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Adaptive capacity of the sea urchin *Heliocidaris erythrogramma* to ocean change stressors: responses from gamete performance to the juvenile

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ABSTRACT: To predict impacts of ocean acidification and warming on the responses of marine populations, it is important to determine an organism's capacity for phenotypic plasticity and genetic adaptation. We determined the effects of near-future acidification and warming across the life cycle of *Heliocidaris erythrogramma* from fertilisation to metamorphosis in the progeny of 16 sire–dam crosses. Sources of variation in tolerance to warming (+3°C) and acidification (–0.3 to –0.5 pH units) were investigated for fertilisation, larvae and juveniles. Across all life stages, maternal legacy was important, with dam identity significantly interacting with stressors. Across the genotypes tested, fertilisation was negatively affected by increased temperature, but not low pH. Larval development was compromised by low pH, but not increased temperature. By the juvenile stage, no impact of warming or acidification was evident, likely due to selective mortality of sensitive individuals, indicating the presence of a subset of resilient progeny. Across all treatments, the juveniles exhibited a similar ability to calcify. The impact of treatments on development was influenced by parental identity, with the offspring of some sire–dam pairs more sensitive than others. That the progeny of some sire-dam pairs showed high stress tolerance indicates the potential for selection of resistant genotypes and adaptation that could facilitate the persistence of *H. erythrogramma* populations. Performance of progeny was not consistent across development, with the impact of stressors differing depending on developmental stage. This shows the importance of assessing climatic change across multiple stages in the life cycle.

KEY WORDS: Sea urchin · Climate change · Quantitative genetics · Ocean acidification · Maternal effects · Adaptation

INTRODUCTION

The world's oceans are changing, due to anthropogenic gas emissions, creating an imperative to assess the potential impacts of climate change on marine populations (Byrne 2012, Bernhardt & Leslie 2013). In response to environmental change, animals can shift their distribution, adjust their phenotype, or genetically adapt (Gienapp et al. 2008, Hoffmann & Sgrò 2011). While shifts in distribution as species track favourable environmental conditions are now a global phenomenon (Burrows et al. 2014, Garcia Molinos et al. 2016, Sunday et al. 2015), this phenotypic response is not an option for all species, such as those that have restricted dispersal, physical barriers and habitat fragmentation (Kinlan & Gaines 2003,

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Hansen et al. 2012). For these species, acclimatisation to changing conditions and genetic adaptation will be essential for survival (Gienapp et al. 2008, Visser 2008). Acclimatisation allows species to adjust to a new or changing environment in their lifetime, with potential for these effects to be passed on to offspring and across generations (Ghalambor et al. 2007, Whitman & Agrawal 2009, Chown et al. 2010).

The ability to genetically adapt to a changing marine environment depends on the existence of additive genetic variance within the population (Billington & Pelham 1991). Existing genetic variation can provide a reservoir of resilience to stressors (Anttila et al. 2013, Kelly et al. 2013, Pespeni et al. 2013), especially if the variation is present for the trait of interest. Within a population, the presence of genetic variance influences the adaptive response of life-history traits, such as fertilisation and larval success, to increased temperature and acidification (e.g. sea urchins and mussels; Sunday et al. 2011, Foo et al. 2012, 2014).

The environment the species experiences can influence the presence and pattern of genetic variation across the range that the species inhabits. Variable conditions experienced among populations across the species range have been shown to result in the presence of locally adapted genotypes (Sanford & Kelly 2011). Thus, species with a broad latitudinal distribution across a range of thermal or pH environments are likely to have populations with an in-built capacity to persist in changing oceans (Bradshaw & Holzapfel 2001).

For free-spawning marine invertebrates, the gametes of the male and female parents can be isolated for experimental matings, making it possible to compare the performance of offspring genotypes in different environments. The North Carolina II quantitative breeding design, where sires and dams are crossed in all combinations, allows variance in traits within a population to be partitioned into additive, maternal, interactive and environmental components, with the potential for genetic adaptation strongly related to the levels of additive genetic variation (Lynch & Walsh 1998). The opportunity to control matings provides a model system to investigate selection in different environments from fertilisation in offspring generated under experimental conditions (Foo et al. 2012, 2014).

Studies have used this breeding design to investigate the responses of the offspring of sea urchins, mussels and macroalgae to climate change stressors and have found significant levels of variation in stress tolerance among genotypes, indicating the potential for adaptation to those stressors (Sunday et al. 2011, Foo et al. 2012, Chirgwin et al. 2015, Clark et al. 2013, Kelly et al. 2013, Lymbery & Evans 2013, Foo et al. 2014). These studies largely involve a single stressor (temperature: Clark et al. 2013, Lymbery & Evans 2013, Chirgwin et al. 2015; acidification: Sunday et al. 2011, Kelly et al. 2013), with 2 studies investigating the response to both stressors in combination (Foo et al. 2012, 2014).

We used the short development time of the sea urchin *Heliocidaris erythrogramma*, with access to the juvenile stage in 3–5 d, as a model system to assess the response of genotypes to both increased temperature and acidification across the life cycle using the North Carolina II design. *H. erythrogramma* is a widely distributed and ecologically important sea urchin endemic to southern Australia (Keesing 2013). Previous studies have investigated the outcome of sire–dam crosses of the species using a single stressor or considering performance at a single life-history stage only. There is evidence for significant additive genetic variance in embryos at metamorphosis (Evans et al. 2007). For embryos fertilised in control conditions and then transferred to increased temperature treatments (+3°C), significant sire × temperature interactions and pair × temperature effects were evident at hatching (Lymbery & Evans 2013). In sire–dam crosses, fertilisation success was reduced (Havenhand et al. 2008) or variable, with some pairs performing better than others (Schlegel et al. 2012) in low pH (–0.4 pH units) conditions. Studies using gametes from multiple parents found that fertilisation in *H. erythrogramma* is resilient to increased acidification and temperature (–0.3 to –0.5 pH units, +4°C) (Byrne et al. 2009, 2010a), with larvae and juveniles sensitive to greater increases in temperature (+4–6°C) but tolerant to 2°C warming (Byrne et al. 2009, 2011). In multistressor studies, juveniles survived temperature increases of +4°C coupled with decreases in pH up to 0.7 units; however, the number of abnormal juveniles increased in response to combined effects of increased temperature (+2–4°C) and reduced pH (–0.3 to –0.5 pH units) (Wolfe et al. 2013).

In this study, the performance of 16 sire–dam crosses of *H. erythrogramma* was followed from gamete performance to the settled juvenile to identify the sources of variation in tolerance to warming (+3°C) and acidification (–0.3 to –0.5 pH units) for fertilisation, larval success and juvenile metamorphosis. We assessed carry-over effects across the life cycle to identify how ocean change stressors during one life stage can influence further development, as

shown for oysters (Hettinger et al. 2012). As calcification in *H. erythrogramma* (Wolfe et al. 2013) and other marine calcifiers is negatively affected by ocean acidification (Byrne & Przeslawski 2013, Kroeker et al. 2013, Przeslawski et al. 2015), we also tested for variation in spine number among genotypes. We took the approach of initiating the stressor treatments with gametes prior to fertilisation, as these cells are known to be highly sensitive to stressors (e.g. Reuter et al. 2011, Schlegel et al. 2012). In contrast to fertilisation in control conditions and subsequent transfer of zygotes to stress treatments, this approach provides a more realistic assessment of stressor responses for free-spawning marine invertebrates in the carry-over effects from the parental (sperm, eggs) to the zygotic genotype. For *H. erythrogramma*, it was predicted that adaptive capacity is likely to stem from existing genetic variation maintained through balancing selection across a large spatial environmental mosaic along the coast of Australia (Keesing 2013), as shown for *Strongylocentrotus purpuratus* (Pespeni et al. 2013).

MATERIALS AND METHODS

Study species, spawning and fertilisation

Heliocidaris erythrogramma was collected (3–5 m depth) near Coffs Harbour, New South Wales (30°15′S, 153°08′E) in April and transferred to large flow-through aquaria (3500 l) shortly after collection. Spawning was induced by injection of 2 ml of 0.5 M KCl. Eggs from each female were placed in separate beakers of fresh, filtered seawater (FSW; 1 µm), and sperm from each male was stored dry at 4°C until use. Egg density was determined in counts of 100 µl aliquots from the egg suspension. Approximately 1000 eggs were placed in rearing containers — 100 ml plastic jars, with mesh sides to allow water to flow through. Positioning of the meshed windows ensured at least 40 ml of water remained in each container at any time as it was constantly renewed. The eggs were supplied with flowing experimental filtered sea water (FSW), with randomly assigned temperature/pH conditions for approximately 20 min before sperm were introduced. Haemocytometer counts of semen samples were used to determine the amount of sperm required to achieve a consistent sperm concentration. Just prior to fertilisation, 1 µl of the semen sample was activated in 1 ml of experimental FSW. The amount of diluted sperm to add into each rearing container to achieve a sperm to egg ratio of 200:1 was determined from the original sperm count. Before addition of sperm, the flow-through system was turned off to allow fertilisation and turned back on after 10 min to remove excess sperm.

Manipulation of temperature and pH

The experiments were conducted in a flow-through water system with a flow rate 7.8 ml min^{-1}, ambient pH of 8.18, temperature of 23.7°C, salinity of 33.6 psu and dissolved oxygen at >90% saturation. Experimental treatments were based on model projections for near-future (2100) surface ocean waters in the Southeast Australia region (IPCC 2013, CSIRO & BOM 2015). The treatments consisted of 2 target temperatures (control 24°C, 27°C) and 3 target pH_{NIST} levels (control 8.1, 7.8, 7.6 pH units), in all combinations (Table S1 in the Supplement at www.int-res.com/articles/suppl/m556p161_supp.pdf). The experiments were conducted in UV-sterilised and filtered (1 mm) FSW that was supplied to three 60 l header tanks. The experimental pH was regulated by injection of pure CO_2 into 2 of these tanks using an automatic CO_2 injection system with 2 pH controllers (Tunze), set at pH 7.6 and pH 7.8. The CO_2 was mixed in these tanks using a vortex mixer (Red Sea). A third header tank was allowed to track ambient pH. All header tanks (control and experimental treatment water) were continuously bubbled with air to promote mixing and to maintain dissolved oxygen at >90%. A constant volume was maintained in the header tanks using a float valve. Water from the header tanks was fed into sub-header tanks (20 l), where it was warmed to the required temperature (+3°C) using an aquarium heater (200 W, Jager) or unmanipulated for the ambient control. Temperature was automatically regulated using temperature sensors in the rearing containers and a temperature controller (Tunze) connected to the heaters. Water from each sub-header tank was continually circulated using 20 W pumps to maintain even temperatures within each treatment. Water was delivered separately into each individual rearing container using irrigation dripper valves.

Temperature, pH and salinity were measured daily in all treatments across random containers (n = 33 per treatment) using a Hach Hqd Portable Multiprobe. The probe was calibrated frequently using NIST buffers pH 4.0, 7.0 and 10.0 (Oakton) with pH on the total scale determined through calibration with TRIS buffers. Temperature was also monitored with this meter. As indicated in Table S1 in the Supplement,

mean (±SE) pH_T in the 3 treatments was 8.10 ± 0.01 (control), 7.80 ± 0.00 and 7.63 ± 0.00, and mean (±SE) temperatures were 23.66 ± 0.08°C (control) and 26.48 ± 0.08°C. Water samples (250 ml) were collected at the beginning and end of the experiment for each treatment, filtered through a 0.45 mm syringe filter, and fixed with 150 µl of saturated HgCl. These water samples (n = 12) were then used to determine total alkalinity by potentiometric titration, using an automatic titrator (Metrohm 888 Titrando) and calibrated against certified reference standards (Dickson et al. 2007). Experimental pCO_2 (Table S1 in the Supplement) was determined from Total Alkalinity (TA), temperature, pH_{NIST} and salinity data using CO2SYS (Pierrot et al. 2006) and the dissociation constants of Mehrbach et al. (1973) as refitted by Dickson & Millero (1987).

The North Carolina II breeding design

Single sire–dam crosses were completed in 2 experimental runs (blocks), with each block using gametes from 4 sires and 2 dams crossed in all combinations. Each block thus resulted in 8 full-sib families (total of 16 families). Each family was exposed to each of 6 treatments. Thus, each block had a total of 144 containers (2 females × 4 males × 3 pH levels × 2 temperature × 3 replicates).

At the control temperature (24°C), *H. erythrogramma* develops to the juvenile stage within 4 d. At 2 h post-fertilisation (hpf), 24 hpf and 96 hpf, a random sample of approximately 50 embryos was pipetted from the containers, placed into tubes, and fixed with 2% formalin in FSW. The first 30–50 embryos randomly selected from each tube were examined microscopically (Leica) and scored for successful development. At 2 hpf, the percentage of fertilised embryos was determined through counts of unfertilised, fertilised and cleavage-stage embryos. At 24 hpf, the percentage of larvae was calculated from counts of normal/abnormal and arrested embryos. At 72 hpf, coralline algae (*Amphiroa anceps*) were added to each container to induce the larvae to settle. At 96 hpf, the percentage of metamorphosed larvae was calculated from counts of normal/abnormal juveniles and arrested embryos. The number of embryos arrested at fertilisation (e.g. fertilisation envelope only) was low (<1%); thus, polyspermy was minimal.

Photographs of juveniles from each replicate across all genotypes and treatments were taken, and the number of spines was counted for at least 10 individuals per replicate.

Statistical analyses

Data on development for each time point and spines were analysed using analysis of variance (ANOVA) conducted in the PERMANOVA routine of Primer V6, with temperature and pH as fixed factors, experimental block as a random factor and sire and dam as random factors nested within blocks. Since some significance tests involved quasi-*F*-ratios (in which significance tests derived from the *F*-distribution are unreliable [Quinn & Keough 2002]), we calculated significance of the *F*-statistics using 9999 permutations of the raw data for all factors (Anderson et al. 2008).

Reaction norms (interaction plots; see Quinn & Keough 2002) were plotted to visualize the interactions between sire and dam genotypes across a range of environments (Lynch & Walsh 1998). For the developmental stages of larvae and metamorphosed larvae — the stage by which the zygotic genome is fully switched on (Howard-Ashby et al. 2006, Tadros & Lipshitz 2009) — genetic correlations of performance (i.e.percent of larvae and percent of metamorphosed larvae) across temperature and pH environments were used to quantify the genotype × environment interaction using variance components derived from restricted error maximum likelihood (REML) estimates calculated in the R package lme4 (available at http://cran.r-project.org/web/packages/lme4/index.html). Variance components for the random factors were calculated in a single analysis with all factors (temperature, pH, block, sires, dams). Genetic correlations were calculated using the causal variance components associated with the sire effects (additive genetic [V_A]) and the interaction effects between sires and each of the environmental factors of temperature (V_{AT}), pH ($V_{A\,pH}$) and both temperature and pH ($V_{AT\,pH}$). Genetic correlations for the same trait averaged over both types of environments (r^*_G), the genetic correlation for the same trait within 1 environmental class (i.e. temperature; $r^*_{G(T)}$) and the genetic correlation within the other environmental class (i.e. pH; $r^*_{G(pH)}$) were calculated using equations from Eisen & Saxton (1983):

$$r^*_G = V_A/(V_A + V_{AT} + V_{A\,pH} + V_{AT\,pH})$$
$$r^*_{G(T)} = (V_A + V_{AT})/(V_A + V_{AT} + V_{A\,pH} + V_{AT\,pH})$$
$$r^*_{G(pH)} = (V_A + V_{A\,pH})/(V_A + V_{AT} + V_{A\,pH} + V_{AT\,pH})$$

Coefficients of variation (CV) were calculated for each treatment across all developmental stages to determine whether stressful treatments can in-

crease the variability in the response of embryos and juveniles. The CV is defined as the standard deviation divided by the mean, and multiplied by 100 to be given as a percentage. It expresses the relative variability of a measurement and is less likely to increase as an artefact of increases in the mean (Quinn & Keough 2002).

Linear regression analyses were performed in Microsoft Excel (2013) to assess the relationship between the performance of individual sire–dam pairs across different life-history stages — fertilisation and larvae and larvae and juveniles. Sire–dam pairs were used for these analyses due to significant interactions among males, females and stressors (see 'Results').

RESULTS

Fertilisation

Increased temperature significantly reduced the percentage of fertilisation, with no significant effect of decreased pH (Table S2 in the Supplement). There was a significant effect of sire identity on the percentage of fertilisation contributing the highest percentage of variation (15.47%). There was a significant interaction between dam identity and temperature (Table S2 in the Supplement), where reaction norms of maternal half-siblings showed that the effect of increased temperature on the percentage of fertilisation differed between the female parents (Fig. 1).

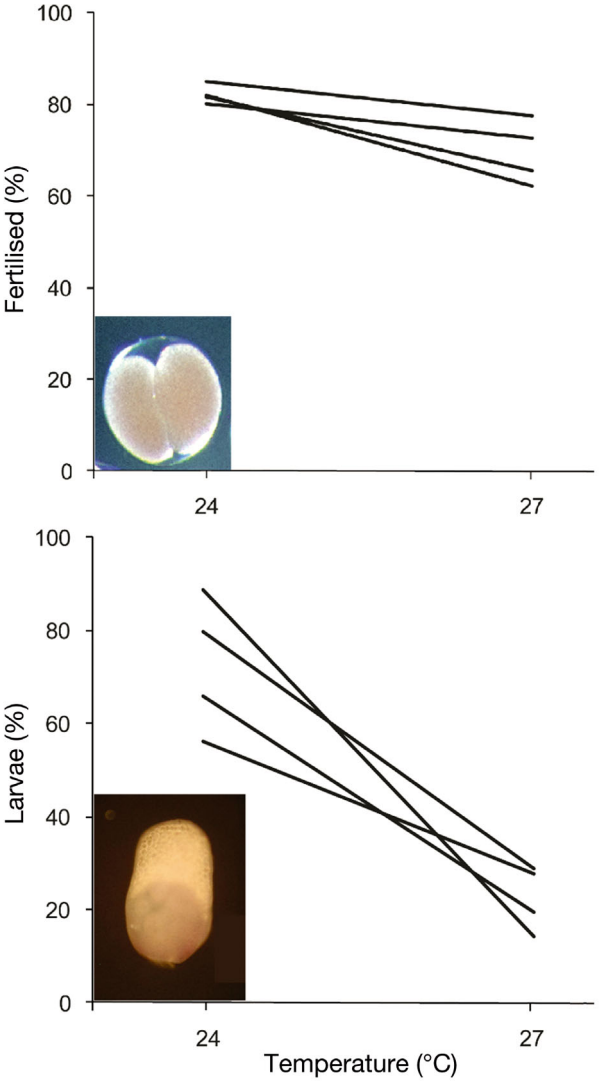

Fig. 1. Reaction norms showing the percentage of fertilised embryos (upper panel) and normal larvae (24 hpf) (lower panel) of *Heliocidaris erythrogramma* maternal half siblings in response to increased temperature pooled for pH. Lines represent the mean percentages for maternal half siblings (n = 4); hpf: hours post-fertilisation

Larvae

Decreased pH, but not increased temperature, significantly reduced the percentage of normal larvae (Table S2 in the Supplement). There was a significant dam × temperature interaction, indicating that dam identity was an important source of variation in determining the percentage of normal larvae in increased temperature, as shown in the reaction norms (Fig. 1). In addition, the significant interactions between sire × dam and sire × dam × pH in the percentage of normal larvae indicate the importance of the parental pair to the developmental success of their progeny, with the latter contributing 9.52% to the variance. The different responses of the offspring of the 16 pairs to decreased pH are shown in the reaction norms (Table S2 in the Supplement; Fig. 2).

The percentage of metamorphosed larvae

On Day 4, when the larvae had settled and metamorphosed, there were no significant effects of increased temperature or decreased pH on the survivors that settled (Table S2 in the Supplement). There was a significant interaction between sire × dam × pH, indicating that success to the settled juvenile stage was significantly affected by the sire–dam pair, as shown in the reaction norms (Fig. 2). There was also a significant interaction between dam × pH × temperature (10.18% of the variance), where in-

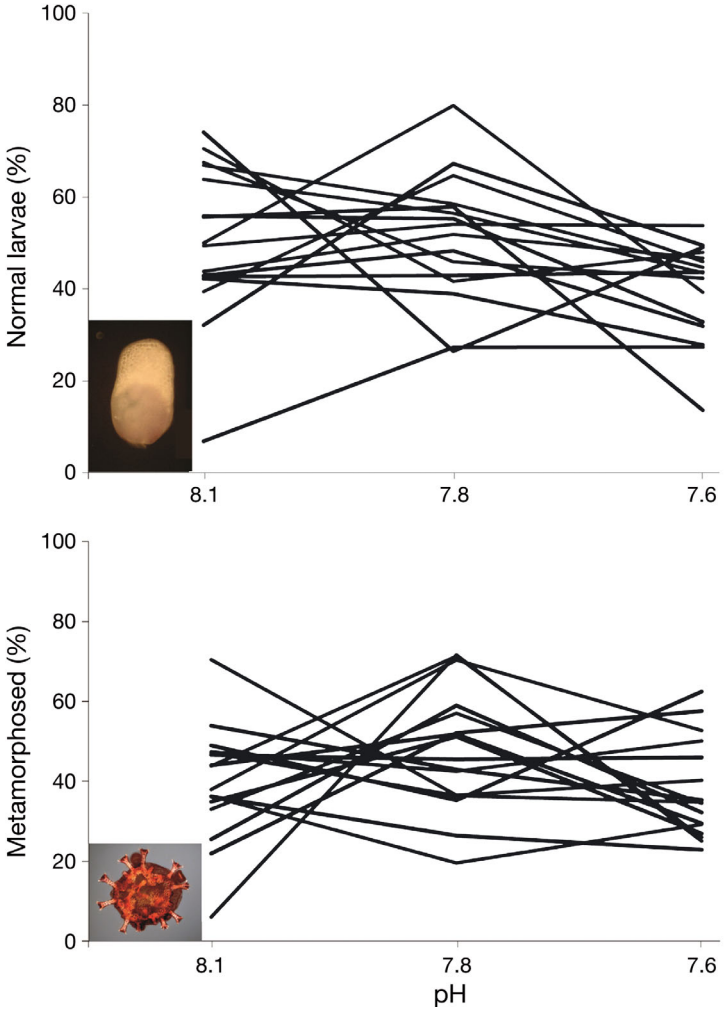

Fig. 2. Reaction norms show the percentage of normal larvae (24 hpf) (upper panel) and metamorphosed juveniles (96 hpf) (lower panel) across *Heliocidaris erythrogramma* offspring of the 16 sire–dam pairs in response to experimental pH levels pooled for temperature. Lines represent the mean percentages for full siblings (n = 16); hpf: hours post-fertilisation

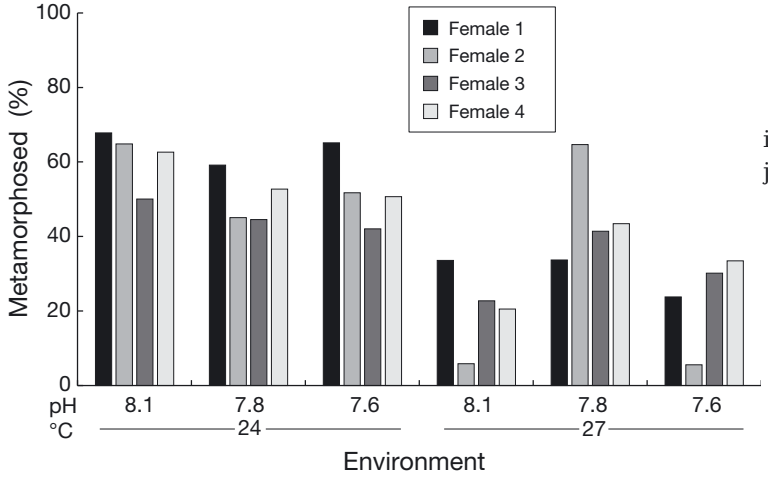

Fig. 3. Histogram displaying the percentage of metamorphosed juveniles for each *Heliocidaris erythrogramma* female across the 6 treatments. The significant interaction between female × pH × temperature shows that settlement was influenced by increased temperature and decreased pH; however, this varied with maternal identity

creased temperature greatly reduced the percentage metamorphosed, with responses dependent on pH level and maternal identity (Fig. 3).

Genetic correlations

The genetic correlation (r^*_G) in the larval trait across the temperature/pH environments based on paternal half siblings was 0. This indicates that genotypes that performed well in a particular combination of temperature and pH did not necessarily perform similarly in other temperature/pH combinations. There were also genetic correlations of 0 across the 2 temperature levels and across the 3 pH levels. Furthermore, genetic correlations for metamorphosed juveniles were all 0. Thus, half siblings that performed well at control temperatures were not necessarily those that performed the best at high temperatures and likewise for pH.

Coefficients of variation

At control temperature, there was only a slight increase in variation from fertilisation to the juvenile stage across all pH levels. Increased temperature greatly increased the variation across all developmental stages. Furthermore, at increased temperature there appears to be a synergistic effect with decreased pH, where pH 7.6 increased the variation seen across developmental stages in comparison with the control pH of 8.1 (Fig. 4).

Spine production

There was a significant effect of maternal identity on the number of spines produced per juvenile. There were also significant effects of

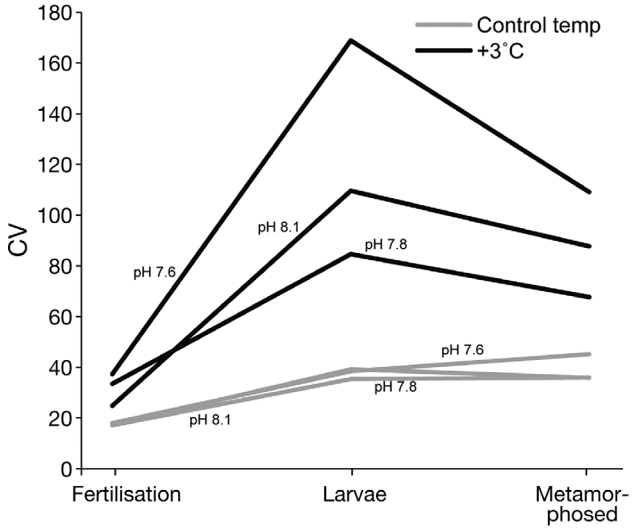

Fig. 4. Effects of increased temperature and decreased pH on the coefficients of variation (CV) of developmental success across fertilisation, larvae and juveniles of *Heliocidaris erythrogramma*

sire × dam, sire × dam × temperature, sire × dam × pH and sire × dam × pH × temperature. This indicates the strong influence of the parental pair on spine number in response to stressors, as shown in the reaction norms (Fig. 5; Table S3 in the Supplement).

Performance across life history stages

In none of the 6 combinations of temperature and pH were there significant relationships between fertilisation success and the percentage of normal larvae (Fig. 6). Genotypes that had a high fertilisation success did not subsequently have the highest percentage of normal larvae. However, the relationships between percentage of normal larvae and subsequent metamorphosis did show that pairs in certain environments performed consistently (Fig. 7). Genotypes that had a high percentage of normal larvae in the control pH/27°C and pH 7.6/27°C environments also had the highest percentage of metamorphosed larvae. Thus, performance of specific genotypes at fertilisation did not predict performance of that genotype at the larval stage; however, performance at the larval stage did predict metamorphosis in 2 environments. There was no correlation found for the pH 7.8/27°C environment. With the removal of 2 outliers, a positive correlation became evident, but this was not followed up for the analysis.

DISCUSSION

Using the short development time of *Heliocidaris erythrogramma* as a model system to investigate effects from gametes to the juvenile stage, we found that stress experienced during early development had potential carry-over effects on metamorphosis. Negative effects of decreased pH and temperature found during fertilisation and larval stages were not evident during settlement, demonstrating that influences of stressors can change across life-history transitions. For *H. erythrogramma*, plasticity is likely to stem from existing genetic variation maintained through balancing selection across a large spatial environmental mosaic along the coast of Australia (Keesing 2013), as shown for *Strongylocentrotus purpuratus* (Pespeni et al. 2013). Species like *H. erythrogramma* that have

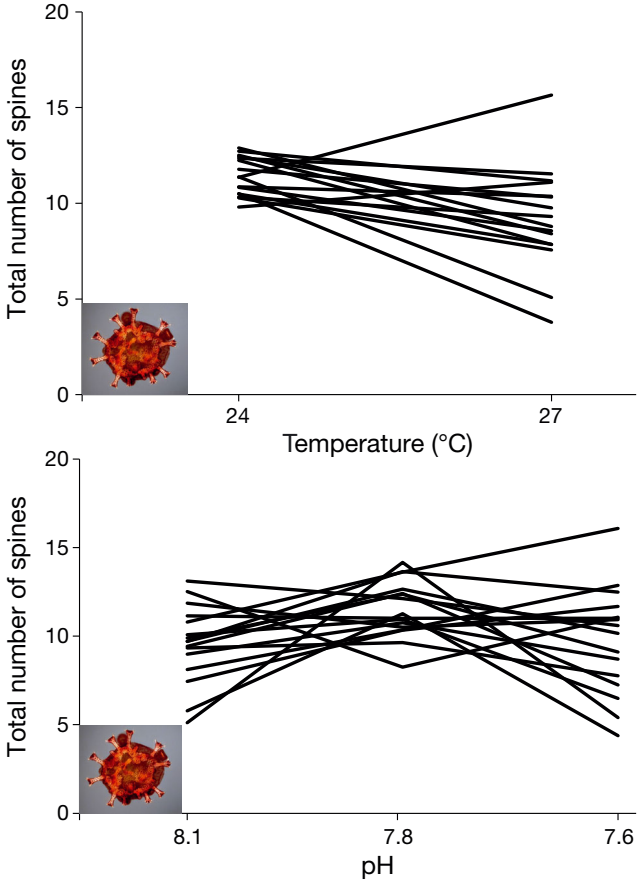

Fig. 5. Reaction norms showing total number of spines present on *Heliocidaris erythrogramma* juveniles (96 hpf) across offspring of the 16 sire–dam pairs in response to experimental temperatures pooled for pH (upper panel) and in experimental pH levels pooled for temperature (lower panel). Lines represent the mean numbers of spines for full siblings (n = 16); hpf: hours post-fertilisation

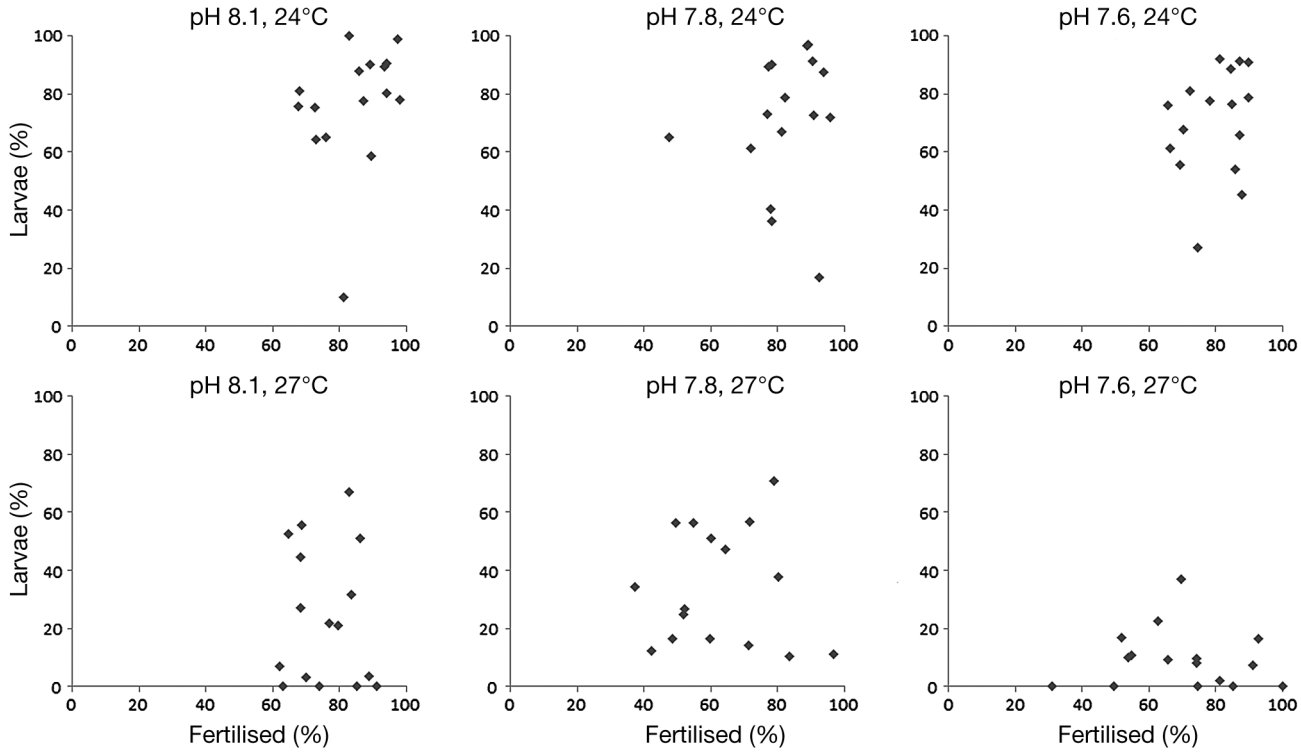

Fig. 6. Scatter plots of the relationship between pair performance at fertilisation (*x*-axis) and at the larval stage (*y*-axis) of *Heliocidaris erythrogramma*. Each point represents the mean performance of an individual pair in each treatment across both stages. No relationships were evident for any of the 6 treatments

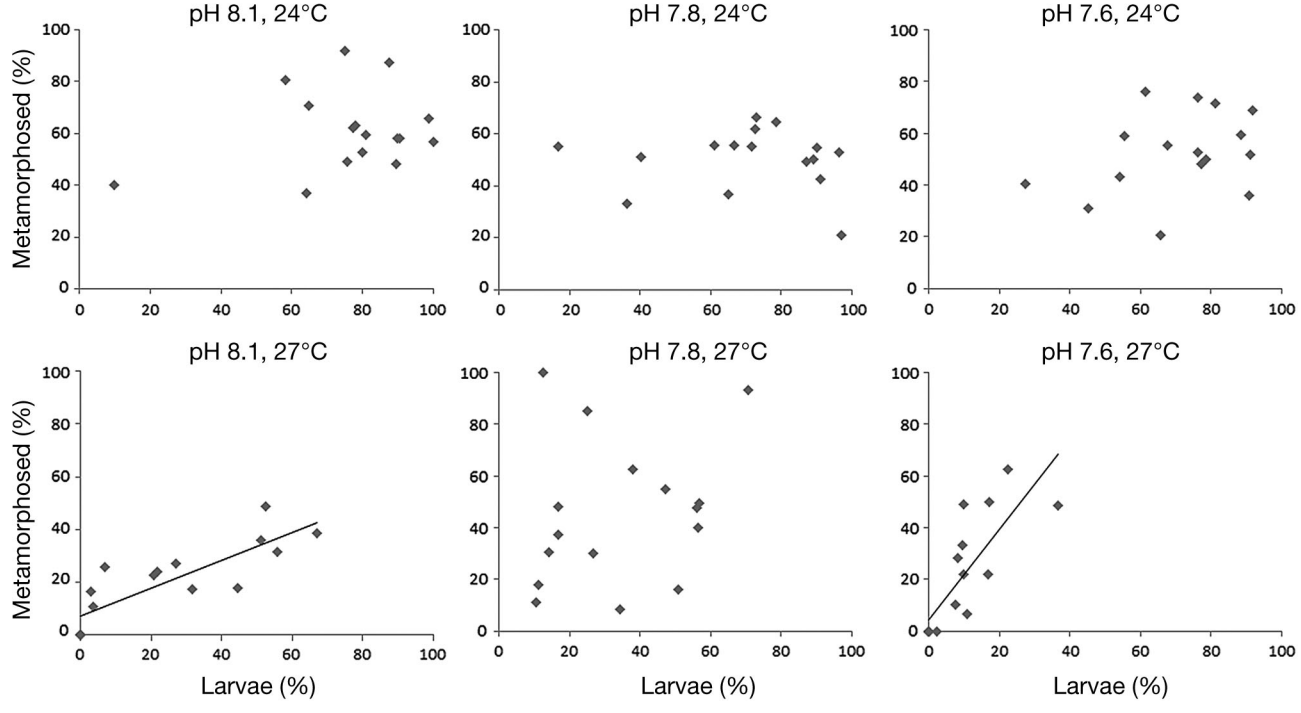

Fig. 7. Scatter plots of the relationship between pair performance at the larval stage (*x*-axis) and as metamorphosed larvae (*y*-axis) of *Heliocidaris erythrogramma*. Each point represents the mean performance of an individual pair in each treatment across both stages. A positive relationship was evident for the pH 8.1/27°C ($R^2 = 0.69$, $p < 0.001$) and pH 7.6/27°C treatments ($R^2 = 0.64$, $p < 0.001$)

broad latitudinal ranges are likely to have substantial adaptive potential in a changing ocean.

At fertilisation, the significant contributions of sire effects were expected because fertilisation success in *H. erythrogramma* and other sea urchins is significantly influenced by sperm traits such as motility, velocity and viability (Gage et al. 2004, Evans & Marshall 2005, García-Gonzalez & Simmons 2005, Evans et al. 2007). The differences in the effects of each stressor varied significantly among females, which remained true throughout the development of *H. erythrogramma*. The significant dam × temperature interaction at fertilisation indicated that eggs of different females were differently affected by warming, whereas the eggs of some females were less affected by +3°C with respect to fertilisation success.

The strong maternal effects found at fertilisation were anticipated due to known variability in egg size, quality and maturity. These attributes have been shown to be important sources of variation in *H. erythrogramma* and other sea urchins (Styan 1998, Marshall et al. 2004, Levitan 2006).

The maternal legacy that continues through the larval stage and metamorphosis may be due to the presence of maternal protective factors (e.g. stress proteins) loaded into sea urchin eggs during oogenesis (Hamdoun & Epel 2007). In addition, *H. erythrogramma* produces a large egg that provides all the nutrition needed to support development to metamorphosis, and eggs are also supplied with maternal transcripts to facilitate rapid development (Raff & Byrne 2006). Over evolutionary history, lecithotrophic larvae have been shown to be more resilient than planktotrophic larvae to extinction driven by climate change (Uthicke et al. 2009). Significant maternal provisioning in marine invertebrates like *H. erythrogramma* with non-feeding larvae may provide a strong buffer against stressors influencing this species' capacity to adapt (Hamdoun & Epel 2007, Byrne 2011).

The interaction of sire and dam with stressors becomes apparent after fertilisation and is influenced by gamete compatibility. At the larval and juvenile stages, the progeny of certain pairs were less sensitive to warming and acidification, with the ability to calcify as normal. Pairs that perform better are likely to be selected for in changing-ocean conditions and seed future populations (Hoffmann &Parsons 1991, Gassmann et al. 2009, Ghalambor et al. 2015). That the progeny of some sire–dam pairs showed high stress tolerance indicates that the survival of resistant genotypes could facilitate the persistence of *H. erythrogramma* populations under stressful conditions.

The ranking of pair performance across life-history stages did not show consistent performance between the fertilisation and larval stages. However, when comparing pairs from larvae to metamorphosis, the pairs that performed the best at the larval stage performed the best at metamorphosis in 2 of the high-temperature treatments. This suggests that increased temperature may impose selection on specific genotypes, possibly revealing pairs that are likely to be selected for under future ocean warming (Ghalambor et al. 2015).

The lack of an overall effect of pH or temperature on spine number across all genotypes tested is similar to that found in other studies of *H. erythrogramma* (Byrne et al. 2009, 2011, Wolfe et al. 2013), where juveniles exhibited a similar ability to calcify across all treatments. The resilience of the juvenile stage is likely due to selective mortality of sensitive individuals at the larval stage. Due to flow-through conditions, dead offspring would have been washed from the system. To discern how differential mortality could have affected the outcome, we would have had to track a known population of individual *H. erythrogramma* as in Byrne et al. (2010b) where increased temperature caused ~70% mortality in the larvae. That a subset of resilient progeny became juveniles and were able to calcify as normal shows the potential for persistence of this species under stressful conditions. However, survivorship data are needed to more fully understand the influence of sensitive and resistant genotypes on overall adaptive capacity.

Genetic correlations were calculated to determine if performance in one stressor would influence the performance of another stressor. As genetic correlations were 0 for both the larval and metamorphosed stages, it appears that the progeny of parents that performed the best in the high-temperature environments did not necessarily perform the best in low-pH environments, indicating there is little overlap in the gene sets that influence performance in response to the 2 stressors. This is not surprising as transcriptome analysis of *H. erythrogramma* shows a marked difference in gene expression between these stages (Byrne et al. 2015). Most importantly, as the genetic correlation is not negative, evolution is not constrained in adapting to both stressors simultaneously, an important prerequisite for survival in a changing ocean. A marked increase in variation among genotypes occurs with an increase in temperature. The coefficients of variation for each trait also showed a slight increase in variation with development, well known for development in *H. erythrogramma* and other marine invertebrates (Pechenik 1987). When de-

creased pH is considered at control temperatures, the variability in the progeny's response does not change compared to the control response. However, at increased temperature, a decreased pH of 7.6 causes an even greater increase in variation. This indicates that at pH 7.6 only, the synergistic effects of increased temperature and decreased pH may make the probability of success more unpredictable as development progresses, resulting in greater selection pressure on genotypes (Hoffmann & Merilä 1999). Interestingly, a deviation from the control pH can also cause a decrease in variation, as seen for pH 7.8 at increased temperature.

The variation in the response of *H. erythrogramma* to ocean stressors seen here may be due to the presence of genetic variation across the metapopulation of this species (Sanford & Kelly 2011, Pespeni et al. 2013). Although the short planktonic stage of *H. erythrogramma* would be expected to limit genetic connectivity, this is not the case (M. Gall unpubl. data), and the species has a very extensive distribution along the coast of Australia (Byrne et al. 2010b, Keesing 2013). Thus, for this species, a short-lived larval stage does not appear to hinder its adaptive potential in a changing ocean. In fact, locally adapted populations spanning different climatic regimes could positively influence variation, enhancing adaptation to ocean change. Northern *H. erythrogramma* embryos have been shown to have a higher thermotolerance than southern populations; thus, the poleward migration of thermotolerant propagules could facilitate population persistence in a warming ocean (Byrne et al. 2010b).

Although early development is impacted by decreased pH and increased temperature, by metamorphosis individuals in the extreme treatments appear similar to those in the control in both development and number of spines. As carry-over effects are evident in the transition from larvae to juveniles (Pechenik 1987, Hettinger et al. 2012), stress experienced during fertilisation and during the larval stage could have had positive carry-over effects on metamorphosis in *H. erythrogramma*. Whether these positive effects can persist into adulthood and into the next generation is not known.

Our results indicate that the effects of environmental stressors and the contributions of sire and dam change throughout the life cycle of a sea urchin, and that carry-over effects across life-history stages could be significant in reducing the impacts of stressors. For *H. erythrogramma*, maternal effects have the strongest influence on the outcome of fertilisation and development. Sire–dam effects become apparent after fertilisation, revealing pairs that are likely to be selected for. In the face of a warming and acidifying ocean, maternal buffering and sire–dam pairs with high tolerance to stressors will allow for adaptation in this species. These are important mechanisms for persisting in an ocean decreasing in pH and increasing in temperature for species like *H. erythrogramma* which exhibit a significant maternal investment in the production of large eggs.

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