similarly, Havenhand et al. (2008) observed a decrease in sperm activity, 11.7% in velocity and 16.3% in motility, for the sea urchin *Heliocidaris erythrogramma*, when exposed to 7.7 pH. These findings support the hypothesis proposed by Kurihara (2008b), that seawater acidification would affect intracellular pH of the egg and sperm and alter sperm motility, fertilization and embryo development. In contrast, Havenhand and Schlegel (2009) did not found significant perturbations in the sperm activity of *C. gigas* when exposed to a pH of 8.1–7.8. Overall these results emphasize that, although biological membranes are extremely permeable to CO_2 (Gutknecht et al., 1977), the sensitivity of marine invertebrates to seawater acidification varies

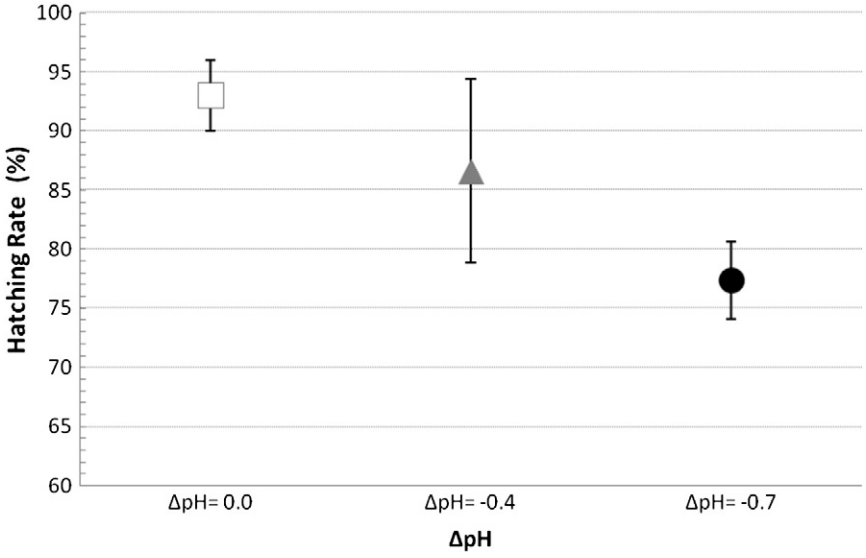


Fig. 1. Hatching rate (%) for *Crassostrea gigas*, 48 h after fertilization, in the control treatment ($\Delta pH = 0.0$), $\Delta pH = -0.4$ and $\Delta pH = -0.7$ treatments (mean ± SE, n = 3).

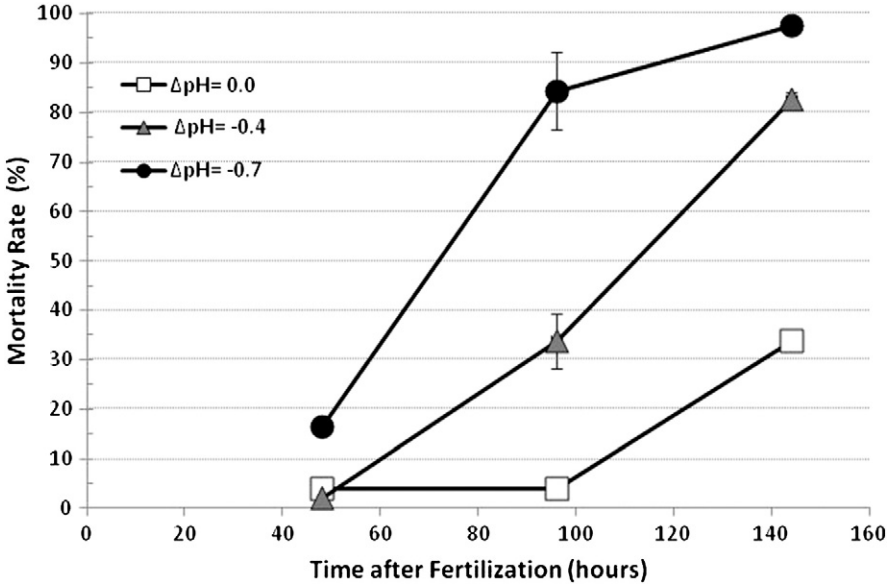


Fig. 2. Evolution of mortality (%) on *Crassostrea gigas* larvae in the control (ΔpH = 0.0), ΔpH = −0.4 and ΔpH = −0.7 treatments (mean ± SE; n = 3).

substantially, among different species and between local populations of the same species (Byrne, 2011; Range et al., 2012; Ross et al., 2011).

Adverse effects were also observed in the present study, regarding the fertilization rate of *C. gigas*, with decreases of 10% in ΔpH = −0.4 and 50% in ΔpH = −0.7. This suggests that the sperm was not functional to penetrate the egg or the egg was not healthy for fertilization or both. These negative effects have been previously documented for the oyster *Saccostrea glomerata*, at a seawater pH of 7.6 and temperature of 18–30 °C (Parker et al., 2009), although in that study acid was used do adjust the pH instead of CO_2.

The hatching rate decreased significantly by 16% in ΔpH = −0.7 when compared to the control treatment, however the effect in hatching rate was not so evident at ΔpH = −0.4. Kurihara et al. (2007) reported a decrease in the hatching rate for *C. gigas* in seawater of pH 7.4. Similar results were obtained for the mussels *Mytillus galloprovincialis* (Kurihara et al., 2008) and *Mytillus edulis* (Gazeau et al., 2010) when exposed to pH values of 7.4 and 7.5, respectively. In contrast, Gazeau et al., 2011 found that the hatching rate of *C. gigas* was not directly affected by changes in pH, aragonite or calcite saturation states of seawater (controlled by $CaCl_2$ addition), but was strongly decreased when total alkalinity, carbonate and bicarbonate ion concentrations were reduced by HCl addition. As emphasized by Gattuso and Lavigne (2009), the carbonate chemistry manipulation used by Gazeau et al. (2011) in T4 (*i.e.*, decreased alkalinity and bicarbonate ion concentration) does not fully mimic the changes associated with ocean acidification. Nevertheless, given the elevated alkalinity of seawater in our experiment (Table 1), the hypothesis proposed in the latter study provides an interesting framework to interpret our results.

The larvae of *C. gigas* exhibited a distinct pattern of mortality in the three treatments. Mortality was higher in the ΔpH = 0.7 treatment, possibly because of the large quantity of abnormal veligers that did not survive. At the end of the experiment, the mortality rate was nearly 100% in this treatment. This suggests that, not only abnormal veliger have difficulties in surviving, but also normal veliger were metabolically

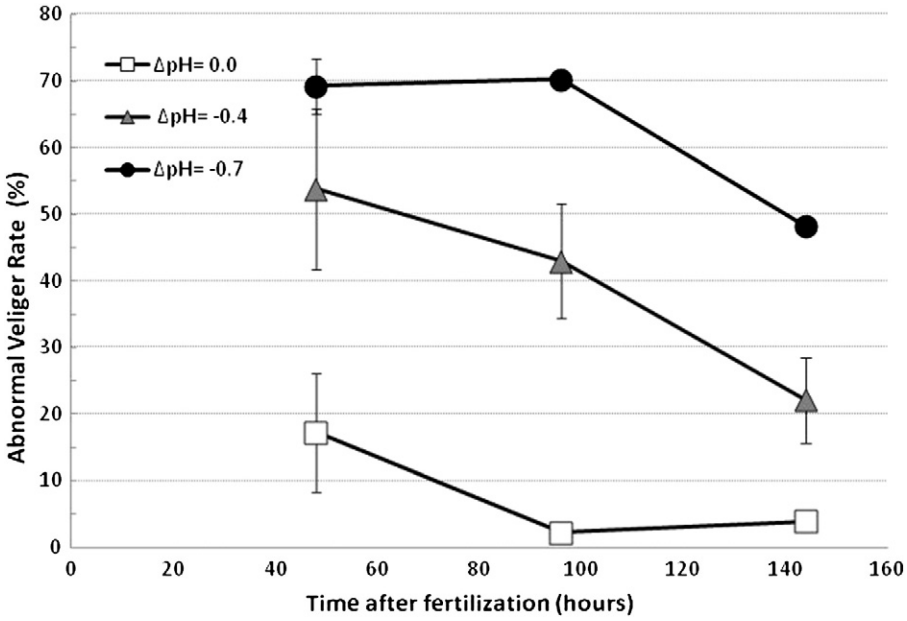
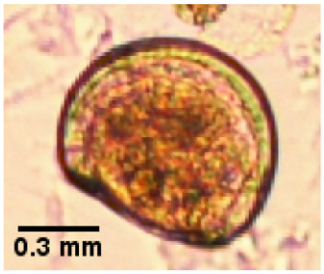
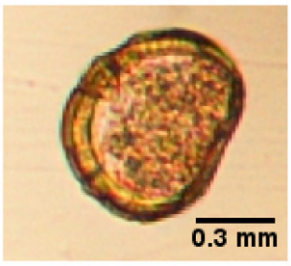
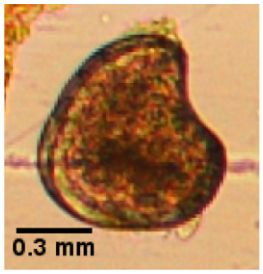
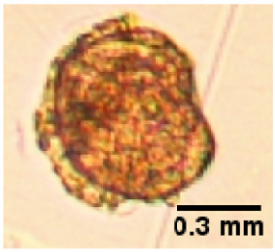


Fig. 3. Abnormalities (%) in *Crassostrea gigas* veliger in the control treatment, (ΔpH = 0.0), ΔpH = −0.4 and ΔpH = −0.7 treatments (mean ± SE; n = 3).

Table 2
Changes in morphology of *Crassostrea gigas* larvae, observed during the experiment.

Normal veliger	Shell degradation	Convex hinge	Mantle protuberances
			

or morphometrically sensitive to high-CO_2 seawater. The large energetic requirements needed to build a shell under sub saturated conditions for may have contributed to this effect. Reductions of larval survival were also described by Parker et al. (2012) for wild *S. glomerata*, with 10% less than the selective bred larvae, and Watson et al. (2009), with a decrease in survival of 72% in pH 7.6, relative to the control (pH 8.1). In contrast, Timmins-Schiffman et al. (in press) found no differences in the mortality of *C. gigas* larvae exposed to 400 and 1000 ppm CO_2, up to 3 days post-fertilization. Recently, Melzner et al. (2011) showed that under environmental stress (high pCO_2 and limited food) the mussel *M. edulis* allocated its energy input to more vital processes such as maintaining its somatic mass. This leads to believe that CO_2 increases larval mortality not only through physical fragilities of the larvae but also indirectly, by shifting its energy requirements (Findlay et al., 2011).

The present study revealed that exposure to high-CO_2 seawater, particularly at more extreme levels ($\Delta pH = -0.7$), leads to increased frequencies of morphological abnormalities of the D-shape *C. gigas* larvae, when compared to larvae in the control treatment. Microscopic observations provided evidences of irregularities on shell formation, such as deformation of the dorsal margin line, convex hinge, asymmetry and fissures on the prodissoconch, suggesting deficiencies in the calcification process. Larval abnormalities have been previously reported in mollusks and echinoderms exposed to seawater acidification by CO_2 (*e.g.* Brennand et al., 2010; Dupont et al., 2008; Gazeau et al., 2007; Kleypas et al., 2006; Kroeker et al., 2010; Kurihara et al., 2007, 2008; Parker et al., 2010). According to these authors, the morphological abnormalities could be due to two possibilities: (a) damage to the embryonic ectodermic cells rendering them unable to produce sufficient amorphous calcium carbonate, which is crucial in the development of a strong and stable shell, or (b) dissolution of the shell due to corrosion by seawater. In our study, the mantle protuberance was predominant at $\Delta pH = -0.7$, mostly during the first 48 h after fertilization. This evidence is thought to be due to an incomplete calcification process, with defaults on the Ca^{+} transport process, enough not to cover the entire mantle of the larva (Kurihara, 2008). This feature may also decrease the swimming capability of the larva and therefore decrease its fitness (Beiras and His, 1994; Kurihara et al., 2008).

In addition to morphological abnormalities, CO_2 acidification inhibited shell growth of *C. gigas* larvae. Length and height differed between the control and CO_2 manipulated treatments, tending to decrease with pH. Other studies with mussel and oyster larvae support this tendency (Kurihara et al., 2007, 2008; Talmage and Gobler, 2009, 2011; Watson et al., 2009). More recently, growth and production of *C. gigas* larvae have been found to be negatively correlated with pH and aragonite saturation of the water supply in a commercial shellfish hatchery (Barton et al., 2012). According to Anil et al. (2001) and Gazeau et al. (2010), smaller size during the initial developing stages of marine organisms affects their fitness in the juvenile stage, reducing the competitive capability and increasing mortality after fixation. In addition, it is assumed that smaller larvae cannot feed as well as larger ones, becoming more susceptible to starvation which compromises

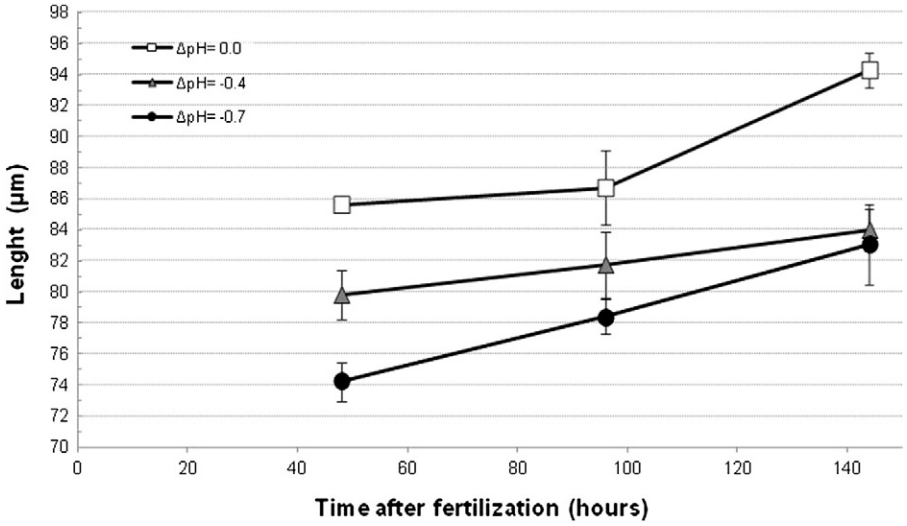


Fig. 4. Shell length (µm) for *Crassostrea gigas* larvae, in the control treatment ($\Delta pH = 0.0$), $\Delta pH = -0.4$ and $\Delta pH = -0.7$ treatments (mean ± SE; n = 3).

Table 3
Crassostrea gigas larvae length, larvae height and growth rate (per hour and per day) in the three treatments (ΔpH = 0.0, ΔpH = −0.4 and ΔpH = −0.7); R^2 corresponds to the statistical coefficient of determination (mean ± SE; n = 3).

144 h after fertilization	ΔpH = 0.0	ΔpH = −0.4	ΔpH = −0.7
Mean larval length (μm)	94.3	84.0	83.0
Larval length growth rate (μm h^{-1})	0.08995	0.04328	0.09185
Larval length growth rate (μm d^{-1})	2.1588	1.03872	2.2044
R^2	0.8411	0.9979	0.999
Mean larval height (μm)	82.5	71.5	71.4
Larval height growth rate (μm h^{-1})	0.10828	0.05926	0.09155
Larval height growth rate (μm d^{-1})	2.59872	1.42224	2.1972
R^2	0.94	0.9926	0.9277

their survival (Anger, 1987; Hart and Strathmann, 1995; Kurihara et al., 2007; Strathmann, 1987). Some of these detrimental effects may, however, be attenuated by the capacity of the organisms to acclimate or adapt to elevated pCO_2 over the next century (Parker et al., 2012), so caution is advised when extrapolating results to natural populations.

The value of total alkalinity (3550 μmol kg^{-1}, Table 1) used to calculate seawater carbonate chemistry parameters was not directly measure during the study, but is the average of 84 previous measurements on the same hatchery (Range et al., 2011, 2012). Although it largely exceeds the typical oceanic alkalinity (2325 μmol kg^{-1}, Gattuso and Lavigne, 2009; Barton et al., 2012), this value was well within the range observed during field sampling in the Ria Formosa lagoon (2415 to 3606 μmol kg^{-1}). The carbonate chemistry of coastal waters is known to be affected by continental runoff and by the mineral composition of the drainage basins (Borges and Gypens, 2010). The Ria Formosa basin is mainly constituted by carbonate rocks, which contribute to the strong mineralization and supersaturation of the groundwater with respect to carbonate minerals (Almeida and Silva, 1987; Stigter et al., 2006). Accordingly, groundwater discharges, evaporation and nutrient exchanges with sediments are probably increasing total alkalinity within this coastal lagoon, at least in areas of restricted exchange, which may locally attenuate some of the detrimental effects of CO_2-induced seawater acidification on marine calcifiers.

5. Conclusion

Global climate change has acidified and warmed the oceans, trends that are projected to continue during this century. Oceans face a serious change to their biogeochemical cycle due to the rapid absorption of CO_2 generated by human activities. The results obtained in this study demonstrate that fertilization, hatching and larval development (survival and growth) of *C. gigas* were negatively influenced by CO_2 driven acidification, as projected to occur in the surface ocean by the year 2100 and beyond. The probability that other bivalves may suffer similar effects is high, since the larval stage is very similar among different species of his group. These effects were apparent despite the naturally elevated alkalinity of seawater from the Ría Formosa, which may contribute to offset the local impacts of ocean acidification. Adaptation could be a possibility but, given the accelerating rate of ocean acidification, it seems questionable if there will be enough time for most species to adapt. In this scenario, coastal ecosystems could be facing severe adjustments in the near future. Given the greater variability of seawater carbonate chemistry in nearshore coastal habitats, relative to the open ocean, these should be priority areas in future investigations to assess the generality and underlying mechanisms determining the sensitivity of marine organisms to ocean acidification.

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