



Long-term mesocosms study of the effects of ocean acidification on growth and physiology of the sea urchin *Echinometra mathaei*



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ABSTRACT

Recent research on the impact of ocean acidification (OA) has highlighted that it is important to conduct long-term experiments including ecosystem interactions in order to better predict the possible effects of elevated $p\text{CO}_2$. The goal of the present study was to assess the long-term impact of OA on a suite of physiological parameters of the sea urchin *Echinometra mathaei* in more realistic food conditions. A long-term experiment was conducted in mesocosms provided with an artificial reef in which the urchins principally fed on algae attached to the reef calcareous substrate. Contrasted pH conditions (pH 7.7 vs control) were established gradually over six months and then maintained for seven more months. Acid–base parameters of the coelomic fluid, growth and respiration rate were monitored throughout the experiment. Results indicate that *E. mathaei* should be able to regulate its extracellular pH at long-term, through bicarbonate compensation. We suggest that, within sea urchins species, the ability to accumulate bicarbonates is related to their phylogeny but also on the quantity and quality of available food. Growth, respiration rate and mechanical properties of the test were not affected. This ability to resist OA levels expected for 2100 at long-term could determine the future of coral reefs, particularly reefs where *E. mathaei* is the major bioeroder.

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1. Introduction

Over the last century, human activities have produced large amounts of CO_2 through fossil fuel burning, intensive agriculture and deforestation. Changes in ocean surface water chemistry (pH and carbonate saturation decrease) has been evidenced and unequivocally linked to the rise of atmospheric CO_2 concentration (IPCC, 2013). This raised concerns about the possible impact of these changes on marine organisms.

Responses of organisms to ocean acidification (OA) greatly differ according to taxa (e.g. Wittmann and Pörtner, 2013). Due to their low metabolism and poor osmoregulation abilities, sea urchins have been considered as victims of OA (Dupont et al., 2010; Melzner et al., 2009; Pörtner et al., 2004). However, studies carried out so far

produced contrasted results. Some adult sea urchins appear to be able to maintain the pH of their coelomic fluid (the main extracellular fluid compartment in echinoderms) when facing a decrease of the seawater pH (Dupont and Thorndyke, 2012; Calosi et al., 2013; Collard et al., 2013, 2014; Moulin et al., 2014; Stumpp et al., 2012; Uthicke et al., 2014). On the contrary, other studies reported an incomplete or ineffective extracellular pH regulation (Catarino et al., 2012; Kurihara et al., 2013; Miles et al., 2007; Spicer et al., 2011). Organisms which cannot avoid extracellular acidosis could be directly affected by OA (Fabry et al., 2008; Melzner et al., 2009; Pörtner et al., 2004; Pörtner, 2008). For those which regulate their extracellular pH, the energetic cost of proton elimination or buffering could modify the allocation of the resources within the organism, leading to reduced calcification and somatic or gonad growth. Indeed, several authors observed a decreased growth when sea urchins were submitted to elevated $p\text{CO}_2$ (Albright et al., 2012; Courtney et al., 2013; Holtmann et al., 2013; Shirayama and Thornton, 2005; Stumpp et al., 2012; Wolfe et al., 2013) while in

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other studies, no effect was reported (Kurihara et al., 2013; Moulin et al., 2014; Uthicke et al., 2013, 2014). Regarding energy allocation to the reproductive organs, it seems that OA leads to a gonad growth decrease and/or a gametogenesis delay (Kurihara et al., 2013; Siikavuopio et al., 2007; Stumpp et al., 2012; Uthicke et al., 2013) but, in some cases, it is only evidenced when temperature is increased in combination with the reduction in seawater pH (Uthicke et al., 2014). When dealing with energy related effects, crucial aspects are the duration of the experiment and the provided food. Until now, only three studies conducted long-term experiments (over 6 months, Dupont et al., 2013; Kurihara et al., 2013; Shirayama and Thornton, 2005). All three reported significant effects either on growth or reproductive output although the latter was modulated according to acclimatization duration (Dupont et al., 2013). Indeed, in this last study, after a four months exposition to elevated $p\text{CO}_2$, the energy allocated to reproduction was decreased but not after a 16 months acclimatization. This highlights the need for more long-term studies. Moreover, previous studies exposed sea urchins brutally to increased $p\text{CO}_2$ levels whereas gradual acclimatization will better mimic future OA. Furthermore, in all studies carried out so far on postmetamorphic sea urchins, food was supplied *ad libitum* and used algae were not submitted to OA before being provided to sea urchins. Yet, the biochemical composition and palatability of algae may be affected by OA (e.g. Borell et al., 2013; Poore et al., 2013). Asnaghi et al. (2013, 2014) reported more severe effects of OA on *Paracentrotus lividus* when fed non-calcified algae instead of calcified algae *Corallina* and OA is known to significantly reduce growth of coralline algae (Kuffner et al., 2008).

Therefore, the goal of the present study was to assess the impact of OA at long-term on a suite of physiological parameters of a sea urchin not fed *ad libitum*. The sea urchin *Echinometra mathaei* was submitted during six months to a gradual pH decrease until reaching a moderate OA level (pH 7.7). Sea urchins were then maintained at the targeted pH for seven more months. Sea urchins were placed in replicated mesocosms provided with an artificial reef consisting of hermatypic scleractinians and reef calcareous substrate with its diverse communities of algae as principal food. The growth, the carbonate chemistry of the coelomic fluid (pH, dissolved inorganic carbon and total alkalinity) and the respiration rate of sea urchins were monitored throughout the experiment. Biomechanical resistance of the skeleton was also tested at the end of the experiment to assess the test's robustness to fish predation.

2. Materials and methods

2.1. Mesocosms design and seawater physico-chemistry

The complete description of the technical setup of the artificial reef mesocosms is presented in Leblud et al. (2014). Briefly, the study was conducted in two separated mesocosms (true replicates). Each mesocosm is composed of one main unit and two experimental aquaria. Both experimental aquaria were connected to the main unit and the water flow was the same for both aquaria ($800 \pm 50 \text{ mL min}^{-1}$). Water was recirculated between the main unit and each aquarium, so that the water from both aquaria was mixed in the main unit. This design allowed the maintenance of the same physico-chemical parameters in both aquaria. Only $p\text{CO}_2$ and pH differed: one aquarium was kept at control conditions (mean target pH in total scale, pH_T , 8.10) and the other was acidified by bubbling CO_2 , controlled by an IKS system (mean target pH_T 7.65). The contrasted conditions of pH were established gradually over six months (approximately -0.03 units of pH_T every two weeks) and then the targeted pH was maintained for seven more months. In our reference field site (La Saline fringing reef, Réunion Island,

$21^\circ 70'S$, $55^\circ 32'E$), pH increased between sunrise and zenith time on average by 0.18 units (± 0.05 , $N = 1344$, from 2011/05/10 to 2011/06/13, pH measured every 15 min, Cuet P., pers. Comm., see also Chauvin et al., 2011). From zenith to sunset, the pH decreased approximately by the same value. In order to get more realistic conditions in our mesocosms, daily variation of pH_T was maintained at a level similar to field values through community respiration/photosynthesis and change in the day/night pH setpoint of the control system (for data, see Leblud et al., 2014).

In each experimental aquarium, a temperature sensor (Aquastar, Germany) and a pH electrode (Aquastar, Germany) were connected to an IKS control system (IKS, Aquastar, Germany) which recorded the values every 20 s. Seawater samples were collected every other day and immediately filtered ($0.22 \mu\text{m}$ GSWP, Millipore). Total alkalinity (A_T) was measured by a potentiometric titration with 0.01 M HCl in NaCl 0.7 M following Dickson et al. (2007) and adapted for a smaller volume (25 mL). Each titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record pH (Consort, Belgium) and a TW Alpha Plus autosampler (SI Analytics, Germany). Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, Dickson, batch 94). Once a day, the electromotive force (e.m.f.) was measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor). The e.m.f. was then converted to pH_T using calibration curves of standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (DelValls and Dickson, 1998; Dickson et al., 2007; DOE, 1994). Salinity and temperature were measured once a day using a conductivity meter pH/Cond 340i WTW (Germany). The intercalibration between these measures (pH_T and temperature) and those recorded by the IKS control system allowed recalculating the true 24 h cycle of pH and temperature and to adjust setpoints of the controllers. The $p\text{CO}_2$ was determined from A_T , pH_T , temperature and salinity data using the program R (R Core Team, 2013) and the package seacarb (Lavigne and Gattuso, 2012) (Lueker et al. (2000) constants for K1 and K2; Perez and Fraga (1987) constant for Kf; Dickson (1990) constant for Ks). Nutrients concentration were also determined throughout the experiment (see Leblud et al. 2014 for methods used).

2.2. Mesocosms community

Sea urchins (*E. mathaei* corresponding to *Echinometra* sp. B) were collected at Réunion Island in the Indian Ocean, from the back-reef of Saint-Pierre fringing reef ($21^\circ 33'S$, $55^\circ 47'E$). This zone, in major part covered with detritic sediment, is characterized by a low algae cover (coralline algae and encrusting pheophyta), a low coral cover (10–15%) and a high sea urchin density (ca. $30 \text{ individuals m}^{-2}$) resulting in low food availability and competition between sea urchins. The experimental community composition was similar to that in the field. In order to determine the species of *Echinometra* to which belonged the individuals used in this experiment, the color of the spine and the appearance of the milled ring as well as that of the skin on the peristome were chosen as criteria. Indeed, a study by Arakaki et al. (1998) assigned violet *Echinometra* presenting dark milled ring and dark skin on the peristome to the species *Echinometra* sp. B. Violet spines are specific to *Echinometra* sea urchins from Mauritius and Réunion Island as it is not found in Okinawa, Indonesia and other parts of the Pacific Ocean. Other *Echinometra* sea urchins were also collected: green *Echinometra* sea urchins with spines without white tips, with dark milled ring and dark skin on the peristome. They were also assigned to *Echinometra* sp. B (Arakaki et al., 1998). Therefore, violet

Echinometra's and green *Echinometra*'s from Réunion Island are two color morphs of *Echinometra* sp. B, which corresponds to the holotype *E. mathaei* (Arakaki et al., 1998). Hermatypic scleractinians and reef calcareous substrate (coral rubble) with its diverse communities of algae, bacteria, archae, fungi and meiofauna were also introduced in the mesocosms. The corals and substrate came from Réunion Island in the back reef of La Saline fringing reef (21°70'S, 55°32'E, depth: 0.5–1.5 m) (substrate, *Acropora muricata*, *Acropora digitifera*, *Pocillopora damicornis*) and from the aquarium market (Dejong, Holland) (*Seriatopora hystrix*, *Acropora tenuis*). Before field collection, permits were obtained from the Marine Nature Reserve of Réunion Island (RNN164) and the "Direction de l'Environnement, de l'Aménagement et du Logement" (DEAL) and CITES permits were checked for aquarium market corals. Organisms were acclimated to the mesocosms in Belgium in control condition for seven months before the beginning of the experiment. Sixteen violet *E. mathaei* (test diameter: 28–40 mm), 0.4 ± 0.04 kg of corals and 22 ± 1 kg of reef substrate were distributed randomly in each experimental aquarium. When a sea urchin died, it was replaced by a green *E. mathaei* of approximately the same size in order to maintain the same biological interactions (e.g. grazing/bioerosive pressure, competition). The color of the sea urchins allowed us to identify urchins which were present from the beginning of the experiment from those who were used as replacements. All analyses were conducted on the violet *Echinometra*'s present since the beginning of the experiment. Sea urchins principally fed on macroalgae and coralline algae that grew on the reef substrate. As mortality occurred, excessive starvation was suspected and additional food (dehydrated *Porphyra* sp., Japanese edible seaweed "Nori") was supplied from the fifth month of the experiment (ca. 0.35 ± 0.05 g every other day).

2.3. Growth and respiration rate measurements

Growth and respiration rates were only measured on violet *Echinometra* sea urchins. The respiration rate was determined every 3 months using homemade intermittent respirometers. The respirometers (1.2 L) consisted of a Plexiglas cylinder with a waterproof gas tight cap provided with a water inlet and outlet. A Clark oxygen electrode (AquaStar, Germany) was immersed inside the respirometer chamber through a sealed aperture. To ensure water mixing and homogeneous distribution of the oxygen, a magnetic stir bar was introduced in each respirometer. Water inflow came from the experimental aquarium from which the sea urchins originated. The respirometers were placed inside a thermostated water bath (25 ± 0.5 °C) on a magnetic stirring unit. Water inflow was open for 1 h and then stopped for 1 h during the respiration measurement phase. Oxygen concentrations were recorded by means of a control system (IKS AquaStar, Germany) every 8 s. The sequence open/closed water inflow was pursued over 24 h before the introduction of sea urchins. Then, two sea urchins were introduced into each respirometer for 48 h. Two urchins were processed at the same time in order to detect a sufficient decrease of oxygen concentration. After the sea urchins were removed, the system was maintained for an additional 24 h. Oxygen electrodes were calibrated with water-saturated air as 100% O₂. Moreover, each probe signal was corrected using a WTW oxymeter and an WTW Oxycal probe, calibrated using 0% O₂ NaSO₂ solution and 100% O₂ water-saturated air. Linear regressions ($R^2 > 0.9$) of seawater oxygen concentration over time were computed during the closed phases. Those values were converted into $\mu\text{mol O}_2 \text{ L}^{-1} \text{ h}^{-1}$ and multiplied by the water volume of the respirometer. Background respiration and oxygen consumption by the electrode itself were assessed by linear regression between the first 24 h (before the sea urchins respiration measurement) and the last 24 h measurements (after

the sea urchins respiration measurement). The correction was then applied to the sea urchins respiration rate measurement. Wet weight of the sea urchins was measured after the respiration rate measurements (± 0.1 g) to avoid the influence of gut content. Indeed sea urchins were fed prior to the respiratory run but not during the measurement, leading to the progressive digestion of the food and its evacuation. At the end of the respiratory run (48 h), the gut was empty. Indeed, twenty sea urchins were dissected after 48 h of starvation and the gut was always observed empty. Respiration rates were normalized by the sea urchins wet weight.

The skeletal weight was assessed by the buoyant weighing method (Jokiel et al., 1978; Moulin et al., 2014) (± 0.005 g) after the respiration rate measurement (no gut content confounding effect). The density of the skeleton was determined from the whole skeleton (spine, test and Aristotle's lantern) of twenty sea urchins coming from the same population using the buoyant weighing method. The growth rate was calculated following an exponential growth curve using the equation:

$$\log Y = \log Y_0 + k \cdot t$$

with Y being the skeletal weight at time t , Y_0 the initial skeletal weight at time $t = 0$, k the growth rate and t the time (in month).

2.4. Carbonate chemistry of the coelomic fluid

The pH_T, the dissolved inorganic carbon concentration (DIC) and the A_T of the CF (respectively pH_{CF}, DIC_{CF} and A_{T-CF}) were measured every 3 months in violet *Echinometra* individuals. Samples of CF (2.5 mL) were collected by puncture through the peristomial membrane (syringe 21G, Terumo, Japan). The e.m.f. of the CF was immediately measured by immersing a microelectrode in a 1 mL aliquot (microelectrode 6.0224.100 coupled to a 827 pH Lab meter, Metrohm, Switzerland). Calibration and e.m.f. values conversion to pH_T were done as previously described (section 2.1). Salinity was assumed to be the same as that of the seawater in the experimental aquaria from which the sea urchins came as the composition of the sea urchins CF is known to be close to that of seawater (Farmanfarmaian, 1966; Russell, 2013). The remainder of the sample was transferred to a microcentrifuge tube filled to the top and closed to avoid sample-air gas exchanges. Samples were always kept on ice to prevent clotting. Sample were then centrifuged for ten minutes (4000 G) using a microcentrifuge (Eppendorf, Germany) at 4 °C. The supernatant was used to determine DIC_{CF} and A_{T-CF}.

A homemade method was developed to determine DIC_{CF}. Air circulation is switched on using a recirculation pump and air containing an initial known amount of CO₂ (provided here by a scuba tank) is introduced into a polypropylene bottle (≈ 20 mL) through

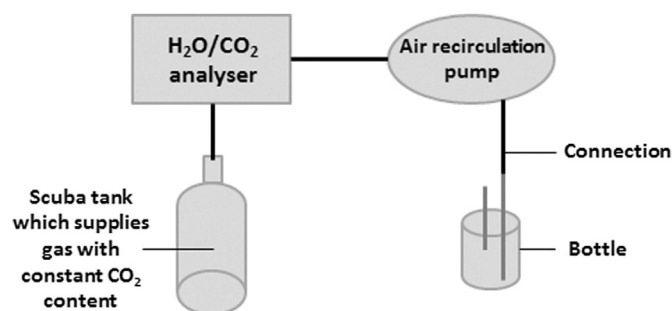


Fig. 1. Diagram of the dissolved inorganic carbon concentration determination setup (opened, as described in the text).

tube connections as illustrated in Fig. 1 (opened system). The amount of CO₂ is measured with a CO₂/H₂O LI-840 analyzer (LI-COR, USA). Then, the bottle is disconnected and orifices are closed in order to avoid gas exchange. One milliliter of sample is weighted (precision ± 0.2 mg) and introduced into the bottle using a syringe. 25 μ l HNO₃ 32.5% is added to the sample and the bottle is reconnected as illustrated in Fig. 2 (closed system), in order to acidify the sample and thus to transform all DIC species into CO₂. Air circulation is turned on. CO₂ partial pressure is recorded by the CO₂/H₂O analyzer until equilibrium is reached using a data acquisition computer. The bottle is maintained at a constant temperature and pressure during the measure. The method was calibrated using standard solutions containing different HCO₃⁻ concentrations (NaHCO₃, normapur[®], VWR) and then corrected by an offset estimated from a DIC standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (batch number 120). Certified reference seawater measurements had a random deviation of 1.5% of the original certified value.

A_{T-CF} was determined by a manual potentiometric titration with HCl 0.02 N in NaCl 0.7 mol kg⁻¹ on 0.5 mL CF sample following Collard et al. (2013). The quality of the method was assessed using certified reference seawater (by Andrew G. Dickson, Scripps Institute of Oceanography, Dickson, batch 9). Certified reference seawater measurements had a random deviation of 1.5% of the original batch value. Results were reported as the difference between the A_{T-CF} and A_T of seawater (ΔA_T). Theoretical A_{T-CF} was also calculated from pH_T, DIC, temperature and salinity data using the seacarb package.

2.5. Biomechanical properties

At the end of the experiment, the sea urchins were dissected. The test skeleton was cleaned from its associated soft tissues in gently shaken solutions of 2.5% and 5.25% NaOCl (Loda, professional quality) for, respectively, 1h30 and 2h30, rinsed in MilliQ water (Millipore) and air-dried. Three ambital and three apical plates coming from three different interambulacral zones from each sea urchin were selected for mechanical tests. The ambital and apical plates chosen were respectively the largest and the smallest ones. The effective length of the plate (length between the two external supporting points) was measured on enlarged photos by image analysis (ImageJ software, National Institutes of Health, USA). Three-point bending tests were performed on ambital plates following Moureaux et al. (2011). Briefly, each ambital plate was placed on a stainless steel stand. A noncutting blade fixed on the load frame of the force stand (Instron 5543) was lowered on the primary tubercle at a speed of 0.01 mm min⁻¹ to bend the plate. Displacement and force were recorded at a frequency of 10 Hz. The Young's Modulus was determined using the equation:

$$\text{Young's modulus } E(\text{Pa}) = \frac{F_{\max} L_e^3}{48 \Delta L I_2}$$

where $F_{\max}$: force at rupture, L_e : effective length, ΔL : displacement, I_2 : second moment of area.

The macro MomentMacro (Ruff C., Johns Hopkins University School of Medicine, MD, USA) for the software ImageJ was used to calculate the second moment of area I_2 using the fracture surfaces of the plates. The apical plates underwent simple compression tests. Each apical plate was placed on a stainless steel block. A second similar block was fixed on the load frame of the force stand (Instron 5543) and was lowered on the main tubercle of the plate at a speed of 0.3 mm min⁻¹ until rupture. Displacement and force were recorded at a frequency of 10 Hz. The Young's Modulus was determined using the equation:

$$\text{Young's modulus (Pa)} = \frac{\text{stress}}{\text{strain}} = \frac{\frac{F}{A}}{\frac{\Delta L}{L_e}}$$

where F : force at rupture, A : area of the tubercle, ΔL : displacement, L_e : effective length.

2.6. Data analysis

Normality and homogeneity of variances were checked before analysis, using respectively Shapiro–Wilk's and Levene's tests. Temperature, A_T and salinity of seawater were analyzed using a paired Student's *t*-test in order to examine possible differences between aquaria within each mesocosm. In order to determine if the growth rate of sea urchins was significantly different from zero, a paired Student's *t*-test was performed. Mortality, growth and biomechanical properties were analyzed using a one-way ANOVA with repeated measures on one factor (factor nominal pH nested in repeated factor mesocosm). The respiration rate and CF acid–base parameters (pH_{CF}, DIC_{CF} and ΔA_T) were treated as the difference between means from control and acidified aquaria in each mesocosm to take into account the paired design of the experiment. During the gradual pH decrease, this difference was fitted using a linear regression with Δ pH (difference of mean seawater pH between control and acidified aquaria at the time of measure) and mesocosm as explanatory variables. The y intercept and the slope correspond to the difference between control and acidified aquaria at the beginning of the experiment and during the gradual pH decrease respectively. After the establishment of the contrasted conditions, this difference was fitted using a linear regression with time and mesocosm as explanatory variables. The slope provided information about the possible changes in this difference when contrasted conditions were clearly established. All tests were conducted using the program R. The level of significance α was set to 0.05.

3. Results

3.1. Physico-chemical parameters in mesocosms

A complete description of the physico-chemical parameters (carbonates system parameters, nutrients and oxygen) changes throughout the experiment is available in Leblud et al. (2014). The pH_T of the experimental aquaria was maintained close to target values, in control conditions as well as in acidified conditions throughout the experiment (Fig. 3, Table 1). For the control aquaria, mean pH was respectively 8.08 ± 0.08 and 8.09 ± 0.09 in the mesocosm A and B. For the acidified aquaria, after the progressive

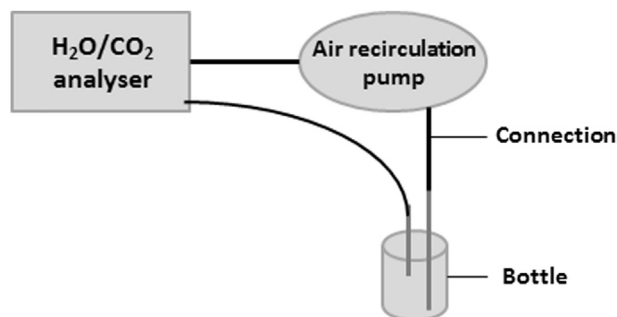


Fig. 2. Diagram of the dissolved inorganic carbon concentration determination setup (closed, as described in the text).

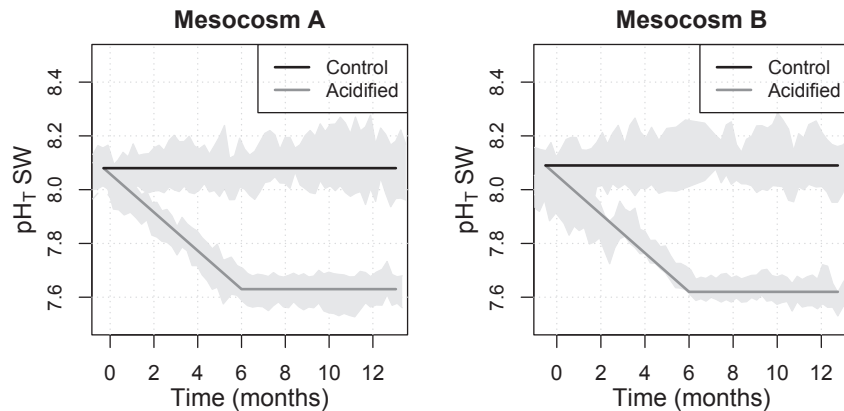


Fig. 3. Seawater pH_T in control (black) and acidified (gray) aquaria during the experiment (months since the beginning of the experiment). The lines represent target pH. The gray zones represent minimal and maximal value (respectively during night and day) measured once a week throughout the experiment.

decrease of seawater pH, mean pH was respectively 7.63 ± 0.03 and 7.62 ± 0.04 in mesocosm A and B. Mean values of A_T , salinity and temperature are presented in Table 1. Temperature varies significantly between contrasted conditions in the mesocosm A (paired t -test, $t_{(9638)} = 11.34$, $p < 10^{-3}$) but not in mesocosm B (paired t -test, $t_{(9832)} = -1.51$, $p = 0.13$). However, mean of the difference is small and in the range of precision of the control system (< 0.1 °C). A_T and salinity did not differ significantly between contrasted conditions in both mesocosms (paired t test, $t_{(210)} \leq 1.88$, $p \geq 0.06$).

3.2. Mortality and growth

Some mortality occurred during the experiment. At the end of the experiment, mortality was 56.25 and 37.5% for the acidified aquaria in the mesocosm A and B respectively. For the control aquaria, mortality was 68.75 and 75% in the mesocosm A and B respectively. Mortality did not differ significantly according to the pH condition (ANOVA, $F_{(1,3)} = 4$, $p = 0.30$). From the fifth month of the experiment, additional food was supplied but mortality continued to take place independently. Sea urchins were not tagged individually and mortality occurred during the whole experiment, except the last three months. Consequently, growth rates were only assessed over the last three months using individual skeleton weight after 10 and 12 months of experiment, i.e. during a period with established contrasted conditions. During this period, the remaining sea urchins were individually recognizable thanks to morphological characteristics (e.g.: size, weight, length of the spines, shape of the test) allowing individual growth rates to be measured. The growth rate of the sea urchins at the end of the experiment was significantly different from 0 (mean $\pm$ SD = $1.1 \pm 1.3\%$ per month, t -test, $t_{(24)} = 4.18$, $p < 10^{-3}$) (Fig. 4). Seawater pH did not have a significant effect on growth rate of sea urchins (ANOVA, $F_{(1,24)} = 0.26$, $p = 0.70$).

3.3. Metabolism

The respiration rate of the sea urchins was measured every 3 months throughout the experiment (Fig. 5). As the sea urchins exhibit greater activity during the night, respiration rate data were separated between night and day data. At the beginning of the experiment, the difference in respiration rates between control and future acidified aquaria was not significant for both mesocosms (Table 2, y-intercepts). During the gradual pH decrease, the pH of the seawater did not affect significantly the respiration rate in both mesocosms and the mesocosm factor was not significant (Table 2,

slopes). After the establishment of contrasted conditions, the respiration rate of the sea urchins did not vary significantly with time (Table 2, slopes).

3.4. Acid–base parameters of the coelomic fluid

pH_{CF} , DIC_{CF} and ΔA_T were measured every three months throughout the experiment. At the beginning of the experiment, pH_{CF} did not differ significantly between the experimental aquaria of both mesocosms (Fig. 6, Table 3, y-intercept). During the gradual pH decrease, the pH of the seawater did not affect significantly the pH_{CF} in both mesocosms and the mesocosm factor was not significant (Fig. 6, Table 3, slope). After the establishment of the contrasted conditions, pH_{CF} did not vary significantly with time (Fig. 6, Table 3, slope). The DIC_{CF} (Fig. 7) and the ΔA_T (Fig. 8) did not differ significantly between the experimental aquaria of both mesocosms before the gradual pH decrease (Table 3, y-intercepts). The DIC_{CF} and ΔA_T were significantly higher in acidified conditions during the establishment of the contrasted conditions independently of the mesocosm considered (Table 3, slopes). The adjusted linear model showed that seawater pH predicted well DIC_{CF} and ΔA_T ($R^2 > 0.94$). Once the contrasted conditions were established, the difference in DIC_{CF} and ΔA_T between the contrasted conditions remained constant throughout the experiment (Table 3, slopes). The difference between the measured and the calculated A_{T-CF} (Fig. 9) did not vary significantly between the experimental aquaria of both mesocosms at the beginning of the experiment (Table 3, y-intercept), or during the gradual pH decrease or after, when contrasted pH conditions were maintained (Table 3, slopes).

3.5. Mechanical tests

Mechanical tests were carried out on ambital and apical plates (Figs. 10 and 11 respectively). The pH of seawater did not have a significant effect neither on the force at rupture F_{max} and on the Young's modulus E for both types of plates (ANOVA, $F_{(1,25)} \leq 38.2$, $p \geq 0.10$) nor on the flexural stiffness El_2 of ambital plates (ANOVA, $F_{(1,25)} = 1.09$, $p = 0.49$).

4. Discussion

Over a 6 months gradual pH_{SW} decrease and subsequent 7 months exposure to a mean ΔpH_{SW} of -0.46 , the pH_{CF} of *E. mathaei* was maintained similar in control and treatment sea urchins. The increase of the ΔA_T was well predicted by the ΔpH_{SW} , indicating

Table 1
Mean seawater conditions during the experiment. Values between brackets are standard deviations. Mean temperature and pH_T were calculated on measurements recorded every 20 s. Mean salinity, A_T and pCO_2 , DIC, CO_3^{2-} , HCO_3^- and Ω (calcite and aragonite) were calculated on values measured every day shortly after mesocosms' sunrise. Mean salinity, temperature and A_T correspond to the data during the whole experiment. Other parameters correspond to the data after establishment of contrasted conditions (last 7 months).

pH condition	Mesocosm	Salinity	Temperature (°C)	A_T ($\mu\text{mol kg}^{-1}$)	pH_T	pCO_2 (ppm)	DIC ($\mu\text{mol kg}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	HCO_3^- ($\mu\text{mol kg}^{-1}$)	Ω_{Cal}	Ω_{Ar}
Control	A	34.3 (0.6)	25.2 (0.3)	2400 (133)	8.08 (0.08)	382 (40)	2074 (130)	234 (21)	1829 (117)	5.67 (0.50)	3.73 (0.33)
	B	34.6 (0.5)	25.2 (0.4)	2421 (237)	8.09 (0.09)	359 (47)	2024 (110)	236 (16)	1778 (107)	5.70 (0.37)	3.76 (0.25)
Acidified	A	34.3 (0.9)	25.2 (0.3)	2409 (177)	7.63 (0.05)	1288 (124)	2335 (194)	100 (9)	2198 (183)	2.41 (0.22)	1.59 (0.14)
	B	34.6 (0.5)	25.2 (0.3)	2432 (175)	7.62 (0.04)	1269 (93)	2286 (134)	97 (6)	2153 (127)	2.35 (0.15)	1.54 (0.10)

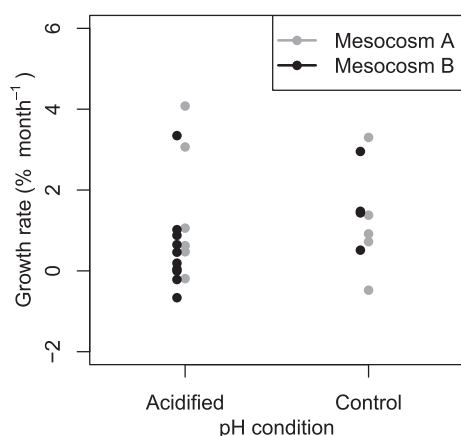


Fig. 4. Skeletal growth (as percentage of the initial skeleton weight) of *E. mathaei* at different pH conditions in mesocosm A (gray) and mesocosm B (black).

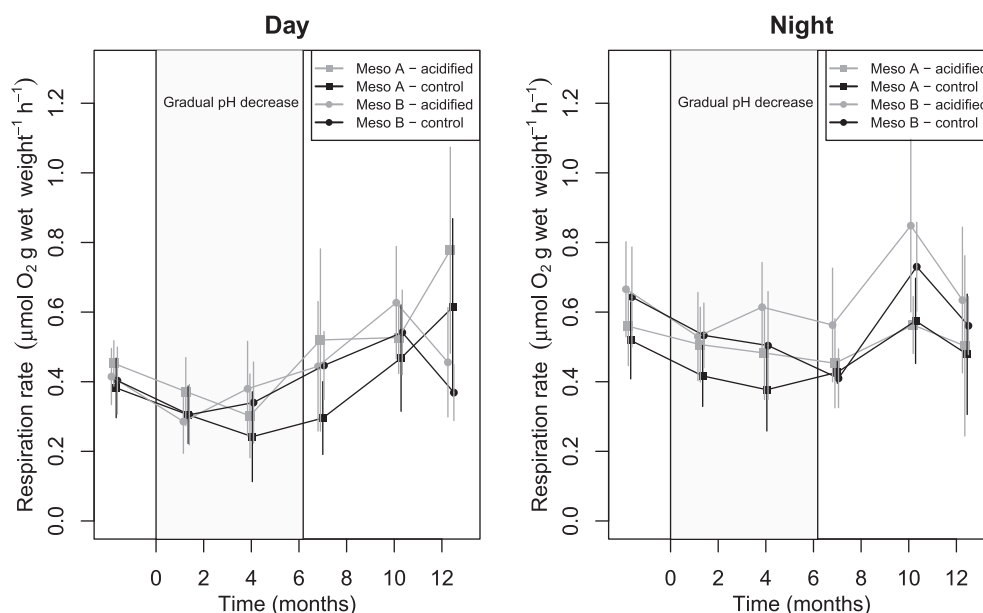


Fig. 5. Respiration rate (mean $\pm$ SD, $n \geq 4$) of *E. mathaei* in the different pH conditions (gray: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) over the course of the experiment (months since the beginning of the experiment) for day or night data.

that the pH_{CF} regulation was conducted by an increase of A_T . The measures of pH_{CF} and DIC_{CF} allowed to calculate the theoretical value of A_T using carbonate system equilibrium equations in seawater, without taking into account biological compounds of the CF. As the difference between the calculated $A_{T-\text{CF}}$ and the measured $A_{T-\text{CF}}$ did not vary significantly with seawater pH, the increase of the

buffering capacity is principally due to an increase of bicarbonates as evidenced by the increase in DIC_{CF} . A compensation by bicarbonates was already observed, at short-term, in *E. mathaei* (Moulin et al., 2014) and in other species (*Psammochinus miliaris*, *Paracentrotus lividus*, *Strongylocentrotus droebachiensis*, *Tripteneustes ventricosus* in Table 4 and *Echinus esculentus* in Spicer et al., 1988). In most of the studies, this compensation by bicarbonates allowed to restore pH_{CF} to control value despite a transient decrease during the first days of exposure at $\text{pH}_{T-\text{SW}} \geq 7.6$ (Table 4). For example, in *P. lividus*, pH_{CF} was decreased after a 24 h exposure to $\text{pH}_{T-\text{SW}} 7.6$ (Calosi et al., 2013) but not after over 7 days (Catarino et al., 2012; Collard et al., 2013, 2014). Similarly, pH_{CF} was fully compensated after over 7 days in almost all regular euechinoid species tested to date when facing OA levels predicted for 2100 (Table 4). On the contrary, the cidaroid *Eucidaris tribuloides* has a naturally low pH_{CF} which does not change when the sea urchin faces acidified conditions (Collard et al., 2014). The basal euechinoid *Arbacia lixula* also has a low pH_{CF} which did not change after 4 days of exposure to

$\text{pH}_{T-\text{SW}} 7.6$ (Calosi et al., 2013). However, a longer exposure should be tested before any conclusion can be drawn for this species. The present results show that *E. mathaei* is able to maintain this compensation at long-term when facing a $\text{pH}_{T-\text{SW}}$ of 7.6. In contrast, a decrease of the pH_{CF} occurred in *Hemicentrotus pulcherrimus* after a 9 month exposure to $\text{pH}_{T-\text{SW}} 7.7$ (Kurihara et al., 2013).

Table 2

Linear models of the difference of respiration rate between acidified and control conditions. The difference in pH between the contrasted conditions was used as a predictive variable during the gradual pH decrease. Time was used as a predictive variable after the establishment of the contrasted conditions. Models were computed for day and night data separately. Parameters of the linear regressions are showed for the mesocosm A (A) and for the difference between the mesocosms A and B (B–A).

Day				Night		
	Estimate	t value	p	Estimate	t value	p
Gradual pH decrease						
A y-intercept	−0.04	−1.07	0.35	−0.07	−2.27	0.09
Slope	0.33	2.02	0.11	−0.03	−0.23	0.83
B–A y-intercept	0.04	0.71	0.52	0.07	0.17	0.17
Slope	−0.30	−1.32	0.26	0.41	0.08	0.08
Model R ²		0.74			0.70	
Contrasted conditions established						
A y-intercept	−0.29	−1.46	0.28	−0.03	−0.43	0.71
Slope	0.01	0.73	0.54	0.001	0.22	0.85
B–A y-intercept	0.40	1.44	0.29	−0.23	2.78	0.11
Slope	−0.03	−1.14	0.37	0.01	1.59	0.25
Model R ²		0.64			0.95	

Interestingly, almost all these contrasted responses match the phylogeny of the sea urchin genera (Fig. 12). *E. tribuloides* and *A. lixula* do not need any regulation due to their naturally low pH_{CF} making the changes in pH_{SW} due to ocean acidification negligible. *P. lividus* and *P. miliaris* are able to regulate their pH_{CF} at $\text{pH}_{\text{T-SW}} \geq 7.6$ but not at lower pH (Catarino et al., 2012; Collard et al., 2014; Miles et al., 2007). *Odontophora* species tested at $\text{pH}_{\text{T-SW}} \geq 7.4$ (*Strongylocentrotus droebachiensis*, *Strongylocentrotus fragilis*, *T. ventricosus*) were shown to regulate their extracellular fluid acid–base status after a minimum acclimation period of 7 days (Collard et al., 2014; Stumpp et al., 2012; Taylor et al., 2014). This hypothesis has to be verified for *E. mathaei*. Based on this phylogenetic hypothesis, *H. pulcherrimus* should be able to regulate its pH_{CF} at long-term but this was not observed by Kurihara et al. (2013). We suggest that the nutrition status is another parameter that should be taken into account. Stumpp et al. (2012) observed that *S. droebachiensis* was not able to compensate its pH_{CF} by an increased bicarbonate ion concentration when fasted. Most other studies involved sea urchins fed *ad libitum* and at rather low

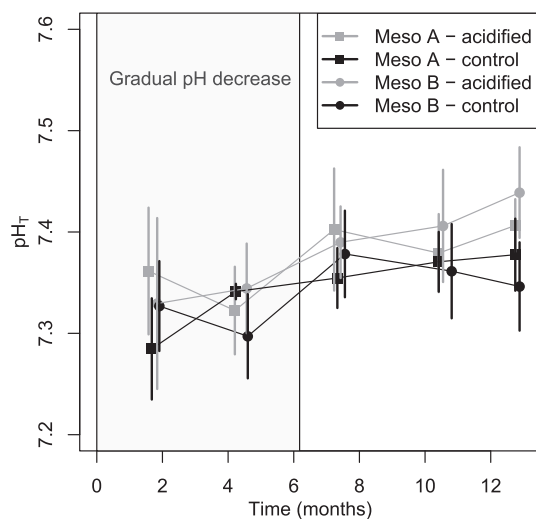


Fig. 6. Coelomic fluid pH_{T} (mean $\pm$ SD, $n \geq 4$) of *E. mathaei* in the different pH conditions (gray: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) over the course of the experiment (months since the beginning of the experiment).

densities. In the study of Kurihara et al. (2013), *H. pulcherrimus* density was very high (100 urchins in a 45 L aquarium) and competition for food could have affected their pH regulation ability. Indeed, intraspecific competition was demonstrated in sea urchins living in large aggregations even when supposedly fed *ad libitum* (see Grosjean et al., 1996 and references therein). Furthermore, food limited sea urchins have a decreased coelomic fluid buffer capacity (Collard et al., 2013). In the present study, sea urchin density (30 individuals m^{-2}) was voluntarily high, probably inducing also competition for food. A high density of *E. mathaei* is encountered in the field, in particular in Réunion Island (up to 74 individuals m^{-2} observed by Conand et al., 1997; field personal observations). McClanahan and Kurtis (1991) showed that the population regulation of *E. mathaei* occurs at the individual level, allowing high population densities through decreased energy availability to all individuals (respiration rate and growth decreased). Furthermore, the sea urchins were not fed *ad libitum*. Indeed, $\Delta\text{A}_{\text{T}}$ of the coelomic fluid of *E. mathaei* maintained in control conditions was lower than that measured in the field (mean $\pm$ SD = $2234 \pm 406 \mu\text{mol kg}^{-1}$, Moulin et al., 2014). This difference is representative of starved sea urchins as observed by Collard et al. (2013). Reduced food availability could explain the low respiration rate and low growth observed during this experiment as compared with other studies where adults *E. mathaei* were fed *ad libitum* and/or studied at shorter term (Moulin et al., 2014; Uthicke et al., 2013, 2014). Indeed, a previous study conducted on starved *Echinometra* showed that starvation led to a decrease of the respiration rate down to values comparable to those measured in the present study (McClanahan and Kurtis, 1991). A low quantity of artificial food was provided from the fifth month of the experiment but this did not modify the pattern of acid–base regulation. Despite the high density/low food availability conditions, *E. mathaei* were able to maintain their pH_{CF} . We suggest that this is explained by the fact that they were able to graze on calcareous substrate and ingest calcium carbonate which could provide buffering bicarbonate ions. Indeed, a previous field study indicated that *E. mathaei* took in less food and a higher percentage of inorganic substratum with increasing population density resulting in signs of starvation (McClanahan and Kurtis, 1991). Holtmann et al. (2013) showed that the intestine wall prevents the loss of bicarbonate ions from the CF to the digestive lumen but hypothesized a bicarbonate transport from the intestine to the CF. Results of Asnaghi et al. (2013) support a role of calcareous food in mitigating the impacts of ocean acidification. It is noteworthy that *H. pulcherrimus* studied by Kurihara et al. (2013) were fed non calcareous *Undaria pinnatifida*. Thus, we suggest that the ability to maintain the pH_{CF} in front of ocean acidification is linked to phylogeny, feeding status and the nature of the available food and/or substrate (calcareous or not). This emphasizes the importance of conducting experiments in realistic environmental conditions, especially feeding conditions.

Even food limited, the respiration and growth rates of *E. mathaei* were not affected by seawater pH. This indicates that individuals that survived until the end of the experiment were resilient in front of ocean acidification. Mortality was indeed important during the experiment. This could be explained by natural population senescence. It is noteworthy that the total duration of the experiment, from collection of the sea urchins in the field to the end of the OA experiment, was 20 months, which is significant in comparison of the longevity of this species. Indeed, *E. mathaei*'s mean longevity is relatively low (ca. 7 years) and the test diameter range studied in this experiment was close to the maximum length (40 mm) observed for this species close to the Equator (Ebert et al., 2008). For this species, when size approaches its maximal value, the growth rate of the individuals tends to zero. Therefore, senescence of the population studied in the present experiment could explain

Table 3
Linear models of the difference of CF parameters (pH, DIC, A_T and difference between measured and calculated A_T) between the acidified and control conditions. The difference in pH between the contrasted conditions was used as a predictive variable during the gradual pH decrease. Time was used as a predictive variable after the establishment of the contrasted conditions. Parameters of the linear regressions are showed for the mesocosm A (A) and for the difference between the mesocosms A and B (B–A).

		pH			DIC			A_T			Measured—calculated A_T		
		Estimate	<i>t</i> value	<i>p</i>	Estimate	<i>t</i> value	<i>p</i>	Estimate	<i>t</i> value	<i>p</i>	Estimate	<i>t</i> value	<i>p</i>
Gradual pH decrease													
A	y-intercept	0.07	0.79	0.51	−17	−0.26	0.82	−1923	−1.08	0.34	245	1.88	0.20
	slope	0.04	0.12	0.92	−3665	−16	0.004	−4290	−5.90	0.004	−125	−0.27	0.81
B−A	y-intercept	−0.05	−0.42	0.72	82	0.90	0.46	250	0.99	0.38	114	0.62	0.60
	Slope	−0.09	−0.19	0.87	−372	−1.16	0.37	64	0.06	0.95	−2	−0.003	0.998
Model R^2			0.15			0.997			0.95			0.51	
Contrasted conditions established													
A	y-intercept	0.19	2.29	0.15	2122	1.96	0.19	2469	4.48	0.046	−213	−0.89	0.47
	Slope	−0.01	−1.79	0.22	−65	−0.62	0.60	−85	−1.61	0.25	71	3.11	0.09
B−A	y-intercept	−0.26	−2.16	0.16	503	0.33	0.77	521	0.67	0.57	651	1.93	0.19
	Slope	0.03	2.29	0.15	−49	−0.33	0.77	−84	−1.12	0.38	−79	−2.43	0.14
Model R^2			0.73			0.44			0.89			0.89	

the relatively low growth rate observed in comparison to other studies conducted on adult and juvenile *Echinometra* (see references above; Shirayama and Thornton, 2005). Additional food (nori) was supplied from the fifth month of the experiment but mortality was not affected and continued to occur. Sea urchins still fed on the substrate. Indeed, supplementary food was added in low quantity (0.02 g dry algae individual^{–1} day^{–1}) in comparison with the grazing rate of *E. mathaei* (0.04–0.24 g algae individual^{–1} day^{–1}; Bronstein and Loya, 2014). Moreover, the buffer capacity of the coelomic fluid in control conditions did not increase in parallel to food addition. This indicates that the sea urchins were still starved despite food addition which may explain the high mortality observed during the experiment resulting in the selection of “starvation resistant” individuals. Such selection could also account for the absence of a pH effect on respiration and growth rates. However, growth and/or calcification of adult regular echinoids are generally not affected by pH_{SW} ≥ 7.7 (Kurihara et al., 2013; Moulin et al., 2014; Ries et al. 2009; Stumpp et al., 2012; Taylor et al., 2014; Uthicke et al., 2013, 2014; Wang et al., 2013). Only two studies conducted on adults showed that growth was affected by OA (Courtney et al., 2013; Holtmann et al., 2013). Similarly, respiration rates did not vary significantly in adults at pH_{SW} ≥ 7.7 (Catarino

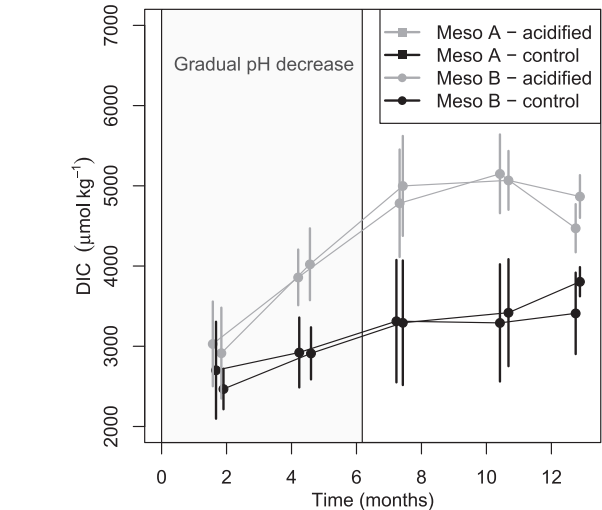


Fig. 7. Coelomic fluid DIC (mean ± SD, $n \geq 4$) of *E. mathaei* in the different pH conditions (gray: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) over the course of the experiment (months since the beginning of the experiment).

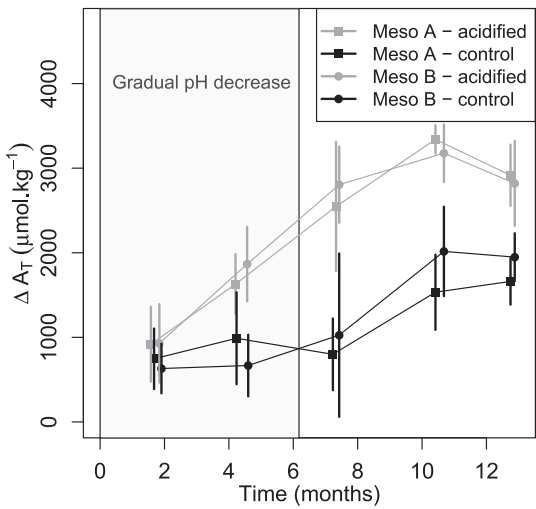


Fig. 8. ΔA_T (difference between the seawater A_T and the coelomic fluid A_T of *E. mathaei*) (mean ± SD, $n \geq 4$) in the different pH conditions (gray: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) over the course of the experiment (months since the beginning of the experiment).

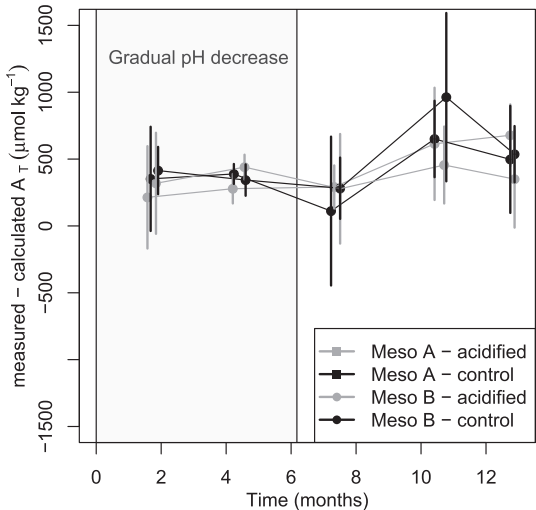


Fig. 9. Difference between the measured and the calculated A_T (using seacarb, see text) of the CF (mean ± SD, $n \geq 4$) of *E. mathaei* in the different pH condition (darkgray: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) over the course of the experiment (months since the beginning of the experiment).

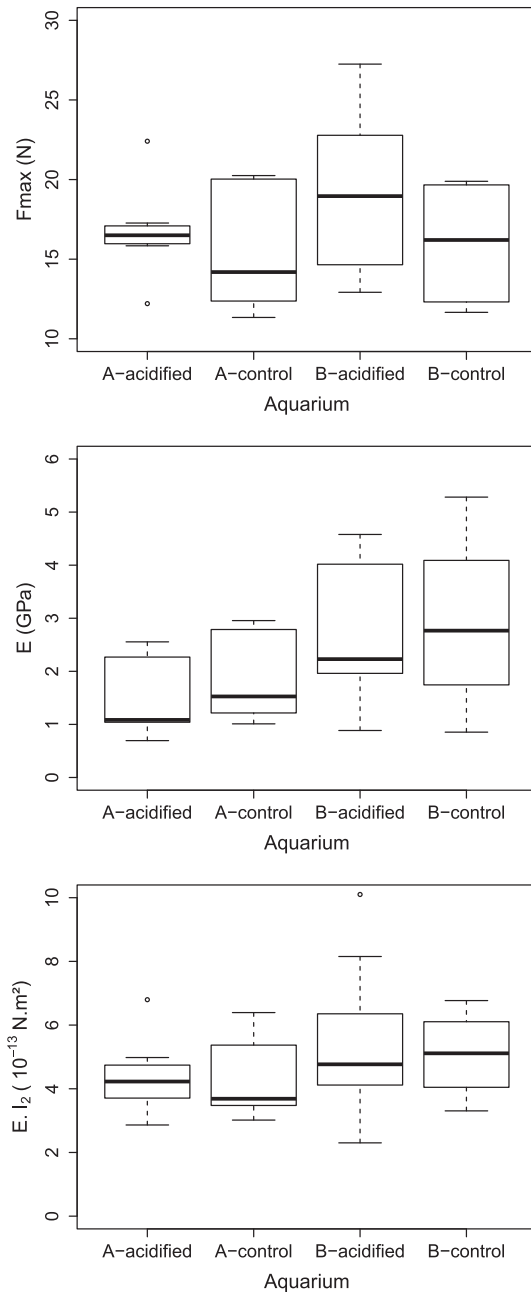


Fig. 10. Force at rupture $F_{\max}$ (top), Young's modulus E (middle) and flexural stiffness EI_2 (bottom) of the skeletal ambital plates of *E. mathaei* coming from the different aquaria (A for mesocosm A and B for mesocosm B) at different pH conditions at the end of the experiment. Boxplots represent the median (blackline), the interquartile range (box), the 1.5 times extended interquartile range from the box edges (whiskers) and the outliers (individual points).

et al. 2012; Kurihara et al., 2013; Moulin et al., 2014; Stumpp et al., 2012; Uthicke et al., 2014; Wang et al., 2014). Only Uthicke et al. (2013) observed a slightly decreased respiration rate at pH_{SW} 7.7 but not at pH_{SW} 7.5. So growth and respiration rates appear as not or only weakly affected by acidification down to pH_{SW} 7.7 in adult sea urchins, including at long-term. This agrees also with a recent field survey conducted on tropical CO_2 seeps where *Echinometra* sp. density was high even at reduced pH_{SW} (Fabricius et al., 2014).

The mechanical properties of the test did not differ significantly between control and treatment sea urchins at long-term. Similarly, no difference in the mechanical properties of the test of *P. lividus*

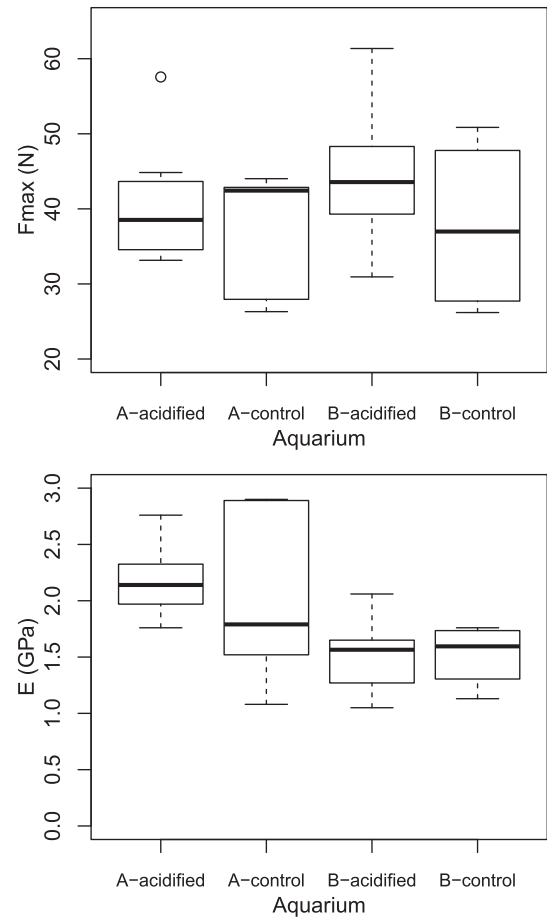


Fig. 11. Force at rupture $F_{\max}$ (top) and Young's modulus E (bottom) of the skeletal apical plates of *E. mathaei* coming from the different aquaria (A for mesocosm A and B for mesocosm B) at different pH conditions at the end of the experiment. Boxplots represent the median (blackline), the interquartile range (box), the 1.5 times extended interquartile range from the box edges (whiskers) and the outliers (individual points).

was observed after one year at pH_{SW} 7.8 (Collard et al., pers comm). This agrees with short-term results obtained on *S. droebachiensis* in which test plate strength was not affected after 45 days of exposure to pH_{SW} 7.7 (Holtmann et al., 2013). Asnaghi et al. (2013) reported that the whole test (crushing) of *P. lividus* was less robust at pH_{SW} 7.7. However, these tests were conducted on dried test and mainly reflect the strength of dried ligaments maintaining the plates together (see Dubois, 2014; Ellers et al., 1998). So results obtained so far indicate that mechanical properties of the test are not affected by a reduced pH_{SW} to levels of about 7.7.

The last principal biological function which could be affected is reproduction. Several previous studies suggested that effects on adults may be small compared to effects on reproduction and larvae (see e.g. Byrne, 2011; Byrne and Przeslawski, 2013). However, few of these studies were conducted at long-term taking into account pre-exposition and acclimation of parents. Kurihara et al. (2013) observed only a delay in gonad maturation and spawning following a 9 months exposure to decreased pH_{SW} but no effect on the maximum number of ova produced by *H. pulcherrimus*. *S. droebachiensis* fecundity was not affected after 16 month exposure to elevated pCO_2 (Dupont et al., 2013). Indeed, the effect of OA on the allocation of energy to reproduction seems to disappear when sea urchins are acclimatized at long-term, a process probably associated to a replenishment of the energy stores (Dupont et al., 2013). In our study, gonad development (observed during

Table 4

Impact of ocean acidification on the pH and the concentration of bicarbonates in the coelomic fluid (CF) of regular echinoid species. pH of seawater is expressed in total scale (pH_{T-SW}).

Species	Exposure time	Tested pH _{T-SW}	pH _{CF} change	Bicarbonate compensation	Reference
<i>Eucidaris tribuloides</i>	1 month	7.65	no–no	yes–yes	Collard et al. (2014)
<i>Arbacia</i>	2–4 days ^d	7.6 ^c	no	no	Calosi et al. (2013)
<i>laxula</i>	24 h	7.6 ^c	no	no	
<i>Paracentrotus lividus</i>	19 days	7.7–7.4	no	–	Catarino et al. (2012)
	2–4 days ^d	7.6 ^c	no	yes	Calosi et al. (2013)
	24 h	7.6 ^c	yes	yes	
	7 days	7.7–7.4	no	yes–yes	Collard et al. (2013)
			–yes		
	1 month	7.65	no	yes–yes	Collard et al. (2014)
		–7.5	–yes ^a		
<i>Psammechinus miliaris</i>	8 days	7.45 ^c	yes	yes	Miles et al. (2007)
<i>Echinometra mathaei</i>	14 + 35 days ^e	7.65	no	yes	Moulin et al. (2014)
	6 + 7 months ^e	7.65	no	yes	Present study
	77 days	7.8	no	–	Uthicke et al. (2014)
<i>Hemicentrotus pulcherrimus</i>	9 months	7.7 ^c	yes	–	Kurihara et al. (2013)
<i>Strongylocentrotus droebachiensis</i>	5 days	7.35 ^c	yes	yes	Spicer et al. (2011)
	3–5 days	7.6 ^c	yes	–	Dupont and Thorndyke (2012)
			–no		
	10 days	7.5–7.1	no	yes–yes	Stumpp et al. (2012)
			–yes		
	45 days	7.7	no–no	yes–yes	
		–7.25	yes	no	
		7.25 ^b			
<i>Strongylocentrotus fragilis</i>	7–31 days	7.5	yes	no–no	Taylor et al. (2014)
<i>Tripneustes ventricosus</i>	1 month	7.7–7.4	no–no	yes–yes	Collard et al. (2014)

^a pH_{CF} was reduced from 7.4 to 6.8 at pH_{T-SW} 7.5 but this decrease was not significant probably due to small sample size (n = 3).

^b Fasted individuals.

^c pH_T was calculated from carbonate system parameters presented in the study using seacarb.

^d In situ transplantation to volcanic vent.

^e Respectively gradual pH decrease + maintenance of contrasted conditions.

dissections for the sampling of the test plates) was zero or low. It is unclear if this was due to feeding conditions or simply because sea urchins were sacrificed out of the spawning season.

According to the general “law of conservation of energy”, pH_{CF} regulation should be energetically costly. However, in sea urchins, the elimination of the CO₂ from respiration (and from calcification) relies only on a favorable diffusion gradient for gas exchange (Farmanfarmaian, 1966). Like in marine organisms for which the acid–base balance was studied, an increase of the seawater pCO₂ directly causes an increase of the extracellular fluids pCO₂. This increase takes place until the diffusion gradient of the gas between the extracellular fluid and sea water is sufficient to restore the diffusion of CO₂ into the external environment (Seibel and Walsh, 2003). This increase of CO₂ in the coelomic fluid leads to a transient pH_{CF} decrease that should be avoided in order to maintain protein and enzyme functioning (Cameron, 1986; Boron, 2004). A transient energetically costly compensatory mechanism, i.e. an increase of bicarbonate ions, allows the restoration of pH_{CF} to previous values. Once this new equilibrium is reached, no more energy is needed to maintain the pH_{CF} as the CO₂ diffuses freely. This

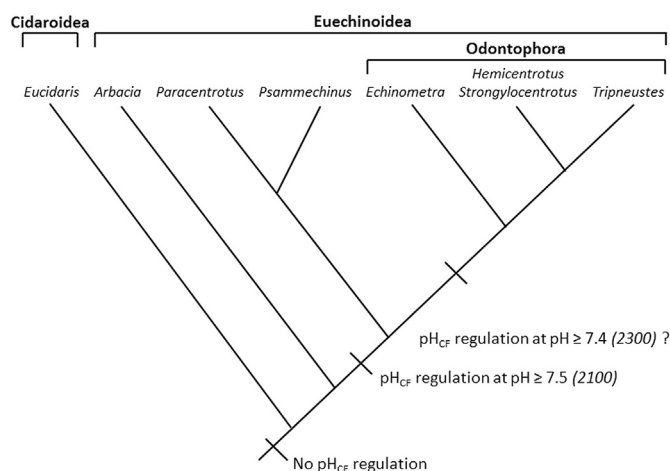


Fig. 12. The phylogeny of sea urchin genera (according to Kroh and Smith, 2010) in relation to their ability to regulate the pH of the coelomic fluid (pH_{CF}) under ocean acidification. Genera represented in the tree are those for which the ability to compensate pH_{CF} in OA conditions was investigated (Table 4 and as discussed in the text).

would explain why adult sea urchins are not affected by OA at long-term (replenishment of energy stores). This hypothesis is only valid if bicarbonate ions cannot diffuse to the external environment. Holtmann et al. (2013) showed that the intestine is a tight epithelium with respect to bicarbonate. Ries et al. (2009) observed a similar tightness of the epidermis. These two studies favor the hypothesis of an accumulation of bicarbonate into the coelomic fluid.

In conclusion, adult *E. mathaei* appear resilient in front of ocean acidification levels expected to occur by 2100 regarding acid–base regulation, growth, respiration rate and test mechanical properties. The assessment of the effects on reproductive output needs long-term experiments. Moreover, the impact of food quality and quantity needs definitely to be taken into account in the design of these future studies. Sea urchins, of which *E. mathaei*, actively participate in the resilience of coral reefs by their grazing activity, preventing the overgrowth of corals by algae (Valentine and Edgar, 2010). On the other hand, sea urchins, in some locations, are the major bioeroders of the coral reef substrate (Bak, 1994; Carreiro-Silva and McClanahan, 2001; Mokady et al., 1996), weakening the reef structure. The ability of *E. mathaei* to resist long-term moderate OA could therefore in turn affect its biological interaction with algae and corals, making it difficult to predict the future of coral reefs.

Acknowledgments

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