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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  $p\text{CO}_2$  exposure on the juvenile growth of the sea urchin *Lytechinus variegatus*, we reared individuals for three months in one of three target  $p\text{CO}_2$  levels: ambient seawater (380  $\mu\text{atm}$ ) and two scenarios that are projected to occur by the middle (560  $\mu\text{atm}$ ) and end (800  $\mu\text{atm}$ ) of this century. At the end of 89 days, urchins reared at ambient  $p\text{CO}_2$  weighed 12% more than those reared at 560  $\mu\text{atm}$  and 28% more than those reared at 800  $\mu\text{atm}$ . Skeletons were analyzed using scanning electron microscopy, revealing degradation of spines in urchins reared at elevated  $p\text{CO}_2$  (800  $\mu\text{atm}$ ). Our results indicate that elevated  $p\text{CO}_2$  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.

### Keywords

echinoderm; carbon dioxide; invertebrate; development;  $\text{CO}_2$ ; pH;  $p\text{CO}_2$

## 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  $\text{CO}_2$  emissions have been absorbed by the oceans (Sabine et al., 2011). Consequently, seawater carbonate concentrations have been depleted by  $\sim 30 \mu\text{mol kg}^{-1}$ , 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  $\text{CO}_2$  (IPCC, 2007).

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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  $\text{CaCO}_3$  shells or skeletons (Doney et al., 2009; Fabry et al., 2008; Kroecker 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 (Kurihara, 2008; Dupont and Thorndyke, 2009). 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  $p\text{CO}_2$  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  $p\text{CO}_2$  (560  $\mu\text{atm}$ ). 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.

The objective of the present study was to evaluate the effect of elevated  $p\text{CO}_2$  exposure on post-metamorphic 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 two 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 near-shore animals is estimated to be approximately 3–4 years (Allain, 1975; Beddingfield and McClinktock, 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  $p\text{CO}_2$  conditions over the course of three months (89 days) and evaluated the effect on post-metamorphic growth. Three  $p\text{CO}_2$  levels were targeted: ambient seawater (~380  $\mu\text{atm}$ ) and two scenarios that are projected to occur by the middle (560  $\mu\text{atm}$ ) and end (800  $\mu\text{atm}$ ) 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 *Lytechinus 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<sup>-1</sup>. No mortality or signs of stress were observed during the acclimation period. On 18 January 2010, 175 d 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  $p\text{CO}_2$  concentration (380  $\mu\text{atm}$ ) and two elevated  $p\text{CO}_2$  concentrations (560 and 800  $\mu\text{atm}$ ) 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  $p\text{CO}_2$ ; consistent bubbling with  $\text{CO}_2$ -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  $p\text{CO}_2$  levels are typically observed near dawn and dusk respectively, water samples were taken between 1200–1300h and represent average daily  $p\text{CO}_2$  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  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{CO}_2$ , and  $\Omega_{\text{arag}}$  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 the Table 2.

### 2.2 Experimental Design

Three individuals (initial weight  $30.4 \pm 3.5$  mg; mean  $\pm$  SD) were randomly assigned to each of twelve exposure tanks (four tanks per treatment x three  $p\text{CO}_2$  treatments) containing seawater as described in Table 2. Tanks were maintained at a constant temperature of  $28 \pm 0.2$  °C. Urchins were fed algae (*Ulva* spp.) *ad libitum* and weighed approximately every two 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  $p\text{CO}_2$  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<sup>®</sup> 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/ $p\text{CO}_2$  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 three minutes 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).

## 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  $\pm$  SD):  $31.17 \pm 4.15$  (380  $\mu\text{atm}$ );  $28.62 \pm 3.67$  (560  $\mu\text{atm}$ );  $31.48 \pm 2.89$  (800  $\mu\text{atm}$ ). 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  $\mu\text{atm}$  weighed, on average, 12% more than those reared at 560  $\mu\text{atm}$  and 28% more than those reared at 800  $\mu\text{atm}$ . Final weights (mg) by treatment were as follows (mean  $\pm$  SD):  $7500 \pm 380$  (380  $\mu\text{atm}$ );  $6690 \pm 324$  (560  $\mu\text{atm}$ );  $5851 \pm 414$  (800  $\mu\text{atm}$ ). A repeated measures two-way ANOVA indicated significant effects of both time ( $F_{6,54}=1957$ ;  $P<0.001$ ) and  $p\text{CO}_2$  ( $F_{2,54}=30.46$ ;  $P<0.001$ ) on urchin weight. A significant interaction between time and  $p\text{CO}_2$  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  $\mu\text{atm}$  > 800  $\mu\text{atm}$ ;  $P<0.05$ ); further separation of treatments was observed by day 68 (380  $\mu\text{atm}$  > 560  $\mu\text{atm}$ ,  $P<0.001$  and 380  $\mu\text{atm}$  > 800  $\mu\text{atm}$ ,  $P<0.001$ ), and full separation (i.e., all treatment means significantly different from one another) was observed by day 89 ( $P<0.001$ ).

### 3.2 Electron Microscopy

Quantitative reductions in weight with increasing  $p\text{CO}_2$  are supported by the SEM photographs, showing a loss of structural integrity (malformation or dissolution) in urchin spines grown under 800  $\mu\text{atm}$   $p\text{CO}_2$  (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  $\mu\text{atm}$ . Individuals grown in this treatment had thinner longitudinal ribs (Fig. 2b). No consistent differences were observed between controls and individuals reared at 560  $\mu\text{atm}$ .

## 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  $p\text{CO}_2$ . Previous studies indicate that numerous physiological and biological processes of several sea urchin species are vulnerable to rising  $\text{CO}_2$  levels, including: fertilization (Havenhand et al., 2008; Kurihara et al., 2004; Kurihara and Shirayama, 2004; Reuter et al., 2010; but see

Byrne et al., 2010a, 2010b; Martin et al., 2011); larval development (Kurihara et al., 2004; Kurihara and Shirayama, 2004; Kurihara, 2008; Clark et al., 2009; Sheppard Brennard et al., 2010; O'Donnell et al., 2010; Martin et al. 2011); 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.

In our study, we observed 100% survival and positive net growth (albeit it slower at elevated  $p\text{CO}_2$ ) 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  $\mu\text{atm } p\text{CO}_2$ . 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  $p\text{CO}_2$  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  $p\text{CO}_2$  levels. Structural dissolution of the magnesian calcite test of the purple-tipped sea urchin, *Psammechinus miliaris*, results in spikes in the concentrations of  $\text{HCO}_3^-$  and  $\text{Mg}^{2+}$ ; 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  $p\text{CO}_2$  is consistent with previous studies conducted on both larval (Kurihara and Shirayama, 2004; Kurihara et al., 2004; Clark et al. 2009) 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/liter) than the crystalline calcite of mature spines (6.7 mg/liter) (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 (implications for urchin survival). 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, 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<sub>2</sub> 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 often 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<sub>2</sub> 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).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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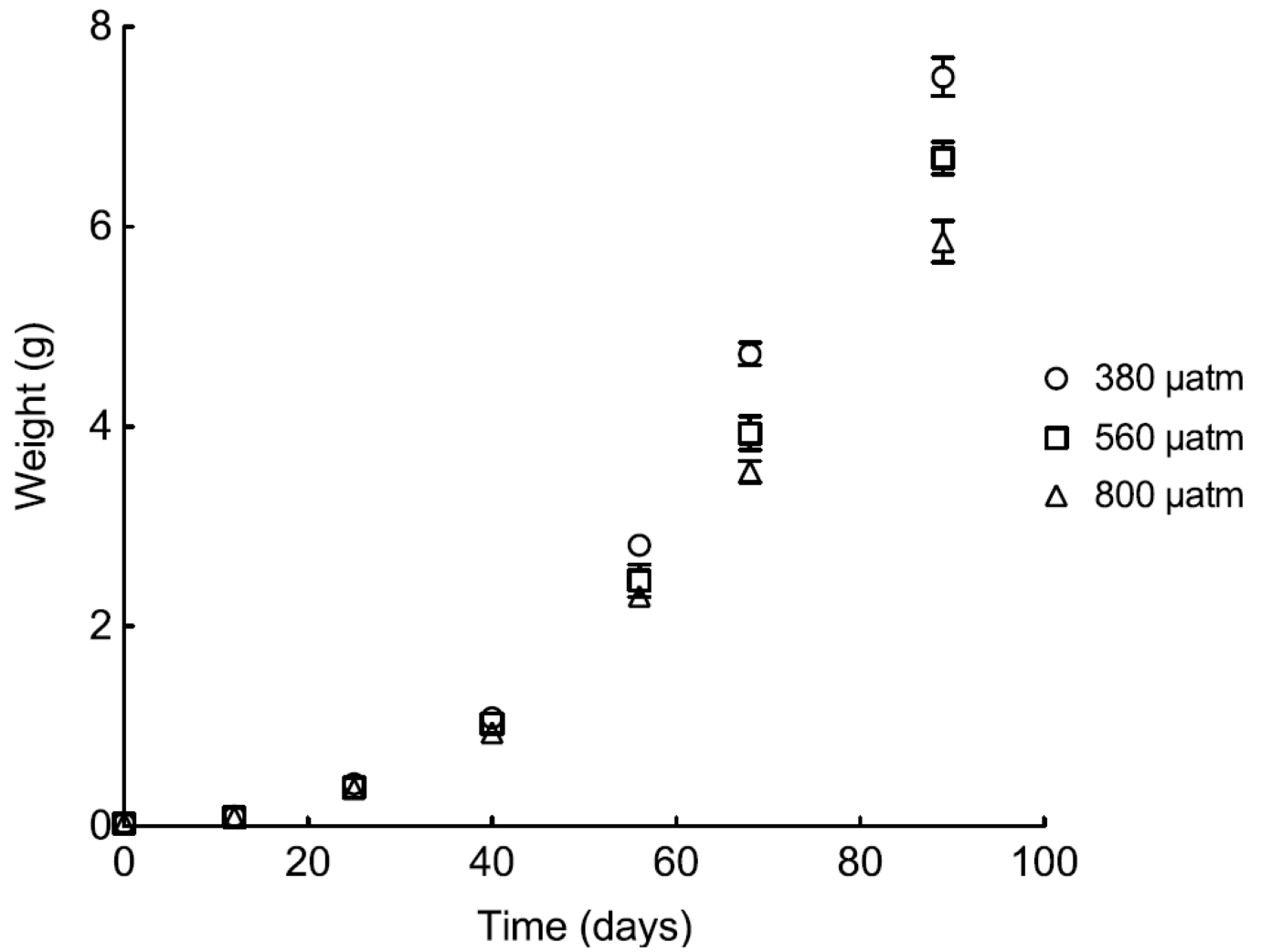
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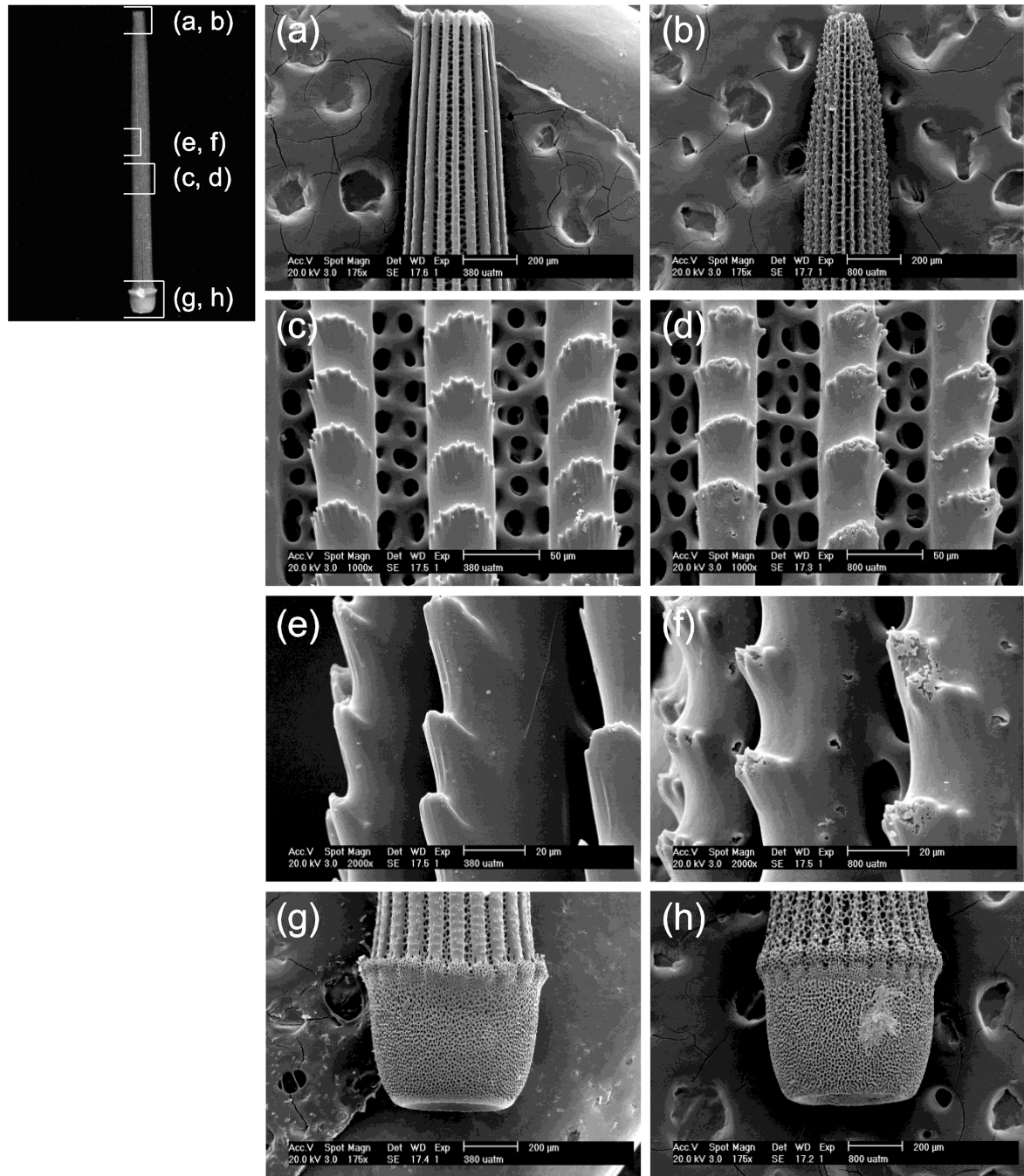
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### Highlights

- We evaluate the effect of near-future ocean acidification scenarios on juvenile urchin growth
- We show that elevated  $p\text{CO}_2$  depresses juvenile urchin growth
- Scanning electron microscopy demonstrates that elevated  $p\text{CO}_2$  alters skeletal integrity



**Figure 1.** Mean weight ( $\pm 1$  SEM) of *Lytechinus variegatus* individuals over time (89 days) by  $p\text{CO}_2$  level. Day 0 represents initial weights taken at the start of the experiment.



**Figure 2.**

Scanning electron micrographs of *Lytechinus variegatus* spines from individuals reared at ambient (380  $\mu\text{atm}$ ) and elevated (800  $\mu\text{atm}$ )  $p\text{CO}_2$  over the course of 89 days. The regions of the spines shown are indicated on the representative spine at 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  $\mu\text{atm}$  (b); (c,d) middle of spine (front view) comparing the fenestrated structure and longitudinal striations of the control (c) with evidence of malformation and/or dissolution at 800  $\mu\text{atm}$  (d); (e,f) middle of spine (high magnification, side view), showing normal skeleton (e) and evidence of malformation and/or

dissolution at 800  $\mu\text{atm}$  (f); (g,h) base of spine and calcareous annular ridge in the control (g) compared to thinning of longitudinal striations at 800  $\mu\text{atm}$  (h).

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 <sub>2</sub> or HCl (ppm)	pH	Temp °C	Exposure period	Effect	Source
<i>Echinometra mathaei</i>	PL, L	HCl & CO <sub>2</sub> (365–10,360)	6.8–8.1		3 d	pH < 7.8, skeletal malformation, reduced larval size & fertilization	Kurihara & Shirayama 2004
<i>Echinometra mathaei</i>	J	CO <sub>2</sub> (ambt., 560)	7.90, 7.94	<30	6 mo	Decreased growth at 12 wk; mortality in 1 of 2 expts.	Shirayama & Thornton 2005
<i>Hemicentrotus pulcherrimus</i>	PL, L	HCl & CO <sub>2</sub> (365–10,360)	6.8–8.0		3 d	pH < 7.8, skeletal malformation, reduced larval size & fertilization	Kurihara & Shirayama 2004
<i>Hemicentrotus pulcherrimus</i>	J	CO <sub>2</sub> (ambt., 560)	7.90, 7.94	<30	6 mo	Decreased growth at 14 wk; mortality in 1 of 2 expts.	Shirayama & Thornton 2005
<i>Helicoidaris erythrogramma</i>	PL	CO <sub>2</sub> (ambt., 1000)	7.7, 8.1	20.5	24 h	Decreased sperm motility, swimming speed, and fertilization	Havenhand et al. 2008
<i>Helicoidaris erythrogramma</i>	PL	CO <sub>2</sub> (230–690)	7.6–8.2	20–26	4 d	No effect of CO <sub>2</sub> on fertilization or development; T effect on development, not fertilization; no T*CO <sub>2</sub> interaction	Byrne et al. 2009
<i>Helicoidaris erythrogramma</i>	PL	CO <sub>2</sub> (370, 1100, 1850)	7.6–8.2	20–26	2 h	No effect of T or CO <sub>2</sub> on fertilization	Byrne et al. 2010a
<i>Strongylocentrotus franciscanus</i>	PL	CO <sub>2</sub> (400, 800, 1800)	7.6, 7.8, 8.0	10	3 h	Decreased fertilization efficiency; increased susceptibility to polyspermy at 1580 ppm; CO <sub>2</sub> effect dependent on sperm concentration	Reuter et al. 2010
<i>Strongylocentrotus franciscanus</i>	PL, L	CO <sub>2</sub> (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 <sub>2</sub> showed reduced expression of heat shock proteins in response to acute T stress (1h); T of max. induction was shifted	O'Donnell et al. 2009
<i>Strongylocentrotus purpuratus</i>	L	CO <sub>2</sub> (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 & Hofmann 2009
<i>Triploneustes gratilla</i>	L	CO <sub>2</sub> (450, 1200, 1900)	8.15, 7.8, 7.6	24, 27, 30	5 d	pCO <sub>2</sub> decreased larval growth; T < 30°C increased growth; interaction of CO <sub>2</sub> * T – 3° warming (24°–27°) diminished CO <sub>2</sub> effect	Sheppard Brennard et al. 2010
<i>Triploneustes gratilla</i> (tropical spp.)	L	CO <sub>2</sub> (395–1119)	6.0–8.1	26	4 d	Decreased calcification and size; no effect on fine skeletal morphology; decreased survival at pH < 7.0	Clark et al. 2009
<i>Pseudoechinus huttoni</i> (temp. spp.)	L	CO <sub>2</sub> (429–1,282)	6.0–8.1	10–15	9 d	Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH < 7.0	Clark et al. 2009

Taxon	Life Stage	CO <sub>2</sub> or HCl (ppm)	pH	Temp °C	Exposure period	Effect	Source
<i>Evechinus chloroticus</i> (temp. spp.)	L	CO <sub>2</sub> (438–1,320)	6.0–8.1	10–15	13 d	Decreased calcification and size; degradation of fine skeletal morphology; decreased survival at pH < 7.0	Clark et al. 2009
<i>Sterechinus neumayeri</i> (polar spp.)	L	CO <sub>2</sub> (521–1,380)	6.0–8.1	–1	7 d	No effect on calcification; no effect on fine skeletal morphology; decreased survival at pH < 7.0	Clark et al. 2009
<i>Lytechinus pictus</i>	L	CO <sub>2</sub> (380, 540, 970)	7.78, 7.87, 7.93	18.5	6 d	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
<i>Paracentrotus lividus</i>	PL, L	CO <sub>2</sub> (400, 700, 1100, 1900, 3600, 6600)	7.0, 7.25, 7.5, 7.7, 7.9, 8.1	20	3 d	No effect on fertilization or larval survival; slowed larval growth at low pH; upregulation of development and biomineralization candidate genes	Martin et al. 2011
<i>Helicoidaris erythrogramma</i>	PL	CO <sub>2</sub> (330–1828)	7.6–8.25	18–26	15 min.	No effect of T or CO <sub>2</sub> on fertilization at the sperm concentration used	Byrne et al. 2010b
<i>Helicoidaris tuberculata</i>	PL	CO <sub>2</sub> (330–1828)	7.6–8.25	18–26	15 min.	No effect of T or CO <sub>2</sub> on fertilization at the sperm concentration used	Byrne et al. 2010b
<i>Triploneustes gratilla</i>	PL	CO <sub>2</sub> (330–1828)	7.6–8.25	18–26	15 min.	No effect of T or CO <sub>2</sub> on fertilization at the sperm concentration used	Byrne et al. 2010b
<i>Centrostephanus rogersii</i>	PL	CO <sub>2</sub> (330–1828)	7.6–8.25	18–26	15 min.	No effect of T or CO <sub>2</sub> on fertilization at the sperm concentration used	Byrne et al. 2010b
<i>Strongylocentrotus purpuratus</i>	L	CO <sub>2</sub> (375, 1264)	7.7, 8.1	14	21 d.	Developmental delay, increased respiration, no effect on feeding rate	Stumpp et al. 2011a
<i>Strongylocentrotus purpuratus</i>	L	CO <sub>2</sub> (399, 1318)	7.7, 8.17	16	7 d.	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
<i>Strongylocentrotus purpuratus</i>	L	CO <sub>2</sub> (ambt., 1000, 1450)	7.5, 7.7, 8.07	15.6	6 d.	Smaller larvae at 1450 ppm after 3 d.; no effect on developmental timing	Yu et al. 2011
<i>Helicoidaris erythrogramma</i>	PL, L, J	CO <sub>2</sub>	7.6, 7.8, 8.2	20, 22, 24	5 d.	T & CO <sub>2</sub> caused abnormal development; 2°C warming diminished negative effects of low pH; decreased number of spines with pCO <sub>2</sub>	Byrne et al. 2011

**Table 2**

Physical and chemical conditions of seawater during growth experiment (mean  $\pm$  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  $10.28 \text{ mmol kg}^{-1}$  of  $Ca^{2+}$  at a salinity of 35 ppt.

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