



Effects of ocean acidification on juveniles sea urchins: Predator-prey interactions



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ABSTRACT

Increasing CO₂ concentration in the atmosphere during the last decades has led to a significant decrease in ocean pH. Organisms that need carbonate to build their calcareous skeletons could be severely affected. In this study we focused on the effects of ocean acidification on juveniles of the sea urchins *Paracentrotus lividus* and *Diadema africanum*. We assessed the effects of decreased pH on two skeletal structures, spines and test, and their impacts on species performance to avoid predation events in the field. Juveniles of both study species were exposed for 100 days to two treatments of pH: a pH of 8.0 (413.2 μatm) and pH of 7.6 (1349 μatm). Our results showed that *D. africanum* juveniles from the acidic treatment were more predated than those kept in the control treatment. These differences were not observed between treatments in *P. lividus*. *Diadema africanum* may be more sensitive to the indirect effects of ocean acidification on predator avoidance than *P. lividus*. However juveniles reared in a pH of 7.6 showed changes in shape in skeletal structures in both species. Considering these results in future scenarios, *P. lividus* may be considered a “winning species”, and *D. africanum* a “losing species” in the climate change stake.

1. Introduction

In the last decades there has been an increase in CO₂ levels in the atmosphere. This increase in atmospheric CO₂, caused mainly by human emissions, has led to a global modification of the seawater carbonate system, and has caused a decrease in ocean pH of up to 0.1 units between the years 1750 and 2000 (Gatusso and Hanson, 2011). It is predicted that there will be a further decrease of ~0.4 pH units before the end of the century (IPCC, 2013). By the end of the 23rd century, there may have been a decrease of ~0.7 units (Caldeira and Wicket, 2003; Orr et al., 2005). When the concentration of CO₂ is raised in seawater, it changes the equilibrium between bicarbonate and carbonate ions, and increases the concentration of hydrogen ions in the water. The new environment then causes an increase in the dissolution rate of deposited calcium carbonates. Organisms that need carbonate to build their calcareous shells and skeletons, such as corals, gastropods and sea urchins are well known to be particularly sensitive to acidic conditions (Gatusso et al., 1999; Kleypas and Langdon, 2006; Anthony et al., 2008; Andersson et al., 2011). Seawater with increased acidity has been found to limit growth and calcification rates of many organisms (Ries et al., 2009; Asnaghi et al., 2013a; Courtney et al., 2013; Wolfe et al., 2013). These changes in seawater chemistry can

modify the morphology of skeletal structures of diverse organisms, such as some gastropods that lose their shells under acidic conditions (Coleman et al., 2014; Kroeker et al., 2014) or algae of a certain genus such as *Corallina*, in which external calcification is hindered resulting in higher rates of herbivory (Brodie et al., 2014). The skeletal structural defenses can be used to deter predators (Vermeij, 1993) and their direct modification as a consequence of environmental changes could indirectly affect predator-prey interactions and disproportionately influence the community structure in the future (Zarnetske et al., 2012).

In recent years, an increasing number of studies have investigated the effects of ocean acidification on sea urchin species (see Dupont and Thorndyke, 2013). However, most existing studies have focused their research on direct effects (see Kroeker et al., 2014; Byrne, 2011) and few studies have evaluated indirect effects of ocean acidification, such as predation or predator-prey interactions in future scenarios of climate change (Ferrari et al., 2011; Gaylord et al., 2015; Ghedini et al., 2015).

Juvenile sea urchins are highly susceptible to ocean acidification (Shirayama and Thornton, 2005; Byrne, 2011; Albright et al., 2012; Dupont et al., 2012; Asnaghi et al., 2013a, 2013b; Wolfe et al., 2013; Byrne et al., 2014), mainly due to the high growth rates of this stage of the life cycle. Growth and calcification are both processes affected by changes in seawater pH. It is thought that the skeletal structure and

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mineralogy of spines and tests can be modified by acidification (Bray et al., 2014). These changes in skeletal structure and composition can increase the vulnerability of certain echinoids to predation and specially at one the most vulnerable life cycle stages as is juvenile stage (Clemente et al., 2007); potentially leading to a decrease in the abundance of species that play key roles in maintaining ecosystem structure (Hernández et al., 2008). Ocean acidification can therefore have indirect effects on the functioning of whole ecosystems (Shirayama and Thornton, 2005; Asnaghi et al., 2013a, 2013b) if abundances of key species are substantially modified.

In this study we addressed the effects of ocean acidification on juvenile sea urchins and the potential indirect influence on major trophic relationships within benthic food webs. We hypothesized that predation pressure will increase on juveniles sea urchins reared at low pH as consequence of low pH on skeletal structures.

We focused our study on juvenile stages of two key sea urchin species, *Diadema africanum* (Rodríguez et al., 2013) and *Paracentrotus lividus* (Lamarck, 1816) which are the most frequent sea urchins species in rocky habitats off the Canary Islands (Hernández et al., 2013). The effects of ocean acidification on juvenile *D. africanum* and *P. lividus* have not been investigated before, except in one single study carried out in the Mediterranean Sea. In that study, Asnaghi et al. (2013b) found a direct relationship between acidification and *P. lividus* growth, vulnerability to predation (test robustness), and fitness.

In the Canary Islands *D. africanum* is the predominant sea urchin in rocky subtidal habitats where their population densities can reach 24 individuals/m², causing the elimination of erect algal beds and the prevalence of unvegetated bottoms as result of its intense grazing. In contrast *P. lividus* is more abundant in intertidal habitats and first meters of the subtidal, most frequently found up to 10 m deep within algal cover and well-illuminated environments (Hernández et al., 2013). This consistent depth-dependent partitioning pattern of distribution of both species on rocky reefs, in which *D. africanum* is the competitive dominant species at the Canary Islands (Tuya et al., 2007), has been related to their distinct morphological characteristics, and consequently, their different resistance to natural hydrodynamic forces (Tuya et al., 2007). Moreover, *D. africanum* is a thermophilus species, which probably constitutes a competitive advantage for its proliferation at the sublittoral latitude of the Islands in contrast to sea urchins of temperate affinities such as *P. lividus*. The interplay of predatory interactions and recruitment dynamics on both sea urchin species have been previously assessed (Girard et al., 2008; Hernández et al., 2010; Clemente et al., 2010, 2011.), finding important species-specific differences, which may be responsible for the huge differences in populations sizes of both species at the Canary Islands. Increases in sea water temperature is known to enhance the survivorship of *D. africanum* recruits (Hernández et al., 2010) and act as a relevant factor that, in conjunction with the overfishing of sea urchin predators (Clemente et al., 2010), favor the recent outbreak of densities of the sea urchin. On the other hand, the Canary Islands are the southern-most limit of distribution of the temperate sea urchin *P. lividus*, and in fact anomalous warm summers have been associated with localized mass mortalities at several intertidal and subtidal locations in the Tenerife Island (Girard et al., 2012).

The specific aims of our study were: 1) determine whether predation pressure in the natural environment differed between groups of juvenile sea urchins reared at two different levels of pH; 2) assess differences in the skeletal morphological structure of the sea urchins incubated at different seawater pH levels in the laboratory and 3) elucidate how these morphological changes, driven by ocean acidification, affect the predator avoidance abilities of the two echinoid species in the field.

2. Material and methods

2.1. Experimental set up

Juveniles of the sea urchins *Paracentrotus lividus* and *Diadema africanum* (4–11 mm in test diameter) were collected by scuba diving at two localities around Tenerife Island, at depths of 2–7 m: *P. lividus* juveniles were collected beneath boulders in La Caleta (28° 16' 20.33" N, 16° 23' 4.43" W), in October 2012, a site with rocky bottoms characterized by algal stands with a mixture of algal species., *Diadema africanum* were collected in September 2012 in Abades (28° 8' 31.05" N, 16° 26' 12.49" W), which was dominated by urchin barrens, by means of looking for juveniles inside crevices in the substrate. Both localities are known to experience high recruitment of the two species during the months of September and October (Hernández et al., 2010; Girard et al., 2008) and have a mean environmental seawater pH of 8.1 ± 0.1 units (Hernández et al., 2015; Hernández, 2016) Collected juveniles were measured with calipers, with a precision of ± 0.10 mm, and then kept in the laboratory for an acclimatization period of 10 days. During this time, sea urchins were fed with a mixture of *Ulva compressa* and *Dyctiota* sp., highly palatable algal species. Two hundred juveniles of each species were divided between two different pH treatments (NBS scale): half were incubated in current environmental pH conditions (pH = 8.1, control treatment), and the other half at a lower pH to represent the future predicted scenario (pH = 7.6, low pH treatment). Previously the juveniles reared at pH 7.6 units were acclimated from pH 8.1 to pH 7.6 during 7 days to avoid a shock-response. The system consisted of two 130 l tanks, one the control (pH = 8.1), and the other the low pH (pH = 7.6). In the low pH treatment, seawater pH was maintained with a computerized system (AquaMedic) that regulated pH by bubbling pure CO₂ directly into the water, to a resolution of ± 0.01 pH units. A seawater temperature of 19 °C was kept constant using thermostat coolers (ECHEIM AQUATIC, 50 W, with a precision ± 0.5 °C), which corresponded to the mean SST in winter months of November–February in Canary Islands. The same seawater conditions were maintained at a constant level in each tank until the end of the experiment, with duration of 100 days. Every 5 days throughout the experiment, the water was completely replaced with fresh filtered and sterilized seawater, prepared at the appropriate pH conditions for each treatment. Salinity, temperature and pH were measured five times per week during the experiment, using a salinometer (COND 315i) and temperature and pH sensors (Metrohm mobile meter with a Primatrode NTC IP pH electrode and temperature sensor). The pH console was calibrated on a weekly basis, with three standards (NBS scale). Seawater total alkalinity (TA) was measured by titration in each treatment on three separate occasions over the course of the experiment (Metrohm, 665 Dosimar, Switzerland), using an open cell titration system and following the Standard Operation Procedure 3b (Dickson et al., 2007). Other seawater carbonate chemistry parameters (pCO₂, calcite saturation state (Ω_c)) and aragonite saturation state (Ω_a) were calculated from TA and pH results, using CO2sys software (Lewis and Wallace, 1998). Calculations for pCO₂, Ω_c and Ω_a, were based on a set of constants K1 and K2 from Mehrbach et al. (1973), refitted by Dickson and Millero (1987). During the 100 day of experiment, all sea urchins were fed ad libitum with a mix of *U. compressa* and *Dictyota* sp.

2.2. Predation experiments

At the end of the 100 day laboratory experiment, a subset of 36 *P. lividus* (in February of 2013) and 36 *D. africanum* (in January of 2013), were transported back to their natural environment for in situ predation experiments. Daytime observations of predation events, separately for each studied species, were made while SCUBA diving Two localities were selected, along the southern coast of Tenerife, Abades and Boca Cangrejo (28° 24' 24.25"N, 16° 18' 44.99" W). Both localities were chosen due to the high abundance of juvenile urchins already in barren

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