PROCEEDINGS OF THE ROYAL SOCIETY B

FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2010.2404
Published online

Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean

Maria Byrne[1,2,*], Melanie Ho[1], Eunice Wong[1], Natalie A. Soars[1], Paulina Selvakumaraswamy[1], Hannah Shepard-Brennand[1], Symon A. Dworjanyn[3] and Andrew R. Davis[4]

[1]*Schools of Medical Sciences, and* [2]*School of Biological Sciences, F13, University of Sydney, New South Wales 2006, Australia*
[3]*National Marine Science Centre, Southern Cross University, New South Wales 2450, Australia*
[4]*Institute for Conservation Biology, University of Wollongong, New South Wales 2522, Australia*

The most fragile skeletons produced by benthic marine calcifiers are those that larvae and juveniles make to support their bodies. Ocean warming, acidification, decreased carbonate saturation and their interactive effects are likely to impair skeletogenesis. Failure to produce skeleton in a changing ocean has negative implications for a diversity of marine species. We examined the interactive effects of warming and acidification on an abalone (*Haliotis coccoradiata*) and a sea urchin (*Heliocidaris erythrogramma*) reared from fertilization in temperature and pH/pCO_2 treatments in a climatically and regionally relevant setting. Exposure of ectodermal (abalone) and mesodermal (echinoid) calcifying systems to warming (+2°C to 4°C) and acidification (pH 7.6–7.8) resulted in unshelled larvae and abnormal juveniles. *Haliotis* development was most sensitive with no interaction between stressors. For *Heliocidaris*, the percentage of normal juveniles decreased in response to both stressors, although a +2°C warming diminished the negative effect of low pH. The number of spines produced decreased with increasing acidification/pCO_2, and the interactive effect between stressors indicated that a +2°C warming reduced the negative effects of low pH. At +4°C, the developmental thermal tolerance was breached. Our results show that projected near-future climate change will have deleterious effects on development with differences in vulnerability in the two species.

Keywords: climate change; ocean warming; ocean acidification; calcification; sea urchin; abalone

1. INTRODUCTION

The gametes of marine invertebrates are being spawned into an ocean simultaneously warming, decreasing in pH and increasing in pCO_2 [1–4]. These stressors exert negative effects on marine biota [5–7] and may have deleterious interactive effects [8,9]. Marine calcifiers appear particularly vulnerable to climate change because ocean acidification reduces the availability of the carbonate ions required to construct their skeletons [10], although this differs among species and life stages [6–8,11–20]. The most fragile skeletons produced by benthic marine invertebrates are those that larvae and early juveniles make to support their bodies [13]. Failure to produce these skeletons in a changing ocean would have negative consequences for a great diversity of marine species. For marine invertebrates, future prospects for the early life-history stages are of particular concern because their sensitivity to climate change stressors may be the bottleneck for persistence of some species in a changing ocean [5–7].

Ocean acidification increases physiological P_{CO2} and so is linked to increased organism hypercapnia (acidosis), a stressor that suppresses metabolism [21–23]. By contrast, increased temperature has a stimulatory affect on metabolism, potentially countering the negative effects of acidification and hypercapnia on calcification and other physiological processes. Small increases in temperature (approx. 2°C) can diminish the negative effects of ocean acidification on calcification in sea urchin larvae and coral growth [9,24]. For some species, ocean acidification has a positive effect on growth, although in a juvenile sea star this was not because of an increase in skeleton [12,17]. The responses of organisms and marine ecosystems to multiple climate change stressors appear to be species-specific and so are difficult to predict [5–7,25–27].

The Mollusca and Echinodermata are significant calcifying marine phyla and have different calcifying systems [28,29]. In mollusc development an aragonite skeleton is produced in the ectoderm tissue layer, while in echinoderm development a magnesium calcite skeleton is produced within mesoderm. Here we investigated the response of skeleton development in a mollusc (abalone *Haliotis coccoradiata*) and an echinoderm (sea urchin *Heliocidaris erythrogramma*) to near-future ocean warming and acidification. These species co-occur in southeast

* Author for correspondence (mbyrne@anatomy.usyd.edu.au).

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2010.2404 or via http://rspb.royalsocietypublishing.org.

Received 3 November 2010
Accepted 2 December 2010

Australia, a climate change hotspot, where the ocean is warming several times faster than the global average [30]. They represent ecologically and commercially important marine invertebrates because they function as grazers that structure habitats and are targeted by fisheries [31,32]. We investigated the interactive effects of near-future (*ca* 2100 [4]) ocean warming, acidification/hypercapnia, and decreased aragonite and calcite saturation states on development in these two species. Owing to uptake of CO_2, surface ocean waters are projected to decrease in pH by 0.4 U by 2100 and 0.7 U by 2300 [1]. In southeast Australia, climate-driven changes to the East Australian Current has resulted in significant warming of coastal waters, with a projected increase of 1–2°C by 2030 and 2–3°C by 2100 [30,33].

Fertilization in *H. coccoradiata* and *H. erythrogramma* is robust to climate change stressors and early development is sensitive to warming [15,34–37]. These species have large eggs (*H. coccoradiata*, 175 µm diameter eggs; *H. erythrogramma*, 440 µm diameter eggs), and their rapid development through lecithotrophic larvae facilitates access to the first calcified stage [37,38]. Owing to their developmental mode, we did not have to feed the larvae, avoiding the potentially confounding influence of the variable responses of feeding larvae to nutrient regime [39]. This mode of development is characteristic of *Haliotis* [37]. The endpoint we used was the 21 h shelled veliger stage. Lecithotrophic development, as seen in *H. erythrogramma*, is reported in approximately 45 per cent of Echinoidea that have been investigated [40]. This species lacks a calcified larva [38] and so we focused on the early juvenile (5 day), one of the least studied stages of marine invertebrate development with respect to changing ocean conditions.

Concerns about future prospects for marine calcifiers have prompted numerous studies on the impacts of climate change on development with a focus on acidification (reviews in [5–7,13]). The most common approach has involved the transfer of larvae or juveniles from present-day to experimental conditions (e.g. 19,41]). A recent study, however, has shown that oyster larvae reared in ocean change conditions from the outset of development (fertilization) were more severely impacted by stressors than those placed in treatments as later-stage embryos [8]. Developmental success requires that all ontogenetic stages be completed successfully, and it is likely that stressor effects are integrated across developmental stages. To identify potential developmental bottlenecks, we reared abalone and urchin embryos from the onset of development (fertilization) in multifactoral experimental treatments. To our knowledge, this is the first study of the interactive effects of ocean warming and CO_2-driven acidification on marine life histories from the beginning of development to the benthic juvenile. We predicted that ocean warming and acidification would have a negative impact on development (e.g. decreased calcification), with the possibility that warming might diminish the negative effects of decreased carbonate availability on growth and calcification. The morphology of the skeleton and shell was used as an indicator of calcification, as in previous studies [5–7,13]. We also predicted that because of its location close to the surface in ectoderm, the *H. coccoradiata* shell might be more vulnerable to stressors than the internal skeleton of *H. erythrogramma*.

2. METHODS

(a) *Study species and collection site*

Haliotis coccoradiata and *H. erythrogramma* were collected during their peak reproductive seasons (October–November and December–January, respectively) [37,42], from Little Bay (33°58′ S, 151°14′ E), an open ocean site near Sydney where they co-occur in shallow-water boulder habitat. The size range used (*H. coccoradiata* shell length 25–35 mm; *H. erythrogramma*, test diameter 65–85 mm) was kept as small as possible to reduce interindividual variability in gamete quality. Animals were transported in ambient sea water in a cool box and placed in aquaria at ambient sea surface temperature (SST). They were used for experiments within days of collection. Sea water for experiments was collected at high tide near the collection site. During the collection period, SST averaged 20°C for abalone and 22°C for urchin as determined from the Physical Oceanography DAAC Ocean ESIP Tool (POET) website (http://poet.jpl.nasa.gov) and a local reference station (www.mhl.nsw.gov.au). These temperatures were used as the control treatments.

Freshly collected filtered sea water (FSW; 1.0 µm) was used for all experiments. Sea water parameters—pH_{NBS}, dissolved oxygen (DO), temperature—were measured using a WTW multimeter (Multiline P4) and total alkalinity (TA) was determined by potentiometric titration (CSIRO Laboratories, Hobart). Sea water was collected twice for the abalone experiments and three times for the sea urchin experiments. Parameters for these water sources (electronic supplementary material, tables S1 and S2) were similar across the five water collections ($\bar{x}$ salinity 36.9 ppt, s.e. = 0.2; $\bar{x}$ pH = 8.18, s.e. = 0.03; $\bar{x}$ TA = 2368, s.e. = 15).

The experiments were designed within the context of the 'business-as-usual' emissions scenario for an estimated increase in surface ocean pCO_2 from present levels of around 380 to 700–1000 ppm by 2100 and greater than 2000 ppm by 2300, levels modelled to drop in surface ocean pH by 0.14–0.41 and 0.30–0.70 U, respectively [2,4,10]. Local waters are projected to increase in SST by 2–3°C by 2100 (CSIRO Model 3.5 in [30,33]). While we do not know the details of future local SST/pH/pCO_2 conditions in the habitat where the study organisms live, we used these modelled estimates to guide our experimental design to reflect projections for 2100 and beyond. The experiments involved three temperature (ambient control, +2°C, +4°C) and three pH (ambient control, pH 7.8, pH 7.6) treatments used in all combinations.

Experimental pH_{NBS} and DO greater than 90 per cent were achieved by bubbling CO_2 gas and air into the FSW until the desired pH was reached. Large volumes (20–30 l) of sea water were equilibrated to each temperature/pH treatment to ensure that embryos from experimental replicates were placed in the same conditions. This water was placed in the 500 ml beakers for fertilization and into the rearing chambers (140 ml glass jars), sealed with minimal headspace. These were placed in water baths set to the experimental temperature. Data from temperature loggers (iBTag model iBCod G, Thermodata) placed in the baths confirmed that the temperature conditions were stable. Experimental pCO_2 and aragonite (Ω_{ar}) and calcite (Ω_{ca}) saturation values for each temperature–pH combination (electronic supplementary material, tables S1 and S2) were determined from TA, pH_{NBS} and salinity data using CO2SYS [43]. For abalone, we used Ω_{ar} to assess mineral availability. For urchin, we consulted data for Ω_{ar} and Ω_{ca} because the

saturation state of echinoderm magnesium calcite is not known, although is likely to be closest to aragonite [44]. The saturation dynamics of the echinoderm skeleton depends on its Mg^{++} content, and this is variable [28,45]. The mean Ω_{ca} ranged from 1.47 (20°C/pH 7.6) to 5.11 (26°C/pH 8.2), while that for Ω_{ar} ranged from 0.95 (20°C/pH 7.6) to 3.76 (26°C/pH 8.2; electronic supplementary material, tables S1 and S2).

(b) *Fertilization and rearing conditions*

For each experimental replicate, a different population of embryos derived from multiple parents (greater than or equal to two to three of each sex) were used. For sea urchin, we used six embryo populations across the multifactoral treatments. For abalone, we used 10 embryo populations. The urchins were induced to spawn by injection of 1–3 ml of 0.5 M KCl and the gametes were collected using pipettes. To induce spawning in abalone, males and females were separately placed in 500 ml of FSW at control temperature with addition of 1.5 ml of 6 per cent hydrogen peroxide. The pooled eggs were transferred into beakers (500 ml) of fresh FSW at control temperature. Urchin sperm were collected dry and abalone sperm were collected on release through the shell pore. Pooled sperm from multiple males were mixed in a small dish, covered and kept cool (4°C) until use. The eggs were checked microscopically for shape and integrity, and sperm were checked for motility before use. Baths were switched between runs to avoid a potential bath effect. The experiments were conducted over a four-week period for *H. erythrogramma* and a six-week period for *H. coccoradiata*.

Prior to fertilization, the eggs (approx. 3 ml^{-1}) were placed in 500 ml of experimental FSW for 15 min. The required amount of sperm to have a final concentration of 10^3 sperm ml^{-1} was determined in haemocytometer counts of the semen samples. Sperm were briefly (1–2 s) activated in experimental water and added to the eggs. After 15 min, the eggs were rinsed two to three times in experimental FSW using reverse filtration to remove excess sperm. At 2 h post-fertilization, approximately 100 *H. coccoradiata* embryos (as determined in counts of 1 ml aliquots of the embryo suspension) were placed into 140 ml jars (less than one embryo per millilitre) filled with experimental FSW. For *H. erythrogramma*, where the embryos are large, we were able to track the fate of an exact number of embryos. Fifty cleaving embryos were selected and transferred into the rearing containers (approx. one embryo per 3 ml). The jars were sealed, leaving minimal headspace.

The larvae were reared to the first calcified stage. For *H. coccoradiata*, this was the 21 h veliger stage, when the larval shell is prominent [37]. From each jar, the first 50 specimens (where available) were scored microscopically (20× magnification). Scoring was based on the presence or absence of a shell. Unshelled specimens also exhibited a range of abnormalities (electronic supplementary material, figure S1), and some were dead. Thus, the scoring incorporated mortality at 21 h and larvae that would not complete development (electronic supplementary material, figure S1). Approximately 50 unshelled veligers from the control temperature 20°C/pH 7.8 treatments from two embryo populations that looked normal except for the absence of a larval shell were transferred to control FSW for 3 days, with daily renewal of FSW and microscopic examination to determine whether calcification would recover. This transfer experiment was not done with specimens from other treatments (e.g. 22°C/pH 7.6, 24°C/pH 7.8, 24°C/pH 7.6) owing to high percentages of mortality and abnormality (electronic supplementary material, figure S1).

Heliocidaris erythrogramma was reared to the 5 day juvenile stage with daily renewal of experimental water. To determine whether the integrative impact of development in experimental conditions for 4 days had an effect on the ability of the larvae to respond to a settlement cue and metamorphose to the juvenile, a small piece (approx. 1–2 mm) of coralline alga (*Amphiroa anceps*) was placed in the containers at the end of day 4. This cue prompts rapid settlement and metamorphosis of *H. erythrogramma* in control conditions [36]. On day 5, the juveniles were scored microscopically (20× magnification) as normal (symmetrical test and spines) or abnormal (arrested or dead embryos/larvae, irregular juvenile profile), as illustrated in [36] and the electronic supplementary material (figure S1). The number of normal juveniles was divided by the number of embryos originally placed in the rearing containers (50) multiplied by 100 to get percentage normal.

After scoring, the *H. erythrogramma* juveniles from each treatment were fixed in 2.5 per cent glutaraldehyde in FSW for 10 min. They were then rinsed twice (10 min each) with phosphate buffer saline (PBS), followed by a rinse in PBS for 10 min, two rinses in 25 per cent ethanol (ETOH) in PBS followed by two rinses in each of 50, 75, 90 and 100 per cent ETOH/PBS. Juveniles were transferred into 0.5 ml of one part benzyl alcohol to two parts benzyl benzoate to clear the tissue for photography under cross-polarized light using an Olympus microscope. This was done promptly to minimize post-fixation change. The first 20–30 juveniles (as available) from each jar were photographed. The number of spines on each juvenile was counted as a measure of calcification. At 5 days, they have up to 20 spines. The mean number of spines in juveniles from each population (e.g. $n = 6$) was used as the data point for analysis.

Sea water parameters (pH_{NBS}, DO, salinity, temperature) were measured at the end (21 h) of the abalone experiments in five haphazardly selected containers per treatment of 10 total replicates. For the sea urchin experiments, these parameters were measured in four to five of the six replicate containers from each treatment on each daily water change. There was minimal change in experimental pH (electronic supplementary material, tables S3 and S4), no change in salinity and temperature, and DO remained more than 80 per cent.

(c) *Statistics*

The data on percentage of calcified abalone larvae, percentage of normal juvenile urchins and number of spines produced were analysed by two-way ANOVA with temperature and pH as fixed factors. Percentage data were arcsine transformed and Cochran's C confirmed homogeneity of variance. Data on spine number were log transformed to achieve homogeneity of variance. Examination of residual plots confirmed normality of *H. erythrogramma* data prior to analyses. Owing to high mortality of abalone embryos in the +4°C (24°C) treatments, the prevalence of zero data points caused heterogeneity of variance and so these data were not used for the ANOVA, although they are illustrated. For abalone experiments, only data from the 20°C and 22°C treatments were analysed. Where significant differences were evident, Newman–Keul's post hoc tests were used to compare difference between means and for *H. erythrogramma* to examine the significant interaction between temperature and pH. Data analyses were performed using NCSS software (v. 17.0).

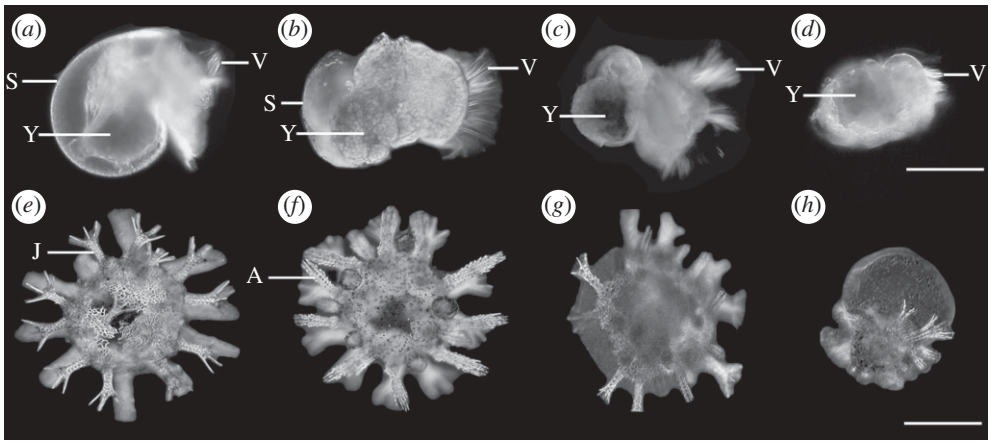

Figure 1. (*a–d*) *Haliotis coccoradiata* larvae (21 h): (*a*) veliger from control treatments, (*b*) larvae from control pH/+2°C and (*c*,*d*) larvae from pH 7.8 treatments. (*e–h*) *Heliocidaris erythrogramma* newly metamorphosed 5-day-old juveniles: (*e*,*f*) juveniles from control treatments and (*g*,*h*) juveniles reared in pH 7.6 and 7.8 treatments (see electronic supplementary material, table S1, for more details). A, adult spine; J, juvenile spine; S, veliger shell; V, velum; Y, yolk mass in developing digestive tract. Scale bars: (*a–d*) 100 μm and (*e–h*) 50 μm.

3. EFFECTS OF OCEAN WARMING AND ACIDIFICATION ON THE PERCENTAGE OF CALCIFIED *H. COCCORADIATA* VELIGERS

In *H. coccoradiata* reared in control conditions, the shell gland is evident by 11 h in trochophore larvae [37]. This is followed by hatching (18.5 h) and formation of the larval shell at 19.5 h. By 21 h, the veligers have a well-developed shell (figure 1*a*).

With increased temperature (+2°C, +4°C) and acidification (−0.4 pH units, −0.6 pH units), abnormal embryos and developmental failure were observed (figure 1*b–d* and electronic supplementary material, table S1). There was a significant reduction in the percentage of calcified veligers (temperature: $p < 0.005$; pH: $p < 0.001$), and Neuman–Keul's tests indicated that the percentage of calcified larvae was significantly lower in the pH 7.6–7.8 treatments and in the +2 to 4°C treatments compared with controls (figure 2 and table 1). There was no significant interaction between factors with a consistent pattern across temperature at each pH (figure 2; temperature × pH: $p = 0.552$).

A 4°C warming breached the thermotolerance of *H. coccoradiata* embryos, with only 20 per cent normal larvae at control pH (figure 2 and table 1). Most of the larvae reared in all pH 7.6 treatments were dead or severely abnormal at 21 h (electronic supplementary material, figure S1).

The sharp drop in Ω_{ar} at pH 7.8 (electronic supplementary material, table S1) may have influenced the prevalence of unshelled veligers in these treatments (figure 1*b–d* and electronic supplementary material, table S1). Some veligers reared in 20°C/pH 7.8 appeared anatomically normal except for the absence of a shell (figure 1*c*). However, they did not form a shell after transfer to control sea water for 3 days.

4. EFFECTS OF OCEAN WARMING AND ACIDIFICATION ON DEVELOPMENT OF THE JUVENILE *H. ERYTHROGRAMMA*

In *H. erythrogramma*, addition of the algal cue on day 4 induced rapid settlement in most larvae and by day 5

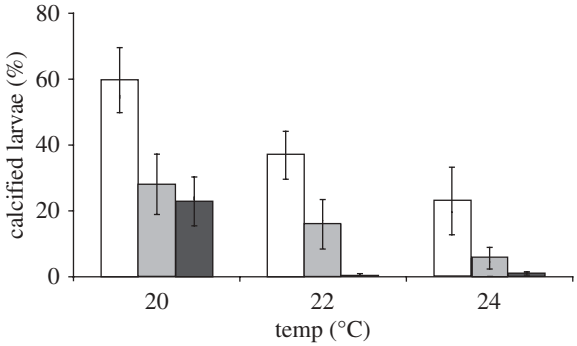

Figure 2. Mean percentage (±s.e.) of calcified *H. coccoradiata* veliger larvae (21 h) in nine treatments (three pH × three temperature). Temperature and pH both significantly affected calcification. Values for pCO_2 and aragonite saturation are provided in electronic supplementary material, table S1. $n = 10$. White bars, pH 8.2; grey bars, pH 7.8; black bars, pH 7.6.

most specimens in the control pH treatments reared at 22°C and 24°C had metamorphosed into juveniles (figure 1*e*,*h*).

Increased temperature (4°C) and lower pH (pH 7.6–7.8) had a significant effect on the percentage of normal juveniles (figure 3 and table 2). Abnormal phenotypes were observed in the more extreme temperature and pH treatments (figure 1*g*,*h* and electronic supplementary material, table S1). Both factors exerted a significant negative effect on development (temperature: $p < 0.001$; pH: $p < 0.001$). There was a significant interaction between factors (temperature × pH: $p < 0.005$). Neuman–Keul's test indicated that the percentage of normal juveniles was higher in the 22°C and 24°C control pH treatments compared with all reduced pH treatments and the +4°C control pH treatment (figures 1 and 3*a*, table 2 and electronic supplementary material, table S1). At +4°C (26°C), development failed (less than 20% normal juveniles) across all treatments (figure 3*a*). This temperature increase breached the thermal tolerance for development in most larvae.

Temperature and pH had a significant effect on spine number (temperature: $p < 0.001$; pH: $p < 0.001$), with

Table 1. Analysis of variance results for percentage of calcified *H. coccoradiata* veliger larvae from 10 independent populations of embryos. Temperature and pH were fixed factors. NK, Neuman–Keul's post hoc test.

factor	sum of squares	mean square	d.f.	*F*-value	*p*-value	NK
temperature	1.705	1.705	1	10.96	<0.005	$22 < 20$
pH	5.096	2.547	2	16.39	<0.001	$(7.6, 7.8) < 8.2$
temperature × pH	0.186	0.093	2	0.60	0.552	
S	13.06	0.155	84			
adjusted total	20.05		89			
total			90			

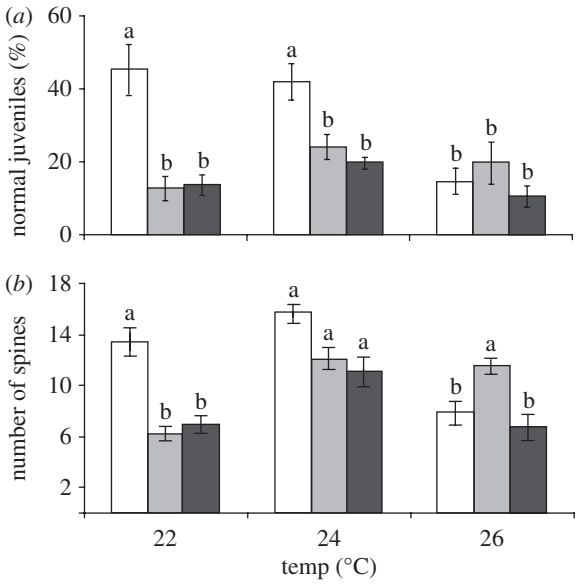

Figure 3. (*a*) Mean percentage (±s.e.) of normal *H. erythrogramma* juveniles in nine treatments (three pH × three temperature). (*b*) Mean number of spines (±s.e.) produced by juveniles in treatments. Values for pCO_2 and aragonite and calcite saturation are provided in electronic supplementary material, table S2. Letters above the bars indicate treatments that did not differ significantly. $n = 6$. White bars, pH 8.2; grey bars, pH 7.8; black bars, pH 7.6.

a significant interaction between factors (temperature × pH: $p < 0.001$; figure 3*b* and table 2). The number of spines produced at +2°C in pH 7.8 and 7.6 did not differ from the number produced at ambient temperature and pH (figure 3*b* and electronic supplementary material, table S1). Newman–Keul's tests indicated that juveniles reared at +2°C and pH 7.6 and 7.8 had more spines than juveniles reared in control temperature at pH 7.6 and 7.8 (figure 3*b*, table 2 and electronic supplementary material, table S1). Thus, a 2°C warming diminished the negative effects of acidification on spine development.

Although the percentage of normal juveniles was lower in the pH 7.6 and 7.8 treatments and reduced Ω_{ar} and Ω_{ca} (electronic supplementary material, table S2), most juveniles possessed skeletal elements, indicating that they had managed to calcify (figures 1 and 3, and electronic supplementary material, table S1). The equivalent of a naked juvenile was not seen for the echinoid. A substantial proportion of larvae (approx. 25%) reared at +2°C produced normal juveniles at pH 7.6 and 7.8 (figures 1 and 3, and electronic supplementary material, table S1). These offspring tolerated near-future warming and acidification.

5. DISCUSSION

Our results show that the early life-history stages of two species from two major benthic calcifying taxa (abalone and sea urchins) reared from fertilization in experimental treatments will be impacted by near-future ocean change. Warming and acidification both exerted deleterious effects, the magnitude of which differed between the species.

Development in the abalone *H. coccoradiata* was vulnerable to near-future levels of warming and acidification (+2°C/pH −0.4 U). Limited thermotolerance appears to be a feature of abalone development [46]. Only a small percentage of embryos formed normal larvae in warm (+2°C to 4°C) and low-pH (pH −0.4 to 0.6 U) conditions. Calcification was inhibited in the low-pH treatments as aragonite became undersaturated, potentially exacerbated by hypercapnic suppression of metabolism. Experimental conditions exceeded the ability of the larvae to control the calcification environment in the shell gland in favour of aragonite accretion. The abalone shell is secreted on the larval surface, so its integrity is likely to be sensitive to water chemistry. Calcification did not recover when unshelled larvae were placed in control sea water. This differs from what has been reported for corals where skeleton-less polyps grown in ocean acidification conditions were able to secrete skeleton on transfer to ambient sea water [47]. Near-future ocean conditions resulted in unshelled *H. coccoradiata* larvae, a condition that prevents survival to the juvenile stage in abalone [48].

The only other mollusc veligers that have been investigated with regard to climate change stressors are those of bivalves. In *Crossostrea gigas*, *Crossostrea ariakensis* and *Mytilus galloprovincialis*, deleterious effects in single-stressor acidification studies are reported at more extreme pH levels ($pH \leq 7.4/\Omega_{ar} \leq 0.8$) than used here [13,18,49]. However, this varies even among congeners, with the veligers of *C. virginica* being more sensitive, producing smaller larval shells (but not unshelled veligers) at pH 7.8 [18]. Calcification in bivalve veligers is less sensitive to acidification than seen here for *H. coccoradiata*. If larvae of other *Haliotis* species are similarly sensitive, this does not bode well for abalone populations, which are of significant economic value and are already in trouble owing to global warming, increasing risk of disease [32]. More data are required to determine whether high sensitivity to climate change stressors is specific to *Haliotis* or also a feature of other gastropod larvae.

Table 2. Analysis of variance results for (*a*) percentage of normal *H. erythrogramma* juveniles, and (*b*) number of spines formed by six independent populations of embryos. Temperature and pH were fixed factors. NK, Neuman–Keul's post hoc test. In the interaction, treatments that are uderlined are not significantly different.

factor	sum of squares	mean square	d.f.	*F*-value	*p*-value	NK
(*a*) percentage normal						
temperature	0.276	0.134	2	9.33	<0.001	26 < (22, 24)
pH	0.496	0.248	2	16.73	<0.001	(7.6, 7.8) < 8.2
temperature × pH	0.314	0.078	4	5.30	<0.005	
S	0.667	0.015	45			
adjusted total	1.754		53			
total			54			
NK: temperature × pH	26,7.6 22,7.8 22,7.6 26,8.2 26,7.8 24,7.6 24,7.8			24,8.2 22,8.2		
(*b*) number of spines						
temperature	2.313	1.156	2	19.90	<0.001	(22, 26) < 24
pH	1.533	0.767	2	13.19	<0.001	7.6 < 7.8<8.2
temperature × pH	2.196	0.549	4	9.44	<0.001	
S	2.616	0.058	45			
adjusted total	8.658		53			
total			54			
NK: temperature × pH	22,7.8 26,7.6 22,7.6 26,8.2		24,7.6, 26,7.8 24,7.8 22,8.2 24,8.2			

Development and calcification in the sea urchin *H. erythrogramma* were significantly affected by experimental treatments, but less so than seen for *H. coccoradiata*. A 2°C warming promoted spine development across all pH levels tested. This level of warming also promotes early metamorphosis and settlement in *H. erythrogramma*, reducing the planktonic larval duration by 25 per cent with the suggested benefits of reduced planktonic mortality [36]. A further 2°C warming breached the thermotolerance of development (approx. 80% dead or abnormal). The decrease in calcification as seen in spine production in the control-temperature (22°C) and low-pH (7.6, 7.8) treatments is similar to that found in single-stressor studies of echinopluteus larvae, where less skeleton is produced at low pH in larvae reared at ambient temperature [9,16,20,41]. Our multi-stressor study showed, however, that interactive effects of these stressors influenced the outcome for development in a warmer and higher pCO_2 ocean. A 2°C warming diminished the negative influence of lowered pH on juvenile calcification in *H. erythrogramma*, similar to that seen for echinoplutei of *Tripneustes gratilla* reared in similar multifactoral experiments [9]. Owing to the interdependence between temperature, mineral saturation state and pH, the mechanism of this facilitation is likely to be complex. The increase in development rate and calcification in *H. erythrogramma* and *T. gratilla* with increased temperature (+2°C) is typical of echinoderm development [50]. However, the decrease in the percentage of normal juveniles at 20–22°C and pH 7.6–7.8, and the more extreme treatments projected by 2100 (approx. 26°C/pH 7.8), will be detrimental to the fitness of *H. erythrogramma*.

A substantial proportion (approx. 20–25%) of *H. erythrogramma* progeny formed normal juveniles in the +2°C/pH 7.6, +2°C/pH 7.8 and +4°C/pH 7.8 treatments. These juveniles were thus able to maintain a high calcium carbonate saturation state at the calcification site to make the test and spines. Owing to enhanced maternal provisioning and deletion of the echinopluteus larva, a developmental stage negatively affected by ocean acidification [9,16,20], *H. erythrogramma* is independent of the need to produce a functional larval skeleton [38]. Development of the lecithotrophic larvae of the sea star *Crossaster papposus* is also robust to low pH conditions [12]. Abbreviated lecithotrophic development may make echinoderms with this life-history mode comparatively more robust to ocean change, a trend evident in the evolutionary history of the phylum through past climate change [40].

Despite the well-known controlling influence of temperature on marine invertebrate development [36,50], recent studies on impacts of climate change on life histories have focused on ocean acidification as a single stressor (reviews in [5–7,13]). Marine propagules of shallow-water species, however, are experiencing simultaneous warming and acidification from the outset of development. It is thus crucial to consider tolerance to multiple stressors and potential interactive effects across ontogenetic stages as this more appropriately reflects the real-world scenario predicted for the oceans in the coming decades. Our experiments provided an estimate of the tolerance of abalone and urchin development to warming and acidification, and for *H. erythrogramma* revealed the interactive effects of stressors.

Ocean warming projected for southeastern Australia [30,33] will breach developmental thermotolerance in the species investigated here with respect to their current tolerance. A significant proportion of *H. coccoradiata* and *H. erythrogramma* embryos may not reach the calcifying stage in the conditions projected for local waters. This will probably also be the case for a suite of co-occurring benthic species. However, unlike the experimental conditions in this study, ocean change will be gradual over the coming decades, creating the possibility that adaptation may occur to facilitate survival of planktonic and benthic stages.

In the short-term (*ca* 2030, 1–2°C increase SST [30]), maintenance of *H. coccoradiata* and *H. erythrogramma* populations may be facilitated by southerly migration of warm-adapted propagules in strong boundary current flow with contraction at northern range limits [36]. Owing to poleward invasion of the sea urchin

Centrostephanus rodgersii along new larval migratory corridors, major changes are already under way in the marine ecosystems of southeastern Australia [31]. In the longer term, however, calcifying species may be caught between the need to move south as the waters warm and the need to avoid acidified water expanding northward in the Southern Ocean [51].

Development can fail at any stage, and it is important to identify where vulnerabilities lie. It is clear that mortality bottlenecks in development and impaired calcification will compromise persistence of *H. coccoradiata* and *H. erythrogramma* in the face of ocean change. Calcification was inhibited (abalone larvae) or impaired (sea urchin juveniles) in conditions projected for 2100 and beyond [4]. These negative impacts will have significant downstream effects on the survival of populations of these ecologically and economically important organisms. Our results underscore the deleterious effects of projected ocean change on these species based on the business-as-usual scenario, and reveal differences in species vulnerability.

Funded by an ARC grant (M.B. and A.D.). Thanks to Dr B Tilbrook, CSIRO, Hobart for assistance with water analysis. The reviewers are thanked for insightful comments that improved the manuscript. Contribution 295, Sydney Institute of Marine Science and 45, Institute for Conservation Biology Wollongong.

REFERENCES

1 Caldeira, K. & Wickett, M. E. 2003 Anthropogenic carbon and ocean pH. *Nature* **425**, 365. (doi:10.1038/425365a)
2 Caldeira, K. & Wickett, M. E. 2005 Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J. Geophys. Res.* **110**, C09S04. (doi:10.1029/2004JC002671)
3 Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J. & Millero, F. J. 2004 Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **305**, 362–366. (doi:10.1126/science.1097329)
4 IPCC (Intergovernmental Panel on Climate Change). 2007 *The 4th Assessment Report of the IPCC*. Cambridge, UK: Cambridge University Press.
5 Byrne, M. 2010 Impact of climate change stressors on marine invertebrate life histories with a focus on the Mollusca and Echinodermata. In *Climate alert: climate change monitoring and strategy* (eds Y. Yu & A. Henderson-Sellers), pp. 142–185. Sydney, Australia: University of Sydney Press.
6 Byrne, M. In press. Impact of ocean warming and ocean acidification on marine invertebrates' life history stages: vulnerabilities and potential for persistence in a changing ocean. *Ocean. Mar. Biol. Ann. Rev.* **49**.
7 Dupont, S., Ortega-Martínez, O. & Thorndyke, M. C. 2010 Impact of near future ocean acidification on echinoderms. *Ecotoxicology* **19**, 449–462. (doi:10.1007/s10646-010-0463-6)
8 Parker, L. M., Ross, P. M. & O'Connor, W. A. 2010 Comparing the effect of elevated pCO_2 and temperature on the fertilization and early development of two species of oysters. *Mar. Biol.* **157**, 2435–2452. (doi:10.1007/s00227-010-1508-3)
9 Sheppard Brennand, H., 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. (doi:10.1371/journal.pone.0011372)
10 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. (doi:10.1146/annurev.marine.010908.163834)
11 Dupont, S., Havenhand, J., Thorndyke, W., Peck, L. & Thorndyke, M. 2008 Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Mar. Ecol. Prog. Ser.* **373**, 285–294. (doi:10.3354/meps07800)
12 Dupont, S., Lundve, B. & Thorndyke, M. C. 2010 Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. *J. Exp. Zool. (Mol. Evol. Dev.)* **314B**, 382–389. (doi:10.1002/jez.b.21342)
13 Kurihara, H. 2008 Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Mar. Ecol. Prog. Ser.* **373**, 275–284. (doi:10.3354/meps07802)
14 Wood, H. L., Spicer, J. I. & Widdicombe, S. 2008 Ocean acidification may increase calcification rates, but at a cost. *Proc. R. Soc. B* **275**, 1767–1773. (doi:10.1098/rspb.2008.0343)
15 Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H. D., Dworjanyn, S. A. & Davis, A. R. 2009 Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proc. R. Soc. B* **276**, 1884–1889. (doi:10.1098/rspb.2008.1935)
16 Clark, D., Lamare, M. & Barker, M. 2009 Response of sea urchin larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among tropical, temperate, and a polar species. *Mar. Biol.* **156**, 1125–1137. (doi:10.1007/s00227-009-1155-8)
17 Gooding, R. A., Harley, C. D. G. & Tang, E. 2009 Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *Proc. Natl Acad. Sci. USA* **106**, 9316–9321. (doi:10.1073/pnas.0811143106)
18 Miller, A. W., Reynolds, A. C., Sobrino, C. & Riedel, G. F. 2009 Shellfish face uncertain futures in high pCO_2 world: influence of acidification in oyster larvae calcification and growth in estuaries. *PLoS ONE* **4**, e5661. (doi:10.1371/journal.pone.0005661)
19 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. (doi:10.1130/G30210A.1)
20 O'Donnell, M. J., Todgham, A. E., Sewell, M. A., LaTisha, M. H., Ruggiero, K., Fangue, N. A., Zippay, M. L. & Hofmann, G. E. 2010 Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. *Mar. Ecol. Prog. Ser.* **398**, 157–171. (doi:10.3354/meps08346)
21 Pörtner, O. 2008 Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar. Ecol. Prog. Ser.* **373**, 203–217. (doi:10.3354/meps07768)
22 Widdicombe, S. & Spicer, J. I. 2008 Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? *J. Exp. Mar. Biol. Ecol.* **366**, 187–197. (doi:10.1016/j.jembe.2008.07.024)
23 Melzner, F., Gutowska, M. A., Lagenbuch, M., Dupont, S., Lucassen, M., Thjorndyke, M. C., Bleich, M. & Pörtner, O. 2009 Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences* **6**, 2313–2331. (doi:10.5194/bg-6-2313-2009)
24 Kleypas, J. A. & Yates, K. K. 2009 Coral reefs and ocean acidification. *Oceanography* **22**, 108–117.
25 Harley, C. D. G., Hughes, A. R., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S.,

Rodriguez, L. F., Tomanek, L. & Williams, S. L. 2006 The impacts of climate change in coastal marine systems. *Ecol. Lett.* **9**, 228–241. (doi:10.1111/j.1461-0248.2005.00871.x)
26 Przeslawski, R., Ahyong, S., Byrne, M., Worheide, G. & Hutchings, P. 2008 Beyond corals and fish: the effects of climate change on non-coral benthic invertebrates of tropical reefs. *Global Change Biol.* **14**, 2773–2795. (doi:10.1111/j.1365-2486.2008.01693.x)
27 Brierley, A. S. & Kingsford, M. J. 2009 Impacts of climate change on marine organisms and ecosystems. *Curr. Biol.* **19**, 602–614. (doi:10.1016/j.cub.2009.05.046)
28 Dubois, P. & Chen, P. 1989 Calcification in echinoderms. In *Echinoderm studies*, vol. 3 (eds M. Jangoux & J. M. Lawrence), pp. 109–178. Rotterdam, The Netherlands: Balkema.
29 Weiss, I. M., Tuross, N., Addadi, L. & Weiner, S. 2002 Mollusc larval shell formation: amorphous calcium carbonate is a precursor phase for aragonite. *J. Exp. Zool.* **293**, 478–491. (doi:10.1002/jez.90004)
30 Poloczanska, E. S. *et al.* 2007 Climate change and Australian marine life. *Ocean. Mar. Biol. Ann. Rev.* **45**, 407–478.
31 Ling, S. D., Johnson, C. R., Ridgway, K., Hobday, A. J. & Haddon, M. 2009 Climate-driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. *Global Change Biol.* **15**, 719–731. (doi:10.1111/j.1365-2486.2008.01734.x)
32 Travers, M.-A., Basuyaux, O., LeGoïc, N., Huchette, S., Nicolass, J.-L., Koken, M. & Paillard, C. 2009 Influence of temperature and spawning effort on *Haliotis tuberculata* mortalities caused by *Vibrio harveyi*: an example of emerging vibriosis linked to global warming. *Global Change Biol.* **15**, 1365–1376. (doi:10.1111/j.1365-2486.2008.01764.x)
33 Poloczanska, E. S., Hobday, A. J. & Richardson, A. J. 2009 *A marine climate change impacts and adaptation report card of marine climate change for Australia*. NCCARF Publication 05/09. Canberra, Australia.
34 Byrne, M., Soars, N. A., Selvakumaraswamy, P., Dworjanyn, S. A. & Davis, A. R. 2010 Sea urchin fertilization in a warm, acidified and high pCO_2 ocean across a range of sperm densities. *Mar. Environ. Res.* **69**, 234–239. (doi:10.1016/j.marenvres.2009.10.014)
35 Byrne, M., Soars, N. A., Ho, M. A., Wong, E., McElroy, D., Selvakumaraswamy, P., Dworjanyn, S. A. & Davis, A. R. 2010 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. (doi:10.1007/s00227-010-1474-9)
36 Byrne, M., Selvakumaraswamy, P., Ho, M. A. & Nguyen, H. D. In press. Sea urchin development in a global change hotspot, potential for southerly migration of thermotolerant propagules. *Deep Sea Res. II*. (doi:10.1016/j.dsr2.2010.06.010)
37 Wong, E., Davis, A. R. & Byrne, M. 2010 Reproduction and early development in *Haliotis coccoradiata* (Vetigastropoda: Haliotidae). *Invert. Rep. Dev.* **54**, 77–87.
38 Byrne, M., Emlet, R. & Cerra, A. 2001 Ciliated band structure in planktotrophic and lecithotrophic larvae of *Heliocidaris* species (Echinodermata: Echinoidea): conservation and change. *Acta. Zool.* **82**, 189–199.
39 Soars, N. A., Prowse, T. A. A. & Byrne, M. 2009 Overview of phenotypic plasticity in echinoid larvae, '*Echinopluteus transversus*' type versus typical echinoplutei. *Mar. Ecol. Prog. Ser.* **383**, 113–125. (doi:10.3354/meps07848)
40 Uthicke, S., Schaffelke, B. & Byrne, M. 2009 A boom-bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol. Mon.* **79**, 3–24. (doi:10.1890/07-2136.1)
41 Kurihara, H. & Shirayama, Y. 2004 Effects of increased atmospheric CO_2 on sea urchin early development. *Mar. Ecol. Prog. Ser.* **274**, 161–169. (doi:10.3354/meps274161)
42 Laegdsgaard, P., Byrne, M. & Anderson, D. T. 1991 Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* (Echinoidea) in New South Wales. *Mar. Biol.* **110**, 359–374. (doi:10.1007/BF01344355)
43 Pierrot, D., Lewis, E. & Wallace, D. W. R. 2006 MS Excel program developed for CO_2 system calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.
44 Morse, J. W., Arvidson, R. S. & Lüttge, A. 2007 Calcium carbonate formation and dissolution. *Chem. Rev.* **107**, 342–381. (doi:10.1021/cr050358j)
45 Borremans, C., Hermans, J., Baillon, S., André, L. & Dubois, P. 2009 Salinity effects on the Mg/Ca and Sr/Ca in starfish skeletons and the echinoderm relevance for paleoenvironmental reconstructions. *Geology* **37**, 351–354. (doi:10.1130/G25411A.1)
46 Gilroy, A. & Edwards, S. J. 1998 Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenly abalone, *Haliotis lavegiata* (Leach). *Aquacult. Res.* **2**, 481–485.
47 Fine, M. & Tchernov, D. 2007 Scleractinian coral species survive and recover from decalcification. *Science* **315**, 1811. (doi:10.1126/science.1137094)
48 Conroy, P. T., Hunt, J. W. & Anderson, B. S. 1996 Validation of a short-term toxicity test endpoint by comparison with longer-term effects on larval red abalone *Haliotis rufescens*. *Environ. Toxicol. Chem.* **15**, 1245–1250.
49 Kurihara, H., Kato, S. & Ishimatsu, A. 2007 Effects of increased pCO_2 on early development of the oyster *Crassostrea gigas*. *Aquat. Biol.* **1**, 91–98. (doi:10.3354/ab00009)
50 Staver, J. M. & Strathmann, R. R. 2002 Evolution of fast development of planktonic embryos to early swimming. *Biol. Bull.* **203**, 58–69. (doi:10.2307/1543458)
51 Richardson, A. J., McKinnon, D. & Swadling, K. M. 2009 Zooplankton. In *A marine climate change impacts and adaptation report card for Australia 2009* (eds E. S. Poloczanska, A. J. Hobday & A. J. Richardson), NCCARF Publication 05/09. Canberra, Australia.