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Adult exposure influences offspring response to ocean acidification in oysters

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Abstract

It is essential to predict the impact of elevated Pco_2 on marine organisms and habitats to anticipate the severity and consequences of future ocean chemistry change. Despite the importance of carry-over effects in the evolutionary history of marine organisms, few studies have considered links between life-history stages when determining how marine organisms will respond to elevated Pco_2, and none have considered the link between adults and their offspring. Herein, we exposed adults of wild and selectively bred Sydney rock oysters, *Saccostrea glomerata* to elevated Pco_2 during reproductive conditioning and measured the development, growth and survival response of their larvae. We found that elevated Pco_2 had a negative impact on larvae of *S. glomerata* causing a reduction in growth, rate of development and survival. Exposing adults to elevated Pco_2 during reproductive conditioning, however, had positive carry-over effects on larvae. Larvae spawned from adults exposed to elevated Pco_2 were larger and developed faster, but displayed similar survival compared with larvae spawned from adults exposed to ambient Pco_2. Furthermore, selectively bred larvae of *S. glomerata* were more resilient to elevated Pco_2 than wild larvae. Measurement of the standard metabolic rate (SMR) of adult *S. glomerata* showed that at ambient Pco_2, SMR is increased in selectively bred compared with wild oysters and is further increased during exposure to elevated Pco_2. This study suggests that sensitive marine organisms may have the capacity to acclimate or adapt to elevated Pco_2 over the next century and a change in energy turnover indicated by SMR may be a key process involved.

Keywords: carbon dioxide, carry-over, climate change, ocean acidification, *Saccostrea glomerata*, Sydney rock oyster

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Introduction

Although it is difficult to predict the impact of elevated Pco_2 on oceans and their inhabitants, it is (Pörtner *et al*., 2004; Dupont *et al*., 2010; Hendriks *et al*., 2010; Kroeker *et al*., 2010) essential to be able to anticipate the severity and consequences of future change. Recent meta-analyses synthesizing the effects among life-history stages and groups of marine organisms (Dupont *et al*., 2010; Hendriks *et al*., 2010; Kroeker *et al*., 2010) aim to comprehensively analyse the exponential growth of studies testing predictions surrounding the acute impacts of CO_2-driven 'ocean acidification' on larval development. In general, meta-analyses and experiments collectively show that larvae of marine organisms are vulnerable to ocean acidification (Kurihara *et al*., 2004; Dupont *et al*., 2008; Findlay *et al*., 2009; Munday *et al*., 2009; Sheppard Brennand *et al*., 2010; Walther *et al*., 2010). In contrast to other phyla like echinoderms where responses are more variable, molluscs larvae are consistently negatively impacted (Kurihara *et al*., 2008a; Parker *et al*., 2009, 2010; Gazeau *et al*., 2010; Talmage & Gobler, 2010). Furthermore, the extent of these effects have been shown to differ even between closely related species (*Crassostrea gigas* and *Saccostrea glomerata*, Parker *et al*., 2010) and in some cases, between populations of the same species (Waldbusser *et al*., 2010; Parker *et al*., 2011).

One of the great unknowns in ocean acidification research is whether marine organisms will be able to adapt to long-term multigenerational exposure. More specifically, whether long-term chronic exposure of adults to elevated Pco_2, can influence the response of their larvae. Indeed, previous studies have found that the rearing environment during the reproductive conditioning of an organism can influence offspring fecundity and survival (Pacific oyster, *C. gigas*, Lannan, 1980; Muranaka & Lannan, 1984; asteroid, *Luidia clathrata*, Hintz & Lawrence, 1994; tropical reef damselfish, *Pomacentrus amboinensis*, McCormick & Gagliano, 2008;

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clam, *Ruditapes decussates*, Matias *et al*., 2009; and clam, *Mercenaria mercinaria*, Przeslawski & Webb, 2009). For example, Muranaka & Lannan (1984) found that the survival of larvae of the Pacific oyster, *C. gigas* was greater when broodstock were conditioned at a salinity of 30 compared to 20. Despite this, studies on the impact of ocean acidification on marine organisms to date, have only considered the impacts on 'adults' or 'larvae', ignoring the potential link between the two life-history stages and the possible carry-over effects that may be passed from adult to offspring (Dupont *et al*., 2010; Hendriks *et al*., 2010; Kroeker *et al*., 2010). A growing body of literature on marine systems highlights the importance of maternal effects on the survival and success of offspring (Bernardo, 1996; Untersee & Pechenik, 2007; Marshall, 2008; Marshall *et al*., 2008; Sanford & Kelly, 2011). Persistent maternal effects induced by the environment in which the adult was held can lead to a variation in the response of offspring (Sanford & Kelly, 2011). For example, in the gastropod, *Crepidula convexa* juveniles released in the laboratory from adults collected from a copper polluted site were more tolerant to copper stress than juveniles released from a reference site (Untersee & Pechenik, 2007). Similar results were also found in the bryozoan, *Bugula nerita* (Marshall *et al*., 2010). The current lack of consideration of the potential for carry-over effects or 'links' across life-history stages and the importance of maternal effects in the response of marine organisms to ocean acidification, greatly limits our ability to predict whether marine organisms and ecosystems will have the capacity to adapt over the next century.

Those studies that have considered the link between other life-history stages (oysters, *S. glomerata*, *C. gigas*, Parker *et al*., 2009, 2010; shrimp, *Palaemon pacificus*, Kurihara *et al*., 2008b; amphipod, *Echinogammarus marinus*, Egilsdottir *et al*., 2009; barnacle, *Semibalanus balanoides*, Findlay *et al*., 2009) generally find negative effects on one life-history stage carried over to the next. Such carry-over begins with the production of gametes; for example, Kurihara *et al.* (2008b) found that egg production in the shrimp, *P. pacificus* was suppressed following long-term exposure of adults to elevated Pco_2 of 1000 ppm. Within the next generation, it continues from fertilization to larval development; for example, Parker *et al.* (2009, 2010) found that the negative effects of elevated Pco_2 such as increased abnormality and reduced survival on larvae of the oysters, *S. glomerata* and *C. gigas*, were greater when fertilization occurred at elevated compared with ambient Pco_2. What these studies do not consider is whether such a carry-over effect from adult to larvae can have positive consequences and provide resilience to the next generation when exposed to elevated concentrations of Pco_2. It is also unknown how differing genotypes in a population express different parental effects and create greater resilience in offspring.

The Sydney rock oyster, *S. glomerata* is an ecologically and economically significant molluscan species occupying intertidal and shallow sub-tidal estuarine habitats along the southeast coast of Australia. In the state of New South Wales (NSW), *S. glomerata* forms the largest and oldest aquaculture industry (White, 2002), generating approximately US$39 million in retail sales each year (O'Connor *et al*., 2008). Acute studies on the impact of ocean acidification on wild populations of *S. glomerata* have shown that the early-life-history stages of this species are extremely vulnerable (Parker *et al*., 2009, 2010). For example, Parker *et al.* (2009, 2010) found that D-veliger larvae of *S. glomerata* suffered 100% mortality after only 2 days of rearing at elevated Pco_2 of 750 μatm and elevated temperature of 30 °C. More recently, newly metamorphosed wild spat were found to have a 64% reduction in shell growth after 4 days at elevated Pco_2 (1000 μatm) when compared with wild spat grown in ambient seawater (Parker *et al*., 2011). In that study, populations of *S. glomerata* that had been produced by selective breeding (to increase growth and overcome pressures such as disease) were more resilient than the wild population to elevated Pco_2.

Herein, we exposed adults of a wild and selectively bred population of the Sydney rock oyster to ocean acidification during reproductive conditioning and measured the development, growth and survival response of their larvae. The hypotheses tested were: (1) Larvae from adults conditioned at elevated Pco_2 (856 μatm) will be more resilient to ocean acidification than larvae from adults conditioned at ambient Pco_2 (380 μatm), and given that selectively bred larvae grew faster and were not as impacted on exposure to elevated Pco_2 in Parker *et al.* (2011) that (2) Selectively bred larvae will be more resilient than the wild larvae. We also measured standard metabolic rate (SMR) as an indicator of fitness and energy turnover and were thereby able to show that energy turnover and allocation to fitness sustaining processes may be a key element in the long-term resistance to ocean acidification. This study is the first of its kind to look at the possibility of a commercially important mollusc to acclimate to the pressures of ocean acidification during trans-generational exposure.

Materials and methods

Selected Breeding Programme

In 1990, a breeding programme was established for the Sydney rock oyster, *S. glomerata* in an effort to combat the

pressures of declining production (Nell *et al.* 2000). Oysters were mass selected initially for faster growth and in subsequent years, disease selection was also added. The base population for the mass selected breeding lines was taken from a combination of wild oysters from the four major Sydney rock oyster growing estuaries across the state of NSW. These included Wallace Lake (32°30′S, 152°29′E), Port Stephens (32°45′S, 152°10′E), the Hawkesbury (33°30′S, 151°12′E) and Georges Rivers (34°00′S, 151°10′E). The base population were mass spawned in the hatchery and the fastest growing offspring were selected and returned to the field once they reached the spat stage with a shell height of 12 mm. Each generation, gravid adults of the mass selected lines that have survived an outbreak of disease are returned to the hatchery. This selection process has been repeated over seven generations. For further information, see Nell *et al.* (2000).

Collection and exposure of adults

Adult Sydney rock oysters, *S. glomerata* of approximately 1.5–2 years of age were collected at the beginning of reproductive conditioning (approximately 1–2 weeks of gametogenesis). Two populations of *S. glomerata* were used in the study. A wild population and a selectively bred population bred for fast growth and disease resistance (selected breeding line B2; seven generations of selection). The wild oysters were collected at random from among naturally occurring oysters in Port Stephens, NSW (32°45′S, 152°10′E). This wild population was chosen because it is historically the major supplier of seed to the NSW oyster industry and thus most representative of farmed stocks in NSW. The selectively bred oysters were collected directly from an Industry & Investment NSW broodstock repository in the Clyde River, NSW (34°78′S, 150°69′E) to ensure they were representative of the B2 line. Three hundred representative oysters from each population (mean flesh weight: wild oysters = 7.15 ± SE 0.32 g, selected oysters = 7.13 ± SE 0.42 g) were transferred in hessian bags to the Industry & Investment NSW, Port Stephens Fisheries Centre (PSFC), Taylors Beach, NSW, Australia. Upon arrival to the hatchery at PSFC, adults of both populations were cleaned to remove mud and fouling organisms. The broodstock were then transferred into 750 L acclimation tanks, where they were held in 40 L trays and maintained under continuously flowing, recirculating seawater (salinity 34.6) at a temperature of 24 °C for 2 weeks. Seawater was collected from Little Beach (152°07′E, 32°72′S), Nelson Bay, NSW, Australia, and was filtered through 1 μm nominal filters prior to delivery into the hatchery. Following the acclimation period, each population was divided equally at random into six 750 L header tanks (50 oysters population^{-1} tank^{-1}). In each tank, there were 50 wild and 50 selectively bred oysters in separate 40 L flow-through trays (flow rate 3 L min^{-1}) supplied by recirculating water from the same 750 L header tank.

There were two Pco_2 levels used in the study: a current atmospheric Pco_2 level of 380 μatm, pH_{NBS} 8.19–8.20; and an elevated atmospheric Pco_2 level predicted for 2100 of 856 μatm, pH_{NBS} 7.89–7.90 (IPCC 2007). The elevated Pco_2 level was maintained using pH negative-feedback systems (Aqua Medic, Aqacenta Pty Ltd, Kingsgrove, NSW, Australia; accuracy ± 0.01). Briefly, CO_2 was bubbled into 1 μm filtered seawater (FSW) in each header tank using a CO_2 reactor to ensure proper mixing. pH was continually monitored by a pH probe connected to a computer. When the set pH level was reached, the delivery of CO_2 was stopped by a solenoid valve. The pH_{NBS} level in the header tanks was measured twice daily using a combined pH electrode (mean pH, 380 μatm = 8.2 ± 0.01 units; 856 μatm = 7.9 ± 0.01 units) and total alkalinity (TA) was measured in triplicate (three water samples) before and after each water change by Gran titration (mean TA = 2308 ± SE 42 μmol kg^{-1}). To determine the pH value corresponding to the desired Pco_2 levels, pH, TA, temperature and salinity of the seawater were added into a CO_2 system calculation programme (CO_2 sys) developed by Lewis & Wallace (1998), using the dissociation constants of Mehrbach *et al.* (1973) (for seawater physiochemical conditions see Table 1).

The adults were fed a combined algal diet of 50% *Chaetoceros muelleri*, 25% *Pavlova lutheri* and 25% Tahitian *Isochrysis aff. galbana* at a concentration of 2×10^9 cells oyster^{-1} day^{-1}. Complete water changes were made every 2 days using pre-equilibrated FSW and oysters were rinsed daily with freshwater. Following 5 weeks of conditioning in the Pco_2 treatments, the oysters reached gravid stage. At that time, the SMR of adults from each population was measured and the remaining oysters were removed from the tanks in preparation for spawning.

Table 1 Seawater physiochemical conditions during the adult and larval exposure experiment

Condition	Salinity	Temperature (°C)	pH_{NBS}	TA (μmol kg^{-1})	Pco_2 (μatm)	DIC (μmol kg^{-1})	$\Omega_{aragonite}$	$\Omega_{calcite}$
Adults								
Ambient	34.6 ± 0.3	24 ± 0.5	8.2 ± 0.01	2308 ± 42	380	2008.3	3.4	5.2
Elevated CO_2	34.6 ± 0.3	24 ± 0.5	7.9 ± 0.01	2308 ± 42	856	2158.1	1.9	2.9
Larvae								
Ambient	34.6 ± 0.3	24 ± 0.5	8.2 ± 0.02	2311 ± 44	380	2010.5	3.4	5.2
Elevated CO_2	34.6 ± 0.3	24 ± 0.5	7.9 ± 0.03	2311 ± 44	856	2160.4	1.9	2.9

Values for Pco_2, DIC, $\Omega_{aragonite}$ and $\Omega_{calcite}$ calculated from salinity, temperature, pH_{NBS} and TA.
TA, total alkalinity; DIC, dissolved inorganic carbon; TA ± SE.

SMR of adults

SMR in wild and selectively bred adult Sydney rock oysters that were conditioned at ambient and elevated Pco_2 was measured using a closed respirometry system. Oysters were placed in individual 500 mL airtight chambers, fitted with a fibre-optic probe (PreSens dipping probe DP-PSt3, AS1 Ltd, Palmerston North, New Zealand) and the time taken to reduce the percentage oxygen saturation of seawater in the chamber from 100% to 80% when oysters were open and filtering was recorded. The probes were calibrated using a two-point calibration (0% and 100%) and all measurements were done at the experimental temperature of 24 °C. Feeding was stopped 24 h prior to the SMR measurements. Following the measurements, oysters were removed from the chambers and dry tissue mass was determined. SMR of each oyster was calculated as follows:

$$\mathrm{SMR} = \frac{V_r\ (\mathrm{L}) \times \Delta C_w O_2 (\mathrm{mg\ O_2 L^{-1}})}{\Delta t(\mathrm{h}) \times \mathrm{bw(g)}},$$

where SMR is the oxygen consumption normalized to 1 g of dry tissue mass (mg O_2 g^{-1} dry tissue mass h^{-1}), V_r is the volume of the respirometry chamber minus the volume of the oyster (L), $\Delta C_w O_2$ is the change in water oxygen concentration measured (mg O_2 L^{-1}), Δt is measuring time (h) and bw is the dry tissue mass (g). Six oysters were tested from each oyster type and Pco_2 combination (2 replicate^{-1}; $n = 3$).

Collection and exposure of embryos and larvae

Eggs and spermatozoa were obtained from each 'oyster type' (wild/selected) and 'adult exposure' (ambient/elevated Pco_2) combination by strip spawning. Gametes were stripped from the gonad of gravid adults into 1 μm nominal FSW set at the same Pco_2 concentration as that in which the adults were conditioned. Eggs and spermatozoa were filtered through a 60 and 45 μm nylon mesh screen, respectively, to facilitate the removal of debris. Eggs from a minimum of 10 females and spermatozoa from a minimum of 10 males were pooled in separate 500 mL containers. Fertilization of each oyster type and adult exposure was undertaken in two 20 L buckets, one set at ambient Pco_2 (380 μatm) and the other set at elevated Pco_2 (856 μatm) (1 μm FSW; 24 °C; salinity 34.6). Eggs were divided equally across the two buckets and were allowed to incubate for 10 min. Following the incubation period, spermatozoa were added to the eggs at a concentration 1×10^6 mL^{-1} (sperm concentration determined using a haemocytometer under a light microscope 100×) to allow fertilization to take place. This resulted in production of eight experimental larval lines: adults from wild populations that were conditioned in ambient Pco_2 and produced larvae in ambient conditions (wild 380 μatm adults, 380 μatm larvae); adults from wild populations that were condition in ambient Pco_2 and produced larvae reared in elevated Pco_2 (wild 380 μatm adults, 856 μatm larvae); adults from wild populations that were conditioned in elevated Pco_2 and produced larvae in ambient conditions (wild 856 μatm adults, 380 μatm larvae); adults from wild populations that were condition in elevated Pco_2 and produced larvae reared in elevated Pco_2 (wild 856 μatm adults, 856 μatm larvae); adults from mass selected populations that were conditioned in ambient Pco_2 and produced larvae in ambient conditions (selected 380 μatm adults, 380 μatm larvae); adults from mass selected populations that were condition in ambient Pco_2 and produced larvae reared in elevated Pco_2 (selected 380 μatm adults, 856 μatm larvae); adults from mass selected populations that were conditioned in elevated Pco_2 and produced larvae in ambient conditions (selected 856 μatm adults, 380 μatm larvae); and adults from mass selected populations that were condition in elevated Pco_2 and produced larvae reared in elevated Pco_2 (selected 856 μatm adults, 856 μatm larvae) (see Fig. 1 for further details).

The larval experiment was set up in 200 L polyethylene tanks. There were three replicate tanks for each treatment. Prior to the experiment, 24 × 200 L tanks were thoroughly washed with Virkon S solution (Antec Corp, North Bend, WA, USA), rinsed with freshwater and left to air dry for 24 h (O'Connor *et al*., 2008). The tanks were then fitted with a tap and air stone and were filled with 1 μm FSW (24 °C; salinity 34.6). The elevated Pco_2 concentration was obtained in 12 of the tanks by manipulation of pH by direct bubbling of CO_2 in seawater controlled by independent pH negative-feedback systems as in the adult experiment (Aqua Medic; accuracy ±0.01). Once the pH was obtained, all 24 tanks were sealed with polyethylene plastic bags to minimize gas exchange, and pH was monitored throughout (mean pH, 380 μatm = 8.2 ± 0.02 units; 856 μatm = 7.9 ± 0.03 units; TA = 2311 ± SE 44; for seawater physiochemical conditions see Table 1). Each tank was gently aerated to keep larvae dispersed in the water column.

Fertilized embryos were added into each tank at a concentration of 15 embryos mL^{-1} (3 million tank^{-1}). After 12 h, the tanks were dropped and sieved through a mesh screen to facilitate the removal of non-developing embryos and reduce the risk of disease. The tanks were then restocked at a concentration of 5 embryos mL^{-1} (1×10^6 embryos tank^{-1}). Larval feeding began with the appearance of the first D-veligers, after approximately 16 h. Larvae were fed an algal diet twice daily consisting of 50% *Chaetoceros calcitrans*, 25% *P. lutheri* and 25% T. *Isochrysis aff. galbana* for the first week of development (O'Connor *et al*., 2008). After this time, *Ch. calcitrans* was gradually replaced with *Ch. muelleri* as the larvae increased in size. Algal concentrations ranged from 1×10^4 cells mL^{-1} at the beginning of the experiment up to 1.16×10^5 cells mL^{-1} at the completion of the experiment. There was a complete water change of each tank every 2 days using pre-equilibrated FSW. At each water change, a subsample was taken from each replicate tank and the mean shell length (antero-posterior measurement) and stage of development (D-veliger, umbonate or eyed larvae) of 10 larvae was measured under the microscope (Leica 100×, Wetzlar, Germany). Larvae remained in the treatments for 19 days, until the appearance of eyed larvae. The number of larvae in each tank was measured at the beginning (following first sieving 12 h after fertilization: initial concentration 1×10^6 embryos per tank) and end of the experiment (day 19) and percentage survival following 19 days of exposure was calculated. The measurements were stopped at this

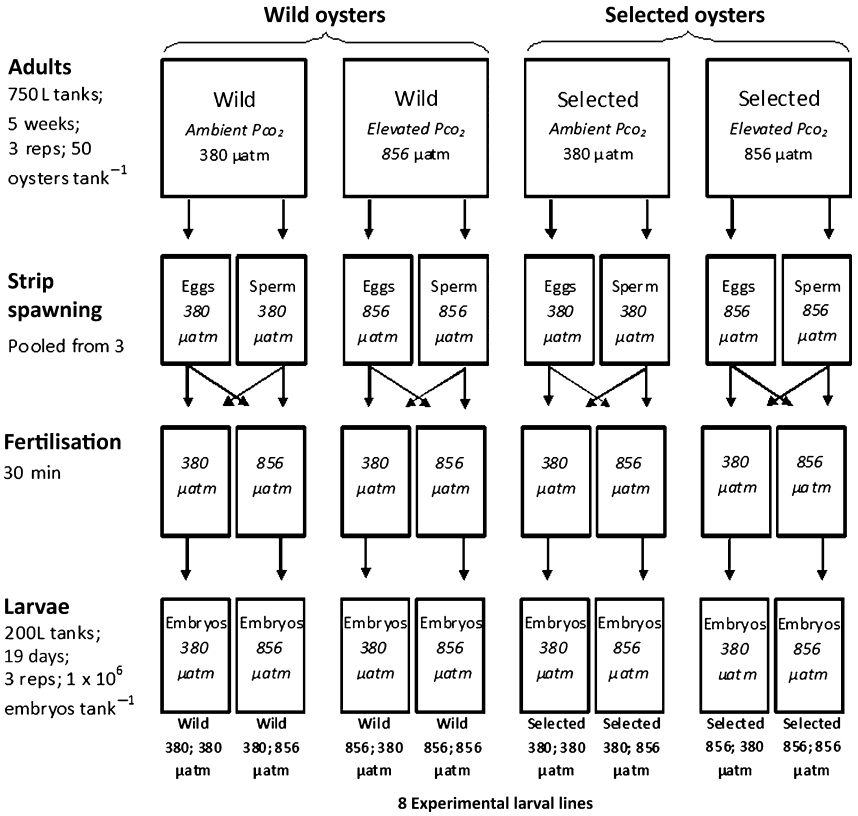

Fig. 1 Flow chart of the experimental design.

stage as after this, size sieving was required to allow the settlement of eyed larvae and the removal of larvae that were not developing.

Data analysis

To determine any significant differences between 'adult exposure', 'oyster type' and 'Pco_2' on larvae of *S. glomerata*, the percentage survival, percentage development to the D-veliger, umbonate and eyed larvae stage and the shell length (anteroposterior measurement) of larvae were analysed using a three-way analysis of variance (ANOVA) using GMAV5 (Underwood, 1997), where 'adult exposure', 'oyster type' and 'Pco_2' were fixed and orthogonal factors. Data were not transformed, as Cochran's test for heterogeneity of variances was not significant. Differences between 'oyster type' and 'Pco_2' on the SMR of adults of *S. glomerata* was determined using a two-way analysis of variance (ANOVA), where 'oyster type' and 'Pco_2' were fixed and orthogonal factors. Cochran's test was used to determine any heterogeneity of variances, and data were transformed if significant. A Student Newman–Keuls (SNK) test was used to detect differences amongst means (Sokal & Rohlf, 1995). As 100% of larvae reached the D-veliger stage after 24 h across each treatment combination, this datum is not presented. These results are not comparable to the 90% larval development obtained in Parker *et al*. (2009, 2010) due to the sieving process used in this study after 12 h to reduce the risk of disease.

Results

Survival of larvae

There was no effect of 'adult exposure' on the percentage survival of larvae after 19 days. There was, however, a significant effect of both 'oyster type' and 'Pco_2' and no interaction. The larvae from the wild oysters had a lower percentage survival than the selectively bred larvae [mean square (MS) = 2410.58, df = 1 × 16, $F = 10.39$, $P < 0.01$; Fig. 2a]. After 19 days under ambient conditions, the average survival of larvae was 82% in the wild oysters and 91% in the selectively bred oysters (Fig. 2a). In addition, elevated Pco_2 caused a significant reduction in the survival of larvae (MS = 3012.74, df = 1 × 16, $F = 12.99$, $P < 0.01$; Fig. 2a). At the elevated Pco_2 level of 856 µatm, the average percentage survival of larvae fell to levels of 48% and 79% in the wild and selectively bred oysters respectively (Fig. 2a).

Development of larvae

There were significant effects of 'adult exposure' and oyster type' and 'Pco_2'on the rate of development to the

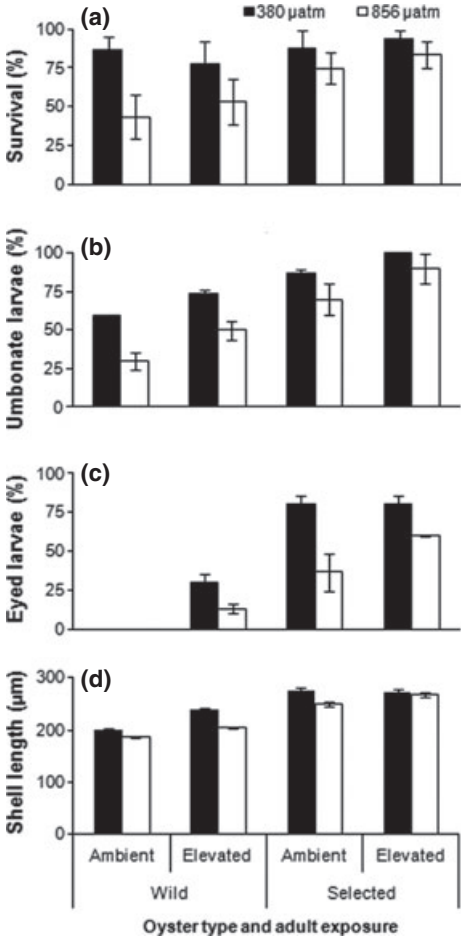

Fig. 2 Effects of ocean acidification on larvae of the Sydney rock oyster, *Saccostrea glomerata*, following exposure of adults to ambient (380 µatm) and elevated (856 µatm) Pco_2; (a) survival of larvae after 19 days; (b) percentage development to the umbonate larval stage after 9 days; (c) percentage development to the eyed larval stage after 19 days; (d) shell length of larvae after 19 days. Black and white bars represent larvae reared at ambient (380 µatm) and elevated (856 µatm) Pco_2, respectively; 24 °C; salinity 34.6; $n = 3$; bars = SEM.

umbonate larval stage after 9 days (Fig. 2b, c). For the eyed larval stage, there was a three-way significant interaction between 'adult exposure × oyster type × Pco_2' after 19 days (Table 2). The rate of development to the umbonate stage for the wild and selectively bred larvae was slower when larvae were reared at elevated compared with ambient Pco_2. After 9 days in the treatments, the percentage of larvae that had reached the umbonate stage was reduced up to 30% and 17% in the wild and selectively bred larvae respectively (Fig. 2b). The rate of development differed between the oyster populations with the wild oysters developing slower than the selectively bred oysters at both ambient and elevated Pco_2 (Fig. 2b). Finally, when larvae were spawned from adults conditioned at elevated Pco_2, they had a faster rate of development than larvae that were spawned from adults conditioned at ambient Pco_2. This occurred when larvae were reared at both ambient and elevated Pco_2 (Fig. 2b; Table 2; SNK).

Similarly, the rate of development to the eyed larval stage was slower in wild larvae compared with selectively bred larvae with a greater percentage of selectively bred larvae reaching the eyed larval stage after 19 days under both ambient and elevated Pco_2 conditions (Fig. 2c; Table 2). Furthermore, larvae reared at elevated Pco_2 (856 µatm) had a slower development rate than those reared at the ambient Pco_2 (380 µatm). On average, the percentage development of eyed larvae after 19 days was 25% (SE ± 7%) lower in larvae reared at 856 µatm compared to 380 µatm. The only experimental line that did not show a difference in development between ambient and elevated Pco_2 was the wild larvae spawned from adults conditioned at ambient Pco_2 as no larvae had reached the eyed larval stage in the ambient or elevated Pco_2 treatments after 19 days (Fig. 2c). Finally, in the wild larvae reared at 380 µatm and selected larvae that were reared at 856 µatm, the rate of development to the eyed larval stage was faster in larvae spawned from adults conditioned at elevated Pco_2 compared with those from adults conditioned at ambient Pco_2 (Fig. 2c; Table 2; SNK).

Shell length of larvae

There was a three-way significant interaction between 'adult exposure × oyster type × Pco_2' on the shell length of larvae of *S. glomerata* after 19 days of development (Table 3). Overall, at each Pco_2 and adult exposure, the selectively bred larvae were larger in size than the wild larvae (Fig. 2d; Table 3; SNK). Furthermore, the shell length of larvae reared at the elevated Pco_2 of 856 µatm was significantly smaller than those reared at the ambient Pco_2 (380 µatm). The exception to this was in the selectively bred larvae that were spawned from adults conditioned at elevated Pco_2, where there was no effect of elevated Pco_2 on the size of larvae after 19 days (Fig. 2d; Table 3; SNK). Larvae that were spawned from adults conditioned at elevated Pco_2 were larger in size than those spawned from adults conditioned at ambient Pco_2 (Fig. 3a, b). The exception to this was in the selectively bred oysters that were reared at ambient Pco_2. Herein, there was no difference in shell length of larvae from adults conditioned at ambient or elevated Pco_2 (Fig. 2d; Table 3; SNK). At the completion of the experiment, the shell length of *S. glomerata* larvae was greatest in the selectively bred larvae that were spawned from adults conditioned at elevated

Table 2 Analysis of mean percentage development to umbonate and eyed larvae in wild and selectively bred larvae of *Saccostrea glomerata* spawned from CO_2-exposed and non-exposed adults and reared at the Pco_2 (375, 856 μatm) treatments for 9 days (umbonate larvae) and 19 days (eyed larvae); $n = 3$ (15/10/10). This was a three-way analysis with adult exposure, oyster line and Pco_2 being fixed and orthogonal

		Umbonate larvae (9 days)			Eyed larvae (19 days)		
Source of variation	df	MS	*F*	*P*	MS	*F*	*P*
Adult exposure (Ad)	1	1666.67	15.38	**	1504.17	19.00	***
Oyster line (Oy)	1	6666.67	61.54	***	17 604.17	222.37	***
Pco_2 (CO_2)	1	2400.00	22.15	***	2204.17	27.84	***
Ad × Oy	1	0.00	0.00	$F = 0$	204.17	2.58	ns
Ad × CO_2	1	66.67	0.62	ns	4.17	0.05	ns
Oy × CO_2	1	266.67	2.46	ns	504.17	8.89	**
Ad × Oy × CO_2	1	0.00	0.00	$F = 0$	74.17	6.37	*
RES	16						
Total	23						
SNK		Elevated > ambient Selected > wild 380 > 856 μatm			Adult exposure: Wild 380 μatm; selected 856 μatm: elevated > ambient Wild 856 μatm; selected 380 μatm: Elevated = ambient Oyster line: Selected > wild CO_2: Ambient wild: 380 = 856 μatm Ambient selected; elevated wild/ selected: 380 > 856 μatm		

Significance level indicated by asterisks,
ns, not significant,
*$P < 0.05$;
**$P < 0.01$;
***$P < 0.001$. $C = 0.35$ ns umbonate larvae, $C = 0.47$ ns eyed larvae.
MS, mean square; SNK, Student Newman Keuls.

Pco_2 and whose larvae were reared at both ambient and elevated Pco_2 (shell length = 380 μatm: 272.00 ± SE 5.51 μm; 856 μatm: 268.38 ± SE 5.32 μm). Shell length was smallest in the wild larvae that were spawned from adults conditioned at ambient Pco_2 and reared at elevated Pco_2 (856 μatm) (shell length = 186.80 ± SE 1.31 μm). Finally, the wild larvae spawned from adults conditioned at elevated Pco_2 and reared at elevated Pco_2 (wild, elevated adults; elevated larvae) were greater in size (shell length = 204.74 ± SE 1.11 μm) than larvae from adults conditioned at ambient Pco_2 that were reared at this Pco_2 (wild, ambient adults; ambient larvae) (shell length = 200.27 ± SE 2.99 μm).

Overall, ocean acidification had negative impacts on larvae of *S. glomerata*, but the impacts were less severe when larvae came from adults reared at elevated Pco_2. Larvae from adults reared at elevated Pco_2 were larger, developed faster, but had similar survival compared to larvae from adults reared at ambient Pco_2. Furthermore, larvae from selectively bred adults were more resilient to elevated Pco_2 than larvae from wild adults.

SMR of adults

There was a significant effect of both 'oyster type' and 'Pco_2' on the SMR of adult *S. glomerata*, with no interaction (Fig. 4). The SMR of the selectively bred oysters was significantly higher than the wild oysters at both ambient and elevated Pco_2 (MS = 0.82, df = 1 × 8, $F = 37.23$, $P < 0.001$; Fig. 4). Exposure of adult *S. glomerata* to elevated Pco_2 for 5 weeks led to a significant increase in SMR in both oyster types (MS = 0.25, df = 1 × 8, $F = 11.33$, $P < 0.01$). This was particularly evident in the selectively bred adults, where SMR was increased by 36% compared with the controls. In the wild adults, the increase in SMR was only 14% (Fig. 4).

Discussion

This study has found that the response of *S. glomerata* larvae to long-term exposure to elevated Pco_2 varies depending on the oyster population and the environment of adults during reproductive conditioning. In our study, larvae of the selectively bred oysters were more resilient to the effects of elevated Pco_2 than wild larvae,

Table 3 Analysis of mean shell length of wild and selectively bred larvae of *Saccostrea glomerata* spawned from CO_2-exposed and non-exposed adults and reared at the Pco_2 (375, 856 μatm) treatments for 19 days; $n = 3$ (15/10/10). This was a three-way analysis with adult exposure being fixed, and oyster line and Pco_2 being fixed and orthogonal

Source of Variation	df	MS	*F*	*P*	SNK
Adult Exposure (Ad)	1	103.94	0.45	***	Adult exposure: Wild 380, 856 μatm; selected 856 μatm:
Oyster line (Oy)	1	2410.58	10.39	***	elevated > ambient Selected 380 μatm: elevated = ambient
Pco_2 (CO_2)	1	3012.74	12.99	***	Oyster line: Selected > wild CO_2: Ambient wild/
Ad × Oy	1	75.83	0.33	**	selected; elevated wild: 380 > 856 μatm
Ad × CO_2	1	168.12	0.72	ns	Elevated selected: 380 = 856 μatm
Oy × CO_2	1	720.21	3.10	ns	
Ad × Oy × CO_2	1	104.32	0.45	**	
Total	23				

Significance level indicated by asterisks,
ns, not significant,
*$P < 0.05$;
**$P < 0.01$;
***$P < 0.001$. $C = 0.31$ ns
MS, mean square; SNK, Student Newman Keuls.

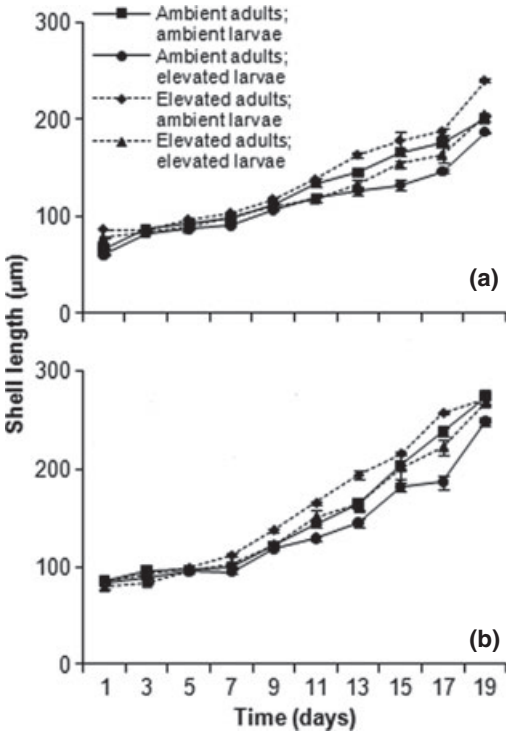

Fig. 3 Mean shell length of (a) 'wild' and (b) 'selected' larvae of the Sydney rock oyster, *Saccostrea glomerata*, spawned from adults exposed to ambient (380 μatm) and elevated (856 μatm) Pco_2 and reared at the Pco_2 (375, 856 μatm) treatments from day 1 to 19; 24 °C; salinity 34.6; $n = 3$; bars = SEM.

but in general larvae that were spawned from adults conditioned at elevated Pco_2 were also more resilient to the effects of elevated Pco_2 than larvae spawned from adults conditioned at ambient Pco_2. For example, when larvae were reared at elevated Pco_2, they were up to 10% larger in size and had a faster rate of development (but similar survival) when they were spawned from adults conditioned at elevated Pco_2 compared with adults conditioned at ambient Pco_2. After 19 days of exposure, wild larvae that were spawned from adults conditioned at elevated Pco_2 and were subsequently reared at elevated Pco_2, were larger in size than wild larvae spawned from adults conditioned at ambient Pco_2 that were reared at ambient Pco_2. This suggests that there are carry-over effects from adults exposed to elevated Pco_2, which may help to compensate or reduce the negative effects of elevated Pco_2 on size and rate of development of mollusc larvae as found in previous acute studies (Kurihara *et al*., 2008a; Parker *et al*., 2009, 2010; Gazeau *et al*., 2010).

Acclimation of offspring due to history of exposure of the adults has been documented for marine invertebrates exposed to environmental stresses such as salinity (Davis, 1958; Bacon, 1971; Muranaka & Lannan, 1984; Hintz & Lawrence, 1994; Allen *et al*., 2008). For example, Bacon (1971) found that when embryos of the barnacle, *Balanus eburneus* were exposed to high or low salinity, the resulting larvae had an increased survival at adverse salinity of a similar level. Furthermore, in the oyster, *Crassostrea virginica*, the optimum salinity and salinity range for development of embryos and larvae was influenced by the salinity at which the adults were held prior to spawning (Davis, 1958). The benefits of exposing adults to elevated Pco_2 during reproductive conditioning in this study were not only seen in larvae that were subsequently reared at elevated Pco_2 but also in larvae that were reared at ambient Pco_2. Across both Pco_2 treatments, larvae spawned from adults condi-

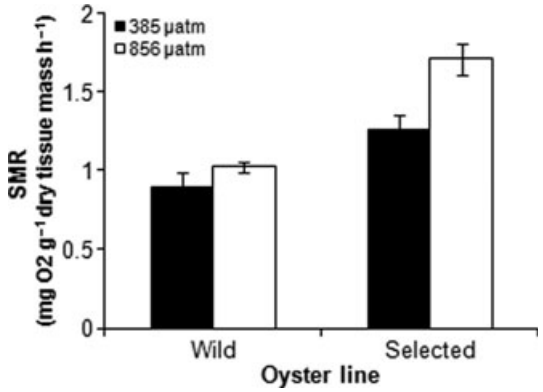

Fig. 4 Standard metabolic rate of wild and selectively bred adults of the Sydney rock oyster, *Saccostrea glomerata*, exposed to ambient (380 µatm) and elevated (856 µatm) Pco_2 for 5 weeks; 24 °C; salinity 34.6; $n = 3$; bars = SEM.

tioned at elevated Pco_2 were generally larger and developed faster than larvae spawned from adults conditioned at ambient Pco_2 (although survival was similar). Changes in phenotypic traits of offspring following exposure of adults to environmental stress, such as those seen here, are often linked to an adaptive maternal effect (Untersee & Pechenik, 2007; Marshall *et al*., 2010; Sanford & Kelly, 2011). Mothers can respond to environmental stress by increasing maternal energy investment per offspring thereby increasing offspring size, a trait which is often considered to be beneficial for offspring (Podolsky & Moran, 2006; Allen *et al*., 2008; Moran & McAlister, 2009). In marine organisms with planktotrophic larval stages such as *S. glomerata*, maternal investment is limited to eggs prior to liberation with larger egg size typically leading to larger sized larvae (Podolsky & Moran, 2006; Moran & McAlister, 2009). Increases in egg size of marine invertebrates have been documented following exposure of adults to environmental stresses including reduced temperature and intraspecific competition (Allen *et al*., 2008; Moran & McAlister, 2009). This adaptive strategy can reduce the time that larvae spend in the water column, reduce their dependence on exogenous food and provide them with a competitive advantage following settlement (Allen *et al*., 2008; Moran & McAlister, 2009). One disadvantage of such an investment, however, is that it can come at a cost to fecundity, with fewer larger eggs produced by a mother in contrast to more numerous smaller eggs (Allen *et al*., 2008). In this study, gametes needed to be obtained from adults via strip spawning, which makes it impossible to accurately determine fecundity.

The effects of elevated Pco_2 may also vary among and within populations. Parker *et al.* (2011) reported that the selectively bred Sydney rock oyster population used in this study was more resilient than the wild population following acute exposure to elevated Pco_2. Herein, we showed that long-term exposure of larvae to elevated Pco_2 led to similar effects. The selectively bred larvae of *S. glomerata* exhibited greater survival and growth and had a faster rate of development than the wild larvae when grown at elevated as well as Pco_2. This demonstrates that there is variation in response to ocean acidification within a population (Waldbusser *et al*., 2010; Parker *et al*., 2011). The differences in the response of the two populations may largely be due to an inherited genetic effect that leads to a higher SMR. SMR of the adults used in this study increased following 5 weeks of exposure to elevated ambient Pco_2. This result was similar to those found on other adult oyster species including *C. gigas* (Lannig *et al*., 2010) and *C. virginica* (Beniash *et al*., 2010) during exposure to elevated Pco_2 and is thought to occur due to a higher energy allocation to homeostasis (Beniash *et al*., 2010). Mechanisms and processes benefiting from a higher SMR would be ion and acid-base regulation, protein synthesis and growth (Pörtner, 2008). A comparison between the wild and selectively bred oysters showed that the SMR of the selectively bred oysters was greater than that of the wild, particularly during exposure to elevated Pco_2. A higher SMR may carry over into larval development and provide selectively bred larvae of *S. glomerata* with a quicker and more complete compensation of homeostatic disturbances induced by elevated Pco_2. We do not know, however, whether the SMR of the larval generation was similar to their parents and whether 'carry-over' effects exist. Elevated SMR may be one of the mechanisms responsible for higher resilience of oysters, and potentially other marine organisms to elevated Pco_2.

The negative effects of elevated Pco_2 on the larvae of *S. glomerata*, as found in this study could have major consequences for oysters at the population level (Gazeau *et al*., 2010). A reduction in the survival of larvae will reduce the number of individuals reaching settlement (Ross, 2001; Ross *et al*., in press). Under natural conditions, juvenile mortality of benthic invertebrates is already thought to exceed 90% (Thorson, 1950). If survival were further reduced during this critical stage of development due to exposure to elevated Pco_2, reduced numbers may also be seen at the population level. In addition to reduced survival, reduced larval size and rate of development will increase the age at metamorphosis, increase the time for predation of larvae and presumably reduce the competitive ability of larvae during settlement (Byrne, 2009). Although exposure of adults to elevated Pco_2 prior to spawning reduced the effects of elevated Pco_2 on the rate of development and size of larvae of *S. glomerata* in this study, it did not

improve larval survival. As a result, we expect that in the absence of genetic adaptation, the effects of elevated Pco_2 on larvae of *S. glomerata* will occur at the population level. Maternal investment may help offspring respond to CO_2-stress, providing phenotypic traits such as larger size, higher energy reserves and higher stress proteins, but in the presence of other environmental stresses such as temperature or reduced food availability, this investment may not be adaptive or even possible. Maternal investment can differ depending on the severity of stress. For example, during intermediate stress, such as the level of Pco_2 used in this study, adults will increase the size of their offspring to provide them with the best chance of success in adverse conditions (Allen *et al*., 2008). If stress becomes too great, however, mothers will conserve energy and produce offspring of smaller size in favour of fecundity (Allen *et al*., 2008). Increases in temperature over the next century will probably lead to increases in the metabolic rate of marine organisms (Munday *et al*., 2008). If we assume that part of the adaptive capacity of larvae of *S. glomerata* to elevated Pco_2 is a higher SMR, then increases in temperature may act synergistically with increased Pco_2 to increase SMR beyond a level that is sustainable and make maintaining homeostasis impossible. A net effect would be a reduction in heat tolerance limits (Pörtner & Farrell, 2008). Similarly, when food availability is high, as in this study, parents have the energy stores available to increase maternal investment and the increased energy demands of increased SMR in adults and larvae can be met. If food availability is reduced, however, such as in areas of poor primary productivity, impacts of ocean acidification could be greater, as there may not be sufficient energy resources to allocate to maternal investment (Allen *et al*., 2008) or to sustain a higher metabolic rate. The ability of adult carry-over effects to increase larval performance during the synergistic exposure to elevated Pco_2 and other environmental stressors such as temperature and food concentration requires further investigation.

Despite the importance of carry-over effects in the evolutionary history of marine organisms, none have directly considered the link between adult and offspring when determining an organism's response to elevated Pco_2. Herein, we show that effects of elevated Pco_2 on larvae of the Sydney rock oyster, *S. glomerata*, were considerably lower following acclimation of adults to elevated Pco_2 during reproductive conditioning. This suggests that previous studies that have investigated the effects of elevated Pco_2 on the larvae of molluscs and other marine organisms may overestimate the severity of their responses. Despite this, the capacity for genetic adaptation may be limited such that elevations in atmospheric Pco_2 over the next century will still have negative ecological and economic consequences for the wild population of *S. glomerata* and potentially other marine invertebrates. In addition, synergistic stressors such as increased temperature and food-limitation may add to the negative effects of ocean acidification. Multi-generational and multi-stressor experiments are needed to anticipate the adaptive capacity of wild *S. glomerata* and other marine organisms over the next century given the current rate of increase of atmospheric CO_2 (Royal Society 2005).

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References

Allen RM, Buckley YM, Marshall DJ (2008) Offspring size plasticity in response to intraspecific competition: an adaptive maternal effect across life-history stages. *American Naturalist*, **171**, 225–337.

Bacon PR (1971) The maintenance of a resident population of *Balanus eburneus* (Gould) in relation to salinity fluctuations in a Trinidad mangrove swamp. *Journal of Experimental Marine Biology and Ecology*, **6**, 187–198.

Beniash E, Ivanina A, Lieb NS, Kurochkin I (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. *Marine Ecology Progress Series*, **419**, 95–108.

Bernardo J (1996) Maternal effects in animal ecology. *American Zoologist*, **36**, 83–105.

Byrne M (2009) 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 Yu J, Henderson A), pp. 142–185. University of Sydney, Sydney.

Davis HC (1958) Survival and growth of clam and oyster larvae at different salinities. *Biological Bulluten*, **114**, 296–307.

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*. *Marine Ecology Progress Series*, **373**, 285–294.

Dupont S, Dorey N, Thorndyke M (2010) What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? *Estuarine, Coastal and Shellfish Science*, **89**, 182–185.

Egilsdottir H, Spicer JI, Rundle SD (2009) The effect of CO_2 acidified sea water and reduced salinity on aspects of the embryonic development of the amphipod *Echinogammarus marinus* (Leach). *Marine Pollution Bulletin*, **56**, 1187–1191.

Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2009) Future high CO_2 in the intertidal may compromise adult barnacle *Semibalanus balanoides* survival and embryonic development rate. *Marine Ecology Progress Series*, **389**, 193–202.

Gazeau F, Gattuso J-P, Dawber C *et al*. (2010) Effect of ocean acidification on the early life stages of the blue mussel (*Mytilus edulis*). *Biogeosciences Discussions*, **7**, 2927–2947.

Hendriks IE, Duarte CM, Álvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: a meta-analysis. *Estuarine, Coastal and Shellfish Sciences*, **86**, 157–164.

Hintz JL, Lawrence JM (1994) Acclimation of gametes to reduced salinity prior to spawning in *Luidia clathrata* (Echinodermata: Asteroidea). *Marine Biology*, **120**, 442–446.

IPCC (2007) *Climate Change 2007: The Physical Science Basis. Contribution of Working Group 1 to the Fourth Assessment Report of the Intergovernmental Panel on Climate*

Change (IPCC). (eds Solomon S *et al*.). Cambridge University Press, Cambridge, UK and New York, USA.

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters*, **13**, 1419–1434.

Kurihara H, Shimode S, Shirayama Y (2004) Sub-lethal effects of elevated concentration of CO_2 on planktonic copepods and sea urchins. *Journal of Oceanography*, **60**, 743–750.

Kurihara H, Asai T, Kato S, Ishimatsu A (2008a) Effects of elevated pCO_2 on early development in the mussel *Mytilus galloprovincialis*. *Aquatic Biology*, **4**, 225–233.

Kurihara H, Matsui M, Furukawa H, Hayashi M, Ishimatsu A (2008b) Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. *Journal of Experimental Marine Biology and Ecology*, **367**, 41–46.

Lannan JE (1980) Broodstock management of *Crassostrea gigas*: I. Genetic variation in survival in the larval rearing system. *Aquaculture*, **21**, 323–336.

Lannig G, Eilers S, Pörtner H-O, Sokolova IM, Bock C (2010) Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas* – changes in metabolic pathways and thermal response. *Marine Drugs*, **8**, 2318–2339, doi: 10.3390/md8082318.

Lewis E, Wallace DWR (1998) *Program Developed for CO_2 System Calculations*. ORNL/CDIAC-105. Carbon dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

Marshall DJ (2008) Transgenerational plasticity in the sea: context-dependent maternal effects across the life history. *Ecology*, **89**, 418–427.

Marshall DJ, Allen RM, Crean AJ (2008) The ecological and evolutionary importance of maternal effects in the sea. In: *Oceanography and Marine Biology: An Annual Review*, Vol 46 (eds Gibson RN, Atkinson RJA, Gordon JDM), pp. 203–250. CRC, Boca Raton, FL.

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea. *Ecology Letters*, **13**, 128–140.

Matias D, Joaquim S, Leitão A, Massapina C (2009) Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *Ruditapes decussates*. *Aquaculture International*, **17**, 257–271.

McCormick MI, Gagliano M (2008) Carry-over effects – the importance of a good start. *Proceedings of the 11th International Coral Reef Symposium*, Ft. Lauderdale, Florida, 7–11 July session number 10, pp. 305–310.

Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RN (1973) Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, **18**, 897–907.

Moran AI, McAlister JS (2009) Egg size as a life history character of marine invertebrates: is it all it's cracked up to be? *Biological Bulletin*, **216**, 226–242.

Munday PL, Jones GP, Pratchett MS, Williams AJ (2008) Climate change and the future for coral reef fishes. *Fish and Fisheries*, **9**, 261–285.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences*, **106**, 1848–1852.

Muranaka MS, Lannan JE (1984) Broodstock management of *Crassostrea gigas*: environmental influences on broodstock conditioning. *Aquaculture*, **39**, 217–228.

Nell JA, Smith IR, McPhee CC (2000) The Sydney rock oyster *Saccostrea glomerata* (Gould 1850) breeding programme: progress and goals. *Aquaculture Research*, **31**, 45–49.

O'Connor WA, Dove MC, Finn B, O'Connor SJ (2008) Manual for hatchery production of Sydney rock oysters (*Saccostrea glomerata*). Final report to Fisheries Research and Development Corporation, Deakin, ACT, Australia. New South Wales Department of Primary Industries – Fisheries Research Report Series, **20**, 55 pp.

Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould 1850). *Global Change Biology*, **15**, 2123–2136.

Parker LM, Ross PM, O'Connor WA (2010) Comparing the effect of elevated pCO_2 and temperature on the reproduction and early development of two species of oysters. *Marine Biology*, **157**, 2435–2452.

Parker LM, Ross PM, O'Connor WA (2011) Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. *Marine Biology*, **158**, 689–697.

Podolsky RD, Moran AL (2006) Integrating function across marine life cycles. *Integrative and Comparative Biology*, **46**, 577–586.

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology Progress Series*, **373**, 203–217.

Pörtner HO, Farrell AP (2008) Physiology and climate change. *Science*, **322**, 690–692.

Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and Earth history. *Journal of Oceanography*, **60**, 705–718.

Przeslawski R, Webb A (2009) Natural variation in larval size and developmental rate of the northern quahog *Mercenaria mercenaria* and associated effects on larval and juvenile fitness. *Journal of Shellfish Research*, **28**, 505–510.

Ross PM (2001) Larval supply, settlement and survival of barnacles in a temperate mangrove forest. *Marine Ecology Progress Series*, **215**, 237–249.

Ross PM, Parker LM, O'Connor WA (in press) The impact of ocean acidification on reproduction, early development and settlement of marine organisms. *Australian Zoologist*.

Royal Society (2005) *Ocean Acidification Due to Increasing Atmospheric Carbon Dioxide*, pp. 1–58. Policy Document 12/05. The Royal Society, London.

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. *Annual Review of Marine Science*, **3**, 509–535.

Sheppard Brennand HS, Soars N, Dworjanyn SA, Davis AR, 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.

Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research*, 3rd edn. WH Freeman and Company, New York.

Talmage SC, Gobler CJ (2010) Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish. *Proceedings of the National Academy of Sciences*, **107**, 17246–17251.

Thorson G (1950) Reproduction and larval ecology of marine bottom invertebrates. *Biological Reviews*, **25**, 1–45.

Underwood AJ (1997) *Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, Cambridge.

Untersee S, Pechenik JA (2007) Local adaptation and maternal effects in two species of marine gastropod (genus *Crepidula*) that differ in dispersal potential. *Marine Ecology Progress Series*, **347**, 79–85.

Waldbusser GG, Bergschneider H, Green MA (2010) Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp. *Marine Ecology Progress Series*, **417**, 171–182.

Walther K, Anger K, Pörtner HO (2010) Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). *Marine Ecology Progress Series*, **417**, 159–170.

White I (2002) Safeguarding environmental conditions for oyster cultivation in New South Wales. Report (Number 010801) for the NSW Healthy Rivers Commission. 83 pp.