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Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816)

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ABSTRACT

The effect of pH ranging from 8.0 to 6.8 (total scale – pH_T) on fertilization, cleavage and larval development until pluteus stage was assessed in an intertidal temperate sea urchin. Gametes were obtained from adults collected in two contrasting tide pools, one showing a significant nocturnal pH decrease (lowest pH_T = 7.4) and another where pH was more stable (lowest pH_T = 7.8). The highest pH_T at which significant effects on fertilization and cleavage were recorded was 7.6. On the contrary, larval development was only affected below pH_T 7.4, a value equal or lower than that reported for several subtidal species. This suggests that sea urchins inhabiting stressful intertidal environments produce offspring that may better resist future ocean acidification. Moreover, at pH_T 7.4, the fertilization rate of gametes whose progenitors came from the tide pool with higher pH decrease was significantly higher, indicating a possible acclimatization or adaptation of gametes to pH stress.

1. Introduction

The continuous increment of anthropogenic carbon dioxide emissions is inducing changes in seawater carbon chemistry, lowering its pH. This phenomenon is known as ocean acidification. Since the industrial revolution, the average surface seawater pH has already been reduced by approximately 0.1 units. Expected surface pH reductions are of around 0.4 units by 2100 and 0.77 units by 2300 (Caldeira and Wickett, 2003, 2005; IPCC, 2007). Some particular environments already present lower pH such as upwelling zones (Feely et al., 2008), coastal areas (Wootton et al., 2008), the deep-sea (Park, 1966; Millero, 1996) and volcanic carbon dioxide vents (Hall-Spencer et al., 2008). Tide pools also undergo significant variations of their physicochemical conditions, including pH (due to pCO_2 fluctuations). Truchot and Duhamel-Jouve (1980) reported in rocky tidal pools (Roscoff, Brittany, France) a night increment of pCO_2, accompanied by a decrease of pH until 7.29. This is caused by the community respiration and absence of photosynthesis. During daytime, the tendency is inverted. These daily fluctuations depend on season, tide duration and algal cover (Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983; Denny and Gaines, 2007). The same authors also reported an increase of total alkalinity at night which was associated with calcium carbonate dissolution due to low pH. Thus, intertidal pools offer an interesting model where organisms are exposed to a succession of pH fluctuations and may possibly present adaptations (linked to genetic variability at a population level) and/or acclimatization (due to phenotypic plasticity at an individual level) processes to face them.

Early development stages of marine invertebrates (fertilization, embryogenesis and larval development) are generally the most sensitive life phases to environmental stresses (Pörtner and Farrell, 2008; Melzner et al., 2009; Dupont et al., 2010). Recruitment success depends on the survival of the embryos and larvae (López et al., 1998) and, consequently, any decrease in embryo and larval survival or delay in development can reduce population long-term viability (Morgan, 1995). Sea urchins are key species in many coastal ecosystems, being important grazers, and the sustainability of their populations is vital (Paine, 1966; Harrold and Pearse, 1987; Leblanc et al., 2005). Several studies showed that fertilization and early development stages of sea urchins can be negatively impacted by ocean acidification which causes a decrease of fertilization and cleavage rates and/or a reduction of the pluteus larva size (Kurihara and Shirayama, 2004; Havenhand et al., 2008; Clark et al., 2009). A down regulation of genes involved in calcification, cellular stress response, metabolism and apoptosis were reported in *Strongylocentrotus* larvae raised in low pH seawater (Todgham and Hofmann, 2009; O'Donnell et al., 2010). However, larvae of *Strongylocentrotus droebachiensis* raised at lower pH (7.9 and 7.7) were more successful in reaching metamorphosis than those raised at control pH (8.0), although it took them longer to reach this stage (Dupont and Thorndyke, 2008). It is noteworthy that a slower development can result in higher plantktonic mortality due to increased predation exposure and desynchronization with algal

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blooms, decreasing recruitment success (Morgan, 1995; Elkin and Marshall, 2007). Nonetheless, in other studies, no effect of ocean acidification was observed on fertilization and embryogenesis (Byrne et al., 2009a,b, 2010). These facts suggest that actually the response of early life-history stages appears to be highly species-specific and differs even in closely related taxa (Dupont and Thorndyke, 2008, 2009; Clark et al., 2009). This fact emphasizes the need for a survey of specific effects of acidification as broad as possible and to understand the origin of the observed differences of sensitivity to ocean acidification between species. It is also essential not to disregard the fact that long-term exposure of adults to lower pH can affect gonad growth (Siikavuopio et al., 2007; Kurihara, 2008), reproductive success and future larval performance (Dupont and Thorndyke, 2008; Kurihara, 2008).

In this work, we studied the sea urchin *Paracentrotus lividus*, an important grazer species (Bulleri et al., 1999) with a broad distribution and that can be found in the whole Mediterranean and North Atlantic coasts of Europe (from Morocco to Scotland), inhabiting intertidal rock pools, seagrass meadows and shallow subtidal shores (Boudouresque and Verlaque, 2001). Furthermore, this species shows a high gene flow over extended distances (Duran et al., 2004; Calderón et al., 2008). The aim of this study was to understand if pH extreme oscillations to which *P. lividus* adults from rock tide pools are submitted could have an influence on fertilization, embryonic and larval development of their progeny. The strategy was to compare the effect of pH on the progeny of individuals collected from the same shore, i.e. same population, but from distinct tide pools: one where night pH was significantly reduced and the other where this decline was not so important.

2. Materials and methods

2.1. Study site and measure of physicochemical parameters

Observations were done in two tide pools, distant of around 2 m, in Aber, Crozon peninsula (48°14′N; 04°27′W, southern Brittany, France), in April 2009, i.e., during the spawning period of *Paracentrotus lividus* populations in this region (Mercier and Hamel, 2009). Intertidal adult individuals occurred in tide pools and showed a sedentary behavior in self burrowed holes. They are thus partially protected from wave action and never get emersed during low tide. Previous tagging experiments (data not shown) confirmed this population sedentary behavior. New recruits are found every year in this population (Catarino and Dubois, personal observation).

The physicochemical parameters of two tide pools were measured every half an hour starting at pool individualization (ebb tide) until its cover (rising tide) during two night and two day low tides: temperature, salinity and pH_{NIST} (National Institute of Standards and Technology), also known as NBS (previous National Bureau of Standards, now NIST) scale. The temperature and pH_{NIST} were measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor) calibrated with pH_{NIST} buffers 4 and 7 (Merck CertiPUR®, Darmstadt, Germany). Even though pH variation within each pool never exceeded 0.1 units, a pH cycle measurement was always done on the same spot. The salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). Sea water samples were collected at the beginning and end of each low tide and immediately filtered (0.22 μm) in order to determine total alkalinity (TA). This was carried out by a potentiometric titration with HCl 0.1 M using a Titrino 718 STAT Metrohm (Switzerland), and calculated using the Gran function (Gran, 1952). Our measurements had a deviation of 0.65% of the standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control laboratory. Aragonite and calcite saturation values (Ω_{ar} and Ω_{cal}, respectively) and pCO_2 were determined from TA, pH_{NIST} and salinity data using the software CO2SYS (Pierrot et al., 2006) and by using the dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and KSO_4 using Dickson (1990).

2.2. Gonad maturity

Ten *P. lividus* individuals with a minimum diameter of 30 mm were collected from each tide pool, transported to the laboratory in tide pool water and dissected on the same day. A piece of gonad was removed and fixed in Bouin's fluid. The gonads were then dehydrated, embedded in paraffin, cut in 7 μm sections (Leica RM 2155 microtome) and stained with Masson's trichrome. The gonad maturity was estimated on a scale of 1–8 based on morphological characteristics according to the method of Spirlet et al. (1998).

2.3. Fertilization and larval development experiments

Thirty individuals were collected from each tide pool and kept at pH_{NIST} 8.13, 12.7 °C and 32.1 PSU until the beginning of the experiment 14 days later. All experiments were conducted in a temperature controlled room at 14 °C and in filtered seawater (0.22 μm) from the study site. The pH of the seawater was adjusted by bubbling CO_2 (Air Liquide) until the required pH was obtained.

The pH_{NIST}, the electromotive force (e.m.f) and the temperature of the seawater were measured at the start and at the end of each experiment with the same pH meter as previously described. These values and sequential measurements of the e.m.f. of the cell using standard buffers of known pH, 2-aminopyridine/HCl (AMP) and tris/HCl (TRIS) were applied on the calculation of the pH expressed in total scale (pH_T) (DOE, 1994; Del Valls and Dickson, 1998; Dickson et al., 2007). The salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). The TA, pCO_2, Ω_{ar} and Ω_{cal} were determined in each vial as described in Section 2.1.

To induce spawning, ca. 1 ml of 0.5 M KCl was injected into the perivisceral cavity of individual sea urchins. Gametes of 5 males and 5 females from each tide pool, selected according to their gamete quantity and quality (shape), were collected in control pH seawater. Gametes of the same sex were gently mixed in order to have a homogeneous batch and to avoid individual variations.

The Lowest Observed Effect Concentration (LOEC; results in nominal pH), i.e. the highest pH at which the considered end point significantly differed from that at control pH (see e.g. Rand, 1995), was calculated for the fertilization rate, cleavage rate, larval morphology and rod size. The LOEC was also determined for other sea urchin species from contrasting environments, based on literature data (Byrne et al., 2009a,b, 2010; Clark et al., 2009; Havenhand et al., 2008; Kurihara et al., 2004; Kurihara and Shirayama, 2004; O'Donnell et al., 2010). Only works in which pH was manipulated by CO_2 addition and for which data for determining a LOEC were available were included.

2.3.1. Fertilization and cleavage

Fertilization was conducted in Petri dishes by mixing diluted sperm and eggs at selected pH_T (control 8.0, 7.6, 7.4 and 6.8). Three replicates by pH for each tide pool were produced. Embryos were randomly sampled from each treatment at different times (10, 20, 30, 60, 90 and 120 min) and fixed in Bouin's fluid and 200 embryos were observed in each replicate using an optical microscope. Fertilization was defined as the presence of an elevated normal fertilization membrane (15 min after gamete mixing) and cleavage as the presence of minimum 2 blastomeres (1 h after gamete mixing). At pH_T 6.8, embryos presenting a very thin membrane not clearly visible or with a fertilization membrane not surrounding completely the embryo were observed. They were counted as