Impacts of ocean acidification on development of the meroplanktonic larval stage of the sea urchin Centrostephanus rodgersii

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The effects of near-future ocean acidification/hypercapnia on larval development were investigated in the sea urchin Centrostephanus rodgersii, a habitat-modifying species from eastern Australia. Decreased pH (−0.3 to −0.5 pH units) or increased pCO2 significantly reduced the percentage of normal larvae. Larval growth was negatively impacted with smaller larvae in the pH 7.6/1800 ppm treatments. The impact of acidification on development was similar on days 3 and 5, indicating deleterious effects early in development. On day 3, increased abnormalities in the pH 7.6/1600 ppm treatment were seen in aberrant prism stage larvae and arrested/dead embryos. By day 5, echinoplutei in this treatment had smaller arm rods. Observations of smaller larvae in C. rodgersii have significant implications for this species because larval success may be a potential bottleneck for persistence in a changing ocean.

Keywords: Centrostephanus rodgersii, hypercapnia, ocean acidification, sea urchin larvae.

Introduction

The climate-change-driven co-varying stressors ocean acidification, hypercapnia, and reduced calcium carbonate saturation state have raised concerns with respect to the impacts of increased anthropogenic CO2 release on marine biota (Orr et al., 2005; Byrne, 2010, 2011; Dupont et al., 2010). These stressors are having negative effects on marine organisms, although the effects vary between species (Dupont et al., 2008, 2010; Byrne, 2010, 2011). Invertebrates that utilize broadcast spawning for reproduction represent the majority of marine taxa, and completion of their life cycle requires success in both benthic and pelagic environments. The pelagic meroplanktonic life stages of benthic marine invertebrates are particularly sensitive to changes in physical and chemical ocean parameters (Pechenik, 1987). Many of these organisms calcify as larvae and are sensitive to decreased carbonate availability and, because of poor acid-base physiological regulatory ability, are also susceptible to ocean hypercapnia (Melzner et al., 2009; Dupont et al., 2010).

Research on the effects of ocean acidification on coastal marine invertebrates has focused largely on species that calcify in the larval and adult life stages (Kurihara, 2008; Byrne, 2010, 2011; Dupont et al., 2010; Kroeker et al., 2010). Several studies show deleterious effects of changing ocean stressors on development, calcification, metabolism, and gene expression in echinoderm and mollusc larvae (Kurihara, 2008; Byrne, 2010, 2011; O’Donnell et al., 2009, 2010; Dupont et al., 2010; Martin et al., 2011; Parker et al., 2011). These effects have serious consequences for species success, because the larval stages may be the bottleneck for the persistence of species. Marine larvae are an integral part of the food chain, and both benthic and pelagic stages are a vital part of their respective ecosystems. Understanding how the early life stages will respond to future ocean-change scenarios provides insight into how species may fare faced with future climate change. As it is clear that marine ecosystems are changing as a consequence of climate alteration, many studies have focused on ecologically important species to assess future climate-change effects on marine ecosystems.

The temperate region of eastern Australia has a dynamic environment influenced by the East Australian Current (EAC; Suthers et al., 2011). With climate-driven strengthening of the EAC causing increased poleward flow of warm water, species range expansion and shifts are evident (Ling et al., 2009). One such example of a southern range extension is that reported for Centrostephanus rodgersii, a sea urchin common in shallow-water coastal habitats of southeast Australia where it is an ecologically important grazer (Andrew and Byrne, 2001). This urchin is an
ecological engineer, playing a keystone species role in the shift between kelp and barren habitats free of foliose macroalgae (Andrew and Byrne, 2001; Ling et al., 2009). Its ecological importance prompted us to investigate the response of its larvae to climate-change stressors. Development in C. rodgersii follows the usual larval form characteristic of the Diadematidae, a major family of echinoids in which echinoplutei have just two arms, following evolutionary loss of the other pairs of arms typical of other sea urchin larvae (Andrew and Byrne, 2001; Soars et al., 2009). These arms are used for swimming and feeding, and an alteration in their development or asymmetrical growth (arm asymmetry) would impair larval function (Soars et al., 2009). Previous studies have shown that fertilization in C. rodgersii is robust to near-future ocean acidification (Byrne et al., 2010). However, the effects of ocean acidification on larval development of this ecologically important species are not known. Here, we investigate the effect of ocean acidification on the early meroplanktonic life stages of C. rodgersii to determine how the larvae might perform in the face of decreasing pH and increasing hypercapnic conditions.

Material and methods

Centrostephanus rodgersii were collected at the peak of their spawning season (Byrne et al., 1998) in July 2010, near Coffs Harbour, New South Wales (30°12.5′S 153°16.1′E) and were maintained in flow-through aquaria (~3500 l) at ambient temperature (~21°C). They were induced to spawn by injection of 2–3 ml of 0.5 M KCl. The eggs of four randomly selected females were spawned into 500 ml beakers of ambient filtered seawater (FSW; 0.2 μm). Sperm were collected dry using a pipette. Before use, the eggs were checked for shape and integrity, and the sperm were checked for motility. The eggs were pooled and fertilized by a mixed sperm sample from five males. In all, 6–7 independent populations of embryos formed the experimental replicates for each treatment.

Approximately 1000 eggs from each egg source (~10 eggs ml⁻¹) were placed in rearing containers (100 ml) that had a window of 45 μm mesh cut each side to maintain a volume of 30 ml and to retain eggs. These containers were placed in each pH treatment (8.1, 7.8, and 7.6), in flow-through experimental FSW (21°C flow rate, ca. 0.13 ml s⁻¹, 300–400 turnovers d⁻¹) for 20 min before the introduction of sperm. Fertilization was completed with 10³ sperm ml⁻¹ determined through haemocytometer counts. The flow-through system was turned off (5 min) during fertilization, then turned back on to remove the excess sperm. Embryos were reared in experimental conditions in separate trials to the prism/78 h early larval stage (ca. 3 d) and the 5-d echinopluteus stage.

Experimental treatments and rearing

The embryos were reared in experimental flow-through FSW at 21°C and three levels of pH (control = pH 8.1, and ~0.3 and ~0.5 pH units). Experimental pH was adjusted using an automatic CO₂ injection system. Two pH controllers (Tunze), set at pH 7.6 and 7.8, were attached to two header tanks (60 l). The controllers, pH probes, solenoid valves, and gas cylinders were connected in series, and pure CO₂ gas was injected into the header when required, where it was dissolved using a vortex mixing device (Red Sea). The header tanks were bubbled with air continuously, to aid mixing and to maintain dissolved oxygen (DO) >90%. A constant volume was maintained in the header tanks using a float valve. The control pH header tank was bubbled with air only. Seawater was delivered to rearing containers using irrigation drip-taps. Temperature, pH_{NIST}, DO, and salinity at the level of the experimental containers with developing embryos and larvae were measured daily with a WTW 3400I multiprobe. Filtered ambient inflow water was not manipulated and had a mean temperature of 21.6°C (s.e. = 0.053, n = 75, range 21.1–22.3°C) and a mean pH_{NIST} of 8.097 (s.e. = 0.006, n = 25, pH range 8.08–8.13). The experimental pH conditions measured at the level of the rearing containers remained stable (pH ~0.3 units: mean 7.85, s.e. = 0.006, n = 25, pH range 7.81–7.88; pH ~0.5 units: mean 7.63, s.e. = 0.006, n = 25, pH range 7.60–7.66). Water samples for total alkalinity were taken at the start of experiments and measured by potentiometric acid titration using the Gran method (experiment 1 (3 d), TA = 2268.0 μmol kg⁻¹; experiment 2 (5 d), TA = 2260.7 μmol kg⁻¹). Mean total alkalinity data from both experiments and pH_{NIST} were used to calculate experimental pCO₂ and calcite and aragonite saturation values using CO2SYS (Lewis and Wallace, 1998; Table 1).

Development and growth

Specimens from each rearing container (n = 20–50, where available) were placed in 1.5 ml tubes containing 10% formaldehyde–FSW for 10 min, followed by a rinse in 70% ethanol (ETOH) in FSW. The first 50 specimens (where available) removed randomly from each tube were photographed using a digital camera mounted on a compound microscope. The percentage of normal development was scored. On day 3, normal development was scored in the presence of normal prism and early larval stages of larvae (Figure 1a). On day 5, normal larvae were defined as echinoplutei with two arms, including larvae with minimal arm asymmetry (Figure 2a–c). Abnormal specimens included arrested embryos, abnormal prisms, larvae with marked asymmetry (one arm ≥30% larger than the other), and armless larvae (Figure 1b–e). Larval growth on day 5 echinoplutei was documented using image analysis software Image J (NIH, USA) by measuring the two post-oral arm rods for the first 20–25 (where available) plutei photographed positioned flat to the plane of focus (Figure 2a). In all, 20–25 larvae were measured per replicate. Total length of calcite rods (TLC) determined as the sum of both arm rods, was used as a proxy for biogenic calcification.

Statistics

Data on the percentage of normal development were analysed with a two-way analysis of variance (ANOVA), with pH (8.1, 7.8, and 7.6) and time (3 and 5 d) as fixed factors. Assumptions of homogeneity of variance and normality were met with an arcsin transformation, as determined with the Levene and the Shapiro–Wilk tests. Percentage difference in arm length (asymmetry) and TLC were analysed with a one-way ANOVA with pH as the fixed factor, and the assumptions of ANOVA were met. A Tukey HSD post hoc test was used to determine the statistical significance of treatment groups. All statistics were carried out using JMP8.

<table>
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<th>Parameter</th>
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<th>Value at pH_{NIST} 7.8</th>
<th>Value at pH_{NIST} 7.6</th>
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<td>2 264.4</td>
<td>2 264.4</td>
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<tr>
<td>pCO₂ (ppm)</td>
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<td>924.3</td>
<td>1 606.4</td>
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<td>Ω_{aragonite}</td>
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<td>1.61</td>
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</table>
Results

The percentage of normal prism stage/early larvae (3 d) and echnoplutei (5 d) decreased significantly (~15%) at both elevated pCO2 treatment groups (F2,37 = 8.20, p = 0.001; Figure 3a and b). In acidified treatments, 3-d arrested and abnormal prism stage larvae were present (Figure 1b and c). By 5 d, embryos that had not reached the larval stage were dead, arrested, or abnormal.

In larvae reared in treatments to day 5, arm length was significantly (~20%) lower in the pH 7.6/1600 ppm treatment (F2,16 = 7.23, p = 0.006; Figure 4a). There was no effect of pH/pCO2 on the percentage of larvae with arm-length asymmetry among the three pH treatment groups (F2,15 = 0.68, p = 0.52; Figures 1d and e and 4b).

Discussion

The results indicate that projected increasing ocean pCO2 will have deleterious effects on larval development in C. rodgersii. The response of C. rodgersii to increasing acidification and hypercapnia was similar in 3- and 5-d larvae, indicating negative impacts early in development. At both time-points, populations reared in ocean acidification conditions were ca. 15% more abnormal than those reared in ambient treatments. This may be because of direct pH effects, or hypercapnia, or both. The presence of arrested, abnormal, and potentially slower developing prism stages on day 3 in the pH 7.6–7.8/900–1600 ppm treatments indicated that development was delayed. This retardation of development is likely the consequence of hypercapnic suppression. In the field, this would increase planktonic larval duration (PLD), influencing population connectivity and augment exposure of larvae to predation in a species with a potential 3–5-month PLD (Lamare and Barker, 1999; Andrew and Byrne, 2001; Soars et al., 2009).

A significant number of larvae had shorter arms/rods in experimental treatments on day 5. Echinopluteal growth was impacted, as indicated by the significantly smaller skeletal rods of larvae reared at pH 7.6. AR, arm rod; BR, body rod.

Figure 1. Five-day cultures: (a and b) normal (a) early and (b) late prism stage, (c) arrested embryo, and (d and e) echnoplutei with arm-length asymmetry.

Figure 2. Representative photographs of 5-d larvae of C. rodgersii at three pH/pCO2 treatments, showing the smaller larval size and reduction in length of the arm skeleton at pH 7.6. AR, arm rod; BR, body rod.
food (Strathmann, 1971). In addition to morphological changes, gene expression in echinoplutei may be up-regulated (e.g. P. lividus; Martin et al., 2011), or down-regulated (e.g. S. franciscanus; O’Donnell et al., 2009; Todgham and Hofmann, 2009) in ocean-acidification conditions.

A decrease in larval arm length would have a negative effect on feeding and swimming capabilities in the larvae of C. rodgersii, because the species only develops two arms (Soars et al., 2009). Arm-rod growth in C. rodgersii was not impaired at pH 7.8/900 ppm, indicating that larval development in this species may be robust to near-future emission scenarios (ca. 2100), similar to previous studies on the sympatric sea urchin species T. gratilla (Sheppard Brennand et al., 2010). The deformities and stunted larval growth of C. rodgersii and other species at pH 7.6/1600 ppm, however, are expected to have a negative impact on larval success. This would have implications on coastal ecosystems where population dynamics are greatly influenced by larval supply (Underwood and Fairweather, 1989).

Further work is needed to determine how additional climate-change stressors such as ocean warming will affect the larvae of C. rodgersii, because ocean warming and acidification are concurrent stressors and are likely to have synergistic effects. Studies on larval and juvenile development in the co-occurring sea urchins Heliocidaris erythrogramma and T. gratilla indicate that a slight increase in temperature (+2°C) has a buffering effect, somewhat compensating for the deleterious effects of ocean acidification (Sheppard Brennand et al., 2010; Byrne et al., 2011a). In addition, other stressors, such as decreased DO and nutrient availability, will accompany ocean warming and acidification. Ocean warming also shortens PLD, resulting in temporal changes in pelagic trophic cascades (O’Connor et al., 2007). For C. rodgersii, however, ocean warming is of particular concern because the species lives in an ocean-warming hotspot (Suthers et al., 2011).

Centrostephanus rodgersii has a broad latitudinal range in eastern Australia, from northern New South Wales to Tasmania, and its successful invasion of Tasmania has been associated with ocean warming (Andrew and Byrne, 2001; Ling et al., 2009). In the sympatric species H. erythrogramma, development of larvae in northern populations is more thermotolerant than that of southern populations, indicating that the northern populations may be a source of thermotolerant propagules for recruitment in southern locations (Byrne et al., 2011b). This would be facilitated by the strengthening poleward flow of the EAC, as seems to be the case for C. rodgersii (Ling et al., 2009). Similarly, for C. rodgersii, it is likely that the thermal history of northern populations has an influence on larval thermal tolerance, facilitating larval survival in southern latitudes. Studies investigating the combined effects of ocean warming and ocean acidification on the development of C. rodgersii would be valuable.

Figure 3. The percentage of (a) normal prisms/early larvae at (a) 3 d and (b) echinoplutei at 5 d shows the significant deleterious effect of pH/CO2 on larvae in low pH treatments. Error bars are standard errors.

Figure 4. (a) Mean post-oral arm lengths ± s.e. of 5-d larvae in the three treatment groups, showing that a significant decrease in larval size at the highest pH/CO2 of 7.6/1700 ppm treatment group (F2,16 = 7.23, p = 0.006). (b) Mean percentage asymmetry ± s.e. calculated from the difference in arm length indicates no difference between pH/CO2 treatments. Letters indicate the post hoc Tukey HSD tests.
of C. rodgersii are clearly needed, to assess how the larvae of this species will respond to climate change. In addition, longer experiments over the larval cycle of C. rodgersii are required to identify the potential synergistic effects of pH and temperature. It is obviously important to assess the impacts of climate change on target species such as C. rodgersii that are ecologically important, and hence to determine which life stages are a bottleneck for persistence in a changing ocean.

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References


