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Larval phenotypic plasticity in the boom-and-bust crown-of-thorns seastar, *Acanthaster planci*

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ABSTRACT: Many echinoderm larvae exhibit phenotypic plasticity: a change in phenotype in response to environmental food levels. We investigated phenotypic plasticity in the larvae of the crown-of-thorns seastar *Acanthaster planci*, an opportunistic boom-and-bust species with larvae that have a strong response to food conditions. The increased predation pressure resulting from outbreaks (population explosions) of *A. planci* is deleterious to coral reefs, but the link between population outbreaks and larval ecology is poorly understood. We hypothesised that the larvae of *A. planci* would have a different morphological profile in the oligotrophic conditions typical of tropical waters than in the eutrophic conditions associated with increased nutrients. We predicted that larvae reared in low food conditions would increase their ciliated band length to enhance feeding potential. Larvae were fed algal concentrations representing starvation (0 cells ml^{-1}), low food (oligotrophic; 1000 cells ml^{-1}), high food (eutrophic; 10 000 cells ml^{-1}) or excessive food (100 000 cells ml^{-1}) conditions. A phenotypic response was evident. Larvae in the 2 high food treatments had a shorter ciliated band length relative to body size. Conversely, larvae in the starvation and low food treatments had longer ciliated bands relative to body size, a change that would enhance particle capture capacity and facilitate larval success. This plastic response of the larvae of *A. planci* could have flow-on effects to adult populations, potentially facilitating population outbreaks.

KEY WORDS: Asteroidea · Bipinnaria · COTS · Feeding larvae · Nutrients

INTRODUCTION

Phenotypic plasticity, a change in phenotype in response to environmental factors, is an important trait that can enhance the success of many marine and freshwater invertebrates (Strathmann et al. 1992, Repka et al. 1999, Klinzing & Pechenik 2000, Agrawal 2001). Plasticity allows species to fine-tune their allometry (the differential growth of body parts) through the allocation of energetic reserves to different features (Strathmann et al. 1992, 1993, Fenaux et al. 1994, Klinzing & Pechenik 2000, Byrne et al. 2008, Soars et al. 2009, Adams et al. 2011, Carrier et al. 2015). In many planktotrophic larvae, larval allometry is affected by food availability (Paulay et al. 1985, Fenaux et al. 1994, George 1999, Morgan 2008, Soars et al. 2009). There appears to be a feedback mechanism between algal cell concentrations in the environment and the disproportionate growth of different body parts (Miner 2007), regulated by changes in molecular signalling and gene expression that facilitate plastic growth (Adams et al. 2011, Carrier et al. 2015).

When reared in low food level conditions, some larvae increase the size of their food-catching structures, thereby expanding their feeding capacity (Strathmann et al. 1992, Fenaux et al. 1994, Hart & Strathmann 1994, Byrne et al. 2008, Soars et al.

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2009). This response occurs in a diversity of taxa, including increased ciliary band length in echinoderm larvae (Strathmann et al. 1992, George 1994, 1999, Reitzel & Heyland 2001, Podolsky & McAlister 2005, Morgan 2008, Sun & Li 2013), larger ciliated velum structures in mollusc larvae (Strathmann et al. 1993, Klinzing & Pechenik 2000, Pechenik et al. 2002), larger filter screens and longer setae in waterfleas (Repka et al. 1999), and a greater number of fan-rays in simuliid fly instars (Lucas & Hunter 1999). Food limitation or starvation also extends the planktonic duration of marine larvae, facilitating greater dispersal but exposing them to a higher risk of predation (Rumrill 1990, Strathmann et al. 1992, Fenaux et al. 1994, George 1996). In contrast, larvae reared in high food conditions can reduce resource allocation to feeding structures, exhibiting a heterochronic shift in developmental timing and allocating more resources to the promotion of early onset of juvenile formation (Strathmann et al. 1992). This reduces the duration of the vulnerable planktonic phase (Lamare & Baker 2001), with potentially positive flow-on effects for benthic life stages.

Larval morphology can be a useful indicator of natural nutrient conditions and provide an ecologically relevant metric for developmental history and dispersal (Hart & Scheibling 1988, James & McCulloch 1990, Strathmann et al. 1992, Fenaux et al. 1994, Bennett et al. 1995, Bertram & Strathmann 1998). The ability of planktotrophic larvae to respond to changes in nutrient availability is well documented for the Echinodermata, including ~21 echinoplutei (Soars et al. 2009), 4 ophioplutei (Podolsky & McAlister 2005), 2 auriculariae (Morgan 2008, Sun & Li 2013), and 2 bipinnariae (George 1994, 1999) species. Phenotypic plasticity has been noted for the bipinnaria larvae of *Pisaster ochraceus* and *Luidia foliolata*, with underfed larvae developing longer and wider bodies, thus increasing ciliated band length (George 1994, 1999). However, phenotypic plasticity is yet to be reported for other asteroid species (Olson 1987, George et al. 1991).

The crown-of-thorns seastar *Acanthaster planci* is an ecologically important corallivore that has contributed to coral reef decline throughout the Indo-Pacific (Birkeland & Lucas 1990, Lourey et al. 2000, Pratchett et al. 2014). *A. planci* has the typical boom-and-bust population pattern seen in many opportunistic echinoderm species (Uthicke et al. 2009). Outbreaks of this seastar have generated much concern for the future of coral reefs, especially in conjunction with other stressors such as cyclones, pollution, coral bleaching and climate change (Brodie & Waterhouse 2012, De'ath et al. 2012, Schaffelke et al. 2012). A wealth of research has focused on elucidating the causes of *A. planci* outbreaks (Pratchett et al. 2014), but links between outbreaks and larval ecology are poorly understood. The larval success–enhanced nutrients hypothesis suggests that waves of successful recruitment by cohorts of well-fed larvae are a key driver of *A. planci* outbreaks (Birkeland 1982, Fabricius et al. 2010, Hock et al. 2014, Pratchett et al. 2014). Recent studies have shown that the larvae of *A. planci* have a strong morphological response to environmental conditions including algal cell concentrations and temperature (Kamya et al. 2014, Lamare et al. 2014, Uthicke et al. 2015, Wolfe et al. 2015). This suggests that these larvae have a high capacity for plastic growth to take advantage of changes in resource (food) availability, a feature seen in many successful outbreaking and invasive species that exhibit rapid population growth (Davidson et al. 2011, Guttal et al. 2012, Quezada García et al. 2015).

Although the larval success–enhanced nutrients hypothesis has received considerable support, particularly for the Great Barrier Reef (GBR), observations of high-density populations of *A. planci* have also been reported in many areas where eutrophy from runoff is unlikely (e.g. French Polynesia, Kayal et al. 2012; Brunei, Lane 2012; the Chagos, Hawaii and Micronesia, Pratchett et al. 2014). Outbreaks of *A. planci* have also been documented for offshore reefs on the GBR that are not influenced by land runoff (Miller et al. 2015). This offers support for the larval resilience hypothesis, which posits that the larvae of *A. planci* are tolerant of low phytoplankton levels, and so are successful in the oligotrophic waters typical of coral reef ecosystems (Olson 1987, Olson & Olson 1989). Phenotypic plasticity of food capture structures may be a key feature of the ability of *A. planci* larvae to fine-tune their allometry with respect to environmental conditions to facilitate success in the planktonic stage, with flow-on effects to adult populations. Thus, it is imperative to develop a better understanding of the larval biology and morphology of this ecologically important organism.

We investigated the ability of the bipinnaria stage of *A. planci* to exhibit phenotypic plasticity in response to different food regimes. It has been demonstrated that seastar larvae reared at optimal or enhanced nutrient conditions (up to a limit) develop faster and have a higher success rate of reaching the juvenile stage (Lucas 1982, Paulay et al. 1985, Strathmann et al. 1993, George 1999, Pechenik 2006, Wolfe et al. 2015). For *A. planci*, phytoplankton concentrations of ~10 000 cells ml^{-1} (~1 µg chlorophyll l^{-1}) are

optimal for larval development in laboratory culture (Fabricius et al. 2010, Uthicke et al. 2015, Wolfe et al. 2015). For the GBR, this food level reflects background conditions in coastal waters and eutrophic conditions offshore following storm or flood events (Wolfe et al. 2015). Here, we reared larvae in a range of food concentrations including conditions representing starvation (0 cells ml^{-1}), low food representing oligotrophic conditions (1000 cells ml^{-1}), high food representing eutrophic conditions (10 000 cells ml^{-1}) and excessive food (100 000 cells ml^{-1}). We predicted that plasticity in response to these different food levels would be manifested in differences in larval allometry. Since the ciliated band is the major system that determines feeding ability, we predicted that the larvae of *A. planci* would exhibit phenotypic plasticity in the development of this structure in response to food levels. Larvae reared in low food conditions were expected to develop longer ciliated bands with respect to larval size, a change that would facilitate greater particle capture. Such phenotypic plasticity in the larvae of *A. planci* may be an important trait influencing population increases, as documented for outbreaking species in terrestrial systems (Davidson et al. 2011, Guttal et al. 2012, Quezada García et al. 2015).

MATERIALS AND METHODS

Specimen collection, fertilisation and rearing

Adult *Acanthaster planci* were collected by SCUBA divers in early November from the GBR near Cairns (16° 55′ S, 145° 46′ E), Australia. Individuals were transported to Coffs Harbour, Australia, and were acclimated in aquaria at the National Marine Science Centre, Southern Cross University, for several weeks at the ambient temperature calculated for the habitat during the time of collection (~27°C; eReefs: www.bom.gov.au/marinewaterquality/).

Two female and 2 male *A. planci* were used for spawning to generate a population of larvae. Ovaries were dissected from the females and rinsed with 1 µm UV-treated filtered seawater (FSW). Ovulation was induced using 10^{-5} M 1-methyl adenine in FSW. The eggs were released and collected after ~60 min, rinsed in FSW and checked microscopically for quality. The average (±SE) egg diameter for each female was 200 ± 2.9 and 205 ± 3.1 µm, respectively (n = 30 $female^{-1}$). The eggs from both females were then combined. Sperm was collected from the testes and checked for motility. The sperm from both males was combined with FSW and the number of sperm was counted using a haemocytometer. The eggs were fertilised at a sperm to egg ratio of 100:1. After ~15 min, fertilisation was checked and confirmed to be >90%, and the eggs were rinsed in FSW to remove excess sperm.

The embryos were reared at ~27°C in two 300 l culture containers in FSW with gentle aeration. After 24 to 36 h, actively swimming gastrulae were siphoned out and divided into 40 rearing containers containing 1000 ml FSW at a density of 1 larvae ml^{-1}. Each container was gently aerated from the base to ensure mixing of the water and to maintain high levels of dissolved oxygen. The experiment was conducted in a temperature-controlled room at 27 ± 0.2°C, which was monitored using a Thermodata logger (iB-Cod Type G). Water changes occurred daily over the course of the experiment by careful reverse filtration, siphoning off ~90% of the water inside containers through an 80 µm mesh filter. To ensure stable conditions across treatment containers, salinity 34.5 ± 0.16 (n = 16), pH 8.28 ± 0.007 (n = 16) and dissolved oxygen (DO) 100.7 ± 0.21% (n = 16) were checked daily before water renewal using a Hach HQd portable temperature-compensated multiprobe.

Experimental feeding treatments

Larvae were fed daily with the tropical microalgae *Proteomonas sulcata*, at 4 cell densities: 0, 1000, 10 000, and 100 000 cells ml^{-1} (n = 10 containers $treatment^{-1}$), representing chlorophyll *a* (chl *a*) levels of 0, 0.1, 1.0, and 10 µg chl *a* l^{-1}. *P. sulcata* is ~7 to 10 µm in length (Hill & Wetherbee 1986), an algal size known to be consumed by the larvae of *A. planci* (Okaji et al. 1997). The 4 food treatments were chosen to simulate conditions of starvation (0 cells ml^{-1}), those reflecting natural background levels of nutrients on the GBR (1000 and 10 000 cells ml^{-1}), and enhanced eutrophic conditions in runoff scenarios on the GBR (100 000 cells ml^{-1}), as determined from chl *a* levels at these algal concentrations (Wolfe et al. 2015). The conversion of algal cells to chl *a* was determined by spectrophotometric analysis of extracted cells across random days throughout the experiment. Chl *a* was extracted using 10 ml of 90% acetone (HPLC grade), with samples kept dark and cool (~4°C) for 18 to 24 h before analysing by spectrophotometry (Jeffery & Humphrey 1975). Chl *a* levels were calculated using the equation chl $a = (11.85 \times A_{664}) - (0.08 \times A_{630})$, where A_{664} and A_{630} are the absorbance of light at the wavelengths

of 664 and 630 nm, respectively, to determine the correlating algal cell density (Jeffery & Humphrey 1975).

Feeding began ~48 h post-fertilisation, once the larvae had a complete digestive tract. Daily water changes and renewal of food ensured that larval feeding did not modify the overall density of *P. sulcata* in each treatment throughout the experiment (see Table A1 in the Appendix). This was further confirmed by spectrophotometric assays of chl *a* levels in treatment water after 24 h and prior to water changes on random days throughout the experiment. Larval density and mortality were monitored across the experiment to achieve a consistent food ration per larva. Water levels were adjusted to ensure that larval density remained at ~1 larva ml^{-1}.

Larval development

By Day 7, the larvae were well-developed bipinnariae (Fig. 1), a stage where developmental plasticity has been recorded for seastars (George 1994, 1999). On Day 7, larvae were randomly collected from each replicate container using a pipette, and were placed in 7% $MgCl_2$ for ca. 15 min to relax. The bipinnariae were then fixed in 4% paraformaldehyde in FSW. The first 10 larvae encountered from each replicate were then promptly photographed using a camera mounted on an Olympus DP26 microscope to avoid post-fixation change. Larval length and width, posterior width, gut surface area and ciliated band length (Fig. 1) were measured using ImageJ software (National Institute of Health). The gut perimeter was traced as indicated in Fig. 1, and the surface area of the gut was calculated in ImageJ. The ciliated bands were traced around the oral and gut hood perimeters, and both larval sides. Total ciliated band length was calculated as the sum of these values. Means from 10 larvae $replicate^{-1}$ were used as the datum for analysis (n = 10). Ratios between ciliated band length and larval length and width were also calculated to examine the relationship between larval size and potential food capture.

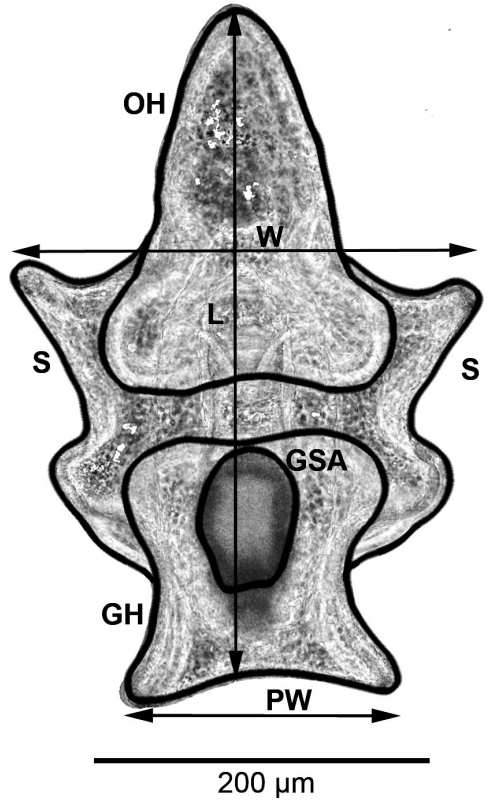

Fig. 1. *Acanthaster planci* bipinnaria on Day 7 showing the morphological traits measured. L: length, W: width, PW: posterior width, GSA: gut surface area. Ciliated band length = OH (oral hood) + GH (gut hood) + S: (larval sides)

The larvae were fed and monitored until Day 16, when we observed juveniles in some treatments. This was spontaneous settlement as no specific cue was added, and biofilm would have been minimal. All containers were thoroughly searched and the juveniles were counted.

Statistical analyses

Data for all size measurements were analysed using a 1-way ANOVA in JMP 9 (SAS Institute), with algal cell density as the fixed factor. These tests were performed to determine how morphological traits differed between feeding treatments. Assumptions of normality (Shapiro-Wilk tests) and homogeneity of variance (Levene's tests) were met for all data, as required for ANOVA (Quinn & Keough 2002). Post hoc Tukey's HSD test was used to determine differences between treatments.

Principal component analysis (PCA) was used to determine which variables contributed most to the differences in larval allometry in response to feeding treatments. Morphological data were square root-transformed before analysis to standardise variances. ANOSIM and pairwise tests were also performed to determine the relationship between morphological variables in response to feeding treatments. Algal concentration was used as a fixed factor with the 5 aforementioned morphological traits used as response variables. All statistical tests were performed using PRIMER v.6 (Clarke & Gorley (2006)).

RESULTS

Individual morphological parameters — univariate analyses

Larval length ($F_{3,38}$ = 7.68, p = 0.0011) and width ($F_{3,38}$ = 7.32, p = 0.0005) differed significantly across food treatments (Table 1, Fig. 2A,B). Post hoc Tukey's HSD test revealed that starved larvae (0 cells ml^{-1}) had the smallest length and width. Larvae fed 1000 and 10 000 cells ml^{-1} were larger in both length and width (Fig. 2A,B). Larvae reared in the highest food treatment (100 000 cells ml^{-1}) were smaller than the 2 mid-food treatments (Fig. 2A,B). Tukey's HSD test revealed similar results for the morphological measurements of posterior width ($F_{3,38}$ = 6.99, p = 0.0006) and ciliated band length ($F_{3,38}$ = 8.13, p = 0.0005) across food treatments (Table 1; Figs. 2C,D & 3). Larvae reared at the highest food treatment had the largest gut surface area ($F_{3,38}$ = 70.84, p = 0.0001), which decreased in size with reduced food ration (Table 1, Fig. 2E).

Ciliated band length differed significantly across treatments, with the longest bands seen in larvae reared at 1000 cells ml^{-1}, and the smallest at 0 and 100 000 cells ml^{-1} (Fig. 2D). Larvae in the no food treatment and those fed the lowest ration (1000 cells ml^{-1}) had a longer ciliated band length relative to length ($F_{3,38}$ = 23.72, p < 0.0001) and width ($F_{3,38}$ = 3.69, p = 0.0207) (Table 1, Figs. 3 & 4A,B). In contrast, larvae fed the highest rations (10 000 to 100 000 cells ml^{-1}) had a significantly smaller ciliated band length relative to larval size (Table 1, Fig. 4A,B).

Spontaneous settlement was observed in the presence of juveniles in containers of larvae fed 100 000 cells ml^{-1} (4 of 10 containers), 10 000 cells ml^{-1} (7 of 10 containers), and 1000 cells ml^{-1} (1 of 10 containers). Spontaneous settlement was highest in larvae reared at 10 000 cells ml^{-1}, while settlement did not occur in the no food treatment (see Fig. A1 in the Appendix).

Table 1. Results of 1-way ANOVAs of the morphological traits of larval *Acanthaster planci* measured with feeding treatment as a fixed factor; (*) indicates significance at p < 0.05

Morphological trait	Source	df	*F*-ratio	p-value
Larval length	Diet	3	7.68	0.0011*
	Residual	35		
	Total	38		
Larval width	Diet	3	7.32	0.0005*
	Residual	35		
	Total	38		
Posterior width	Diet	3	6.99	0.0006*
	Residual	35		
	Total	38		
Gut surface area	Diet	3	70.84	0.0001*
	Residual	35		
	Total	38		
Ciliated band length	Diet	3	8.13	0.0005*
	Residual	35		
	Total	38		
Ciliated band: larval length	Diet	3	23.72	<0.0001*
	Residual	35		
	Total	38		
Ciliated band: larval width	Diet	3	3.69	0.0207*
	Residual	35		
	Total	38		

Larval allometry — multivariate analysis

PCA indicated that well-fed larvae differed morphologically from larvae in the low food treatments (Fig. 5). Larvae fed at 10 000 and 100 000 cells ml^{-1} had a larger gut surface area but a smaller width and ciliated band length. While larval morphology was variable within feeding treatments, there was a significant effect of algal density on the overall allometry of the larvae (ANOSIM; p = 0.001). Pairwise comparison showed strong differences between the high food treatment and the low (R = 0.776) and no (R = 0.824) food treatments. The first PC explained 65.3% of variation between factors, however there was no clear differentiation based on feeding density for PC1 (Fig. 5). Data from the 2 high food treatments (10 000 and 100 000 cells ml^{-1}) clustered in the positive half for PC2, which accounted for 18.9% of total variation with a clear differentiation of larval width and ciliated band length towards lower algal treatments (Fig. 5).

DISCUSSION

The larvae of *Acanthaster planci*, a key species in the ecological dynamics of coral reefs, exhibited phenotypic plasticity in the development of feeding structures in response to different food levels. Food levels on a per-larva basis were tightly controlled in this study, which is likely to have facilitated the detection of developmental plasticity of ciliary band growth. How this capacity to adjust body form may contribute to the success of larvae in nature remains unknown. We suggest that the plastic response of *A. planci* larvae to variation in food availability is an

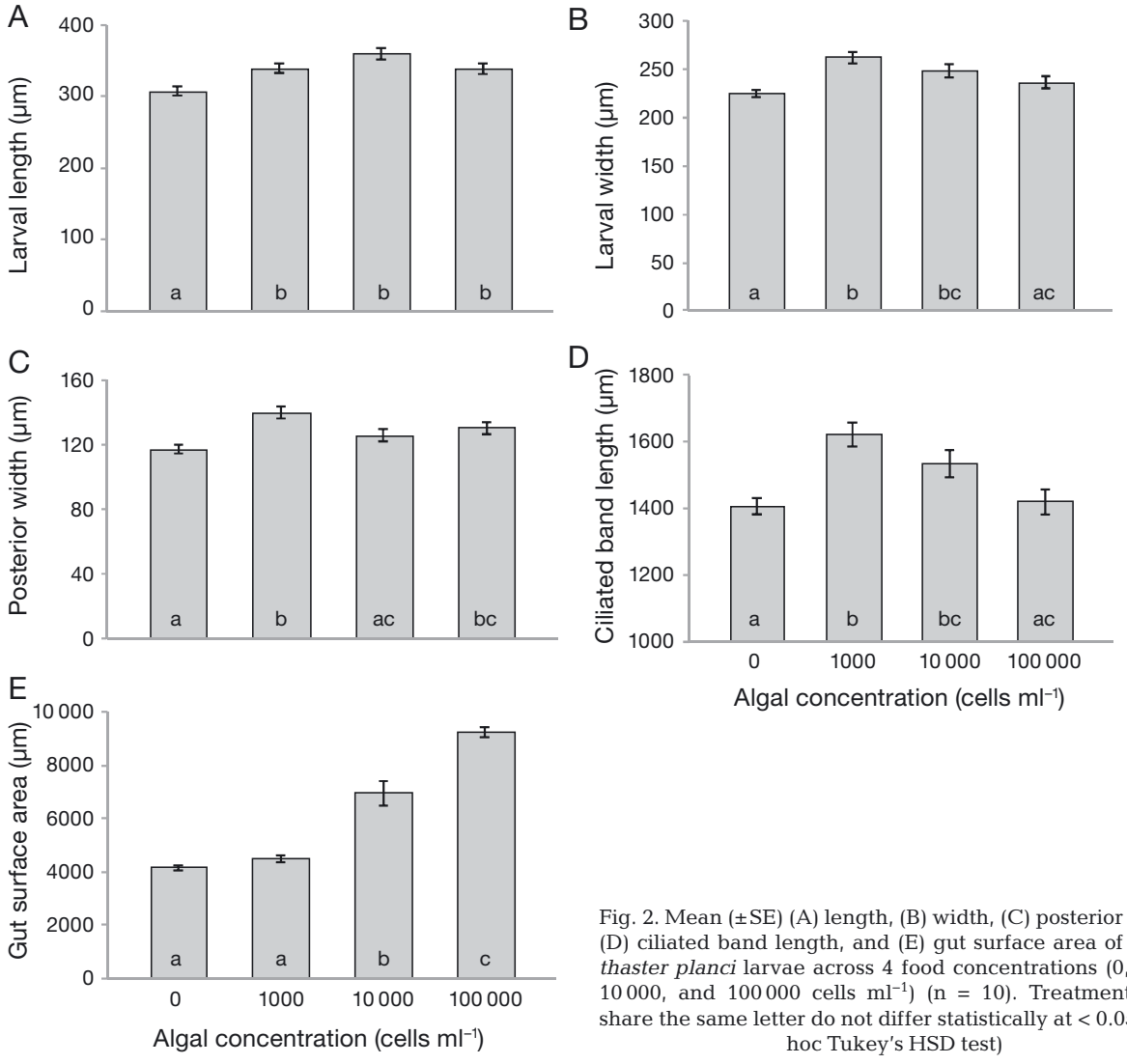

Fig. 2. Mean (±SE) (A) length, (B) width, (C) posterior width, (D) ciliated band length, and (E) gut surface area of *Acanthaster planci* larvae across 4 food concentrations (0, 1000, 10 000, and 100 000 cells ml^{-1}) (n = 10). Treatments that share the same letter do not differ statistically at < 0.05 (post hoc Tukey's HSD test)

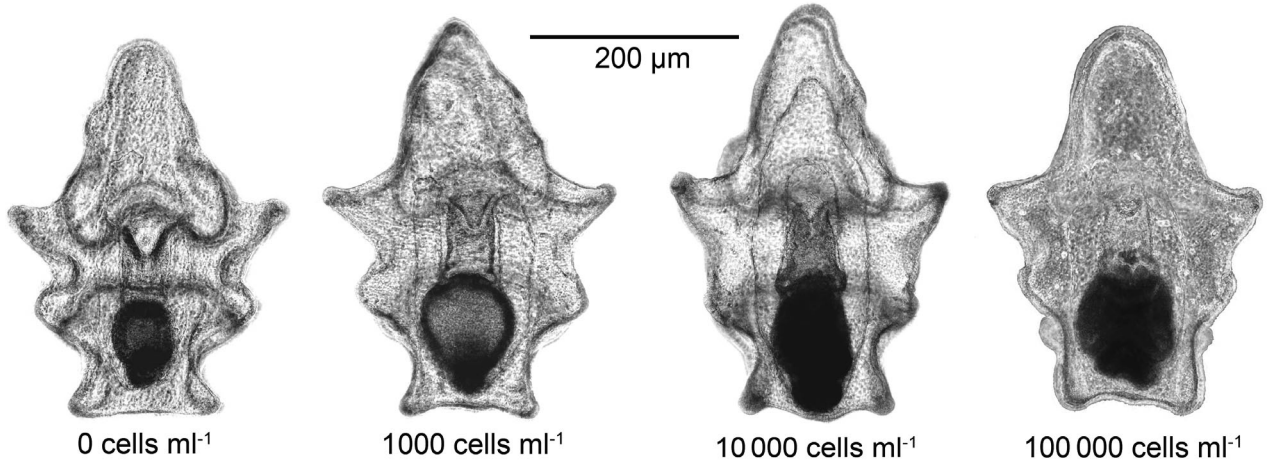

Fig. 3. *Acanthaster planci* larvae from each food concentration. Note contours and undulating profile of the larval body in the 0 and 1000 cells ml^{-1} food treatments, increasing the length of the ciliated bands

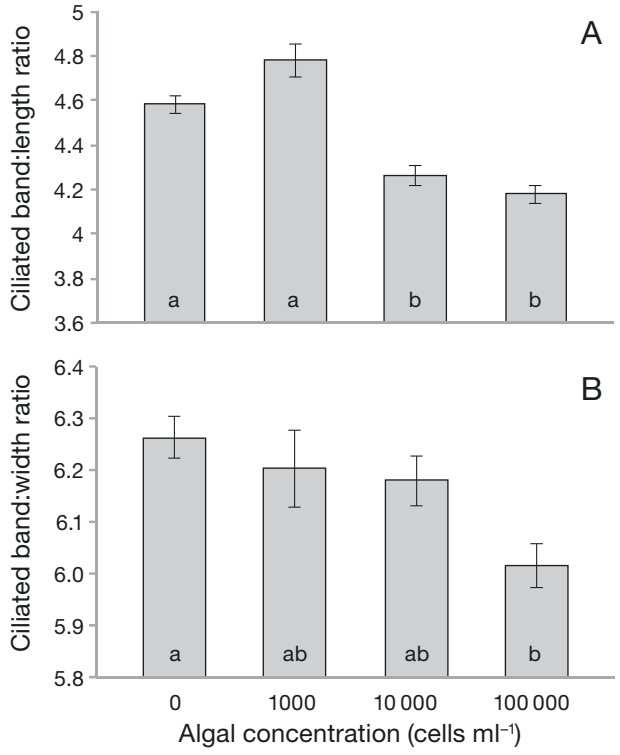

Fig. 4. (A) Mean (±SE) ratio of ciliated band length to larval length and (B) ciliated band length to larval width of *Acanthaster planci* larvae across 4 food concentrations (0, 1000, 10 000, and 100 000 cells ml^{-1}) (n = 10). Treatments that share the same letter do not differ statistically (post hoc Tukey's HSD test)

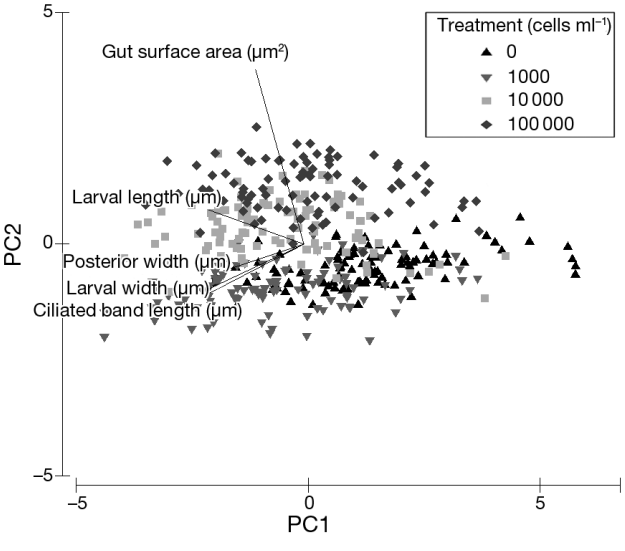

Fig. 5. Principal component analysis (PCA) plot of the 5 morphological traits measured for *Acanthaster planci* larvae on Day 7 across 4 feeding concentrations (0, 1000, 10 000, and 100 000 cells ml^{-1}) (n = 100 treatment^{-1})

important feature for establishing outbreak populations, as documented for outbreaking and invasive species in terrestrial systems (Davidson et al. 2011, Guttal et al. 2012, Quezada García et al. 2015). Phenotypic plasticity is likely to be one of the many traits of the larvae of *A. planci* that influences its feeding biology and overall success.

A. planci larvae that were fed a low algal concentration (0 or 1000 cells ml^{-1}) exhibited disproportionate growth of their ciliary band feeding structures relative to length and width. This is similar to that documented for the planktotrophic larvae of echinoids, ophiuroids, holothuroids, and other asteroids, where food limited larvae have longer ciliary bands thereby increasing their capacity for food capture (George 1999, Sewell et al. 2004, Podolsky & McAlister 2005, Morgan 2008, Soars et al. 2009, Sun & Li 2013). These observations for larvae across phylogenetically diverse groups support the notion that larval phenotypic plasticity is a trait characteristic of the Echinodermata, although not all species demonstrate this response (Soars et al. 2009).

A. planci larvae in our higher food treatments (10 000 and 100 000 cells ml^{-1}) were larger than food-limited larvae. Similar results have been documented in a plethora of studies of the growth of planktotrophic larvae of echinoderms and other invertebrates in laboratory culture, as well as for this species in recent studies (Uthicke et al. 2015, Wolfe et al. 2015). Although *A. planci* larvae in the higher food treatments were larger than those that were food limited, they had a smaller ciliated band length with respect to larval size. When food supply is plentiful, larvae do not need to increase their ciliated band structures to achieve maximum food capture. Instead, well-fed larvae can shift allocation of nutrients to focus on development of juvenile structures, such as the rudiment, leading to earlier metamorphosis (Strathmann et al. 1992, George 1999, Pechenik 2006). This was supported by the presence of juveniles after only 16 d in high food treatments. Whether food limited or enriched, the ability to exhibit such developmental responses to changes in food availability would be expected to enhance fitness, supporting increased survival into adulthood and reducing the risks associated with developing in the plankton (Rumrill 1990, Strathmann et al. 1992). Phenotypic plasticity of *A. planci* larvae may contribute to the opportunistic nature of these larvae and facilitate their success on coral reefs.

Phenotypic plasticity of planktotrophic larvae may be influenced by egg size, the nature of egg energetic reserves, and the length of the facultative feed-

ing period (FFP) (George 1996, Reitzel & Heyland 2001, Podolsky & McAlister 2005, Prowse et al. 2008, Moran et al. 2013). During the FFP, the larvae are supported by maternal nutrients, which allows them to develop in the absence of exogenous food. This reduces the risk of starvation in the crucial transition period between embryonic development and the onset of a feeding larva (Byrne et al. 2008). Early larval success is therefore influenced by maternal condition and the quality of the eggs produced (George 1996, Bertram & Strathmann 1998). The length of the FFP of echinoderm larvae increases with egg size, and so species that produce larger eggs and higher nutrient levels (Moran et al. 2013) have a longer buffer period to facilitate early larval development in the absence of food (McEdward 1986, Miner et al. 2005, Pechenik 2006, Byrne et al. 2008). Species with a greater dependence on early feeding (i.e. smaller egg size) are therefore predicted to have a greater phenotypic plasticity and are able to adjust their phenotype to environmental conditions earlier in development (Herrera et al. 1996, George 1999). The phenotypic response of sand dollar and brittlestar species with smaller eggs, for example, is greater than closely related species with larger eggs (Reitzel & Heyland 2001, Podolsky & McAlister 2005).

The question remains as to why *A. planci* has such disproportionate success compared with other tropical asteroids (Pratchett et al. 2014). *A. planci* produces large eggs (≥180 µm diameter) compared to other tropical asteroids with planktotrophic larvae, such as *Linckia laevigata* (150 µm) and *L. guildingii* (110 µm) (Emlet et al. 1987). This also contrasts with other tropical echinoderms that have a similar planktonic life history but produce smaller eggs (<100 µm) (Prowse et al. 2009, Falkner et al. 2015). Thus, the composition of the large eggs of *A. planci* larvae would be predicted to convey a long larval FFP with significant buffering against starvation during early development. This would facilitate early larval success in the oligotrophic low food conditions typical of reef waters. A long FFP would also assist in bridging development under low nutrient conditions, while phenotypic plasticity would assist in fine-tuning allocation to growth before the maternal resources are exhausted and feeding becomes essential (Prowse et al. 2008, 2009), or in response to pulses of elevated nutrients. Moreover, it appears that the pre-feeding stages of echinoderms can gain information from the environment through the detection of chemical or physical cues from algal cells before they develop the ability to feed, prompting an early plastic response when necessary (Miner 2007, Adams et al. 2011), as seen for *A. planci* larvae in the no- and low food treatments here.

While most studies of phenotypic plasticity in echinoderm larvae have used a range of algal concentrations, we also examined the effects of starvation (no food) on the growth and development of *A. planci* larvae. Starved larvae are typically smaller and have delayed development compared to well-fed cohorts (Lucas 1982, Allison 1994, Bertram & Strathmann 1998, George 1999, Sewell et al. 2004, Sun & Li 2014). Larvae reared at starvation or very low nutrient levels may not be able to develop beyond early larval stages once the FFP has passed, experiencing high mortality in the plankton and/or becoming arrested at a point of no return when maternal nutrients are exhausted (Prowse et al. 2008). While it remains to be tested whether starved larvae can recover from nutrient limitation, the plastic growth documented here for starved larvae and those fed at algal densities similar to background levels that appear typical for the GBR (~1000 cells ml^{-1}) suggests that *A. planci* larvae are resilient to the low nutrient conditions typical of tropical oligotrophic waters. This may explain *A. planci* outbreaks in areas that do not correlate to runoff-induced eutrophy on the GBR (Miller et al. 2015) and elsewhere (e.g. Kayal et al. 2012, Lane 2012, Pratchett et al. 2014). In these instances, it has been suggested that the larvae may be benefiting from alternate nutrient sources (Olson & Olson 1989) or oceanographic processes such as upwelling (Lane 2012, Miller et al. 2015). It has also been suggested, however, that the natural levels of alternative food sources are insufficient for the larvae of *A. planci* (Hoegh-Guldberg 1994).

Algal concentrations of 10 000 cells ml^{-1} (representing ~1 µg chl l^{-1}) are optimal for the growth and performance of *A. planci* larvae in laboratory culture (Uthicke et al. 2015, Wolfe et al. 2015). In the present study, larvae reared in the highest food treatment were likely to be overfed, which has deleterious consequences (Wolfe et al. 2015). Very high levels of chlorophyll (≥10 µg l^{-1}), observed in nature during highly eutrophic runoff scenarios, may cause increased mortality and reduced larval success (Wolfe et al. 2015). However, it is not known whether starved or poorly fed *A. planci* larvae can avail of short-lived pulses of extreme eutrophy to enhance their nutritive state and hence their success. Overall, the plastic response of *A. planci* larvae to high food availability (to a limit) would allow a shift in allocation to other aspects of development, thereby reducing the larval period and increasing overall success to settlement (Strathmann et al. 1992, Wolfe et al.

2015). We observed spontaneous settlement in the high food treatments, particularly at the optimal food level (10 000 cells ml^{-1}). Since larvae reared in these conditions also have the greatest settlement success and juvenile size (Wolfe et al. 2015), it is likely that there was a heterochronic shift to the settlement-stage brachiolaria larvae and faster development of the juveniles, as seen for sea urchins (Strathmann et al. 1992, Fenaux et al. 1994). This suggests that increased nutrients enhance the growth and survival of *A. planci* larvae (e.g. Fabricius et al. 2010, Pratchett et al. 2014), but that there is an upper limit where overfed larvae suffer higher mortality (Wolfe et al. 2015).

It has been suggested that food is required through late larval stages to facilitate successful metamorphosis for echinoderm (Allison 1994, Sun & Li 2014) and mollusc larvae (Pechenik et al. 2002). Variation in food availability at different stages of larval development may affect larval fitness and overall survival (Allison 1994, Fenaux et al. 1994, Hentschel & Emlet 2000, Pechenik et al. 2002, Pechenik 2006, Sun & Li 2014). Chlorophyll levels are naturally variable in nature depending on proximity to the coastline and influxes from upwelling, runoff, and storm events (Devlin et al. 2001, Brodie et al. 2007, Lane 2012, Schaffelke et al. 2012). There may be an interesting interplay between food availability and the FFP for the larvae of *A. planci*, whereby larvae buffered by maternal nutrients could take advantage of short-lived pulses of increased food supply, thereby accelerating development to brachiolaria and juvenile stages. The larvae could also exhibit a plastic response to natural fluctuations in eutrophy by enhancing their ability to capture scarce food. The opportunistic and plastic nature of this larva suggests particular resilience to eutrophic and oligotrophic conditions, supporting the larval success–enhanced nutrients hypothesis. However, additional research is needed to examine the larval response to maternal energetic lipids, natural fluxes in eutrophy during early stages of development, the dynamics of nutrient utilisation, and larval gene expression (e.g. Prowse et al. 2008, Carrier et al. 2015).

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Appendix

Table A1. Mean (±SE) chlorophyll *a* concentrations (µg l^{-1}) between initial (I) feeding concentration and 24 h after (F) feeding (n = 5)

	0 cells ml^{-1} (0 µg l^{-1})	1000 cells ml^{-1} (0.1 µg l^{-1})	10 000 cells ml^{-1} (1.0 µg l^{-1})	100 000 cells ml^{-1} (10 µg l^{-1})
I	0.04 ± 0.06	0.12 ± 0.03	1.06 ± 0.08	10.1 ± 0.79
F	−0.13 ± 0.07	0.04 ± 0.07	0.95 ± 0.24	9.70 ± 0.53

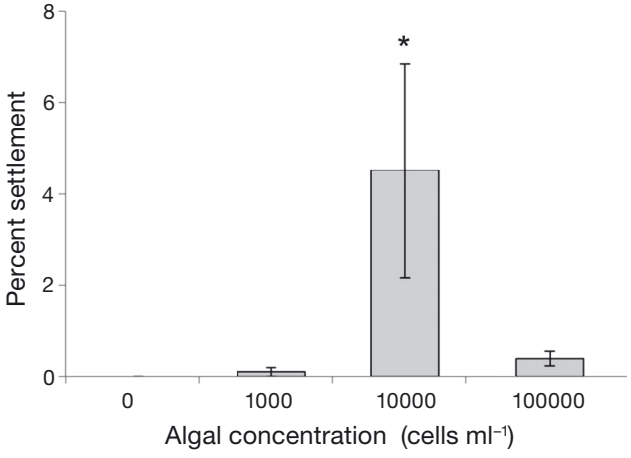

Fig. A1. Average percent larvae spontaneously settled in treatment containers on Day 16; (*) indicates significantly different treatments at $p < 0.05$ (post hoc Tukey's HSD test)

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