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Larvae of the coral eating crown-of-thorns starfish, *Acanthaster planci* in a warmer-high CO₂ ocean

Running head: COTS development in a warm and high CO₂ ocean

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Abstract

Outbreaks of crown-of-thorns starfish (COTS), *Acanthaster planci*, contribute to major declines of coral reef ecosystems throughout the Indo-Pacific. As the oceans warm and decrease in pH due to increased anthropogenic CO₂ production, coral reefs are also susceptible to bleaching, disease and reduced calcification. The impacts of ocean acidification and warming may be exacerbated by COTS predation, but it is not known how this major predator will fare in a changing ocean. Because larval success is a key driver of population outbreaks, we investigated the sensitivities of larval *A. planci* to increased temperature (2-4 °C above ambient) and acidification (0.3-0.5 pH units below ambient) in flow-through cross-factorial experiments (3 temperature × 3 pH/pCO₂ levels). There was no effect of increased temperature or acidification on fertilisation or very early development. Larvae reared in the optimal temperature (28 °C) were the largest across all pH treatments. Development to advanced larva was negatively affected by the high temperature treatment (30 °C) and by both experimental pH levels (pH 7.6, 7.8). Thus, planktonic life stages of *A. planci* may be negatively impacted by near future global change. Increased temperature and reduced pH had an additive negative effect on reducing larval size. The 30 °C treatment exceeded larval tolerance regardless of pH. As 30 °C sea surface temperatures may become the norm in low latitude tropical regions, poleward migration of *A. planci* may be expected as they follow optimal isotherms. In the absence of acclimation or adaptation, declines in low latitude populations may occur. Poleward migration will be facilitated by strong western boundary currents, with possible negative flow-on effects on high latitude coral reefs. The contrasting responses of the larvae of *A. planci* and those of its coral prey to ocean acidification and warming are considered in context with potential future change in tropical reef ecosystems.

Introduction

Coral reefs throughout the world are being negatively impacted by anthropogenic stressors such as pollution, overfishing, ocean warming and acidification (Wilson *et al.*, 2006; Hoegh-Guldberg *et al.*, 2007; Anthony *et al.*, 2008; Carpenter *et al.*, 2008; Hoegh-Guldberg & Bruno, 2010; Hughes *et al.*, 2010). Due to these stressors, coral cover has declined across the Indo-Pacific and the Caribbean (Gardner *et al.*, 2003; Bellwood *et al.*, 2004; Bruno & Selig, 2007). Ocean warming is a major threat to tropical marine ecosystems (Poloczanska *et al.*, 2007; Przeslawski *et al.*, 2008), where due to shallow spatial gradients, rates of warming are predicted to be greater than in higher latitudes (Burrows *et al.*, 2011). Coral community calcification has declined over the last 40 years, likely due to increased temperature and decreased ocean pH and carbonate mineral saturation (De'ath *et al.*, 2009; Cooper *et al.*, 2012; Silverman *et al.*, 2012). The degradation of coral reefs in the Indo-Pacific due to anthropogenic stressors has been exacerbated by outbreaks of the coral eating crown-of-thorns starfish (COTS), *Acanthaster planci* (Bruno & Selig, 2007; Pratchett *et al.*, 2009,2011; Osborne *et al.*, 2011; De'ath *et al.*, 2012; Baird *et al.*, 2013). While corals are expected to continue to decline under predicted ocean warming and acidification (Hoegh-Guldberg, 1999; Hoegh-Guldberg & Bruno, 2010), there is a major gap in knowledge as to how *A. planci*, the most important predator of corals, will fare under co-occurring increased ocean temperature and acidification. This knowledge gap is addressed here.

All physiological processes and organism performance in the marine environment are dependent on organism thermal tolerance levels (Pörtner, 2010). Increased temperature above tolerance levels has deleterious impacts on physiology, phenology, planktonic larval performance, physiology and biogeography of marine invertebrates (Negri *et al.*, 2007; O'Connor *et al.*, 2007; Sheppard Brennan *et al.*, 2010; Byrne *et al.*, 2011; Putnam *et al.*,

2013). Thermotolerance of planktonic life phases will strongly influence species survival and distribution in a warming ocean (Byrne *et al.*, 2011). For marine larvae, increased temperature causes faster progression through developmental stages up to thermal limits when growth is stunted and mortality occurs (O’Conner *et al.*, 2007; Nguyen *et al.*, 2012; Hardy *et al.* 2013). Many tropical species live on the edge of their thermotolerance limits (Hoegh-Guldberg *et al.*, 2007; Wernberg *et al.*, 2011,2012), with the expectation that local decline or extirpation and poleward shifts in distribution to cooler climates will occur, as seen in the range expansion of corals in Japan (Yamano *et al.*, 2011).

Ocean acidification is expected to be deleterious to marine biota by causing hypercapnic alteration of metabolism, disruption of acid-base homeostasis, energetic constraints and stress to calcification systems (Miles *et al.*, 2007; Pörtner, 2008, 2010; Stump *et al.*, 2012). Growth of marine calcifiers is predicted to be negatively affected by reduced mineral saturation that accompanies ocean acidification (Doney *et al.*, 2012) and many studies show a negative effect on growth of calcifying larvae (Parker *et al.*, 2010; Byrne, 2011; Byrne *et al.*, 2013a; Byrne & Przeslawski, 2013), although slight warming can reduce this effect (Sheppard Brennan *et al.*, 2010; Byrne *et al.*, 2013b). Reduced size and calcification of sea urchin larvae (*Tripneustes gratilla*) reared in near future levels of pH were buffered by increased temperature (+ 3 °C) up to a thermal threshold (+ 6 °C) (Sheppard Brennan *et al.*, 2010). It is suggested that non-calcifying larvae, such as *A. planci*, may have differential survival as the oceans reduce in pH, the potential “winners” in the climate change stakes (Byrne, 2011; Kroeker *et al.*, 2013).

The negative effects of contemporary and projected ocean warming have been shown to exceed those of acidification for many marine invertebrates especially with regard to survivorship of developmental stages (Byrne *et al.*, 2009; Nguyen *et al.*, 2012; Arnberg *et al.*, 2013; Chua *et al.*, 2013). Most ocean change research however has focused on acidification as

a single stressor (Hofmann *et al.*, 2010; Byrne, 2011; Wernberg *et al.*, 2012). Consideration of the combined effects of warming and acidification is essential to assess species responses to global change (Byrne, 2011; Pörtner, 2010; Wernberg *et al.*, 2012; Byrne & Prezlawski, 2013). Simultaneous warming and acidification of the oceans is expected to have deleterious effects on the sensitive planktonic life stages of benthic invertebrates with negative flow-on effects on populations and community structure (Harley *et al.*, 2006; Brierley & Kingsford, 2009; Byrne, 2011).

This study examined the effects of predicted near future ocean acidification and warming on development of *A. planci*. Outbreak populations of *A. planci* contribute to major declines in coral reefs throughout the Indo-Pacific (Birkeland & Lucas 1990; Fabricius 2013). In the Great Barrier Reef (GBR), model predictions suggest that in the absence of predation by this starfish, coral cover would increase by $\sim 1\% \cdot y^{-1}$ (De'ath *et al.*, 2012). The boom and bust population cycle of *A. planci* appears largely driven by opportunistic larval success in response to increased phytoplankton levels due to eutrophication (Birkeland 1982; Uthicke *et al.*, 2009; Fabricius 2013). Because larval success is a key driver of population outbreaks, it is essential to understand how development in *A. planci* responds to concurrent ocean acidification and warming to predict how population dynamics of this predator may be affected.

Thus far, research on the impacts of simultaneous exposure to increased temperature and acidification on non-calcifying marine invertebrate larvae has involved five species (one echinoid, three asteroids, two corals) (Byrne *et al.*, 2009; Nguyen *et al.*, 2012; Byrne *et al.*, 2013c; Chua *et al.*, 2013). For asteroids, development to the bipinnaria larva of temperate starfish *Patiriella regularis* was more negatively affected by increased temperature than acidification (Byrne *et al.*, 2013c). However, with long-term rearing decreased pH had a negative effect, reducing larval size, as also found for *Odontaster validus* (Byrne *et al.*, 2013c;

Gonzalez-Bernat *et al.*, 2013). In contrast, lecithotrophic (non-feeding) larvae of starfish and other species, including corals, appear to be more tolerant to increased acidification (Dupont *et al.*, 2010; Nguyen *et al.*, 2012; Chua *et al.*, 2013; Putnam *et al.*, 2013).

We investigated the response of planktonic stages of *A. planci* to increased temperature and acidification in context with near-future ocean conditions including ‘business as usual scenarios’ for 2100 (2-4 °C warming, - 0.3-0.5 pH units) (Caldeira & Wickett, 2005; IPCC, 2007). In eastern Australia, the distribution of *A. planci* parallels that of corals, extending from low latitude GBR reefs to high latitude reefs in Northern New South Wales and Lord Howe Island. Through the distribution of COTS, large outbreak populations occur in the warm, more tropical parts of its range (spawning at > 26°C), while low density populations occur on cooler high latitude reefs (spawning at ~ 24°C) (De Vantier & Deacon, 1990; Johnson & Babcock, 1994; Harriott, 1995). Research on the thermal biology of COTS indicates that development to the late brachiolaria larva and juvenile stage has an optimal temperature of ~ 28 °C (Lucas, 1973; Yasuda *et al.*, 2010), although early bipinnaria larvae tolerate 31 °C (Lamare *et al.*, 2014). As tropical regions warm, there is an expectation that *A. planci*, along with other coral reef species, may decline in low latitude regions and expand their range through poleward migration, tracking optimal isotherms (Figueira & Booth, 2010; Yamano *et al.*, 2011; Baird *et al.*, 2013). Research on acidification indicates increased abnormality of *A. planci* larvae at pH 7.6 (Uthicke *et al.*, 2013).

For the response of *A. planci* development to near future ocean conditions we addressed three hypotheses: 1) Early development is broadly tolerant to acidification and warming as observed for many echinoderms (Byrne, 2011); 2) Larval development occurs up to a thermal threshold (~ 4 °C above ambient), as observed for echinoplutei reared under similar conditions (Sheppard Brennan *et al.*, 2010) and; 3) As a non-calcifying larva *A. planci* is more robust to near future acidification than calcifying echinoderm larvae (see Byrne

et al., 2013a). The larvae of *A. planci* and corals are in the plankton at the same time, experiencing similar changing environmental conditions. Thus, it is important to assess how the life stages of both the predator and prey may respond to a changing ocean, and this is also considered.

Material and methods

Study species, gamete collection, fertilisation and rearing

Adult *A. planci* were collected on SCUBA from the Great Barrier Reef near Cairns, Northern Queensland (16°55S, 145°46E), Australia and individually air transported to Coffs Harbour, Northern New South Wales, Australia. The starfish were acclimated for a week in flowing seawater in the aquarium system at the National Marine Science Centre, Southern Cross University at ambient temperatures for the habitat at time of collection (~ 26 °C; <http://data.aims.gov.au/aimsrtds/yearlytrends.xhtml>; Nov. 2012).

Gonads were dissected from *A. planci* (three males and two females, 20-30 cm in diameter). The testes immediately released sperm which was collected and stored dry at 4 °C until use. The ovaries were rinsed in 1 µm filtered seawater (FSW) to remove immature eggs and then placed in 10⁻⁵ M 1-Methyl-Adenine in seawater to induce ovulation. After approximately 1 h, the eggs were collected, rinsed in FSW, and checked microscopically for quality (i.e. shape, integrity, germinal vesicle breakdown). The eggs from the two females were mixed in approximately equal proportions. Before being used, the sperm from each male was activated using a small amount of seawater and checked for motility. The sperm from the three males was then mixed in approximately equal proportions. The number of sperm in the sperm stock was counted using a haemocytometer.

Experiments were done in 100 mL plastic rearing containers supplied with flow through experimental seawater. Each container had a mesh (45 µm) covered window that retains larvae but allows water to flow through and maintains 40 mL of water in the container at all times. Approximately 500 eggs were placed in rearing containers that were supplied with flowing experimental seawater (3 mLs.min⁻¹). Eggs were placed in experimental water

for 10 min prior to fertilization. The sperm solution was diluted so that the addition of 1 μL of the solution to the rearing container achieved a sperm to egg ratio of approximately 50:1. Before addition of sperm, the flow-through seawater supplying each container was turned off to allow fertilisation and turned back on after 10 min to remove excess sperm. Larvae were reared for ten days to the late bipinnaria/early brachiolaria stage. On day three when the digestive tract was developed, the larvae were fed the tropical microalgae *Proteomonas sulcata* ($25\text{-}37 \times 10^3 \text{ cells.mL}^{-1}$) once per day from day 3, then twice per day from day 5 and finally three times a day as the larvae approached the brachiolaria stage on day 7. During feeding the flow of seawater to containers was turned off for approximately 90 min. Thus the algae were present for a very small portion of time and there was little or no change in pH (Online Resource Table S1).

Experimental treatments

Three temperatures (26 °C, 28 °C, 30 °C) and three pH_{NIST} treatments (8.1, 7.8, 7.6) crossed in all combinations were used, with ten replicate containers per treatment. These temperatures represent the low and high average sea surface temperatures that the larvae encounter in the GBR during the summer spawning season (December - January) from 25-26 °C to 28-29 °C (Berkelmans, 2002).

The thermotolerance of development in *A. planci* depends on the recent thermal history of the parents (Johnson & Babcock, 1994). Recent sea surface temperature for the habitat was 26 °C, the average for austral summer months in Northern Australia, where the *A. planci* were sourced for this study (Navy Metoc 2013:<http://www.metoc.gov.au/products/data/ausstt.php>). This was considered the control temperature. The control water was at pH_{NIST} 8.1. The combination treatments of 29-30 °C

and pH 7.6 represent upper threshold for near future warming of SST and acidification (IPCC, 2007; Lima & Wethey, 2012). The high temperature level, 30 °C is representative of predicted warming in low latitude tropical regions, and occurs during heat waves (Wernberg *et al.*, 2011; Lima & Wethey, 2012).

The experiments were conducted in a purpose built flow-through seawater system with filtered (1 µm) and UV sterilized water delivered independently into each individual rearing container using irrigation dripper valves. The experimental pH was regulated by injection of pure CO₂ into the seawater as it passed through reservoirs in the system at ~ 60 L.h⁻¹ using an automatic CO₂ injection system, mixed using a vortex mixer (Red Sea) and continuously bubbled with air to aid mixing and to maintain dissolved oxygen > 90%. The pH_{NIST} in sections of the system was regulated according to water chemistry conditions in the rearing containers with two pH controllers (Tunze) set at pH 7.6 and pH 7.8, with a third section allowed to track ambient pH (mean pH 8.1). This water was fed into subsequent reservoirs (flow rate ~ 20 L.h⁻¹) where it was warmed to the required temperatures, 26 °C, 28 °C and 30 °C, using aquarium heaters (200 W, Eheim Jager). Temperature was automatically regulated using temperature sensors in a rearing container and a temperature controller (Tunze) connected to the heaters. Water from reservoirs to drippers was continually re-circulated back to the reservoirs using 20 W pumps to maintain even temperatures within each treatment.

Temperature, pH and salinity in the rearing containers in all treatments were measured daily using a Hach Hqd Portable temperature compensated multiprobe, calibrated with high precision buffers (Oakton). Water samples (200 mL) were collected daily during the experiment, filtered through a 0.45 µm syringe filter, and fixed with 10 µL of saturated HgCl₂. These water samples were used to determine total alkalinity by potentiometric titration using a Metrohm 888 Titrand using certified reference standards (Dickson *et al.*, 2007). *p*CO₂ and

Ω_{Calcite} were calculated using CO2SYS (Pierrot *et al.*, 2006), using the dissociation constants of Mehrbach (1973) as refitted by Dickson and Millero (1987) from measures of salinity, temperature, pH_{NIST} and total alkalinity (TA) (Online resource Table S2).

The experimental temperatures were (1) control: Mean 26.3 °C, SE = 0.06, $n = (10)$; (2) Mean 27.8 °C, SE = 0.04, $n = (10)$ and (3) Mean 29.8 °C, SE = 0.04, $n = (10)$. The pH_{NIST} levels were (1) control 8.1, SE = 0.01, $n = (10)$; (2) Mean 7.8, SE = 0.003, $n = (10)$ and (3) Mean = 7.6, SE = 0.003, $n = (10)$ and are detailed in Table S2.

Fertilisation and development

At 2 and 24h, approximately 100 specimens were taken from each replicate and fixed in a 1.5 mL sample tube with 10% formalaldehyde in FSW. The percentage of fertilisation and normal embryos were microscopically determined. Fertilisation was scored based on the presence of elevated fertilisation envelope and cell division. Gastrulation was scored based on the presence of a normal development and a well-formed archenteron.

At days 4 and 10, larvae from each rearing container were haphazardly collected with a pipette and placed in 1.5 mL sample tubes with 7% MgCl_2 for ca. 15 min to relax them. They were then fixed with ~ 0.25 mL 10% formalaldehyde-FSW solution. Shortly after fixation, 20 specimens randomly sampled from each tube and photographed with a camera mounted on a microscope (Olympus DP26) to avoid post fixation changes. Care was taken to position larvae flat to the plane of focus. The length and width of each larva was measured as indicated in Fig 1a. Stomach length was measured from the end of the esophagus to the beginning of the hindgut (Fig. 1a). The length and width ratio of each larva was calculated to determine if experimental treatments affected larval form. Measurements were made using the software Image J (NIH, USA). The mean of these measurements (from 20 larvae) from each

replicate was used as the data point for analysis. The larvae from each replicate were also microscopically scored for normal development at days 4 and 10. Normal larvae were defined as having a well-rounded preoral lobe and a complete gut (Fig. 1a). Abnormal larvae had a distorted irregular profile or arrested development (Fig. 1b).

Statistical analysis

Data were analysed with two-way Analysis of Variance (ANOVA) using the PERMANOVA routine of Primer 6 with PERMANOVA+ extension (v6.1.11). The factors 'Temperature' and 'pH' were fixed and fully orthogonal. Post-hoc pair-wise tests were used to determine the difference in means among treatments. The significance level was taken as $P < 0.05$.

Results

Fertilization and gastrulation

Fertilisation was high (> 80 %) across all treatments with no effect of temperature or pH (Temp: $F_{(2,81)} = 0.17$, $p = 0.844$; pH: $F_{(2, 89)} = 0.32$, $p = 0.74$; Fig. S1). Normal development to the gastrula stage (24 h) was also high (> 80 %) across all treatments with no effect of temperature or pH (Temp: $F_{(2, 89)} = 0.26$, $p = 0.78$ pH: $F_{(2, 81)} = 0.22$, $p = 0.81$; Fig. S1). There was a non-significant reduction in gastrulation across the 30 °C treatments with the greatest decrease at pH 7.6/30 °C (15 % reduction with respect to 28 °C). There was no significant interaction between temperature and pH/pCO₂ on fertilisation or early development.

Larval development

Day 4

By day four, larvae were early bipinnariae (Fig. 2a). The percentage of normal four-day old larvae showed significant responses to both temperature and pH (Temp: $F_{(2, 44)} = 4.09$, $p < 0.01$; pH: $F_{(2, 44)} = 2.38$, $p < 0.01$; Table 1a, Fig. 3). Post-hoc pair-wise tests indicated there was a decrease in the percentage of normal larvae at pH 7.8 (average 8.0 % decrease) and pH 7.6 (average 9.8 % decrease) compared to control pH and no difference between pH 7.8 and pH 7.6. ($8.1 > 7.8 = 7.6$, $p < 0.05$; Fig. 3). For temperature post-hoc pair-wise tests indicated that there was a significant decrease (5.4 %) in the percentage of normal larvae at 28 °C, and a

further decrease (10.8 %) at 30 °C (26 °C > 28 °C > 30 °C, $p < 0.01$; Fig. 3). There was no interaction between temperature and pH/pCO₂ on normal larval development.

With respect to larval growth (Fig. 2a), there was a significant effect of temperature and pH on larval length (Temp: $F_{(2, 44)} = 9.36$, $p < 0.01$; pH: $F_{(2, 44)} = 4.68$, $p < 0.01$; Table 1b, Fig. 4a) and width (Temp: $F_{(2, 44)} = 11.08$, $p < 0.01$; pH: $F_{(2, 44)} = 3.52$, $p < 0.05$; Table 1c, Fig. 4b) with no interaction between the two stressors. Post-hoc pair-wise tests indicated that larvae were longer (2.2 %) and wider (1.5 %) at 28 °C compared to 26 °C, but shorter (6.4 %) and narrower (6.8 %) at 30 °C compared to 28 °C (length: 26 °C < 28 °C > 30 °C, $p < 0.05$; width 26 < 28 > 30 °C, $p < 0.01$; Fig. 2a, 4a). Post-hoc pair-wise tests indicated that larvae were shorter and narrower at pH 7.8 (mean 5.4 %, and 3.1 % respectively) and 7.6 (mean 5.1 %, and 3.4 % respectively) compared to control pH, but there was no difference between pH 7.8 and 7.6 (length: 8.1 > 7.8 = 7.6, $p = 0.02$; width: 8.1 > 7.8, $p = 0.01$ and 7.6 (length: 8.1 > 7.6, $p = 0.02$; width: 8.1 > 7.6, $p = 0.01$, Fig. 2a, 4). There was an independent and additive effect of increased temperature and reduced pH on larval size. Larvae in 30 °C/7.6 pH treatments were 4.6 % smaller than controls and 2.5 % smaller than larvae in 28 °C/7.6 pH treatments. There was no effect of temperature or pH on stomach length in bipinnaria at day 4 (Temp: $F_{(2, 44)} = 2.09$, $p = 0.06$; pH: $F_{(2, 44)} = 1.89$, $p = 0.08$). There was no interaction between effects of temperature and pH/pCO₂ on early larvae growth (Table 1).

There was no effect of temperature and pH on larval form (length:width ratio) (Temp: $F_{(2, 44)} = 1.08$, $p = 0.33$; pH: $F_{(2, 44)} = 1.06$, $p = 0.38$; Table 1d). There was no interaction between temperature and pH/pCO₂ on length:width (Table 1d).

Day 10

By day 10, larvae were late bipinnaria or early brachiolaria (Fig. 2b). Developmental arrest was evident in the 30 °C treatments with high mortality in all replicates so these treatments were omitted from the analysis. In the remaining treatments (two temperature × three pH) decreased pH ($F_{(2, 29)} = 2.58$, $p < 0.01$) but not temperature had a significant effect on development (Table 1a, Fig. 3). Post-hoc pair-wise tests indicated that there was a decrease in the percentage of normal larvae at pH 7.8 (10.5 %) and pH 7.6 (24.3 %) compared to controls (8.1>7.8>7.6, $p < 0.01$; Table 1a, Fig. 3). For the 28 °C treatment there was a slight increase (mean 6.4 %) in the percentage of normal larvae but this wasn't significant.

With regard to larval size there was a significant effect of pH on larval length ($F_{(2, 35)} = 4.73$, $p < 0.05$; Table 1b, Fig. 4a) and width ($F_{(2, 35)} = 6.30$, $p < 0.01$; Table 1c, Fig. 4b) with no effect of temperature (Table 1). Post-hoc pair-wise tests indicated that larvae were shorter and narrower at pH 7.8 and 7.6 compared to control pH (length and width: 8.1>7.8 = 7.6; $p < 0.05$; Table 1). There was no effect of temperature or pH on stomach length after seven days of feeding (Temp: $F_{(1, 35)} = 2.88$, $p = 0.09$; pH: $F_{(2, 35)} = 0.53$, $p = 0.59$).

There was a significant effect of pH but not temperature on the ratio between length and width ($F_{(2, 35)} = 7.69$, $p < 0.01$, Table 1d, Fig. 4c). Post-hoc pair-wise tests indicated that this ratio decreased in larvae reared at pH 7.8 and pH 7.6 which did not differ from each other (8.1>7.8 = 7.6, $p < 0.05$, Table 1d). Thus larvae in the low pH treatments were thinner.

Discussion

Our results show the negative impacts of near-future increased ocean temperature (2-4 °C above ambient) and acidification (0.3-0.5 pH units below ambient) on development of *A. planici* to the 10 day advanced larval stage. The 28 °C treatment approximated the optimum temperature for larval development, with increased abnormality and mortality by day 10 at 30 °C, as noted elsewhere for populations on the GBR (Lucas 1973; Johnson & Babcock, 1994). Thus, as hypothesized, advanced larvae have a threshold ~ 4 °C above ambient, although early development (to day 3) is more tolerant (Lamare *et al.*, 2014, this study). Larval development was also suppressed by acidification with smaller larvae in the pH 7.6 and 7.8 treatments. Although the results indicate that non-calcifying asteroid larvae are more tolerant of acidification than calcifying echinoderm larvae (see Byrne *et al.*, 2013a,b), providing partial support for hypothesis #3, they nonetheless succumb to near-future ocean acidification (Byrne *et al.*, 2013c; Gonzalez-Bernat *et al.*, 2013, Uthicke *et al.*, 2013, this study).

During the dispersive phase of *A. planici*, surface temperatures ~ 30 °C may become the norm in the GBR (Hobday & Lough 2011). With regard to ocean acidification, *A. planici* larvae are also likely to experience conditions approaching pH 7.6-7.8 while in the plankton (IPCC 2007; Hobday & Lough 2011), although the dynamics of acidification at the level of reefs will vary locally due to community metabolism and the variable influence of buffering (Shaw *et al.*, 2013). These dynamics are not well understood (Gagliano *et al.*, 2010; Shaw *et al.*, 2013).

It appears that *A. planici* larvae from the region in this study (GBR) may experience sub-optimal conditions due to changing climate unless the adults can undergo a phenotypic switch in spawning time to avoid exposure of larvae to high summer temperatures. However, like many species on the GBR, *A. planici* has a fairly fixed annual spawning cycle in the GBR,

a cycle that appears to be entrained by number of seasonally coincident factors including photoperiod, temperature, lunar cues and tides (Babcock & Mundy 1992). In addition, as many invertebrates, including *A. planci*, spawn predictably at or around the same time each year on the GBR, it is likely that pheromonal cues are also involved (Branch *et al.*, 1975; Babcock *et al.*, 1992; Babcock & Mundy 1992). From what we know about the histology of gametogenesis in GBR populations of *A. planci*, and control of reproduction in asteroids, in areas with distinct seasonal cues (Lucas 1973; Pearse & Eernisse 1982; Pearse & Walker 1986; Babcock & Mundy 1992; Byrne *et al.*, 2013d; Mercier & Hamel 2013), a major shift in gametogenic events in response to increased temperature seems unlikely. Proliferation of oogonia, onset of the vitellogenic phase and terminal oocyte growth in many asteroids is entrained by photoperiod with the time of spawning often fine tuned by temperature, lunar period and tides (Babcock & Mundy 1992; Mercier & Hamel 2013). A synthesis of spawning data for *A. planci* on the GBR indicates spawning is cued by lunar or tidal cycles (Babcock & Mundy 1992). Although temperature might influence spawning time, this factor does not explain the synchronous spawning pattern observed for *A. planci* on the GBR (Babcock & Mundy 1992).

Across the distribution of *A. planci*, from southern Japan to southern Australia, regional differences in spawning (Birkeland & Lucas 1990) and projected climatic change (Hobday & Pecl 2013) highlight the importance to consider local population biology and climate to assess the impacts of global change on larval stress tolerance in this ecologically important species. In contrast to the GBR, the seasonality of spawning in *A. planci* diminishes in regions with minimal environmental change, close to the equator (Cheney, 1974; Birkeland & Lucas 1990). In Micronesia and Guam for instance, spawning in *A. planci* is prolonged and may be continuous (Cheney, 1974; Birkeland & Lucas 1990). In these regions, temporal fluctuations in temperature may be particularly important for induction of spawning. It may be

that low latitude populations of *A. planci*, that spawn at ~ 30° C (or above) are living close to developmental thermal tolerance limits, or have larvae that are physiologically acclimatised to this temperature. For echinoderms, it is well known that the temperature at which eggs develop influences the thermo tolerance of larvae (Byrne, 2011). The increased temperature projected for equatorial regions (Hoegh-Guldberg *et al.*, 2007; Burrows *et al.*, 2011) are likely to be a source of stress for *A. planci* larvae.

Development of *A. planci* showed stage specific responses to simultaneous exposure to warming and acidification. There was no negative effect of these stressors on early development (fertilisation to gastrulation), supporting our first hypothesis. Fertilisation in *A. planci* under an optimal sperm to egg ratio (50:1) was robust to warming and acidification. The tolerance of fertilisation using gametes from multiple parents to warming and acidification is also reported in similar studies of echinoderms, including the starfish *Patiriella regularis* (Byrne, 2011, 2012; Byrne *et al.*, 2013c). This is suggested to be due to increased sperm competition from multiple sires and enhanced chances of compatible gamete binding (Byrne, 2011, 2012; Challener *et al.*, 2013). Gametes of *A. planci* may experience temperatures between 24 °C to 30 °C (Johnson, 1992; Johnson & Babcock, 1994) so a broad thermal tolerance of fertilisation and early development might be expected. Fertilisation and early embryos in many echinoderms are tolerant to increased temperature (~ 4 °C) (Byrne, 2010), potentially due to the presence of protective maternal factors (Hamdoun & Epel, 2007).

Although decreased pH might reduce the percentage of fertilisation at lower sperm concentrations, (Gonzalez-Bernat *et al.*, 2013; Uthicke *et al.*, unpub. data), the multiple parent-synchronous spawning, characteristic of *A. planci*, suggests that sperm limitation and gamete incompatibility may not be a major impediment to fertilisation in this species, at least for the most important outbreak populations. *A. planci* are extremely fertile and have high fertilisation rates due to mass spawning of aggregated starfish (Babcock & Mundy, 1992).

High fertilisation rates in *A. planci* are recorded for spawners 30-60 m apart (Babcock & Mundy, 1992) and as far as 100 m downstream from spawning males (Babcock *et al.*, 1994).

Development to the gastrula stage of *A. planci* tolerated a 4 °C increase in temperature with normal gastrulation at 30 °C across all pH treatments (70-80 % gastrulation). In contrast, gastrulation in *Asterias amurensis* was reduced at 2 °C above ambient (Lee *et al.*, 2004). For *P. regularis* the combination of 2 °C above ambient and pH 7.6 was lethal to gastrulae (Byrne *et al.*, 2013c).

There was a high percentage of normal bipinnaria across all temperature-pH treatments on day 4, but by day 10, 30 °C exceeded the larval tolerance with mortality and arrested development evident. This threshold for successful development, ~ 4 °C above ambient, is similar to that in other marine invertebrate larvae including asteroid and echinoid species (Lee *et al.*, 2004; Sheppard Brennan *et al.*, 2010; Byrne, 2011; Byrne *et al.*, 2013c). At the optimal temperature for development (28 °C), the percentage of normal *A. planci* larvae was reduced in low pH treatments (also see Uthicke *et al.*, 2013) and larvae were smaller than those reared in control conditions. The larvae reared in pH 7.6 also exhibited the largest deviation from isometric growth, a change that would compromise feeding and swimming efficiency (Strathmann & Grünbaum, 2006; Chan *et al.*, 2011).

The embryos and larvae of *A. planci* that survived temperature-driven mortality, succumbed to the negative effects of acidification in later development, as observed in several cross-factorial warming-acidification studies of marine larvae (Byrne, 2011; Sheppard Brennan *et al.*, 2010; Byrne *et al.*, 2013c; Byrne & Przeslawski, 2013). The stunting effect of low pH levels (pH 7.6-7.8) on larval growth may involve several mechanisms including hypercapnic alteration of metabolism, energy constraints in acid base regulation and teratogenic (mortality and abnormality) effects, as suggested for sea urchin echinoplutei (Sheppard Brennan *et al.*, 2010; Byrne, 2011,2012; Stumpp *et al.*, 2011; Uthicke *et al.*, 2013;

Byrne *et al.*, 2013a). Our study, and recent research with starfish larvae (Byrne *et al.*, 2013c; Gonzalez-Bernat *et al.*, 2013; Uthicke *et al.*, 2013), does not support recent meta-analyses that suggest that non-calcifying larvae (like *A. planici*) will be more resilient to ocean acidification than calcifying larvae (Hendriks *et al.*, 2010; Kroeker *et al.*, 2013). The stunting effect of acidification on growth in bipinnaria larvae, for which carbonate mineral saturation is not likely to have direct effects, shows the strong influence of increased $p\text{CO}_2$ on growth (Byrne *et al.*, 2013c; Gonzalez-Bernat *et al.*, 2013).

As planktonic larvae of marine invertebrates are sensitive to a plethora of environmental perturbations (Pechenik, 1987), the impacts of other interactive factors also need to be considered (Byrne & Przeslawski, 2013). Increased temperature and decreased salinity had a synergistic effect on larval development in *A. planici* with completion of development at low salinity (22 ‰) and optimal temperature treatments (Lucas, 1973). Food availability is also a key environmental factor for the success of these larvae (Fabricius *et al.*, 2010).

Success of *A. planici* is inextricably linked to the success of its coral prey. The larvae of both predator and prey are in the plankton at the same time (see Harrison, 2011) and how these larvae will respond to climatic change will have a major impact on coral reef ecosystems. Future success of these larvae may be influenced by their contrasting nutritional needs. Species like *A. planici* with feeding larvae are highly sensitive to the vagaries of their planktonic food supply, and are suggested to have a high-risk-high-gain life history, and be more sensitive to climatic change (Uthicke *et al.*, 2009). On the other hand, species like corals with non-feeding larvae where maternal provisions support development to the juvenile stage are suggested to have a buffered life history, and be less vulnerable to climatic change (Uthicke *et al.*, 2009). In contrast to *A. planici*, non-feeding starfish larvae are not affected by near-future ocean acidification (Dupont *et al.*, 2010, Nguyen *et al.*, 2012). Acidification

actually benefitted the larvae of *Crossaster papposus* with doubled growth rates at pH 7.6-7.7 compared with controls (Dupont *et al.*, 2010).

A comparison of how the larvae of predator and prey may fare in a changing ocean is evident from studies on the effects of near future (and beyond) acidification and warming on coral development (Anlauf *et al.*, 2011; Albright & Mason, 2013; Chua *et al.*, 2013). Coral planulae are robust to these stressors (Albright *et al.*, 2010; Chua *et al.*, 2013; Howells *et al.*, 2013; Medina-Rosas *et al.*, 2013) and many species, including the larvae of *Acropora*, the preferred prey of *A. planici* (De'ath & Moran, 1998; Pratchett, 2007), develop normally to the juvenile stage at levels of warming and acidification (Anlauf *et al.*, 2011) that are deleterious to *A. planici* larvae. Thus, in addition to having the benefit of the buffered non-feeding larval life history, coral planulae have a greater tolerance to increased temperature and acidification than *A. planici* larvae and so may be a comparative “winner” in climate change stakes, at least with regard to the planktonic life stage.

That said, it will be important to determine the sensitivities of benthic juvenile and adult stages to increased temperature and acidification, to more fully understand the potential outcomes for *A. planici* and associated biota. The first calcification stages, the early metamorphic juvenile of *A. planici* and other echinoderms may be vulnerable to acidification due to their high magnesium calcite skeleton (McClintock *et al.*, 2011). Studies thus far, however, indicate that juvenile starfish are not negatively affected by near future acidification (Gooding *et al.*, 2009; Dupont *et al.*, 2010; Nguyen & Byrne 2014). Coral juveniles (primary polyp) by contrast, are highly sensitive as near-future acidification reduces growth by almost a third (Anlauf *et al.*, 2011). Thus, with respect to life cycle and climatic change, larval *A. planici* appear more sensitive than larval corals, but the opposite appears to be the case for the juvenile stage. How these different life history sensitivities will play out to affect their future success is not clear, but will undoubtedly be determined by ecosystem wide effects of global

change. Decreased calcification of reef building corals caused by global change (De'ath *et al.*, 2009; Cooper *et al.*, 2012; Silverman *et al.*, 2012) will reduce habitat and food source for *A. planici* and a great diversity of species.

Temperature is a key factor in determining the distribution of corals and, by association, COTS as a specialist coralivore. As the ocean warms, the developmental tolerance of *A. planici* and corals may be pushed closer to their upper limits and so poleward migration of these and other tropical species, to follow optimal ocean isotherms, is expected (Sunday *et al.*, 2012). In Japan, corals have migrated north ca. 14 km.y⁻¹ since the 1930's, and in Australia, corals have migrated south to temperate latitudes (Yamano *et al.*, 2011; Baird *et al.*, 2013). These shifts are being facilitated by the East Australia Current and Kuroshio Current (Yamano *et al.*, 2011; Baird *et al.*, 2013), which are intensifying and shifting poleward, causing sea surface warming two to three times greater than the mean surface warming rate (Wu *et al.*, 2012). In both hemispheres *A. planici* occurs in cooler waters near the limit of coral distribution (Yamaguchi, 1987; De Vantier & Deacon, 1990). Increasing populations of *A. planici* have been reported in the most southern coral reefs at Lord Howe Island and outbreaks are reported for nearby Elizabeth and Middleton reefs (De Vantier & Deacon, 1990; Johnson & Babcock, 1994; Harriott, 1995). Population genetics indicate that high latitude populations are generated by dispersal of propagules from warmer climes (Yasuda *et al.*, 2009). The successful recruitment and survival of *A. planici* in high latitudes reefs and cool tolerance of early development (to 25 °C, Lamare *et al.*, 2014) indicates that populations of this starfish may continue to expand on these reefs, especially in the Lord Howe reefs which are in an ocean warming hotspot (Hobday & Pecl, 2013).

Increased population size of *A. planici*, together with increased temperatures approaching the optimum for larval development, would be expected to enhance reproductive success of higher latitude populations. Whether this enhanced success will generate outbreaks

will depend on the coincidence of other factors, such as the timing of phytoplankton blooms that are modeled to enhance the success of larvae from outbreak source populations (Fabricius *et al.*, 2010). In addition, increased larval mortality or compromised larval health may not prevent outbreaks in the future, because large populations of adult *A. planci* are modeled to sustain outbreaks even in conditions where larval survival is low (Fabricius *et al.*, 2010).

The timing of phytoplankton blooms in response to land runoff following tropical storms appears key to larval success and COTs outbreaks, and so climate-driven change in the intensity and temporal pattern of precipitation events (Mumby *et al.*, 2011; Hobday & Lough 2011) may alter the runoff - to outbreak cascade. The timing of plankton blooms may change in a warmer ocean resulting in a trophic mis-match between the presence of *A. planci* larvae and their food source, as indicated by climate-driven change observed in the phenology of zooplankton and phytoplankton (Hays *et al.*, 2005; Montes-Hugo *et al.*, 2009; Beaugrand, 2012). Increased temperature is also a significant stressor to diatoms (Tatters *et al.*, 2013), a major food source for *A. planci* larvae. Thus, it seems that phytoplankton diversity and food levels for *A. planci* may change in the future. However, more data are needed on responses of planktonic communities from tropical waters to climate change. Although the stress markers used here (morphology) provided a useful assessment of the tolerance of *A. planci* development to ocean change stressors, other factors (i.e. precipitation events, phytoplankton, sub-lethal effects, changes in settlement cues such as coralline algae - see Doropoulos & Diaz-Pulido 2013; Uthicke *et al.*, 2013) may also alter the dynamics of *A. planci* populations. As the phenology of *A. planci* reproduction may not be able to track changes in the timing of conditions favourable for larvae, outbreaks may be less likely to occur in low latitude reefs where large populations currently exist.

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Figures

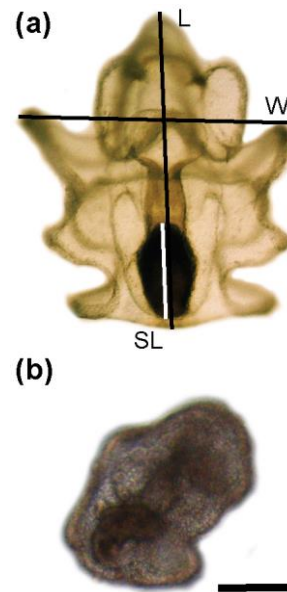


Fig. 1 Late bipinnaria larvae of *Acanthaster planci* as (a) normal larva with lines showing length (L), width (W) and stomach length (SL) measurements and (b) abnormal larva with incomplete gut development. Scale bar = 100 μm .

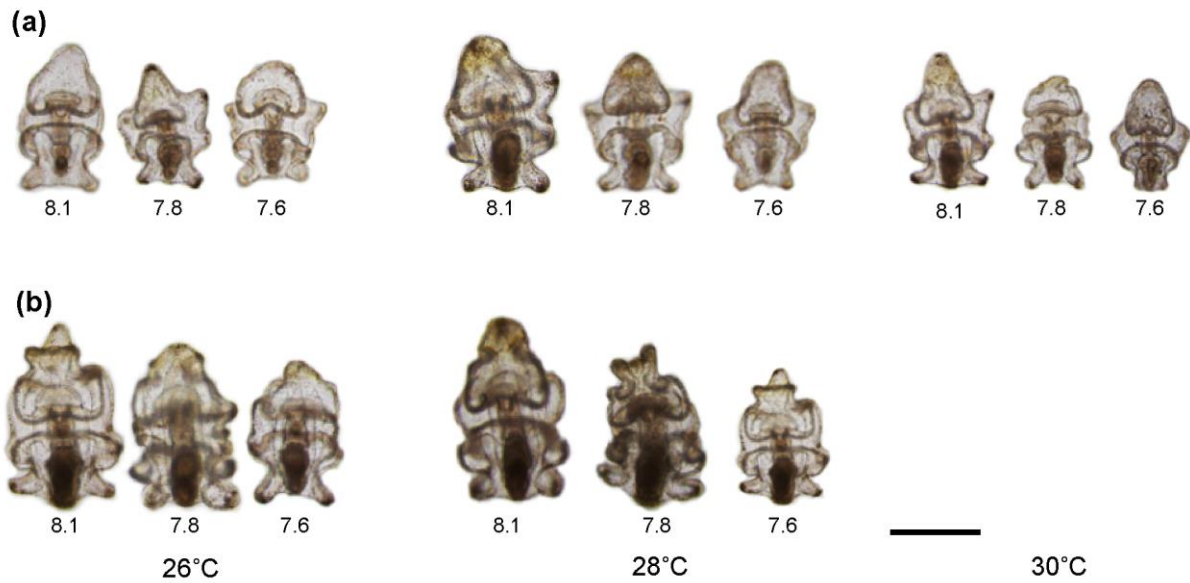


Fig. 2 *Acanthaster planci* larvae at (a) day four in nine treatments (three temperature \times three pH) and (b) day ten in six treatments (two temperature \times three pH). Larvae reared in control pH and 28 °C had optimal growth. Larval size was reduced in pH 7.8/7.6 treatments although development appeared normal. Larval size decreased with increased abnormalities in extreme temperature (30 °C) and pH (7.6) at day four. The thermal threshold (30 °C) was breached by day ten with no surviving larvae in any pH treatments. These are representative photographs of larvae from 3-5 replicates per treatment. Scale bar = 100 μ m.

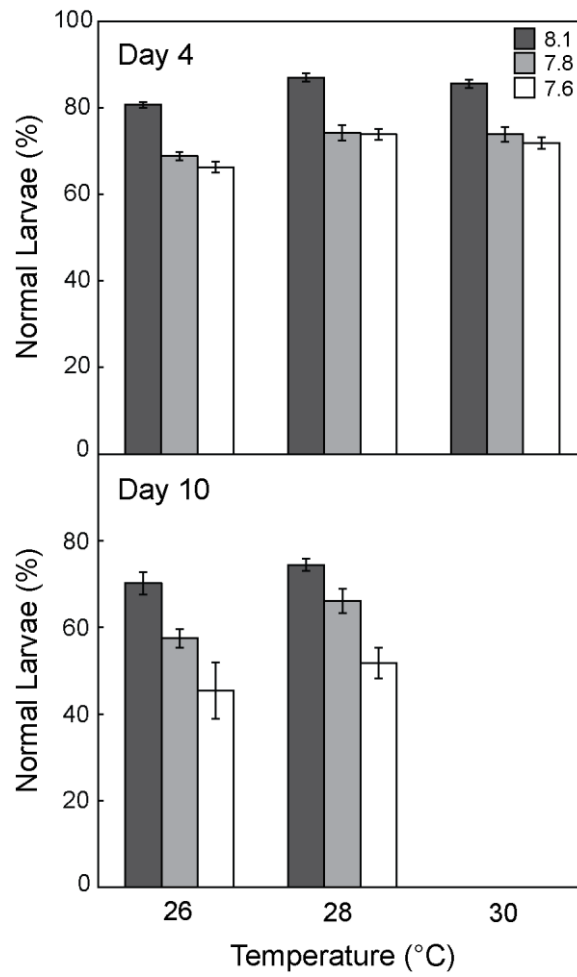


Fig. 3 Percentage of normal *Acanthaster planci* larvae at day four (top) in nine treatments (three temperature \times three pH) and day ten (bottom) in six treatments (two temperature \times three pH). Values are means \pm SE; $n = 5$.

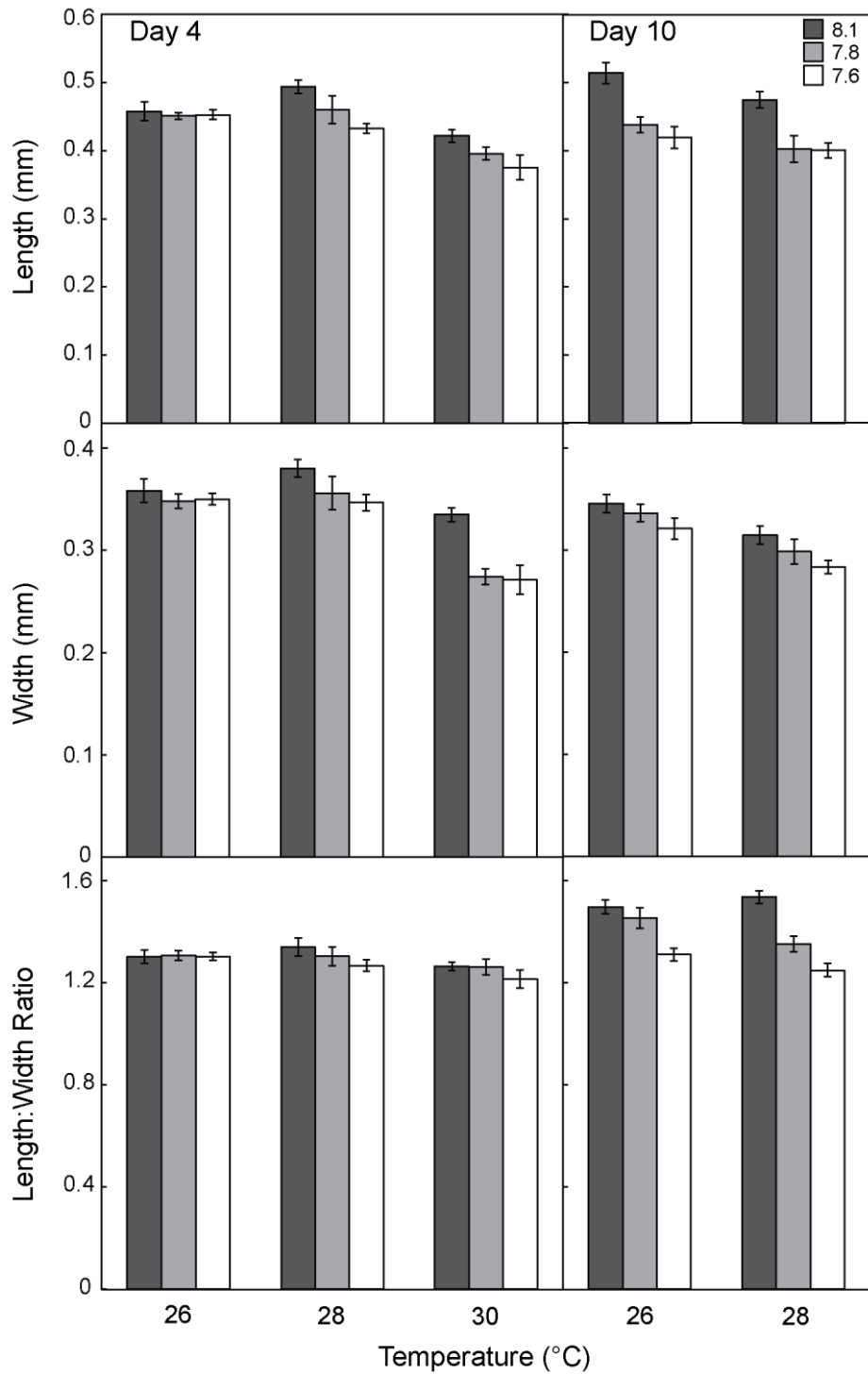


Fig. 4 Larval (a) length, (b) width and (c) length to width ratio of *Acanthaster planci* on day 4 (three temperature \times three pH) and day ten (two temperature \times three pH). Values are means \pm SE; $n = 3-5$ (day 4). $n = 5$ (day ten).

Table 1 ANOVA of data on normal development, length, width and the ratio of length:width for *Acanthaster planci* larvae measured on days four (nine temperature-pH treatments) and ten (six temperature-pH treatments); $n = 3-5$. Bold indicates the factors that are significant. Post-hoc analyses are pair-wise tests.

Parameters	Source	Day 4					Day 10				
		df	MS	F	p-value	Post hoc	df	MS	F	p-value	Post hoc
(a) Normal (%)	Temp	2	445.4	14.095	0.0001	26 > 28 > 30	1	14.175	0.1148	0.743	
	pH	2	375.17	11.872	0.0002	8.1 > 7.8 = 7.6	2	318.49	2.5794	0.0001	8.1 > 7.8 = 7.6
	Temp x pH	4	138.57	3.211	0.056		2	1.48	1.2002	0.9893	
	Residual	37	31.6				24	123.47			
(b) Length	Temp	2	0.0249	9.3623	0.001	26 > 28 > 30	1	0.0072	1.4686	0.2498	
	pH	2	0.0125	4.6827	0.012	8.1 > 7.8 = 7.6	2	0.023	4.7348	0.015	8.1 > 7.8 = 7.6
	Temp x pH	4	0.0061	2.2901	0.0629		2	0.0042	0.8614	0.4775	
	Residual	36	0.00266				30	0.0049			
(c) Width	Temp	2	0.0153	11.078	0.001	26 > 28 > 30	1	0.0168	3.2487	0.0869	
	pH	2	0.00485	3.517	0.043	8.1 > 7.8 = 7.6	2	0.0326	6.3011	0.0138	8.1 > 7.8 = 7.6
	Temp x pH	4	0.00165	1.1972	0.3127		2	0.0164	3.1725	0.0709	
	Residual	36	0.00138				30	0.0052			
(d) Length:width	Temp	2	1.1103	1.0842	0.3347		1	0.0032	0.2528	0.6074	
	pH	2	1.0574	1.0325	0.3796		2	0.0977	7.6882	0.006	8.1 > 7.8 = 7.6
	Temp x pH	4	0.9297	0.9078	0.5415		2	0.0286	2.2517	0.1209	
	Residual	36	1.0241				30	0.0127			

Supporting information

Table S1 Mean changes (%) in pH and alkalinity of sea water treatment conditions after 90 min of feeding with micro algae *Proteomonas sulcata*. $n = 5$.

Treatments		pH		Alkalinity	
Temperature (°C)	pH	Before	After	Before	After
26	8.10	8.00 (0.002)	8.01 (0.00)	2237.43 (1.63)	2250.53 (1.89)
	7.80	7.81 (0.002)	7.84 (0.00)	2247.03 (0.68)	2254.13 (0.9)
	7.60	7.60 (0.00)	7.61 (0.002)	2263.43 (1.10)	2253.39 (1.15)
28	8.10	8.00 (0.002)	8.01 (0.007)	2246.57 (1.71)	2249.76 (1.74)
	7.80	7.80 (0.002)	7.85 (0.00)	2247.30 (0.88)	2251.30 (1.68)
	7.60	7.61 (0.002)	7.66 (0.00)	2252.96 (1.36)	2249.02 (1.75)
30	8.10	8.00 (0.002)	8.01 (0.034)	2247.84 (1.00)	2252.87 (0.53)
	7.80	7.81 (0.002)	7.85 (0.002)	2249.18 (1.39)	2256.40 (1.19)
	7.60	7.61 (0.002)	7.67 (0.007)	2246.05 (2.47)	2258.36 (1.34)

Table S2 Mean seawater treatment conditions: $p\text{CO}_2$ values (μatm) and calcite saturation states (ΩCa) analysed using data for total alkalinity (mean $2328.74 \pm 4.73 \mu\text{mol.kg}^{-1}$), salinity (mean 35.18 ± 0.08) and CO2SYS (Pierrot et al., 2006), $n = 10$.

Temperature ($^{\circ}\text{C}$)	26			28			30		
pH	8.1	7.8	7.6	8.1	7.8	7.6	8.1	7.8	7.6
$p\text{CO}_2$	477.0 (5.86)	1060.5 (8.73)	1894.7 (12.96)	480.3 (5.88)	1072.2 (8.92)	1919.3 (12.79)	484.4 (6.03)	1088.0 (9.25)	1953.1 (13.19)
ΩCa	4.85 (0.04)	2.72 (0.01)	1.69 (0.01)	5.03 (0.05)	2.84 (0.01)	1.48 (0.01)	5.29 (0.05)	3.01 (0.02)	1.87 (0.01)

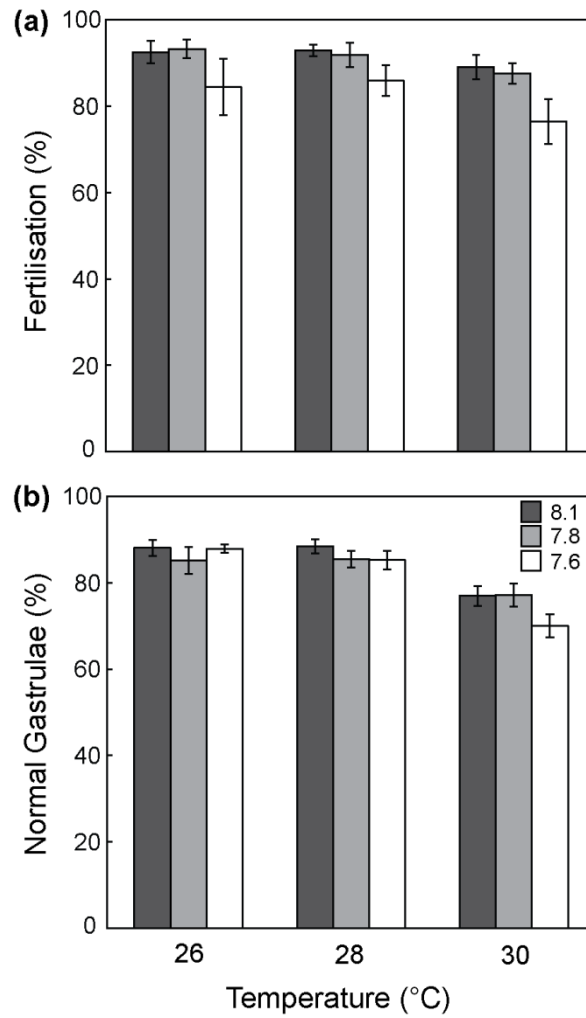


Fig. S1 Percentage normal (a) fertilised and (b) hatched gastrula of *Acanthaster planci* in nine treatments (three temperature \times three pH). Values are means \pm SE; $n = 10$.