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Juvenile growth of the tropical sea urchin *Lytechinus variegatus* exposed to near-future ocean acidification scenarios

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ABSTRACT

To evaluate the effect of elevated pCO_2 exposure on the juvenile growth of the sea urchin *Lytechinus variegatus*, we reared individuals for 3 months in one of three target pCO_2 levels: ambient seawater (380 μatm) and two scenarios that are projected to occur by the middle (560 μatm) and end (800 μatm) of this century. At the end of 89 days, urchins reared at ambient pCO_2 weighed 12% more than those reared at 560 μatm and 28% more than those reared at 800 μatm. Skeletons were analyzed using scanning electron microscopy, revealing degradation of spines in urchins reared at elevated pCO_2 (800 μatm). Our results indicate that elevated pCO_2 levels projected to occur this century may adversely affect the development of juvenile sea urchins. Acidification-induced changes to juvenile urchin development would likely impair performance and functioning of juvenile stages with implications for adult populations.

1. Introduction

As a result of oceanic uptake of atmospheric carbon dioxide, the world's oceans are slowly becoming more acidic (ocean acidification, OA). Approximately 30% (148 Pg C) of anthropogenic CO_2 emissions have been absorbed by the oceans (Sabine et al., 2011). Consequently, seawater carbonate concentrations have been depleted by ~30 μmol kg^{-1}, simultaneously reducing the pH of the ocean's surface waters by 0.1 units relative to the pre-industrial era (a 30% increase in acidity) (IPCC, 2007). Further reductions of 0.3–0.5 pH units are projected to occur by the end of this century as the oceans continue to absorb anthropogenic CO_2 (IPCC, 2007).

Documenting and predicting the response(s) of various marine organisms to changing ocean chemistry has been of recent concern in the scientific community. Mounting experimental evidence suggests that ocean acidification may hold negative consequences for a variety of marine organisms (Gattuso and Hansson, 2011), primarily calcifiers that depend on the delicate balance of inorganic carbon species to form their $CaCO_3$ shells or skeletons (Doney et al., 2009; Fabry et al., 2008; Kroeker et al., 2010).

Sea urchins are one of the most heavily studied groups of organisms with respect to ocean acidification, and early life history stages are thought to be particularly vulnerable to changing water chemistry (Dupont and Thorndyke, 2009; Kurihara, 2008). To date, the majority of work evaluating the effects of ocean acidification on early life history stages of urchins has focused on pre-metamorphic processes including pre-larval (e.g., fertilization, embryonic development) and larval stages (Table 1). While increasing information is available on the effects of ocean acidification on pre-metamorphic life history stages of sea urchins, there is a clear lack of information regarding the sensitivity of post-metamorphic (i.e., juvenile) stages. Only two studies to date have evaluated the effect(s) of elevated pCO_2 exposure on juvenile urchin growth. Shirayama and Thornton (2005) reported decreased juvenile growth of two species of urchin, *Echinometra mathaei* and *Hemicentrotus pulcherrimus*, during six-months exposure to elevated pCO_2 (560 μatm). Increased mortality was also reported in one of two trials. Byrne et al. (2011) reported an increased number of abnormal *Heliocidaris erythrogramma* juveniles exposed to depressed pH (−0.2 and −0.4 pH units) and elevated temperature (+2 °C and +4 °C). Metamorphosis marks the transition from a planktonic larva to a benthic juvenile and as such, larvae and juveniles differ substantially with respect to their morphology, physiology, and ecology and may differ in susceptibility to environmental stressors such as ocean acidification. It is, therefore, important to better understand post-metamorphic susceptibility to OA to couple with our growing knowledge of pre-metamorphic effects.

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Table 1
Compilation of recent studies investigating the effects of ocean acidification (pH change) on early life history stages of sea urchins. This listing is restricted to comprehensive studies for which methods, data, and statistical analyses are available and is focused on studies conducted over the last decade, under the context of near-future ocean acidification scenarios. Life stage abbreviations are as follows: PL = pre-larval (fertilization and/or embryogenesis); L = larval; J = juvenile.

Taxon	Life stage	CO_2 or HCl (ppm)	pH	Temp °C	Exposure period	Effect	Source
Echinometra mathaei	PL, L	HCl and CO_2 (365–10,360)	6.8–8.1		3 days	pH<7.8, skeletal malformation, reduced larval size and fertilization	Kurihara and Shirayama, 2004
Echinometra mathaei	J	CO_2 (ambt., 560)	7.90, 7.94	<30	6 months	Decreased growth at 12 week; mortality in 1 of 2 expts.	Shirayama and Thornton, 2005
Hemicentrotus pulcherrimus	PL, L	HCl and CO_2 (365–10,360)	6.8–8.0		3 days	pH<7.8, skeletal malformation, reduced larval size and fertilization	Kurihara and Shirayama, 2004
Hemicentrotus pulcherrimus	J	CO_2 (ambt., 560)	7.90, 7.94	<30	6 months	Decreased growth at 14 week; mortality in 1 of 2 expts.	Shirayama and Thornton, 2005
Heliocidaris erythrogramma	PL	CO_2 (ambt.,1000)	7.7, 8.1	20.5	24 h	Decreased sperm motility, swimming speed, and fertilization	Havenhand et al., 2008
Heliocidaris erythrogramma	PL	CO_2 (230–690)	7.6–8.2	20–26	4 days	No effect of CO_2 on fertilization or development; T effect on development, not fertilization; no T∗CO_2 interaction	Byrne et al., 2009
Heliocidaris erythrogramma	PL	CO_2 (370, 1100, 1850)	7.6–8.2	20–26	2 h	No effect of T or CO_2 on fertilization	Byrne et al., 2010a
Strongylocentrotus franciscanus	PL	CO_2 (400, 800, 1800)	7.6, 7.8, 8.0	10	3 h	Decreased fertilization efficiency; increased susceptibility to polyspermy at 1580 ppm; CO_2 effect dependent on sperm concentration	Reuter et al., 2010
Strongylocentrotus franciscanus	PL, L	CO_2 (380, 540, 970)	7.87, 7.98, 8.04	15, 19, 21, 23, 25, 27, 29, 31	~96 h	Gametes (larvae) fertilized (reared) at elevated CO_2 showed reduced expression of heat shock proteins in response to acute T stress (1 h); T of max. induction was shifted	O'Donnell et al., 2009
Strongylocentrotus purpuratus	L	CO_2 (380, 540, 1020)	7.88, 7.96, 8.01	15	<72 h	Decreased gene expression in 4 major cellular processes: biomineralization, cellular stress response, metabolism, apoptosis	Todgham and Hofmann, 2009
Tripneustes gratilla	L	CO_2 (450, 1200, 1900)	8.15, 7.8, 7.6	24, 27, 30	5 days	pCO_2 decreased larval growth; T<30 °C increased growth; interaction of CO_2∗T − 3° warming (24°–27°) diminished CO_2 effect	Sheppard Brennand et al., 2010
Tripneustes gratilla (tropical spp.)	L	CO_2 (395–1119)	6.0–8.1	26	4 days	Decreased calcification and size; no effect on fine skeletal morphology; decreased survival at pH<7.0	Clark et al., 2009
Pseudechinus huttoni (temp. spp.)	L	CO_2 (429–1282)	6.0–8.1	10–15	9 days	Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH<7.0	Clark et al., 2009
Evechinus chloroticus (temp. spp.)	L	CO_2 (438–1320)	6.0–8.1	10–15	13 days	Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH<7.0	Clark et al., 2009
Sterechinus neumayeri (polar spp.)	L	CO_2 (521–1380)	6.0–8.1	−1	7 days	No effect on calcification; no effect on fine skeletal morphology; decreased survival at pH<7.0	Clark et al., 2009
Lytechinus pictus	L	CO_2 (380, 540, 970)	7.78, 7.87, 7.93	18.5	6 days	Altered larval size, shape; down-regulation of genes central to energy metabolism, biomineralization; up-regulation of genes involved in ion regulation and acid–base balance pathways	O'Donnell et al., 2010
Paracentrotus lividus	PL, L	CO_2 (400, 700, 1100, 1900, 3600, 6600)	7.0, 7.25, 7.5, 7.7, 7.9, 8.1	20	3 days	No effect on fertilization or larval survival; slowed larval growth at low pH; upregulation of development and biomineralization candidate genes	Martin et al., 2011
Heliocidaris erythrogramma	PL	CO_2 (330–1828)	7.6–8.25	18–26	15 min	No effect of T or CO_2 on fertilization at the sperm concentration used	Byrne et al., 2010b
Heliocidaris tuberculata	PL	CO_2 (330–1828)	7.6–8.25	18–26	15 min	No effect of T or CO_2 on fertilization at the sperm concentration used	Byrne et al., 2010b
Tripneustes gratilla	PL	CO_2 (330–1828)	7.6–8.25	18–26	15 min	No effect of T or CO_2 on fertilization at the sperm concentration used	Byrne et al., 2010b
Centrostephanus rodgersii	PL	CO_2 (330–1828)	7.6–8.25	18–26	15 min	No effect of T or CO_2 on fertilization at the sperm concentration used	Byrne et al., 2010b
Strongylocentrotus purpuratus	L	CO_2 (375, 1264)	7.7, 8.1	14	21 days.	Developmental delay, increased respiration, no effect on feeding rate	Stumpp et al., 2011a
Strongylocentrotus purpuratus	L	CO_2 (399, 1318)	7.7, 8.17	16	7 days	Developmental delay, altered gene expression — upregulation of metabolic genes that induce developmental delay, down regulation of calcification genes, altered ion regulation	Stumpp et al., 2011b
Strongylocentrotus purpuratus	L	CO_2 (ambt., 1000, 1450)	7.5, 7.7, 8.07	15.6	6 days	Smaller larvae at 1450 ppm after 3 days; no effect on developmental timing	Yu et al., 2011
Heliocidaris erythrogramm	PL, L, J	CO_2	7.6, 7.8, 8.2	20, 22, 24	5 days	T and CO_2 caused abnormal development; 2 °C warming diminished negative effects of low pH; decreased number of spines with pCO_2	Byrne et al., 2011

The objective of the present study was to evaluate the effect of elevated pCO_2 exposure on juvenile urchin growth using the tropical Atlantic variegated sea urchin, *Lytechinus variegatus*, as a model organism. *L. variegatus* is an ecologically important species with a broad geographical range. It is found in both intertidal and shallow sub-tidal habitats, ranging from North Carolina and Bermuda southward to Brazil, and throughout the Caribbean and the Gulf of Mexico (Mortensen, 1943; Watts et al., 2001). This species serves as a primary grazer in many seagrass habitats where it plays an important role in energy transfer between trophic levels and can influence the structure of animal communities (Greenway, 1995; Watts et al., 2001). *L. variegatus* has an echinopluteus larva that remains in the plankton for a minimum of 2 weeks but can stay for several months if the appropriate metamorphic cues are not encountered. Upon encountering suitable settlement cues, larvae metamorphose into benthic juveniles. Individuals typically start feeding within 2 days of metamorphosis and mature at a diameter of approximately 40 mm, or around the age of 1 year (Moore et al., 1963). The life-span of nearshore animals is estimated to be approximately 3–4 years (Allain, 1975; Beddingfield and McClintock, 2000), but deep-water animals may live longer.

To evaluate the effect of near-future ocean acidification scenarios on juvenile urchin growth, we reared *L. variegatus* individuals (<6 months old) under controlled pCO_2 conditions over the course of 3 months (89 days). Three pCO_2 levels were targeted: ambient seawater (~380 μatm) and two scenarios that are projected to occur by the middle (560 μatm) and end (800 μatm) of this century (IPCC, 2007).

2. Materials and methods

Juvenile urchins (<6 months old) were sourced from the University of Miami's Experimental Hatchery (UMEH). UMEH regularly collects adult specimens of *L. variegatus* from wild populations (<5 m depth) offshore of Key Biscayne, Florida (ocean-side; 25°41′20″N, 80°9′21″W) to breed in-house. Details regarding the collection and breeding protocols are provided in the supplemental information. Urchins were approximately 5 months old at the time of retrieval. In the hatchery, urchins are maintained at 23 °C. We, therefore, acclimated them to the experimental temperature (28 °C) over the course of 2 weeks at a rate of approximately 0.4 °C day^{-1}. No mortality or signs of stress were observed during the acclimation period. On 18 January 2010, 175 day old juveniles were introduced to experimental conditions.

2.1. Seawater chemistry

Seawater was supplied from a source inlet in the nearby Bear Cut (Virginia Key, FL) and pumped into a 63,000-gallon settling tank and subsequent sand filter to remove particulate matter. Seawater was then supplied to indoor tanks wherein the carbonate system was manipulated prior to introduction to the experimental aquaria. One ambient pCO_2 concentration (380 μatm) and two elevated pCO_2 concentrations (560 and 800 μatm) were chosen for the study, based on near-future projections determined by the Intergovernmental Panel on Climate Change (IPCC) (IPCC, 2007). Seawater chemistry was manipulated via direct bubbling with carbon dioxide-enriched air. The control was bubbled with outside air. All treatments experienced natural fluctuations in pCO_2; consistent bubbling with CO_2-enriched air superimposed an acidification effect on top of diurnal variability.

Discrete water samples from treatment aquaria were analyzed for total alkalinity (TA) and pH on a weekly basis to verify distinct treatments. Because highest and lowest pCO_2 levels are typically observed near dawn and dusk respectively, water samples were taken between 1200 and 1300 h and represent average daily pCO_2 levels. TA was determined in duplicate using an automated, open-cell Gran titration (Dickson et al., 2007, SOP3b), and accuracy was checked against certified seawater reference material (A. Dickson, Scripps Institute of Oceanography). pH (total scale) was determined using an Orion Ross combination pH electrode (Thermo Scientific) calibrated at 25 °C against a seawater TRIS buffer (Dickson et al., 2007, SOP6a). Concentrations of HCO_3^-, CO_3^{2-}, CO_2, and Ω_{arag} were computed from TA, pH, temperature, and salinity using the program CO2SYS (E. Lewis, Brookhaven National Laboratory), with dissociation constants for carbonate determined by Mehrbach et al. (1973), as refit by Dickson and Millero (1987) and dissociation constant for boric acid determined by Dickson (1990). Chemical and physical conditions that persisted during the experiment are provided in Table 2.

2.2. Experimental design

Three individuals (initial weight 30.4 ± 3.5 mg; mean ± SD) were randomly assigned to each of twelve exposure tanks (four tanks per treatment × three pCO_2 treatments) containing seawater as described in Table 2. Tanks were maintained at a constant temperature of 28 ± 0.2 °C. Urchins were fed algae (*Ulva* spp.) ad libitum and weighed approximately every 2 weeks using an analytical balance. Measurements were taken a total of 7 times on days 0, 12, 25, 40, 56, 68, and 89 of the experiment. To obtain accurate weights, excess water was gently removed with laboratory wipes, and organisms were immediately placed on the scale. Care was taken to minimize the time out of water to reduce stress on the animals. Mean urchin weight per tank was compared using a repeated measures two-way ANOVA with time and pCO_2 as main effects. Bonferroni post-hoc comparisons were used to determine which treatments and time intervals differed from each other. Statistical analyses were conducted using GraphPad Prism® 5.0 statistical software.

2.3. Scanning electron microscopy

Upon termination of the 89-day growth experiment, urchins were sampled for use in scanning electron microscopy (SEM) to examine for potential effects of seawater pH/pCO_2 on fine-scale skeletal morphology. Three urchins from each treatment (N = 3) were randomly chosen and bleached in 10% sodium hypochlorite overnight to remove organic material. Skeletons were rinsed three times with tap water and once with distilled water to remove bleach residue. Skeletal subsamples were mounted on aluminum stubs using carbon adhesive tabs and coated with palladium for 3 min using a Cressington 108 Auto sputter coater. Samples were imaged in an FEI XL-30 environmental scanning electron microscope (ESEM) to assess the presence of skeletal abnormalities at low pH (e.g. malformation or dissolution of the skeleton).

Table 2
Physical and chemical conditions of seawater during growth experiment (mean ± SD, N = 8 weekly samples). All measurements are based upon duplicate or triplicate analyses of pH (total scale) and total alkalinity (TA) for each sampling period. pH_T, pCO_2, HCO_3^-, CO_3^{2-}, CO_2, TCO_2, and $\Omega_{calcite}$ were calculated using CO2SYS. Calcium concentration was calculated based on 10.28 mmol kg^{-1} of Ca^{2+} at a salinity of 35 ppt.

	Salinity	T (°C)	TA (μmol kg^{-1})	pH_T	pCO_2 (μatm)	HCO_3^- (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	CO_2 (μmol kg^{-1})	TCO_2 (μmol kg^{-1})	Ca^{2+} (mmol kg^{-1})	$\Omega_{calcite}$
Ambient	35.2 ± 0.6	28.1 ± 0.4	2467 ± 30	8.10 ± 0.06	367 ± 64	1799 ± 78	275 ± 22	10 ± 2	2084 ± 59	10.3 ± 0.2	6.6 ± 0.5
Mid CO2	35.2 ± 0.7	28.2 ± 0.5	2446 ± 35	7.96 ± 0.04	542 ± 66	1928 ± 53	213 ± 15	14 ± 2	2155 ± 45	10.3 ± 0.2	5.2 ± 0.4
High CO2	35.4 ± 0.8	28.1 ± 0.1	2447 ± 42	7.83 ± 0.03	765 ± 58	2039 ± 41	168 ± 11	20 ± 2	2227 ± 41	10.4 ± 0.2	4.1 ± 0.3

3. Results

3.1. Post-metamorphic growth and survivorship

Mean initial urchin weight did not differ significantly between treatments ($F_{2,9} = 0.761$, $P > 0.05$). Initial weights (mg) by treatment were as follows (mean ± SD): 31.17 ± 4.15 (380 µatm); 28.62 ± 3.67 (560 µatm); 31.48 ± 2.89 (800 µatm). We observed 100% survival of all sea urchins over the course of the 89-day experiment. Upon termination of the experiment, urchins reared at 380 µatm weighed, on average, 12% more than those reared at 560 µatm and 28% more than those reared at 800 µatm. Final weights (mg) by treatment were as follows (mean ± SD): 7500 ± 380 (380 µatm); 6690 ± 324 (560 µatm); 5851 ± 414 (800 µatm). A repeated measures two-way ANOVA indicated significant effects of both time ($F_{6,54} = 1957$; $P < 0.001$) and pCO_2 ($F_{2,54} = 30.46$; $P < 0.001$) on urchin weight. A significant interaction between time and pCO_2 was also detected ($F_{12,54} = 11.55$; $P < 0.001$). Post-hoc tests using the Bonferroni correction revealed that no significant differences were detected between treatments during the first 40 days of the experiment. Mean weights began to separate by day 56 (380 µatm > 800 µatm; $P < 0.05$); further separation of treatments was observed by day 68 (380 µatm > 560 µatm, $P < 0.001$ and 380 µatm > 800 µatm, $P < 0.001$), and full separation (i.e., all treatment means significantly different from one another) was observed by day 89 ($P < 0.001$) (Fig. 1).

3.2. Electron microscopy

Quantitative reductions in weight with increasing pCO_2 are supported by the SEM photographs, showing a loss of structural integrity (malformation or dissolution) in urchin spines grown under 800 µatm pCO_2 (Fig. 2). Qualitative differences in skeletal morphology were primarily evident in the spines, in contrast to the tests. Evidence of skeletal malformation and/or dissolution was apparent along the length of the spines of individuals reared at 800 µatm. Individuals grown in this treatment had thinner longitudinal ribs (Fig. 2b). No consistent differences were observed between controls and individuals reared at 560 µatm.

4. Discussion

This study demonstrates that juvenile growth, as determined by increase in wet weight, and skeletal integrity of *L. variegatus* are negatively affected by exposure to elevated pCO_2. Previous studies indicate that numerous physiological and biological processes of several sea urchin species are vulnerable to rising CO_2 levels (Dupont et al., 2010), including: fertilization (Havenhand et al., 2008; Kurihara and Shirayama, 2004; Kurihara et al., 2004; Reuter et al., 2010; but see Byrne et al., 2010a, 2010b; Martin et al., 2011); larval development (Clark et al., 2009; Kurihara, 2008; Kurihara and Shirayama, 2004; Kurihara et al., 2004; Martin et al., 2011; O'Donnell et al., 2010; Sheppard Brennand et al., 2010); gene expression in larvae (Martin et al., 2011; O'Donnell et al., 2010; Stumpp et al., 2011a, 2011b; Todgham and Hofmann, 2009); physiology (Miles et al., 2007); and juvenile/adult growth (Ries et al., 2009; Shirayama and Thornton, 2005). Given the range of life history stages and physiological processes that may be negatively impacted by ocean acidification (OA), carryover effects, and/or compounding effects on successive life history stages are likely.

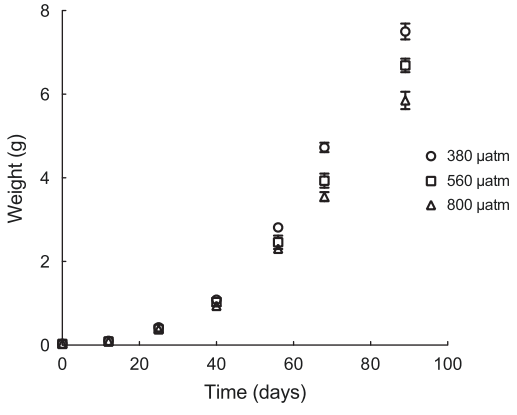

Fig. 1. Mean weight (± 1 SEM) of *Lytechinus variegatus* individuals over time (89 days) by pCO_2 level. Day 0 represents initial weights taken at the start of the experiment.

In our study, we observed 100% survival and positive net growth (albeit it slower at elevated pCO_2) of all individuals over the course of the 89-day experiment. Significant differences in weight between treatments were not observed during the first 40 days of the experiment. Growth rates between treatments began to separate at 56 days (week 8), and full separation (i.e., all treatment means significantly different from one another) was observed at 89 days (week 13). The lack of significant differences during the first 7 weeks of the experiment is most likely due to the slower growth rates observed early on in the experiment, rendering it difficult to detect significant differences between treatments. Our results are consistent with Shirayama and Thornton (2005), who documented decreased juvenile wet weight of two urchin species, *Echinometra mathaei* and *Hemicentrotus pulcherrimus*, following a six-month exposure to 560 µatm pCO_2. Similar to our study, treatment weights did not diverge until part-way through the experiment at weeks 12–16, depending on the species.

While the precise physiological mechanism behind reductions in sea urchin growth at elevated pCO_2 is not yet known, it has been demonstrated that urchins are poor regulators of internal pH (Johansen and Vadas, 1967; Miles et al., 2007; Spicer, 1995). It has been suggested that some marine invertebrates, such as mussels (Lindinger et al., 1984; Michaelidis et al., 2005) and sea urchins (Miles et al., 2007), use passive shell dissolution to support acid-based regulation at high internal pCO_2 levels. Structural dissolution of the magnesian calcite test of the purple-tipped sea urchin, *Psammechinus miliaris*, results in spikes in the concentrations of HCO_3^- and Mg^{2+}; such 'compensation events' occur episodically during exposure to acidified conditions and may serve to temporarily, yet often insufficiently, buffer internal change in pH (Miles et al., 2007).

Whether the skeletal degradation observed in this experiment was evidence of dissolution used by the organism to partially abate changing external seawater chemistry is unknown. Qualitative differences in skeletal morphology were primarily evident in the spines, in contrast to the test, with dissolution/malformation evident along the longitudinal ribs (sensu D'yakonov, 1969, Fig. 2). The degradation of skeletal components (primarily spines/rods) in urchins at elevated pCO_2 is consistent with previous studies conducted on both larval (Clark et al., 2009; Kurihara and Shirayama, 2004; Kurihara et al., 2004) and adult (Ries, 2010) life history stages. Urchin spines form via a transient amorphous calcium carbonate (ACC) precursor (Beniash et al., 1997; Politi et al., 2004) that is 30 times more soluble (200 mg/l) than the crystalline calcite of mature spines (6.7 mg/l) (Brecevic and Nielsen, 1989; Politi et al., 2004). The presence of this transient ACC phase may render growing and/or regenerating urchin spines particularly vulnerable to acidified conditions.

Compromised structural integrity of urchin spines, in conjunction with reductions in overall weight, are likely to weaken the supportive and protective skeleton of urchins, rendering them more susceptible to predation and/or mechanical damage. Urchins use their spines for protection and motility, and spines play a critical role in preventing structural damage to tests by absorbing energy and spreading impact over a broader area (Strathmann, 1981). Following injury, spine reparation can indirectly affect other vital biological functions, as energy channeled into spine repair is unavailable for test growth, grazing,

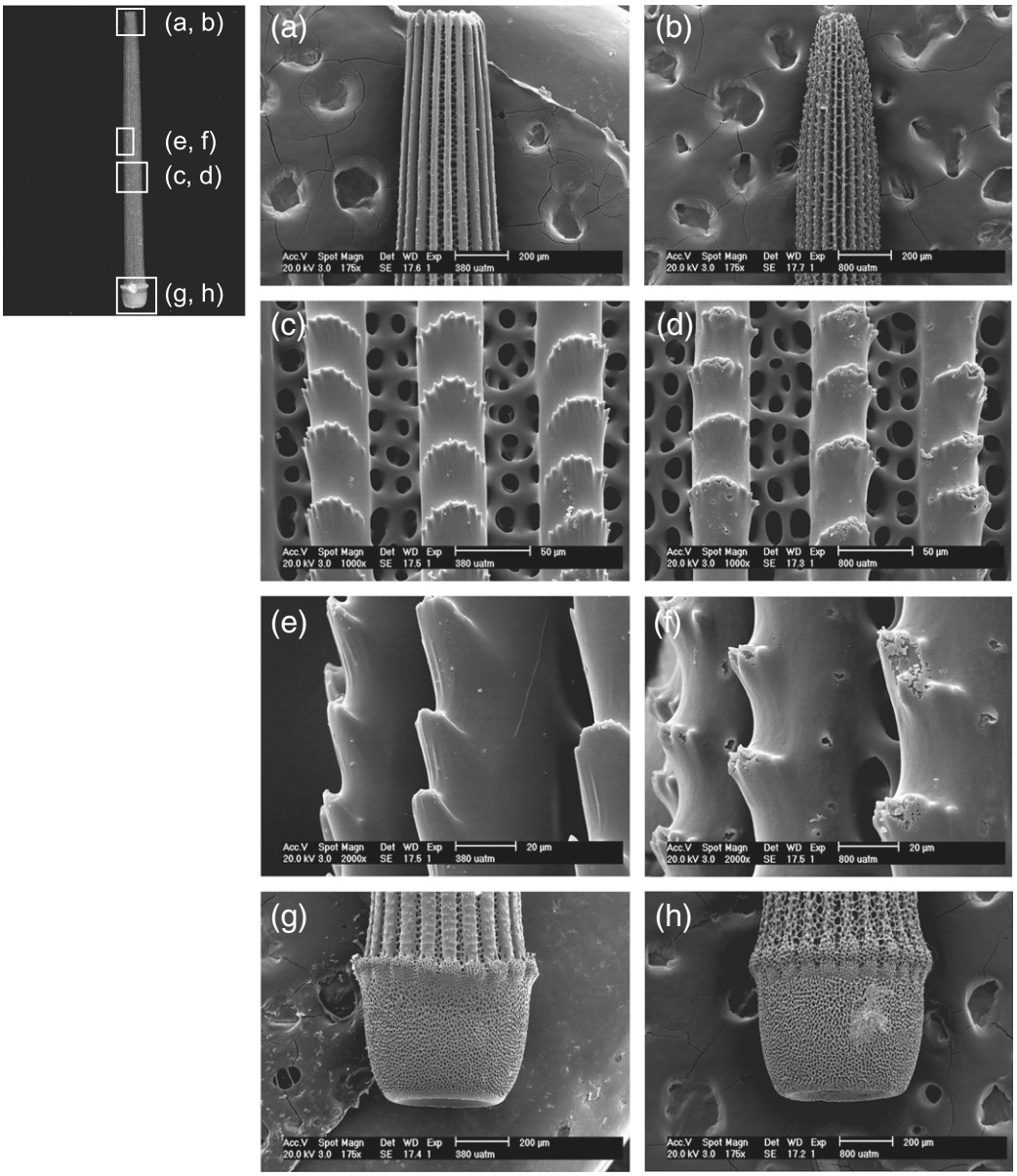

Fig. 2. Scanning electron micrographs of *Lytechinus variegatus* spines from individuals reared at ambient (380 μatm) and elevated (800 μatm) pCO_2 over the course of 89 days. The regions of the spines shown are indicated on the representative spine at the left. Scale bars for each view are indicated on SEM micrographs. (a, b) lateral view of spine tip, showing the control (a) in contrast to thinning of longitudinal striations at 800 μatm (b); (c, d) middle of the spine (front view) comparing the fenestrated structure and longitudinal striations of the control (c) with evidence of malformation and/or dissolution at 800 μatm (d); (e ,f) middle of the spine (high magnification, side view), showing normal skeleton (e) and evidence of malformation and/or dissolution at 800 μatm (f); (g, h) base of the spine and calcareous annular ridge in the control (g) compared to thinning of longitudinal striations at 800 μatm (h).

reproduction, motility, etc. (Ebert, 1968). Additionally, strength under impact increases with size, such that smaller urchins are less resistant to predation pressure as well as other biological and mechanical damage (Ebert, 1968).

As a present-day analog for near-future ocean acidification scenarios, a recent study investigated community composition along pH gradients near a natural CO_2 vent [normal pH (8.1–8.2) to low pH (mean 7.8–7.9, minimum 7.4–7.5)] and found significant reductions in the abundance of sea urchins and other calcifying organisms (e.g. scleractinian corals and coralline algae) approaching the vent (Hall-Spencer et al., 2008). Sea urchins are the dominant grazer in many shallow, marine communities and often determine community structure (Lawrence, 1975; Lawrence and Sammarco, 1982) by acting as bioturbators and keystone species (Brown, 1997; Karlson, 1999). It is, therefore, essential to better understand how they will be affected by changes occurring in the chemistry of the global oceans. Future experimental work should take care to continue the use of ecologically, biologically, and physiologically relevant pCO_2 scenarios and experimental timescales. Additional efforts should be made to evaluate synergistic effects with other stressors (e.g., warming) to determine if, and how, these stressors alter the acidification response. Whenever possible, studies should employ a combined mechanistic approach (e.g. molecular and experimental) to allow the investigator(s) to determine if an organism is physiologically compensating for changing external conditions (e.g., metabolically or through the regulation of gene expression).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2012.05.017.

References

Allain, J.Y., 1975. Contribution a la biologie de *Lytechinus variegatus* (Lamarck) de la baie de Carthagene (Colombie) (Echinodermata; Echinoidea). Union Oceanogr. Fr. 7, 57–62.

Beddingfield, S.D., McClintock, J.B., 2000. Demographic characteristics of *Lytechinus variegatus* (Echinodermata: Echinoidea) from three habitats in a North Florida Bay, Gulf of Mexico. Mar. Ecol. 21, 17–40.

Beniash, E., Aizenberg, J., Addadi, L., Weiner, S., 1997. Amorphous calcium carbonate transforms to calcite during sea urchin larval spicule growth. Proc. R. Soc. Lond. B 264, 461–465.

Brecevic, L., Nielsen, A.E., 1989. Solubility of amorphous calcium carbonate. J. Cryst. Growth 98, 504–510.

Brown, B.E., 1997. Disturbances to reefs in recent times. In: Birkeland, C. (Ed.), Life and Death of Coral Reefs. Chapman & Hall, New York.

Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H., Dworjanyn, S., Davis, A., 2009. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. Proc. R. Soc. Lond. B Biol. Sci. 276, 1883–1888.

Byrne, M., Soars, N., Selvakumaraswamy, P., Dworjanyn, S.A., Davis, A.R., 2010a. Sea urchin fertilization in a warm, acidified and high pCO_2 ocean across a range of sperm densities. Mar. Environ. Res. 69, 234–239.

Byrne, M., Soars, N.A., Ho, M.A., Wong, E., McElroy, D., Selvakumaraswamy, P., Symon, A.D., Davis, A.R., 2010b. Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. Mar. Biol. 157, 2061–2069.

Byrne, M., Ho, M., Wong, E., Soars, N.A., Selvakumaraswamy, P., Shepard-Brennand, H., Dworjanyn, S.A., Davis, A.R., 2011. Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. Proc. R. Soc. Lond. B Biol. Sci. 278, 2376–2383.

Clark, D., Lamare, M., Barker, M., 2009. Response of sea urchin pluteus larvae (Enchinodermata: Echinoidea) to reduce seawater pH: a comparison among a tropical, temperate, and a polar species. Mar. Biol. 156, 1125–1137.

D'yakonov, A.M., 1969. Fauna of Russia and adjacent countries. Echinoidea, volume I. IPST Press, Jerusalem.

Dickson, A.G., 1990. Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15°K. Deep-Sea Res. 37, 755–766.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 34, 1733–1743.

Dickson, A.G., Sabine, C.L., Christian, J.R. (Eds.), 2007. Guide to best practices for ocean CO_2 measurements. : PICES Special Publication, 3. North Pacific Marine Science Organization.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Ann. Rev. Mar. Sci. 1, 169–192.

Dupont, S., Thorndyke, M.C., 2009. Impact of CO_2-driven ocean acidification on invertebrates early life history — what we know, what we need to know and what we can do. Biogeosci. Discuss. 6, 3109–3131.

Dupont, S., Ortega-Martinez, O., Thorndyke, M., 2010. Impact of near-future ocean acidification on enchinoderms. Ecotoxicology 19, 449–462.

Ebert, T.A., 1968. Growth rates of the sea urchin *Strongylocentrotus purpuratus* related to food availability and spine abrasion. Ecology 49, 1075–1091.

Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. International Council for the Exploration of the Sea (ICES). J. Mar. Sci. 65, 414–432.

Gattuso, J.P., Hansson, L., 2011. Ocean acidification: background and history. In: Gattuso, J.P., Hansson, L. (Eds.), Ocean Acidification. Oxford University Press, New York, pp. 1–17.

Greenway, M., 1995. Trophic relationships of macro fauna within a Jamaican seagrass meadow and the role of echinoid *Lytechinus variegatus* (Lamarck). Bull. Mar. Sci. 56, 719–736.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454, 96–99.

Havenhand, J.N., Buttler, F.R., Thorndyke, M.C., Williamson, J.E., 2008. Near-future levels of ocean acidification reduce fertilization success in a sea urchin. Curr. Biol. 18, R651–R652.

IPCC, 2007. Climate Change 2007: The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York.

Johansen, K., Vadas, R.L., 1967. Oxygen uptake and responses to respiratory stress in sea urchins. Biol. Bull. 132, 16–22.

Karlson, R.H., 1999. Dynamics of Coral Communities. Boston Kluwer Academic Publishers, Dordrecht.

Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13, 1419–1434.

Kurihara, H., 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284.

Kurihara, H., Shirayama, Y., 2004. Effects of increased atmospheric CO_2 on sea urchin early development. Mar. Ecol. Prog. Ser. 274, 161–169.

Kurihara, H., Shimode, S., Shirayama, Y., 2004. Sub-lethal effects of elevated concentration of CO_2 on planktonic copepods and sea urchins. J. Oceanogr. 60, 743–750.

Lawrence, J.M., 1975. The relationships between echinoids and marine plants. Oceanogr. Mar. Biol. Annu. Rev. 13, 213–286.

Lawrence, J.M., Sammarco, P.W., 1982. Effects of feeding: Echinoidea. In: Jangoux, M., Lawrence, J.M. (Eds.), Echinoderm Nutrition. AA Balkema, Rotterdam.

Lindinger, M.I., Lauren, D.J., McDonald, D.G., 1984. Acid–base balance in the sea mussel, *Mytilus edulis*. III. Effects of environmental hypercapnia on intra- and extracellular acid–base balance. Mar. Biol. Lett. 5, 371–381.

Martin, S., Richier, S., Pedrotti, M.L., Dupont, S., Castejon, C., Gerakis, Y., Kerros, M.E., Oberhänsli, F., Teyssié, J.L., Jeffree, R., Gattuso, J.P., 2011. Early development and molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO_2-driven acidification. J. Exp. Biol. 214, 1357–1368.

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.

Michaelidis, B., Ouzounis, C., Paleras, A., Pörtner, H.O., 2005. Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar. Ecol. Prog. Ser. 293, 109–118.

Miles, H., Widdicombe, S., Spicer, J.I., Hall-Spencer, J., 2007. Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris*. Mar. Pollut. Bull. 54, 89–96.

Moore, H.B., Jutare, T., Bauer, J.C., Jones, J.A., 1963. The biology of *Lytechinus variegatus*. Bull. Mar. Sci. 13, 25–53.

Mortensen, T., 1943. A monograph of the Echinoidea: III.2 Camarodonta. I. Orthopsidae, glyphocyphidae, temnopleuridae and toxopneustidae. CA Reitzel Publisher, Copenhagen.

O'Donnell, M.J., Hammond, L.M., Hofmann, G.E., 2009. Predicted impact of ocean acidification on a marine invertebrate: elevated CO2 alters response to thermal stress in sea urchin larvae. Mar. Biol. 156, 439–446.

O'Donnell, M.J., Todgham, A.E., Sewell, M.A., Hammond, L.M., Ruggiero, K., Fangue, N.A., Zippay, M.L., Hofmann, G.E., 2010. Ocean acidification alters skeletogenisis and gene expression in larval sea urchins. Mar. Ecol. Prog. Ser. 398, 157–171.

Politi, Y., Arad, T., Klein, E., Weiner, S., Addadi, L., 2004. Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. Science 306, 1161–1164.

Reuter, K.E., Lotterhos, K.E., Crim, R.N., Thompson, C.A., Harley, C.D.G., 2010. Elevated pCO_2 increases sperm limitation and risk of polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. Global Change Biol., http://dx.doi.org/10.1111/j.1365-2486.2010.02216.x

Ries, J.B., 2010. Shell-shocked: marine creatures exhibit varied responses to ocean acidification. Earth 46–53.

Ries, J.B., Cohen, A.L., McCorkle, D.C., 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology 37, 1131–1134.

Sabine, C.L., Feely, R.A., Wanninkhof, R., Takahashi, T., Khatiwala, S., Park, G., 2011. Global oceans [The Global Ocean Carbon Cycle] in state of the climate in 2010. Bull. Am. Meteorol. Soc. 92 (6), S100–S105.

Sheppard Brennand, H.S., Soars, N., Dworjanyn, S.A., Davis, A.R., Byrne, M., 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. PLoS One 5, e11372.

Shirayama, Y., Thornton, H., 2005. Effect of increased atmospheric CO_2 on shallow water marine benthos. J. Geol. Res. 110, 108–115.

Spicer, J.I., 1995. Oxygen and acid–base status of the sea-urchin *Psammechinus miliaris* during environmental. Mar. Biol. 124, 71–76.

Strathmann, R.R., 1981. The role of spines in preventing structural damage to echinoid tests. Paleobiology 7, 400–406.

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M.C., Dupont, S.T., 2011a. CO_2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. Comp. Biochem. Physiol. A 160, 331–340.

Stumpp, M., Wren, J., Melzner, F., Thorndyke, M.C., Dupont, S.T., 2011b. CO_2 induced seawater acidification impacts sea urchin larval development II: gene expression patterns in pluteus larvae. Comp. Biochem. Physiol. A 160, 320–330.

Todgham, A.E., Hofmann, G., 2009. Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO_2-driven seawater acidification. J. Exp. Biol. 212, 2579–2594.

Watts, S.A., McClintock, J.B., Lawrence, J.M., 2001. The ecology of *Lytechinus variegates*. In: Lawrence, J.M. (Ed.), Edible Sea Urchins: Biology and Ecology. Elsevier Science Press, Amsterdam.

Yu, P.C., Matson, P.G., Martz, T.R., Hofmann, G.E., 2011. The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: Laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO_2/pH. J. Exp. Mar. Biol. Ecol. 400, 288–295.