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Effect of ocean acidification on growth, gonad development and physiology of the sea urchin *Hemicentrotus pulcherrimus*

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ABSTRACT: Ocean acidification, due to diffusive uptake of atmospheric CO_2, has potentially profound ramifications for the entire marine ecosystem. Scientific knowledge on the biological impacts of ocean acidification is rapidly accumulating; however, data are still scarce on whether and how ocean acidification affects the reproductive system of marine organisms. We evaluated the long-term (9 mo) effects of high CO_2 (1000 µatm) on the gametogenesis, survival, growth and physiology of the sea urchin *Hemicentrotus pulcherrimus*. Hypercapnic exposure delayed gonad maturation and spawning by 1 mo, whereas it had no effect on the maximum number of ova, survival or growth. After 9 mo of exposure, pH (control: 7.61, high-CO_2: 7.03) and Mg^{2+} concentration (control: 50.3, high-CO_2: 48.6 mmol l^{-1}) of the coelomic fluid were significantly lower in the experimental urchins. In addition, a 16 d exposure experiment revealed that 1000 µatm CO_2 suppressed food intake to <30% of that of the controls. These data suggest that the ocean condition predicted to occur by the end of this century disrupts the physiological status of the sea urchin, possibly through reduced energy intake, which may delay reproductive phenology of the species. Taking into account earlier studies reporting negative impacts of ocean acidification on the early development of the same species, these results imply that ocean acidification will threaten *H. pulcherrimus* at a community level.

KEY WORDS: CO_2 · Ocean acidification · Sea urchin · Reproduction · Feeding · Physiology

INTRODUCTION

There is wide recognition that the increasing concentration of atmospheric CO_2 is acidifying oceans through diffusional entry of the gas into surface seawater (termed ocean acidification; Caldeira & Wickett 2003, IPCC 2007). Increasing acidity of seawater shifts carbonate equilibria so that carbonate ions bind to H^+ ions to form bicarbonate ions, thereby reducing the $CaCO_3$ saturation state of seawater (Orr et al. 2005). Not surprisingly, most ocean acidification studies have used dominant marine calcifiers, e.g. coccolithophores, corals, mollusks and foraminiferans, and revealed reductions in calcification rates (Riebesell et al. 2000, Kleypas et al. 2006, Gazeau et al. 2007, Hofmann et al. 2010), but there are notable exceptions to this general finding (Ries et al. 2009, Kroeker et al. 2010). Meanwhile, more recent studies have demonstrated a much wider spectrum of biological consequences invoked by ocean acidification, which includes immune responses, growth, physiology and behavior (Bibby et al. 2008, Kurihara et al. 2008, Melzner et al. 2009, Domenici et al. 2012).

Given that living in a high CO_2, acidified environment constitutes stress to marine inhabitants, increasing CO_2 could have profound ramifications on

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the biological process by affecting the energy balance of the organism. The energy budget of living organisms follows the 'law of conservation of energy', and the energy input (food source) is equal to the energy output, which is divided between growth (production of tissue), reproduction (production of gametes), maintenance metabolism and excretory loss (Sibly & Calow 1986). Under low energy input or stress conditions, this allocation is suggested to follow the principles of maximizing fitness, and studies have shown that allocation to maintenance tends to take precedence over growth or reproduction (Zera & Harshman 2001, Schneider 2004, Koojiman 2010, Sokolova et al. 2012). Recent studies have indicated that an acidified environment involves potential energy costs for metabolic adaptations and calcification (Melzner et al. 2009, Lannig et al. 2010, Sokolova et al. 2012, Stumpp et al. 2012), thus one might expect that reallocation of energy between somatic and gonad growth will occur in acidified conditions. However, though there are several studies evaluating the effect of ocean acidification on growth rate, to our knowledge, there is a limited number of studies that have examined the effect of high CO_2 on the reproductive potential of marine organisms. Wood et al. (2008) reported that egg size was not affected in regenerating arms of ophiuroid *Amphiura filiformis* cultured in seawater pH 7.7, 7.3 and 6.8 for 40 d. Exposure of adult copepods (*Acartia steueri* and *A. tsuenis*) to high CO_2 (2000 µatm) also did not affect their egg production (Kurihara et al. 2004, Kurihara & Ishimatsu 2008). However, Fitzer et al. (2012) recently demonstrated that the body length of the copepod *Tisbe battagliai* decrease at pH 7.67, while the naupliar production was significantly higher at pH 7.67 compared with pH 7.82, and the authors suggested that copepods preferentially reallocate resources to maintain reproduction at the expense of somatic growth during the maturation stage. Gonad dry mass and growth of the sea urchin *Strongylocentrotus drobachiencis* reared under 1007–1431 and 2800–3800 µatm pCO_2 for 45 d was found to be significantly affected, while ammonium excretion increased (Stumpp et al. 2012). These studies suggest that allocation of energy under stress conditions may differ between species, and evaluation of the responses of different traits, including energy intake, metabolism, somatic and gonad growth, is fundamental for better understanding the energetic strategy of the organism under acidified stress conditions.

In the present study, adult sea urchins *Hemicentrotus pulcherrimus* were reared for 9 mo under control (atmospheric CO_2 concentration 380 µatm and high-CO_2 (1000 µatm) conditions to evaluate the effects of hypercapnia on survival, somatic growth, gonad index, gametogenesis cycle, egg production and several physiological parameters, including oxygen uptake rate, pH, and Ca^{2+} and Mg^{2+} ion concentrations of the coelomic fluid. Additionally, a short-term (16 d) experiment was conducted to evaluate the effects of 1000 µatm CO_2 on feeding rate. The CO_2 condition was adopted in accordance with the A1FI emission scenario (atmospheric CO_2 concentration 825 to 1250 µatm by the year 2100; IPCC 2007).

MATERIALS AND METHODS

Origin of animals

Adult sea urchins *Hemicentrotus pulcherrimus* (test diameter 30–40 mm) were collected in September 2007 (long-term experiment) and October 2008 (short-term experiment) from a subtidal rocky shore (<1 m depth) near the Asamushi Marine Biological Station, Tohoku University in Sendai, Japan, and transported to Institute for East China Sea Research (ECSER), Nagasaki University, where the experiments were conducted. *H. pulcherrimus* spawns in December through April and is in spent stage during June to November along the Japanese coasts (Fuji 1960). All sea urchins were reared in a 3 stock aquaria (capacity 45 l) for more than 1 mo; the aquaria were provided with running seawater (100 ml min^{-1}) to allow recovery from the transportation before the experiments began. During acclimation, the sea urchins were fed on the sea alga *Undaria pinnatifida* every other day.

Long-term CO_2 exposure

Experimental system

Following acclimation, sea urchins were reared in 2 sets of recirculating systems (flow rate 10 l min^{-1}), each consisting of an aquarium (capacity 45 l) and a header tank (capacity 72 l). Natural seawater was pumped from 20 m depth from the coast in front of ECSER, filtered, and continually supplied to each header tank at a rate of 100 ml min^{-1}. Seawater in the header tank of the control and experimental setups was bubbled at a rate of 10 l min^{-1} with outdoor air and with CO_2-enriched air containing 1000 µatm CO_2 supplied by a mass-flow controller (SEC-E50MK3 for air, and SEC-E40 for CO_2 HORIBA

STEC), respectively. The light regime was controlled with a time switch (Hitachi AW-700), where the cycle was adjusted every 2 wk to simulate the natural photoperiod. Seawater salinity, pH (NBS scale) and temperature of the aquaria were measured daily with a refractometer (Atago 100-S), a pH meter (Mettler Toledo MP125) and a thermometer (SS SATO, SK-1250MCIIIα), respectively. Seawater alkalinity was determined with a PHM290 pH meter and an ABU901 autoburette (Radiometer). Seawater carbonate chemistry was calculated using CO2SYS (Lewis & Wallace 1998).

Two hundred specimens were chosen randomly from the stock, divided into 2 groups of 100 individuals of similar size and reared for 9 mo (the initial mean test diameters ± SD of the control and high-CO_2 groups were 35.7 ± 3.1 and 35.2 ± 3.0 mm, respectively). The experiment was conducted from October 2007 to June 2008 so that the sea urchins were exposed to experimental conditions through all gametogenesis cycles (from the spent stage in 2007 until the next spent stage in 2008). The sea urchins were fed on 1 g sea algae *Undaria pinnatifida* per individual every other day. Feces were removed from the aquaria 1 d after feeding.

Growth rate and gonad development

Dead animals were counted every day and removed from the aquarium. Size (test diameter) and wet weight of all sea urchins were measured monthly. The size was determined with calipers to the nearest 0.1 mm and was standardized using the initial mean value of each group determined in October 2007. The wet weight was measured to the nearest 0.01 g after blotting the sea urchins. Each month, 10 sea urchins were sampled randomly from each aquarium and dissected into test, masticating apparatus (Aristotle's lantern), gonad and the rest of the soft body. Hence, numbers of sea urchins in each aquarium decreased with time. Wet weights of the whole body, calcium carbonate test and Aristotle's lantern, and gonad of these animals were measured to the nearest 0.01 g. The soft-body wet weight and gonad index (GI) were calculated as soft-body wet weight = whole-body wet weight − (test + Aristotle's lantern + gonad wet weights), and GI (%) = (gonad wet weight) (whole-body wet weight)$^{-1}$ × 100), respectively. After the measurements, one of the 5 gonads per individual was fixed in Bouin's solution for histological determination of maturation stages and quantification of ova in cross-sections of the ovary. Paraffin sections 5 µm thick were prepared using an RM2135 microtome (Leica Microsystems) and stained with hematoxylin and eosin. Stages of the gametogenesis cycle of both males and females were judged according to Fuji (1960): stage I (spent recovering stage); stage II (growing stage); stage III (premature stage); stage IV (mature stage); and stage V (spent stage). The number of ova (mature eggs) was counted for all sampled females in February and March, when most individuals were at either the premature or mature stage. In January, April, May and June, counts were done for 3 females only. Each gonad was cut into 3 parts of approximately the same length along the longitudinal axis and the number of ova was counted in the cross-sections of 7 to 11 discontiguous slides from the second, central part of the ovary.

Respiration rate

At the end of the long-term experiment, the respiration rate of each of 13 individuals reared for 9 mo under control or high-CO_2 seawater was measured using a closed respirometric chamber (240 ml). A sea urchin was transferred individually into the chamber continuously supplied with control or high-CO_2 seawater and habituated to the condition for 24 h. After the acclimation, the seawater flow was stopped, and 0.5 ml seawater samples were taken at Time 0, 30 and 60 min for the determination of dissolved oxygen concentration. Seawater in the chamber was continuously mixed with a magnetic stirrer and seawater temperature was kept at 22°C. The oxygen concentration of the seawater samples was measured using an oxygen electrode (Model 1302, Strathkelvin) connected to an oxygen meter (Model 782, Strathkelvin). Preliminary determinations without sea urchins verified negligible bacterial oxygen consumption. The oxygen saturation remained above 85% throughout the measurements. After the measurements, body wet weight was determined as described above.

pH and ion concentration

To evaluate coelomic fluid pH, [Ca^{2+}] and [Mg^{2+}] of sea urchins after 9 mo exposure, 1.0 ml perivisceral coelomic fluid was sampled by puncturing the peristominal membrane with a syringe. The pH of the 500 µl perivisceral fluid was immediately measured after sampling using a micro pH electrode (Mettler Toledo, InLab Micro). The rest of the sample was

frozen for later analyses of [Ca^{2+}] and [Mg^{2+}]. Prior to ion determinations, 500 µl of perivisceral fluid samples were diluted in 100 ml milli-Q water and [Ca^{2+}] and [Mg^{2+}] were measured using the ICP-AES (ULTIMA2, HORIBA).

Short-term exposure experiment

To evaluate the effect of ocean acidification on the feeding rate of individual sea urchins, an additional short-term experiment was conducted. A total of 20 sea urchins (mean ± SD wet weight 21 ± 4.9 g) were kept individually in mesh (80 µm)-covered plastic chambers (capacity 500 ml) placed in 2 aquaria (10 chambers per aquaria). Each chamber was continuously supplied with well-aerated, temperature-controlled (18°C) seawater from one of 2 header tanks. Seawater overflowed from the top of the chambers to the aquaria, and was recirculated to the header tanks. An additional heater was placed in each aquarium. After a week of acclimation, the seawater CO_2 condition in each of the 2 header tanks was adjusted to either 380 µatm CO_2 (control) or 1000 µatm CO_2 (high-CO_2). Temperature and pH were checked daily for aquarium seawater. Feeding rates were determined for all individuals as follows. During the 16 d period (5 to 21 January 2009), the sea urchins were fed with a known weight of *Undaria pinnatifida* every other day. The alga was first dried at 60°C for 24 h, and measured to the nearest 0.001 g before being given to the animals. Residual food was collected on the following day, dried and weighed in the same manner to evaluate the food intake after correcting for dissolution of the food in seawater in 24 h without a sea urchin. Seawater environmental parameters were measured as described in the long-term experiment and carbonate chemistry was calculated using CO2SYS (Lewis & Wallace 1998) (Table 3).

Statistical analysis

The software packages SPSS (version 11.0), R (R Development Core Team 2011) and OpenBUGS (www.openbugs.info) were used to conduct the analyses. ANCOVA was performed using SPSS to compare size after standardizing the data using the mean initial body length of each group determined in October 2007, while a 2-way ANOVA was applied to compare GI (arcsine transformed). To elucidate whether the experimental conditions affected the number of ova (N) and shifted the month (t) during peak ova number, a non-linear regression of the form, $N = \gamma/\mathrm{sqrt}(2\pi\sigma^2)\ \exp(-(t - M)^2/(2\sigma^2))$ was conducted, where γ is the maximum number of ova, M is the month when the maximum number of ova was estimated and σ is the period when 68% of the total number of ova are observed. This model was fitted using a Markov Chain Monte Carlo routine based on a Gibbs sampler in OpenBUGS. Four chains were run to approximate convergence, and a total of 4000 uncorrelated samples were saved from the simulations. Priors for the parameters were drawn from a normal distribution, whose parameters were drawn from non-informative uniformly distributed hyperprior. Student's t-test was performed to evaluate the effect of CO_2 on respiration rate, coelomic fluid pH, [Ca^{2+}] and [Mg^{2+}].

RESULTS

Seawater chemistry

The seawater temperature fluctuated seasonally between 12 and 26°C during the 9 mo experimental period (Fig. 1A). The mean seawater pHs during the long-term experiment in the control and high-CO_2 conditions were 8.10 ± 0.05 and 7.83 ± 0.05, respec-

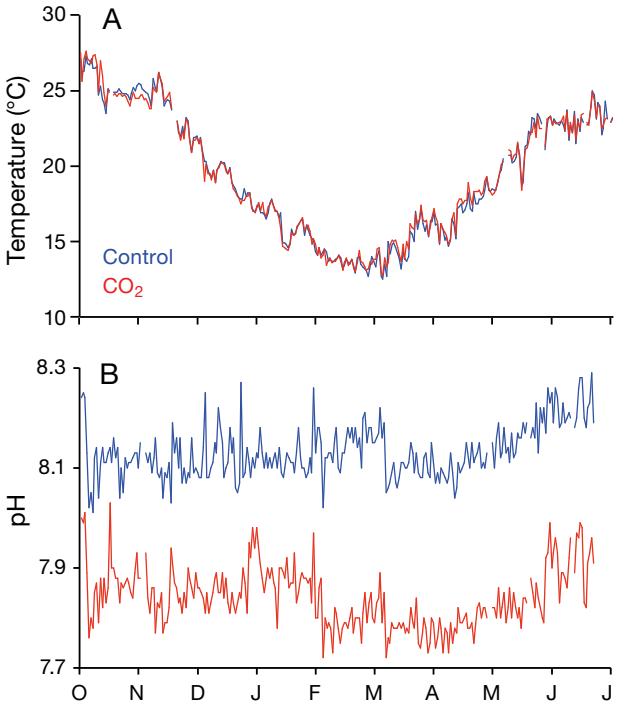

Fig. 1. (A) Seawater temperature and (B) pH in the control (CO_2 380 µatm) and high-CO_2 (CO_2 1000 µatm) treatments during the 9 mo experiment

Table 1. Values (mean ± SD or range) of seawater chemistry during the experimental period. Seawater pH, temperature and salinity were measured daily for each aquarium. Alkalinity was measured 3 times and the average value (2198 µEq kg^{-1}) was used to calculate total CO_2 (TCO_2), concentrations of HCO_3^-, CO_3^{2-} and CO_2, and saturation states of calcite (ΩCa) and aragonite (ΩAr) in CO2SYS (Lewis & Wallace 1998). Dissociation constants K1 and K2, and Ω solubility were adopted from Mehrbach et al. (1973) and Mucci (1983), respectively

Condition	pH	Temperature (°C)	TCO_2 (mmol kg^{-1})	pCO_2 (µatm)	HCO_3^- (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	CO_2 (mmol kg^{-1})	ΩCa	ΩAr	Salinity
Control	8.1 ± 0.1	18.5 (12–26)	1977 ± 35	429 ± 56	1805 ± 56	158 ± 23	15 ± 2.2	3.8 ± 0.6	2.4 ± 0.4	35.0 ± 0.4
CO_2	7.83 ± 0.05	18.5 (12–26)	2096 ± 29	919 ± 122	1976 ± 40	89 ± 16	31 ± 5.3	2.1 ± 0.4	1.4 ± 0.3	34.9 ± 0.4

tively (Table 1, Fig. 1B). The seawater carbonate chemistry in control and high-CO_2 conditions is shown in Table 1.

Survival, growth, gonad development and egg reproduction

No individuals died during the 9 mo experimental period in either condition. The standardized size of sea urchins did not differ significantly between conditions throughout the experimental period (Fig. 2A, ANCOVA, Table 2). The wet weights of the calcium carbonate body (test + Aristotle's lantern) and the soft body were not affected by treatment (Fig. 3). The GI was not significantly affected by high CO_2, but there was an interaction between month and experimental condition (Fig. 2B, 2-way ANOVA, Table 2). Because the gonad size of sea urchins does not necessarily relate to the progress of gametogenesis alone (Walker et al. 1998, 2007), we examined the percentage distribution of each gonad developmental stage, and found that germinal stages were more advanced in the control individuals compared with the high-CO_2 groups at the same sampling times in January through

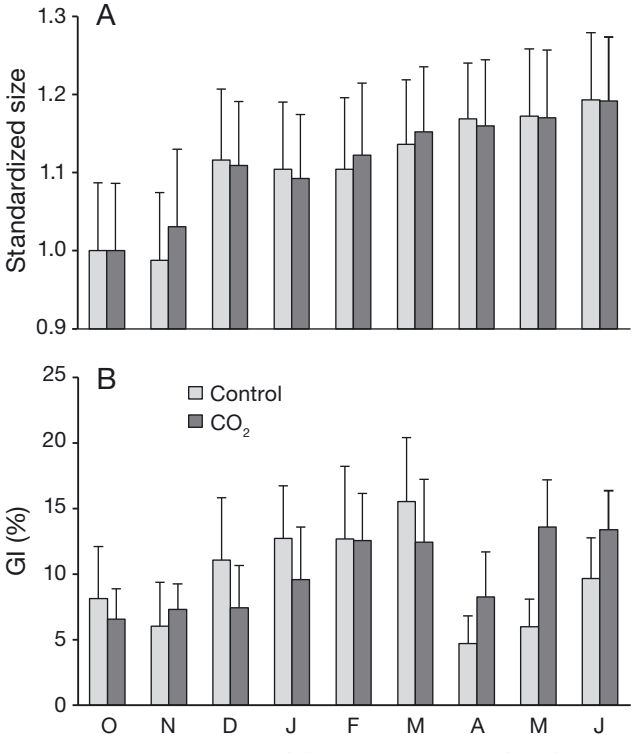

Fig. 2 *Hemicentrotus pulcherrimus*. Mean ± SD (A) standardized test size and (B) gonad index (GI; N = 10) of sea urchins reared under control and high-CO_2 conditions. Size was standardized by dividing the test diameter data of each group measured every month by the mean value of each group determined in October 2007

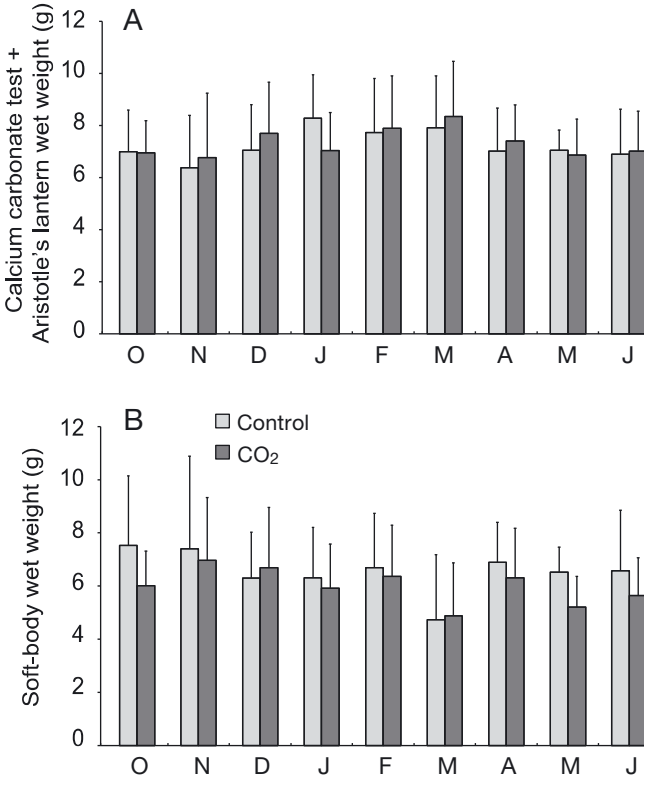

Fig. 3. *Hemicentrotus pulcherrimus*. Mean (A) calcium carbonate test + Aristotle's lantern wet weight and (B) soft-body wet weight (= whole body wet weight – (gonad wet weight + Aristotle's lantern wet weight)) of sea urchins reared under the control and high-CO_2 conditions. N = 10

Table 2. Effects of CO_2 and exposure time on standardized sea urchin size (ANCOVA), and arcsine-transformed gonad index (GI) (2-way ANOVA)

Source	Type III SS	df	MS	*F*	p
Standardized size					
Corrected model	4.005*	3	1.335	166.858	0.000
CO_2	4.88×10^{-5}	1	4.88×10^{-5}	0.006	0.938
Time	3.852	1	3.852	481.522	0.000
CO_2 × Time	0.004	1	0.004	0.520	0.471
Residuals	9	1125	0.008		
Total	1322.079	1129			
Arcsine GI					
Corrected model	0.164**	17	0.010	6.740	0.000
CO_2	0.002	1	0.002	1.212	0.272
Time	0.107	8	0.013	9.359	0.000
CO_2 × Time	0.055	8	0.007	4.811	0.000
Residuals	0.232	162	0.001		
Total	2.132	180			

*R^2 = 0.308 (adjusted R^2 = 0.306), **R^2 = 0.414 (adjusted R^2 = 0.353)

April (Fig. 4). The number of ova peaked in February in the control sea urchins (Fig. 5A,Bii,Civ), whereas it peaked 1 mo later in the high-CO_2 urchins (Fig. 5A,Bii,Cvi). No significant difference was detected for the maximum numbers of ova and period of time when the ova were observed between the control and high-CO_2 conditions (Fig. 5A,Bii,Biii). Spawning was observed on 16 February and 13 March in the control and high-CO_2 conditions, respectively.

Respiration, pH and ion concentration of the coelomic fluid

Respiration rates of the sea urchins exposed for 9 mo under high-CO_2 conditions (0.783 ± 0.35 µmol l^{-1} h^{-1} g^{-1}) were slightly but not significantly higher compared with the controls (0.588 ± 0.24 µmol l^{-1} h^{-1} g^{-1}) (Student's *t*-test, p = 0.22; Fig. 6). Coelomic fluid pH of high-CO_2 sea urchins (7.03 ± 0.29) was significantly lower (*t*-test, p < 0.001) compared with the control (7.61 ± 0.11; Fig. 6). The [Mg^{2+}] of the coelomic fluid was significantly lower in the high-CO_2 sea urchins (48.66 ± 1.82 mmol l^{-1}) compared with the control sea urchins (50.38 ± 1.38 mmol l^{-1}, Student's *t*-test, p = 0.03; Fig. 6), whereas [Ca^{2+}] was not affected (control: 9.67 ± 0.38; CO_2: 9.96 ± 0.31, Student's *t*-test, p = 0.06, Fig. 6).

Feeding

The seawater carbonate chemistry of the short-term experiment is shown in Table 3. Feeding rate was significantly suppressed by high CO_2 (repeated-measures 2-way ANOVA, $F_{1,18}$ = 22.33, p < 0.001) and there was a significant effect of time ($F_{7,126}$ = 4.14, p < 0.001). The interaction of CO_2 concentration and time was significant ($F_{7,126}$ = 3.30, p = 0.003; Fig. 7).

DISCUSSION

The present study demonstrated that long-term exposure to 1000 µatm CO_2 delayed both gametogenesis and spawning of the sea urchin *Hemicentrotus pulcherrimus*, and significantly decreased pH and [Mg^{2+}] of the coelomic fluid. However, survival,

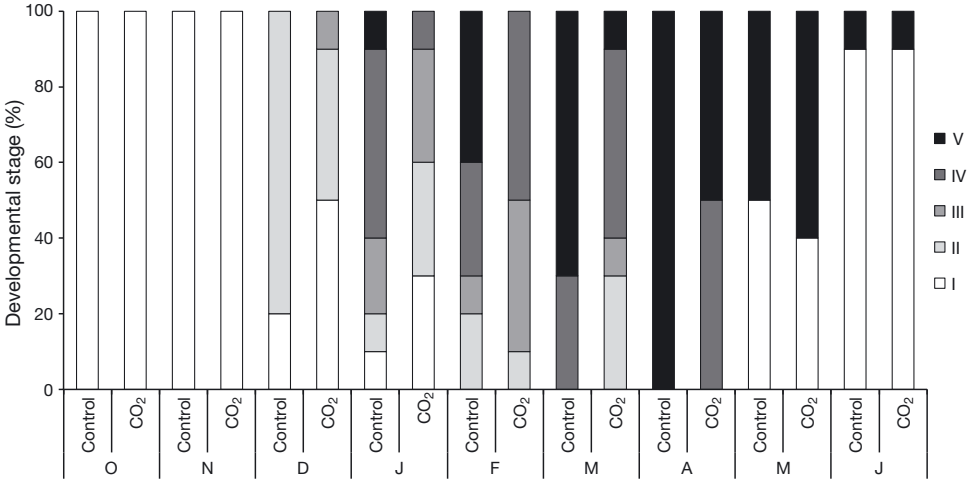

Fig. 4. *Hemicentrotus pulcherrimus.* Percent distribution of the gonad developmental stages reared under control and high-CO_2 conditions (N = 10). The gametogenesis stages were determined according to Fuji (1960): stage I (spent recovering stage); stage II (growing stage); stage III (pre-mature stage); stage IV (mature stage); and stage V (spent stage)

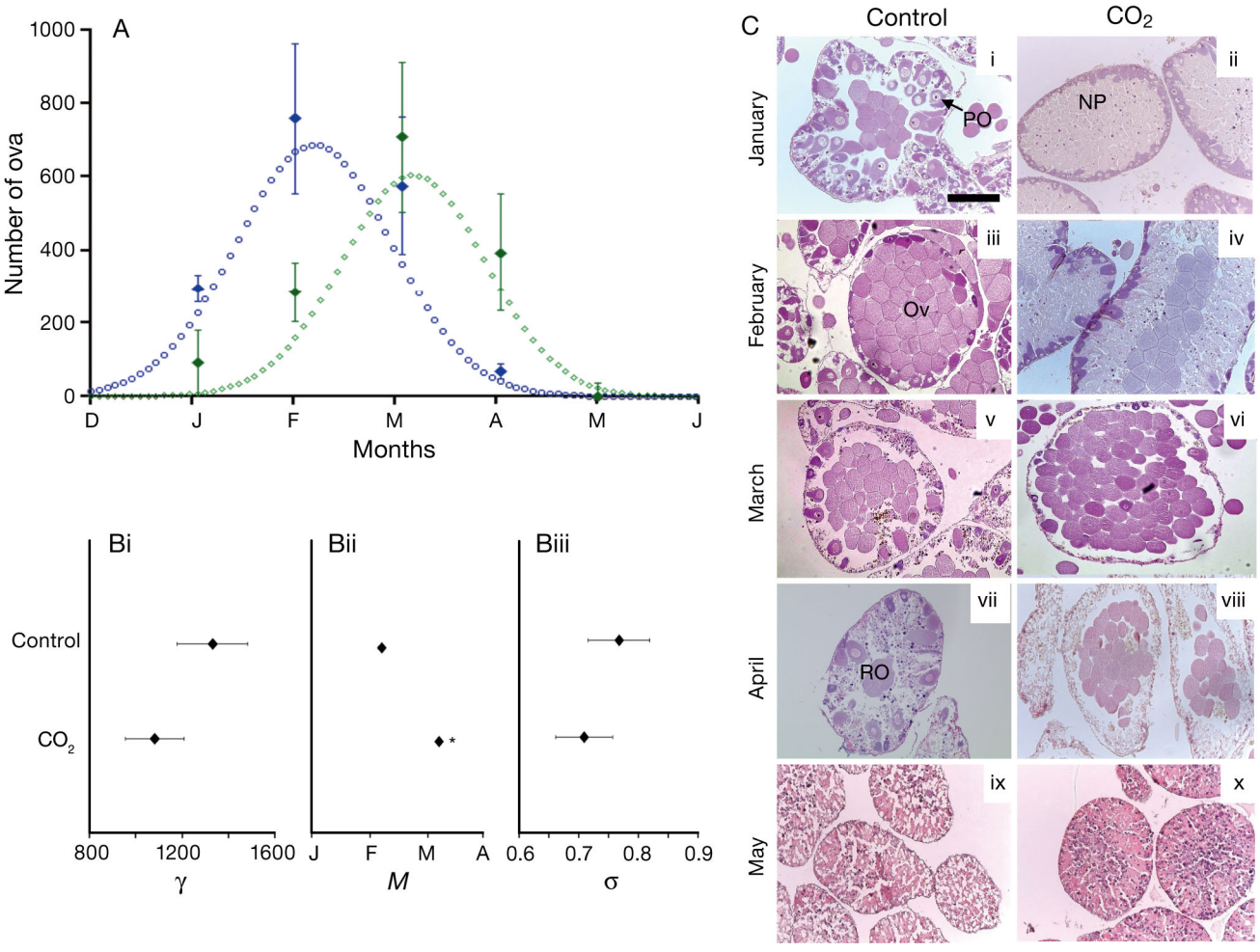

Fig. 5. *Hemicentrotus pulcherrimus.* (A) Mean (±SD) number of ova per cross-section of sea urchin ovary reared under control (blue) and high-CO_2 conditions (green). N = 3 in January, April, May, N = 3 to 6 in February, March. (Bi) Maximum number of ova, γ, (Bii) mean month during the maximum number of ova, *M*, and (Biii) the mean period that the ova were observed, σ, in the control and high-CO_2 conditions. *Significant difference from control. (C) Photomicrographs showing ovarian development in the control and high-CO_2 groups. Ovarian gametogenesis stages were determined according to Fuji (1960) (see Fig. 4 legend): stage I (ix,x); stage II (ii); stage III (i,iv); stage IV (iii,vi); and stage V (v,vii,viii). Scale (200 µm) applies to all photomicrographs. NP: nutritive phagocyte; PO: primary ooctyte; Ov: ovum (mature egg); RO: residual oocyte

growth and respiration rate were not affected by high CO_2. In addition, the short-term experiment demonstrated that exposure to 1000 µatm CO_2 suppressed food intake of this sea urchin.

Effects on gonad growth

Long-term (9 mo) exposure to 1000 µatm CO_2 delayed both gametogenesis and spawning of *Hemicentrotus pulcherrimus* by 1 mo without affecting the maximum number of ova. The timing of spawning and larval release is considered to converge to when biotic and abiotic environmental conditions are most suitable for the survival of spawning females, embryos, larvae and settling stages (Morgan 1995). Hence, a temporal change in gametogenesis and spawning may result in reduced fitness of larvae due to the reduced survival rate at the sub-optimal temperature and phytoplankton food condition (Morgan & Christy 1994).

Gametogenesis of sea urchins is known to be governed by a number of factors including environmental conditions (e.g. temperature, photoperiod) and nutritional status of individuals (Walker & Lesser 1998, Yamamoto et al. 1988), and when energy intake is limited, animals often decrease reproductive effort (Schneider 2004). Siikavuopio et al. (2007) demonstrated that hypercapnia (5000–6000 µatm CO_2)

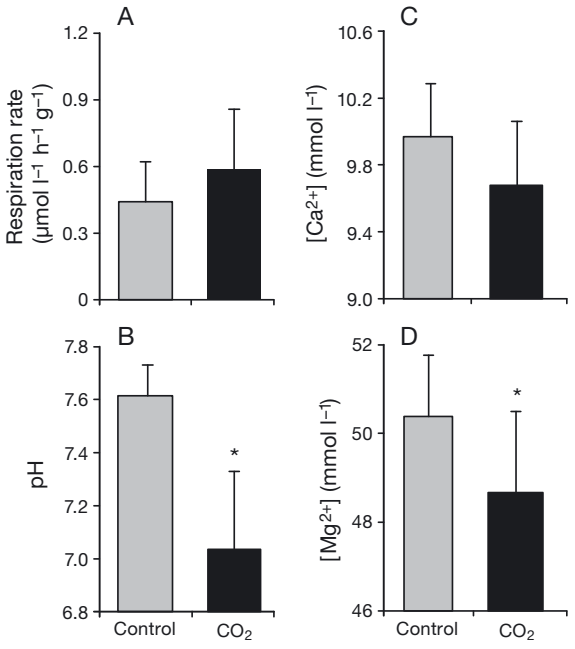

Fig. 6. *Hemicentrotus pulcherrimus.* Mean ± SE (A) respiration rate, (B) coelomic fluid pH, (C) $[Ca^{2+}]$ and (D) $[Mg^{2+}]$ of sea urchins reared under control and high-CO_2 conditions for 9 mo. N = 13. *Significant difference from control

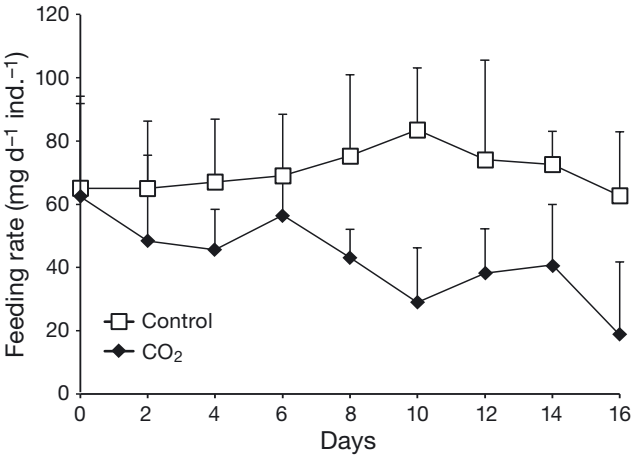

Fig. 7. *Hemicentrotus pulcherrimus.* Mean ± SE feeding rate of sea urchins reared under control and high-CO_2 conditions. N = 10

suppressed both gonad growth and food conversion efficiency of the sea urchin *Strongylocentrotus droebachiensis*. The finding of a significant positive correlation between gonad growth and food intake in *S. droebachiensis* (Christiansen & Siikavuopio 2007) supports the speculation by Siikavuopio et al. (2007). In the short-term experiment in the present study, we also observed a reduction in food intake in high-CO_2 urchins (Fig. 7). Since the effect of CO_2 on food intake was measured in a short-term experiment, we were not able to determine whether the suppression of food intake lasted for the whole experiment; however, from a recent study we found that the food intake of *Hemicentrotus pulcherrimus* declined with time and remained significantly lower compared with controls for several months (R. Yin et al. unpubl. data). Similarly, decreased ingestion and gonad dry weight were also found in *S. drobachiensis* reared for 45 d under 2800–3800 µatm CO_2, (Stumpp et al. 2012). Before gametogenesis begins, the major yolk protein (MYP) synthesized from proteins of ingested food is stored in the nutrient phagocytes (NP), which are the somatic cells within the germinal epithelium (Walker et al. 2005). MYP is consumed for egg and sperm production during gametogenesis, and also serves as a nutrient source for early development after fertilization (Unuma et al. 2003). Since both MYP production and NP growth are stimulated by the availability of food (de Jong-Westman et al. 1995, Lawrence et al. 1997), we speculate that the reduced feeding under the high-CO_2 conditions disrupted MYP synthesis, leading to a delay in ovum growth to maturity. The mechanisms underlying the feeding suppression by CO_2 remain unknown; reduced feeding efficacy due to partial weakening or dissolution of the masticating apparatus (Aristotle's lantern) might be involved because sea urchin teeth are composed of high-Mg calcite, a highly acid-soluble form of $CaCO_3$ (Killian et al. 2011). A more recent study revealed that the movement speed of *H. pulcherrimus* during food searching was reduced by high CO_2 compared with the control (R. Yin et al. unpubl. data).

Table 3. Values of seawater chemistry during the short-term experiment. pH and temperature were measured daily for each tank. Salinity was measured daily and adjusted to 35 ± 0.5 by adding distilled when necessary. Alkalinity (2198 µEq kg^{-1} seawater) was used to calculate the other parameters in CO2SYS. Dissociation constants K1 and K2 were adopted from Mehrbach et al. (1973)

Condition	pH	Temperature (°C)	TCO_2 (mmol kg^{-1})	pCO_2 (µatm)	HCO_3^- (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	CO_2 (mmol kg^{-1})
Control	8.20 ± 0.02	18.2 ± 0.11	1947 ± 13	348 ± 23	1758 ± 20	177 ± 8	11.9 ± 0.8
CO_2	7.85 ± 0.02	18.0 ± 0.15	2096 ± 8	873 ± 48	1976 ± 10	89 ± 4	29.7 ± 1.6

Effects on physiology: respiration, coelomic fluid pH, Ca^{2+} and Mg^{2+} concentrations

We found that 1000 µatm CO_2 did not affect respiration rate, which is consistent with the results observed in the sea urchin *Strongylocentrotus droebachiensis* reared for 45 d under 1007–1431 µatm (Stumpp et al. 2012). Metabolic suppression under high-CO_2 conditions has been proposed as an adaptive strategy to suppress ATP demand (Fabry et al. 2008). However, metabolic responses of marine organisms are highly variable between species; several studies reported no effect (Gutowska et al. 2008, Melzner et al. 2009) or even an increase (Wood et al. 2008, Beniash et al. 2010) under high CO_2 conditions.

A significant reduction in coelomic fluid pH (0.6 units) was observed in *Hemicentrotus pulcherrimus* exposed to 1000 µatm CO_2 (pH 7.83) in our study. Miles et al. (2007) reported the lack of acid–base compensation capacity of the sea urchin *Psammechinus miliaris* reared under high-CO_2 conditions (pH 7.44) for 8 d. A reduction of the coelomic fluid pH was also observed in *S. droebachiencis* reared in 1353 µatm CO_2 seawater for 5 d (Spicer et al. 2011). Melzner et al. (2009) suggested that less active organisms such as echinoderms and bivalve mollusks have less capacity to regulate the acid–base balance, and are therefore more vulnerable to high CO_2. On the other hand, full compensation of coelomic fluid acidosis was reported in *S. drobachiensis* exposed for both 10 and 45 d in 1007–1431 µatm CO_2 (Stumpp et al. 2012). The authors suggested that the sea urchin was pre-adapted to high CO_2 due to natural variability in pCO_2 by upwelling in its habitat.

In addition to the significant decrease in coelomic fluid pH, we also observed a subtle but significant decrease in coelomic fluid [Mg^{2+}] in high-CO_2 sea urchins (Fig. 6). This contrasts with previous findings that demonstrated an increase in coelomic fluid [Mg^{2+}] in *Psammechinus miliaris* (Miles et al. 2007) and an increase in [Ca^{2+}] in *Strongylocentrotus drobachiensis* exposed to high-CO_2 conditions (Spicer et al. 2011). Increases of [Mg^{2+}] and [Ca^{2+}] have been suggested to be due to test dissolution, which functions as a compensation mechanism against extracellular acidosis. However, since the weight of calcium carbonate body or [Ca^{2+}] was not affected by high CO_2 in this study, it seems unlikely that *Hemicentrotus pulcherrimus* relies on test dissolution to restore coelomic fluid pH. The change in [Mg^{2+}] under elevated CO_2 conditions seems to be highly species specific; a decrease (in *H. pulcherrimus*, present study), an increase (in *P. mirabilis*; Miles et al. 2007) and no change (in *S. drobachiensis*; Spicer et al. 2011) have been reported for various species, and the reason for this variability is unclear.

Effects of survival and growth

Our study demonstrated that neither survival nor growth rate of *Hemicentrotus pulcherrimus* (adult specimens, mean initial body weight 14.5 g) was affected when reared for 9 mo under high-CO_2 conditions (CO_2 1000 µatm, pH 7.83). In contrast, when the same species, with a mean initial body weight of 0.84 g, was exposed for 26 wk to 200 µatm above ambient CO_2 (experimental: 560 µatm, seawater pH 7.897–7.902; control: 360 µatm, seawater pH 7.936–7.945), it showed reductions in both survival and growth (Shirayama & Thornton 2005). Similar to our findings for *H. pulcherrimus*, growth and survival rates of the green sea urchin *Strongylocentrotus droebachiensis* (mature specimens, initial body weight 51.3 g) were unaffected when exposed to very high CO_2 (pH 6.89, pCO_2 8000 µatm) for about 2 mo (Siikavuopio et al. 2007). Additionally, a recent study reported that the growth rate of the sea star *Pisaster ochraceus* (juvenile, initial body weight 3–7 g) was significantly stimulated when reared for 2 mo under high-CO_2 conditions (CO_2 780 µatm, pH 7.79) compared with the control (CO_2 380 µatm, pH 7.88) (Gooding et al. 2009). These differences may be attributable to the difference in life stages (juvenile vs. mature), experimental period (2–9 mo) and/or species-specific tolerance to high CO_2 conditions. However, it should also be noted that the pH levels of control seawater varied between studies, possibly due to different seawater alkalinity or failure in controlling seawater carbonate chemistry or both, which makes it difficult to directly compare the results of these studies.

Effects on energy balance and sea urchin population

In the present study, ocean acidification reduced the energy intake of *Hemicentrotus pulcherrimus*, while energy of maintenance (respiration) and growth were not affected. Therefore, we suggest that the energy cost of reduced feeding increases the time until sea urchins acquire enough amount of energy to become fully mature and produce mature

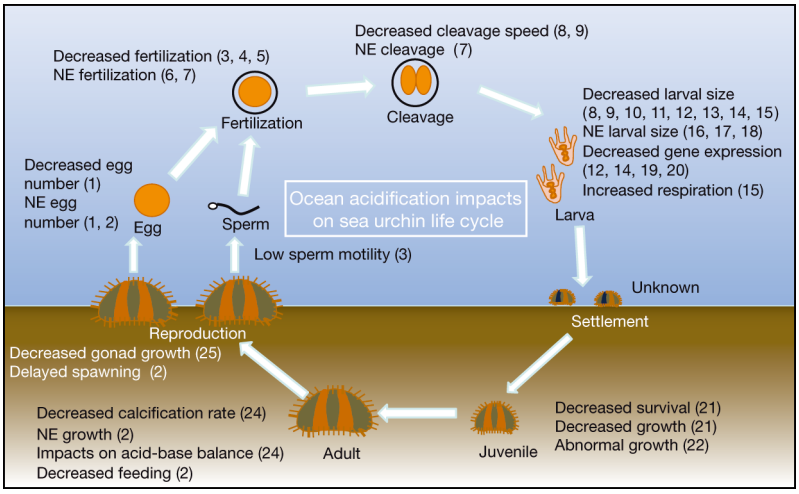

Fig. 8. Effect of ocean acidification on the sea urchin life cycle. NE: no effect. Numbers show references—1: Dupont et al. (2012); 2: present study; 3: Havenhand et al. (2008); 4: Byrne et al. (2010a); 5: Reuter et al. (2011), 6: Byrne et al. (2009); 7: Byrne et al. (2010b); 8: Kurihara & Shirayama (2004); 9: Kurihara et al. (2004); 10: Clark et al. (2009); 11: Sheppard Brennand et al. (2010); 12: O'Donnell et al. (2010); 13: Sunday et al. (2011); 14: Stumpp et al. (2011a); 15: Stumpp et al. (2011b); 16: Yu et al. (2011); 17: Martin et al. (2011); 18: Catarino et al. (2012); 19: Todgham & Hofmann (2009); 20: Kurihara et al. (2012); 21: Shirayama & Thornton (2005); 22: Byrne et al. (2011); 23: Ries et al. (2009); 24: Miles et al. (2007); 25: Stumpp et al. (2012)

eggs. Additionally, though fecundity (number of eggs) of *H. pulcherrimus* was not affected by ocean acidification, potential impacts on egg quality (e.g. egg size, nutrient content) are suggested. A number of studies have documented the influence of adult nutrition on egg 'quality' in marine invertebrates, and egg size has been revealed to have a direct correlation with maternal nutrition (Thompson 1983, George et al. 1990, Jaeckle 1995). Bayne et al. (1978) demonstrated that stressed mussels release fewer and lower quality eggs compared to unstressed individuals. Earlier studies on *H. pulcherrimus* reported negative impacts of ocean acidification on early development, including delayed cleavage and smaller larval size (Kurihara & Shirayama, 2004, Kurihara et al. 2012). Taking into account that the population size of sea urchins (and many other marine invertebrates) is largely determined by the number of offspring, fertilization success and embryonic survival (Kurihara 2008), these results imply that ocean acidification will threaten *H. pulcherrimus* at a community level. Negative impacts have been reported for most, if not all, sea urchin species (Fig. 8; Byrne et al. 2009, 2010b, Dupont et al. 2010). Different vulnerabilities of each life stage to elevated CO_2 might lead to restructuring of local sea urchin fauna. Extrapolating from the observation that drastic phase shifts ensued when the size of sea urchin populations was increased (Estes & Palmisano 1974, Jackson et al. 2001), or when sea urchins declined in abundance on coral reefs due to disease outbreaks (Hughes et al. 2007), changing sea urchin populations as a result of ocean acidification can be envisaged as having far-reaching ecological consequences.

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