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Early metamorphosis is costly and avoided by young, but physiologically competent, marine larvae

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ABSTRACT: Dispersing organisms often cannot assess habitat quality directly, so they employ proxies (cues) to choose habitats that maximise fitness. Theory suggests organisms should settle as soon as they find appropriate cues in order to reduce physiological costs and mortality risk incurred whilst searching. We propose that for planktotrophic marine larvae, when resources are plentiful, development of adult structures during an extended larval phase provide post-metamorphosis benefits that offset the costs of remaining in the plankton. To test this, we measured fitness consequences of metamorphosis in response to habitat cues at a range of developmental maturities in 2 sea urchin larvae, *Tripneustes gratilla* and *Centrostephanus rodgersii*. We found larvae that were capable of responding to cues and settling accrued significant benefits if they extended their pelagic development. Compared to more developed larvae, larvae without adult structures took longer to metamorphose, and after metamorphosis were 11 to 25% smaller, 0.1 to 6 times more likely to lack defensive structures and 3 to 13 times more likely to have abnormal morphology. Most early settlers died within 1 wk compared to >40% survival for more developed larvae. We found larvae avoid the costs of early metamorphosis by only responding to low concentrations of cues in the water column once they have adult structures. Our results contrast with models of habitat selection that suggest organisms should settle in habitat quickly to minimise search costs. Incorporating the trade-off between the benefits of larval development and search costs into current models of habitat selection may provide new insights into how fitness consequences affect habitat selection.

KEY WORDS: Delayed metamorphosis · Development · Fitness · Habitat selection · Search costs

INTRODUCTION

Habitat selection can be a key determinant of Darwinian fitness (Jaenike & Holt 1991). For many organisms (e.g. amphibians, insects, marine invertebrates), a mobile life history stage chooses habitats for subsequent sessile stages with fitness consequences that can carry over to ensuing generations (Whitham 1980). Models of how dispersive life stages use habitat cues to choose habitat for sessile stages highlight that once they are physiologically capable, individuals should settle immediately on finding a cue indicating a high-quality habitat (Stamps et al. 2005, Elkin & Marshall 2007). This is because fitness should decline as dispersal time is extended as a result of accumulating mortality risk and the physiological cost of searching (Stamps et al. 2005, Elkin & Marshall 2007). To limit mortality risk and search costs, marine larvae can shorten pelagic duration by reducing specificity for cues that signal optimal habitat (Doyle 1975, Ward 1987), but incur costs such as reduced growth, survival and fecundity associated with living in sub-optimal habitats (Huey 1991, Huk & Kuhne 1999, Munday 2001). Organisms that incur low search costs or substantial disadvantage by settling in sub-optimal habitats should be inclined to maintain specificity (Stamps et al. 2005, Elkin & Marshall 2007).

Characteristics of dispersive life stages, such as ability to feed and vulnerability to predation, should affect the way in which they trade-off search costs

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against habitat choice (Elkin & Marshall 2007). One such characteristic of planktotrophic marine larvae might be their ability to continue to develop more advanced adult structures and store energy if they do not settle when they are first able to. For example, when exposed to chemicals that signal high-quality adult habitat, echinoderm larvae can metamorphose early in their development (e.g. Moss & Tong 1992, Davis 1994, Yazaki 1995, Mercier et al. 2000, Bishop & Brandhorst 2001, Takahashi et al. 2002, Dworjanyn & Pirozzi 2008, Sutherby et al. 2012). However, in the absence of settlement cues and if sufficient food is available, echinoderm larvae develop adult structures such as pedicellariae, spines and tube feet (Hinegardner 1969, Smith et al. 2008). Adult structures are energetically costly for invertebrate larvae to produce, are not used by larval stages and can reduce larval performance (Strathmann et al. 1992, Grunbaum & Strathmann 2003), but it is possible that these structures provide benefit during and after settlement. The possibility that larvae can actively avoid metamorphosing to develop adult structures and increase energy stores is underlined by the fact that the larvae of many marine species appear to have a specific biochemical mechanism to delay metamorphosis: nitric oxide (NO) signalling (Froggett & Leise 1999, Bishop & Brandhorst 2001, Bishop et al. 2001, Pechenik et al. 2002, Bishop & Brandhorst 2003, Pechenik et al. 2007, Romero et al. 2013). If, as current theory suggests, marine larvae should respond to settlement cues as soon as possible to minimise search costs (Stamps et al. 2005, Elkin & Marshall 2007), why then would larvae have mechanisms to prevent metamorphosis in the presence of settlement cues and consume resources by continuing to develop adult structures after they are able to metamorphose?

We hypothesise that (1) larvae that are capable of responding to settlement cues but are yet to develop adult structures may incur costs by metamorphosing in response to settlement cues compared to larvae that have developed adult structures; and (2) the presence of these costs should result in behaviours that allow larvae to avoid chemical cues that induce settlement before these structures are developed. We test these hypotheses using larvae of 2 ecologically important sea urchins, the tropical Indo-Pacific *Tripneustes gratilla* and the temperate Australasian *Centrostephanus rodgersii*. Both of these sea urchins are important habitat modifiers, denuding areas of foliose macroalgae at high densities (Valentine & Edgar 2010, Byrne & Andrew 2013), and have been implicated in climate change-driven range extensions that may result in ecological phase shifts (Ling 2008, Ling et al. 2009, Valentine & Edgar 2010). To establish whether there are costs associated with settlement early in larval development, *T. gratilla* and *C. rodgersii* larvae were exposed to known settlement cues at different stages of larval development. The time required to accomplish metamorphosis and post-settlement fitness were measured. We found significant costs of early settlement in response to cues. To test if larvae have a mechanism to avoid these costs, we measured behavioural responses of larvae at different developmental stages to waterborne settlement cues.

MATERIALS AND METHODS

Effect of larval development on settlement, post-metamorphic development and post-settlement survival

Sea urchin larvae were cultured according to Mos et al. (2011) (*Tripneustes gratilla*) and Swanson et al. (2012) (*Centrostephanus rodgersii*). Larvae with multiple pedicellariae and/or tube feet appeared in the cultures from Day 19 (*T. gratilla*) or Day 30 (*C. rodgersii*). For each experiment, larvae of the same age were sorted into stages based on the presence of larval and adult anatomical structures. For *T. gratilla* there were 4 stages: 'Stomach', the least developed stage with no rudiment or a rudiment <50% of the size of the stomach; 'Rudiment', possessing a rudiment equivalent to the size of the stomach; 'Pedicellariae I', possessing a stomach, rudiment and 1 or 2 pedicellariae, but no tube feet protruding from the vestibule; and 'Pedicellariae II', the most developed stage, possessing a stomach, rudiment, 3 or 4 pedicellariae and/or tube feet protruding from the vestibule (Fig. 1a). For *C. rodgersii* there were 3 stages. The first 2 stages, 'Stomach' and 'Rudiment', were the same as for *T. gratilla*, but all larvae possessing pedicellariae and tube feet protruding from the vestibule were grouped because these structures were developed simultaneously (designated 'Pedicellariae'; Fig. 1b).

Settlement assays tested the effects of larval development stage on metamorphosis and post-settlement survival. *T. gratilla* were induced to settle using pieces (~15 mm^2) of the geniculate coralline seaweed *Corallina officinalis* as a cue (Dworjanyn & Pirozzi 2008, Mos et al. 2011). *C. rodgersii* were induced to settle using ecologically relevant cues: pieces of the brown seaweed *Sargassum linearifolium* (~15 mm^2),

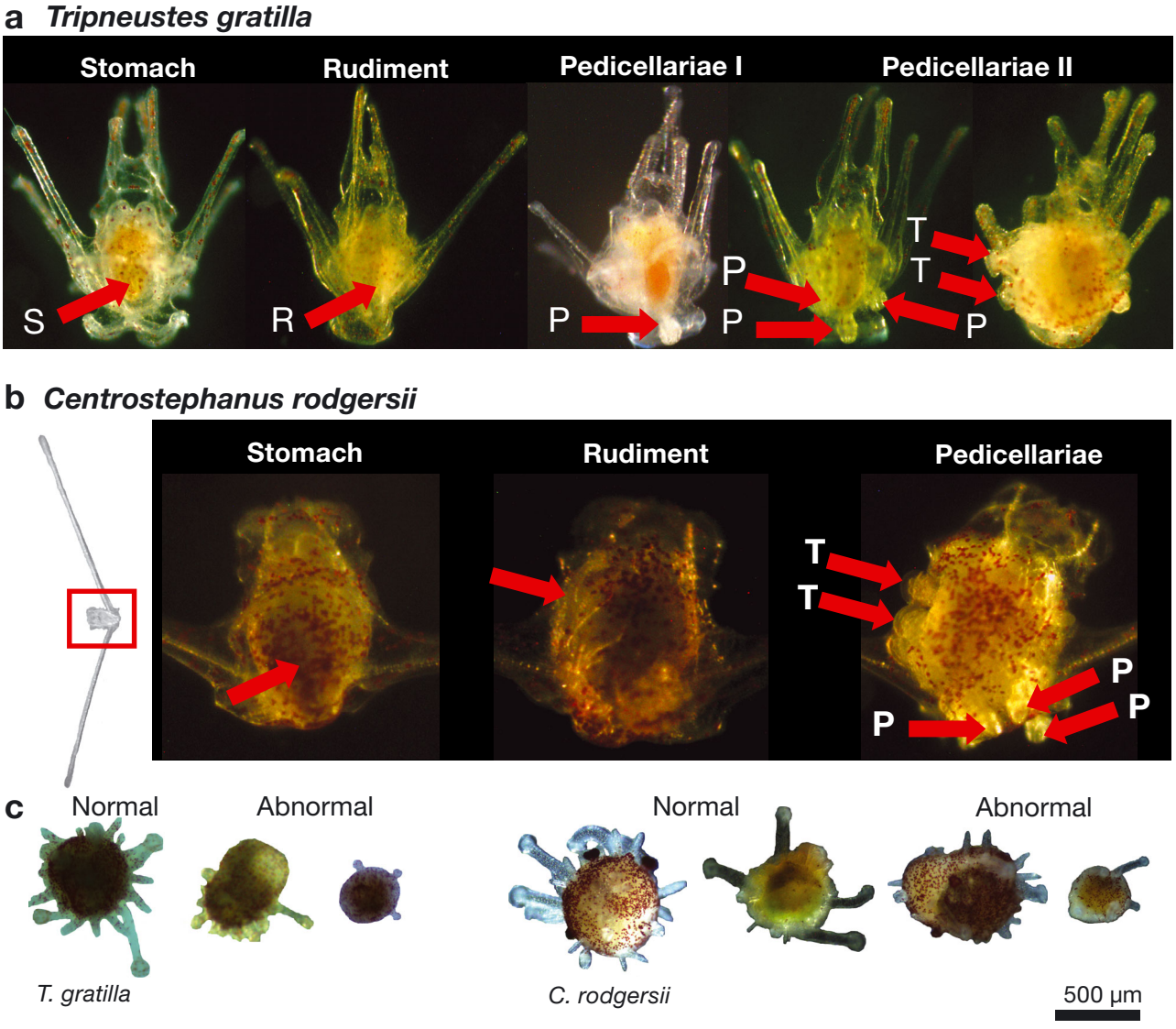

Fig. 1. Morphology of larval and juvenile sea urchins *Tripneustes gratilla* and *Centrostephanus rodgersii*. (a) *T. gratilla*: Stomach, Rudiment, Pedicellariae I and II identify 4 nominal larval stages. Red arrows and letters indicate the distinguishing larval structures of each stage. (b) *C. rodgersii*: Stomach, Rudiment and Pedicellariae identify 3 nominal larval stages. Red arrows and letters indicate the distinguishing larval structures of each stage. (c) Normal and abnormal morphologies of *T. gratilla* and *C. rodgersii* juveniles. S: stomach; R: rudiment; P: pedicellaria; T: tube foot

pieces of *C. officinalis* (~15 mm^2) or histamine dihydrochloride (Sigma-Aldrich) at a concentration of 0.1, 1, 10 or 100 µM in autoclaved seawater (ASW; filtered 0.5 µm, autoclaved and left to stand for >48 h) (Swanson et al. 2012). The pieces of the seaweeds were excised from independent fronds of multiple individuals and 1 piece was used in each replicate. Settlement assays were done in plastic Petri dishes (36 mm Ø, Sarstedt) containing 4.0 ml of ASW, 20 to 30 larvae and a cue. A total of 10 (*T. gratilla*) or 7 (*C. rodgersii*) replicates were used for each treatment. ASW-only controls were used in all assays. Assays were conducted in a temperature-controlled laboratory (25°C *T. gratilla*, 23°C *C. rodgersii*) under 16 h light:8 h dark photoperiod.

Settlement was assessed for *T. gratilla* every 6 h for a total of 48 h, and for *C. rodgersii* at 24, 48 and 72 h after the start of the settlement assay. Larvae were classed as metamorphosed if they had everted their rudiment, were attached to a surface via tube feet and had partially or fully absorbed their larval structures. Settlement rates were calculated as the percentage of larvae that metamorphosed out of the total number of larvae added to each replicate. Settled urchins were scored as dead or alive, and on the presence of spines and abnormalities. Individuals

were abnormal if they had irregular test shape, abnormal colouration and were >50% smaller than other juveniles in the same treatment (Fig. 1c). Test diameters of juveniles were measured from digital photographs as the average of 2 perpendicular lengths at the longest axis using ImageJ (NIH). For *C. rodgersii*, only *C. officinalis* and 100 µM histamine treatments were assessed, because of low settlement rates to 0.1, 1 and 10 µM histamine treatments and high mortality rates of newly settled juveniles for all larval stages in the *S. linearifolium* treatment after 72 h. *C. rodgersii* juveniles in the Stomach stage treatment were not scored because all were dead by 72 h.

To follow post-settlement survival, juveniles were transferred in seawater via pipette into new containers consisting of a 100 ml plastic jar with a window covered with 250 µm mesh that maintained the water level at 30 ml, inoculated with the diatom *Nitzschia closterium* to form a layer of living cells on the bottom (Mos et al. 2011). This method of moving post-larvae has no discernible effect on survival (e.g. Wolfe et al. 2013). *T. gratilla* were haphazardly pooled into 3 replicates per treatment. Replicates were supplied with preheated (*T. gratilla* 25.1 ± 0.4°C, mean ± SD) or ambient (*C. rodgersii* 19.8 ± 0.9°C) flow-through (180 ml h^{-1}) filtered seawater (FSW; 1.0 µm) and kept under 16 h light:8 h dark photoperiod. Aliquots (1 ml) of *N. closterium* (~2 to 3 × 10^6 cells ml^{-1}) were added daily from liquid cultures as food (Mos et al. 2011). Survival was microscopically assessed daily for *T. gratilla* and every 2 d for *C. rodgersii* to 14 and 29 d, respectively.

Effects of larval development on response to settlement cues

Response of larvae to the presence of dissolved settlement cues in the water column was tested for early (rudiment, no pedicellariae) and advanced (rudiment and 1 to 4 pedicellariae) larvae. *T. gratilla* were exposed to 0, 1, 10, 25, 50 or 100% *C. officinalis*-conditioned seawater in FSW. *C. officinalis*-conditioned seawater was made by adding 0.5 g *C. officinalis* per 100 ml of FSW and aerating for 24 h prior to use (Mos et al. 2011). *C. rodgersii* were exposed to 0, 1, 10 or 100 µM histamine dihydrochloride in FSW. Actively swimming *T. gratilla* and *C. rodgersii* larvae were sorted using a microscope (early or advanced) and added to replicates using a pipette. A total of 20 *T. gratilla* larvae per replicate were added to seven 125 ml cylindrical plastic containers (115 height × 40 Ø mm) per treatment, containing a total of 100 ml. *C. rodgersii* larvae (15 to 20), which are larger than *T. gratilla,* were added to 5 replicate containers (185 height × 120 Ø mm), containing a total of 1.75 l. The number of larvae actively swimming after 1 h was recorded, with results expressed as a percentage of the FSW control treatments. The number of larvae swimming in the FSW controls were (mean ± SE) 100 ± 0% early *T. gratilla*, 98.6 ± 1.4% advanced *T. gratilla* and 98.5 ± 1.5% each for early and advanced *C. rodgersii*. All swimming assays were conducted in darkness to avoid confounding effects of light on swimming behaviour (see Supplement 1 at www.int-res.com/articles/suppl/m559p117_supp.pdf).

The response of larvae to the presence of settlement cues created in the laboratory (described above) was compared to larval responses to seawater collected in marine habitats near Coffs Harbour, Australia. Seawater was collected on 21 January 2011 (*T. gratilla*) and 1 October 2010 (*C. rodgersii*) at approximately 1 m depth and 2 m above a macroalgae-dominated rocky reef, comprised of a mix of fleshy seaweeds (predominantly *Ecklonia radiata* and *Sargassum* spp.) and geniculate coralline seaweeds (predominantly *Corallina* spp. and *Amphiroa* spp.). These seaweeds act as settlement cues for *T. gratilla* (Dworjanyn & Pirozzi 2008, Mos et al. 2011) and *C. rodgersii* (Swanson et al. 2012). Adult sea urchins *C. rodgersii*, *Heliocidaris erythrogramma* and *Phyllacanthus parvispinus* are found in this habitat, and adult *T. gratilla* have been found in similar habitats within 10 km of the collection site (B. Mos pers. obs.). Seawater was also collected from approximately 1 m depth and 2 m above the substratum of a sandy beach located >250 m from the nearest known macroalgae-dominated rocky reef habitat to examine if larvae responded to seawater from a habitat unsuitable for adults. Water samples were filtered using filter paper (Whatman, grade 1) to remove large solids and used in swimming assays within 2 h of collection. The seawater was added to seven 125 ml plastic containers (115 height × 40 Ø mm) per treatment, containing a total of 100 ml. A FSW-only treatment was also used as a negative control (filtered to 1 µm and UV sterilized). A total of 10 *T. gratilla* or *C. rodgersii* larvae were added to each container and their position in the water column was recorded after 1 h, with results expressed as a percentage of the FSW control treatments. The number of larvae swimming in the FSW controls were 100 ± 0% early *T. gratilla*, 98.6 ± 1.4% advanced *T. gratilla*, 50.0 ± 2.1% advanced *C. rodgersii* and 84.0 ± 4.7% early *C. rodgersii*.

Statistical analysis

Data were analysed using permutational analysis of variance (PERMANOVA; Anderson 2001). A repeated-measures ANOVA was run for the *T. gratilla* settlement after 6, 18 and 48 h, with time and larval stage as fixed factors. For the *C. rodgersii* settlement assay and all of the swimming assays, 2-way PERMANOVAs were conducted using larval stage and settlement cue as fixed factors. Analyses of untransformed data were conducted using Euclidean distance, utilising approximately 9999 permutations of the raw data. Post hoc pairwise tests were performed if PERMANOVA results indicated that there were significant differences between treatments with 3 or more levels. For post hoc pairwise tests, a Monte Carlo procedure was used where the number of unique permutations was low. All statistical analyses were conducted using Primer 6 (Primer-E) with PERMANOVA$^+$ extension software (v.6.1.11).

RESULTS

Effect of larval development on settlement rate

All 4 *Tripneustes gratilla* stages settled in response to *Corallina officinalis* (Fig. 2), but there was no settlement in ASW-only controls (not presented). *T. gratilla* larvae at late larval stages settled at higher rates than larvae that were less developed (Fig. 2). Settlement in all treatments was significantly different after 6 h (Table S3 in Supplement 2 at www.int-res.com/articles/suppl/m559p117_supp.pdf; followed by post hoc pairwise tests, Pedicellariae II = Pedicellariae I > Rudiment > Stomach) but was not different between Pedicellariae stages after 18 h (Pedicellariae II = Pedicellariae I > Rudiment > Stomach). After 48 h, larvae with pedicellariae (>96 %) settled at higher rates than larvae with a large rudiment (80 %), which settled at a higher rate than larvae with a small rudiment or no rudiment (27 %) (Table S3; post hoc pairwise tests, Pedicellariae II = Pedicellariae I > Rudiment > Stomach). *T. gratilla* at late larval stages settled in response to *C. officinalis* faster than larvae that were less developed (Fig. 2). For example, all Pedicellariae II stage larvae had settled within 6 h; however, of the Stomach stage larvae that settled, 44 % settled after 6 h.

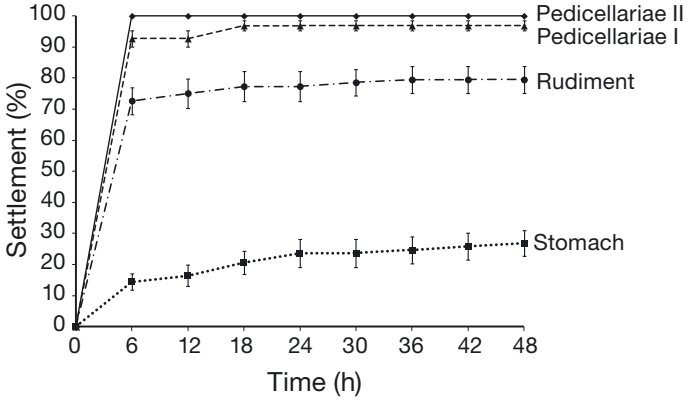

Fig. 2. Effect of larval development on settlement of *Tripneustes gratilla* in response to *Corallina officinalis* over 48 h. Stomach, Rudiment, Pedicellariae I and II identify 4 nominal larval stages (Fig. 1a). Settlement in all treatments was significantly different after 6 h (Table S3 in Supplement 2 at www.int-res.com/articles/suppl/m559p117_supp.pdf; followed by post hoc pairwise tests, Pedicellariae II = Pedicellariae I > Rudiment > Stomach), but was not different between Pedicellariae stages after 18 h (Pedicellariae II = Pedicellariae I > Rudiment > Stomach). Data are mean ± SE; n = 10

All *Centrostephanus rodgersii* stages settled to *C. officinalis*, *Sargassum linearifolium*, and 10 and 100 µM histamine, but not to ASW, or 100 nM or 1 µM histamine (Fig. 3, Table S4 in Supplement 2). Settlement rates varied with larval stage, with higher rates of settlement by more developed larvae (Fig. 3, Table S4). Larvae with pedicellariae settled at higher rates than those with only a rudiment, which settled at a higher rate than those larvae that did not have a rudiment (Fig. 3, Table S4; post hoc pairwise tests, Pedicellariae > Rudiment > Stomach), except for the 10 µM histamine treatment where settlement was not

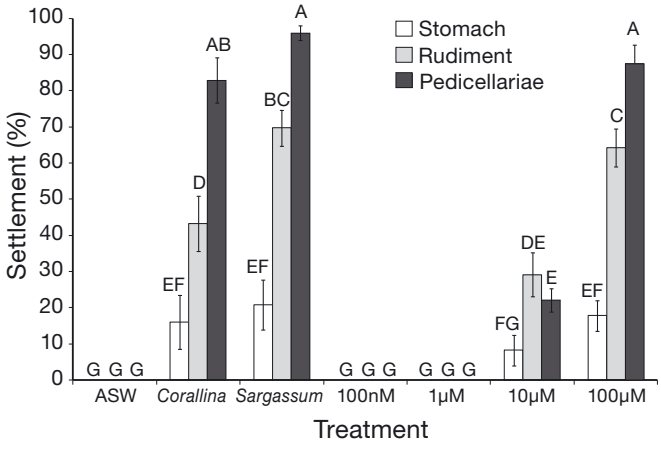

Fig. 3. Effect of larval development on settlement of *Centrostephanus rodgersii* in response to seaweeds and histamine after 72 h. ASW: autoclaved seawater; *Corallina*: *Corallina officinalis*; *Sargassum*: *Sargassum linearifolium*. Numbers on *x*-axis denote molar concentration of histamine dihydrochloride in ASW. Stomach, Rudiment and Pedicellariae identify 3 nominal larval stages (Fig. 1b). Bars with the same letters are not statistically different (Table S4 in Supplement 2 at www.int-res.com/articles/suppl/m559p117_supp.pdf; followed by post hoc pairwise tests). Data are mean ± SE; n = 7

different between the Rudiment and Pedicellariae stages (Fig. 3, Table S4; post hoc pairwise tests, Pedicellariae = Rudiment > Stomach).

Effect of larval development on post-metamorphic development

T. gratilla larvae that metamorphosed in response to *C. officinalis* at late larval stages had more spines, were bigger and had fewer abnormalities as juveniles than larvae that were less developed when they metamorphosed (Fig. 4a,b,c). There were significantly more juveniles that possessed spines in the Pedicellariae II treatment (98%) than in the Pedicellariae I treatment (82%), which had more than in the Rudiment (26%) and Stomach (16%) treatments, which did not differ (Fig. 4a; $F_{3,36}$ = 50.273, p < 0.0001; post hoc pairwise test, Pedicellariae II > Pedicellariae I > Rudiment = Stomach). Juveniles in the Pedicellariae II treatment (0.50 mm) were significantly larger than those in the Pedicellariae I treatment (0.45 mm), which were larger than in the Rudiment and Stomach treatments (both 0.37 mm) (Fig. 4b; $F_{3,34}$ = 37.099, p < 0.0001; post hoc pairwise test, Pedicellariae II > Pedicellariae I > Rudiment = Stomach). Abnormalities occurred at significantly lower rates in the Pedicellariae II treatment (7%) than in the Pedicellariae I treatment (29%), which was significantly lower than in the Rudiment (66%) and Stomach (72%) treatments, which did not differ (Fig. 4c; $F_{3,34}$ = 18.72, p < 0.0001; post hoc pairwise test, Pedicellariae II < Pedicellariae I < Rudiment = Stomach).

No *C. rodgersii* juveniles in the Stomach stage treatment survived to 72 h, so post-metamorphic development was not assessed for this treatment. *C. rodgersii* larvae that metamorphosed at late larval stages had more spines, were bigger and had fewer abnormalities as juveniles than larvae that were less developed when they metamorphosed (Fig. 4a,b,c). Larvae responded in the same manner regardless of whether they were induced to settle by histamine or *C. officinalis* (Fig. 4a,b,c, Table S5 in Supplement 2). There were significantly more juveniles that possessed spines in the Pedicellariae stage treatments (>95%) than in the Rudiment stage treatments (82 to 85%). Juveniles in the Pedicellariae stage treatments (0.65 to 0.67 mm) were significantly larger than those in the Rudiment stage treatments (0.59 to 0.60 mm) (Fig. 4b, Table S5). Abnormalities occurred at significantly lower rates in the Pedicellariae stage treatments (<2%) than in the Rudiment stage treatments (21 to 23%) (Fig. 4c, Table S5).

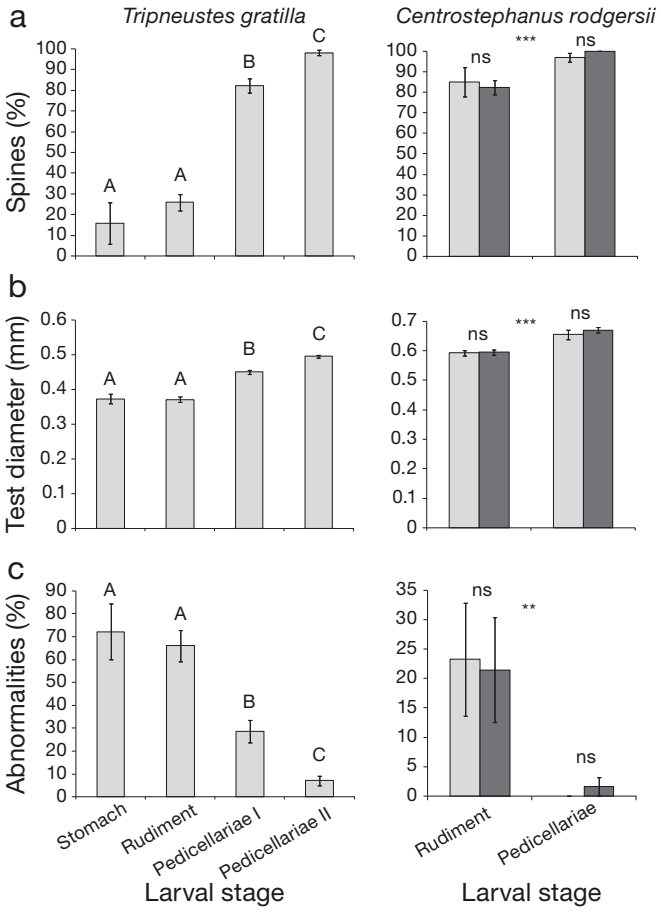

Fig. 4. Effect of larval development on (a) presence of spines, (b) test diameter and (c) occurrence of abnormalities for *Tripneustes gratilla* and *Centrostephanus rodgersii* 48 or 72 h, respectively, after exposure to *Corallina officinalis* (light grey bars) or 100 µM histamine (dark grey bars). Stomach, Rudiment and Pedicellariae (I and II) identify nominal larval stages (Fig. 1). Left panels: bars sharing uppercase letters are not significantly different from each other (PERMANOVA). Right panels: Significant differences between larval stages are indicated by **p < 0.01 and ***p < 0.001 (2-way PERMANOVA); treatment groups did not differ significantly (ns; Table S5 in Supplement 2 at www.int-res.com/articles/suppl/m559p117_supp.pdf). Data are mean ± SE; n = 7 to 10

Effect of larval development on post-settlement survival

T. gratilla juveniles from larvae that metamorphosed at late larval stages had higher survival to 14 d than juveniles from larvae that were less developed when they metamorphosed (Fig. 5a). There was rapid decline in the survival of juveniles from Stomach and Rudiment stage larvae after 3 d, falling to 8 and 34%, respectively, by Day 5 (Fig. 5a). In contrast, survival of juveniles from Pedicellariae I and II stage larvae to 14 d was always greater than 53% (Fig. 5a).

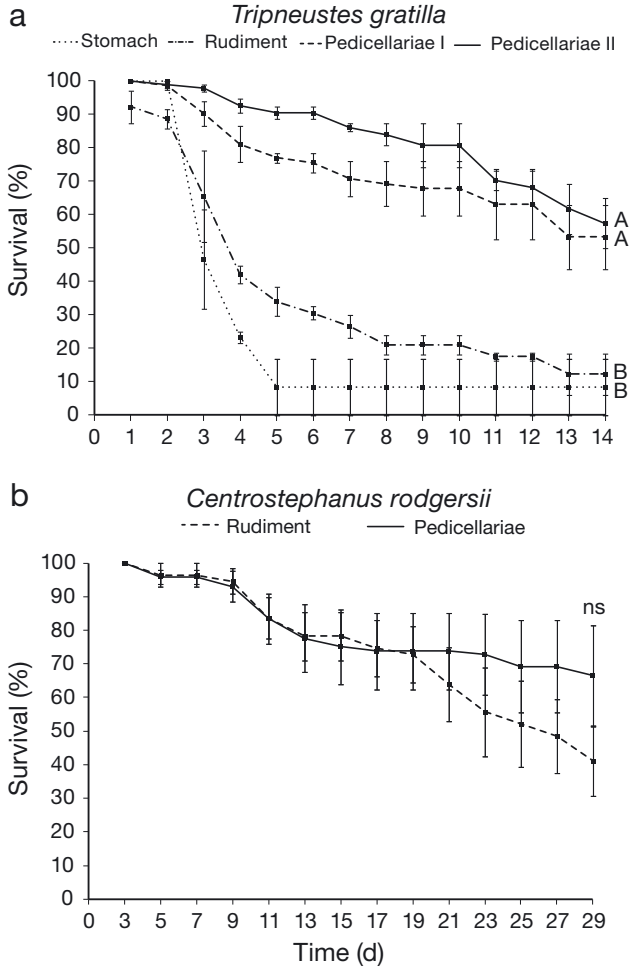

Fig. 5. Effect of larval development on post-settlement survival of (a) juvenile *Tripneustes gratilla* to 14 d and (b) juvenile *Centrostephanus rodgersii* to 29 d for larvae induced to metamorphose by 100 µM histamine. Stomach, Rudiment and Pedicellariae (I and II) identify nominal larval stages (Fig. 1). (a) Capitalised letters denote significant differences in survival between larval stages at Day 14 (PERMANOVA, followed by post hoc pairwise tests). (b) ns: no significant difference in survival between larval stages at Day 29 (PERMANOVA). All *C. rodgersii* juveniles from Stomach stage larvae were dead by 3 d. Data are mean ± SE; n = 3 to 7

At Day 14, survival was significantly higher in the Pedicellariae I and II treatments (53 and 56%, respectively) than in the Stomach and Rudiment treatments (8 and 12%, respectively) (Fig. 5a; $F_{3,8}$ = 10.72, p = 0.0086; post hoc pairwise tests, Pedicellariae II = Pedicellariae I > Rudiment = Stomach).

C. rodgersii juveniles from larvae that metamorphosed at late larval stages had higher survival to 29 d than juveniles from larvae that were less developed when they metamorphosed (Fig. 5b). All larvae that metamorphosed in the Stomach treatment were dead within 3 d, regardless of whether they were exposed to 100 µM histamine or *C. officinalis*. There was no difference in the survival rates of juveniles from Rudiment and Pedicellariae stage larvae at Day 29 for larvae induced to metamorphose by 100 µM histamine (Fig. 5b; $F_{2,18}$ = 10.26, p = 0.0023; post hoc pairwise tests, Pedicellariae = Rudiment > Stomach) or *C. officinalis* (71.8 ± 11.4 and 66.3 ± 12.5%, respectively; $F_{2,16}$ = 18.17, p < 0.0003; post hoc pairwise tests, Pedicellariae = Rudiment > Stomach).

Effect of larval development on response to settlement cues

Early and advanced larvae had different responses when exposed to settlement cues in the water column (Fig. 6). Early *T. gratilla* larvae continued to swim in the water column regardless of the concentration of *C. officinalis*-conditioned seawater that they were exposed to, but advanced larvae moved to

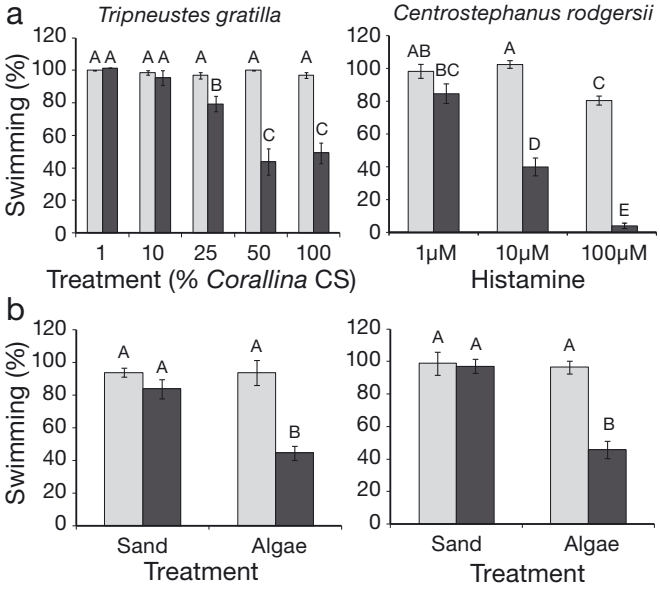

Fig. 6. Response of early (light grey bars) and advanced (dark grey bars) *Tripneustes gratilla* and *Centrostephanus rodgersii* larvae to (a) dissolved settlement cues and (b) seawater samples while swimming. Early larvae possessed only a rudiment. Advanced larvae were more developed and possessed pedicellariae and/or tube feet protruding from the vestibule. Results are expressed as the quantity of larvae in the water column calculated as a percentage of larvae actively swimming in control treatments after 1 h. % *Corallina* CS: percentage of *Corallina officinalis*-conditioned seawater in 1 µm FSW. Sand and Algae respectively denote sandy beach and macroalgae-dominated rocky reef habitats from which seawater samples were collected. Bars with the same letters are not statistically different (Table S6 in Supplement 2 at www.int-res.com/articles/suppl/m559p117_supp.pdf). Data are mean ± SE; n = 5 to 7

the bottom in increasing numbers as the concentration of conditioned seawater increased above 25% (Fig. 6a, Table S6 in Supplement 2; followed by post hoc pairwise tests). Early *C. rodgersii* larvae exposed to 1 and 10 µM histamine continued to swim in the water column, but some larvae (19%) moved to the bottom of the containers at the highest concentration of histamine (100 µM) (Fig 6a). Advanced larvae moved to the bottom of the containers in increasing numbers as the concentration of histamine increased from 1 to 100 µM (Fig. 6a, Table S6; followed by post hoc pairwise tests).

Early and advanced *T. gratilla* and *C. rodgersii* larvae responded differently when exposed to seawater samples collected from sandy beach or macroalgae-dominated rocky reef habitats (Fig. 6b, Table S6; followed by post hoc pairwise tests). Early larvae continued to swim in the water column regardless of the source of the seawater that they were exposed to (Fig. 6b). Advanced larvae exposed to seawater from the sandy beach habitat also remained in the water column; however, 55% of advanced larvae exposed to seawater from macroalgae-dominated habitat moved to the bottom of the containers (Fig. 6b).

DISCUSSION

Models of the dispersal phase of marine organisms highlight the negative fitness consequences of prolonged dispersal that result from accumulating predation risk and the physiological cost of searching (Stamps et al. 2005, Elkin & Marshall 2007). To avoid these costs, larvae should settle as soon as they are able to respond to settlement cues and a suitable habitat is available (Stamps et al. 2005, Elkin & Marshall 2007). However, minimising the length of the larval phase may not be the only optimal strategy. For example, a short larval phase has the disadvantage of reduced dispersal (e.g. Miller 1993). In this study we demonstrate that, in the presence of food, there are other considerable benefits for larvae that delay settling to develop adult structures, even if they are capable of metamorphosing in response to settlement cues.

Costs for early settlement of larvae

We found support for our first hypothesis that there are costs for settling early in larval development, manifested as immediate and deferred reductions in fitness. *Tripneustes gratilla* larvae that had developed a rudiment, but no adult structures, took up to 48 h to settle, whilst more developed larvae that possessed multiple pedicellariae or visible tube feet settled within 6 h. Similarly, Yazaki (1995) found larvae of the sea urchin *Hemicentrotus pulcherrimus* with well-developed spines and tube feet completed metamorphosis faster than larvae that did not have these structures present within the rudiment. Slow metamorphosis is hazardous as it may increase exposure to predation (Arnold & Wassersug 1978, Leis et al. 2011), burial in sediments and dislodgement or ingestion by grazers (Hadfield 2000). *T. gratilla* larvae without adult structures likely metamorphosed more slowly to develop structures such as tube feet required as juveniles (Cameron & Hinegardner 1974).

A deferred cost for early settlement by larvae was evident as reduced post-settlement survival. Juvenile *T. gratilla* metamorphosed from larvae that possessed adult structures had >7-fold higher post-settlement survival after 14 d compared to juveniles that metamorphosed from less developed larvae (i.e. Stomach and Rudiment stages). Despite metamorphosing, all *Centrostephanus rodgersii* larvae that settled without a well-developed rudiment (Stomach stage) were dead within 3 d, whereas more developed larvae (Rudiment and Pedicellariae stages) had significantly higher post-settlement survival after 29 d (>41%).

Differences in post-settlement survival in this study could be because energy reserves can increase with larval development (Byrne et al. 2008). The amount of energy stored by sea urchins as larvae is a key determinate of survival following metamorphosis, as they do not feed for the first 7 to 20 d (Chia & Burke 1978, Gosselin & Jangoux 1998, Vaitilingon et al. 2001, Byrne et al. 2008). The time required to complete metamorphosis and post-settlement size is also linked to larval energy reserves (Downie et al. 2004, Emlet & Sadro 2006). Additionally, post-larvae that have developed adult structures as larvae should have lower demands on energy reserves and bolstered survival rates compared to post-larvae that draw on larval reserves to develop these structures during the early non-feeding period on the benthos.

Early settling larvae also experienced other potential costs. Juvenile *T. gratilla* from larvae that metamorphosed without adult structures (i.e. Rudiment stage) were 25% smaller, 9 times more likely to be abnormal and 4 times more likely to lack spines compared to juveniles from larvae that possessed multiple pedicellariae and/or tube feet (Pedicellariae II stage). Similarly, juvenile *C. rodgersii* from larvae

that metamorphosed at an early developmental stage (Rudiment stage) were 11% smaller and 14 times more likely to be abnormal compared to juveniles from more developed larvae that possessed pedicellariae or visible tube feet (Pedicellariae stage). These costs could have substantial negative effects on long-term survival and fitness. Reduced size at juvenile stages increases vulnerability to early post-settlement predation (Miller & Emlet 1999, Marshall et al. 2003) and is correlated with reduced adult size, growth, fecundity and survival (Emlet & Hoegh-Guldberg 1997, Marshall et al. 2003). Absence of spines reduces fitness as spines have important adhesion, locomotion and defence functions (Strathmann 1981). Similarly, abnormalities decrease survival through impairment of normal physiological and morphological functions (Byrne et al. 2011).

We found that *T. gratilla* and *C. rodgersii* gain post-settlement fitness benefits if they delay metamorphosis to develop adult structures before metamorphosing. In contrast, studies that examined the effects of delayed metamorphosis on other planktotrophic larvae found short delays (days) have little or a negative effect on post-settlement growth and survival (Pechenik & Eyster 1989, Pechenik et al. 1993, 1996, Davis 1994, Qian & Pechenik 1998, McEdward & Qian 2001, Takami et al. 2002), whilst lengthy delays (weeks to months) have an increasingly detrimental effect on post-settlement fitness (Highsmith & Emlet 1986, Qian et al. 1990, Pechenik & Rice 2001, Takami et al. 2002, Rahman et al. 2014; but see Miller 1993). These studies may not have detected fitness benefits for short delays in metamorphosis because they used larval age to measure 'delay' rather than directly measuring developmental stage. Larval age is not directly related to attainment of competence (Bishop et al. 2006), larval development (e.g. Olson et al. 1993, Smith et al. 2008) or larval food reserves (Marshall et al. 2008). A cohort of larvae at a particular time point will have a range of developmental stages (e.g. Pechenik 1987, Hadfield & Strathmann 1996, Lamare & Barker 1999, Sewell et al. 2004, Jackson et al. 2005, Smith et al. 2008). Therefore post-settlement fitness consequences of delayed settlement may be missed when using larval age to measure 'delay', although we recognise it is not possible to differentiate the developmental stages of some marine larvae. To our knowledge, the only other studies that have compared the effects of delayed metamorphosis on larvae at different developmental stages also found post-settlement fitness benefits for delayed settlement by the lecithotrophic *Haliotis iris* (Moss & Tong 1992) and the planktotrophic *H. pulcherrimus* (Yazaki 1995; also see Yazaki 2002) and *Dendraster excentricus* (Hodin et al. 2015).

Elkin & Marshall (2007) defined 2 phases of dispersal: an obligate phase, which ends when larvae become competent to settle, and an ensuing facultative phase. We assume that competence begins when larvae are first able to initiate metamorphosis in response to cues (also see Coon et al. 1990, Bishop et al. 2006, Sutherby et al. 2012), corresponding to when larval *T. gratilla* and *C. rodgersii* have prominent rudiments but before tube feet are developed. With this definition in mind, our interpretation of the results is that if larvae extend their facultative phase, they gain the benefits of possessing adult structures. However, if competence is defined as the ability to respond to cues and successfully recruit to the benthos (Rodriguez et al. 1993), our results could provide evidence that the obligate phase of dispersal is longer than widely appreciated. In reality, however, the onset of competence is more complex than either definition implies (Hodin et al. 2015). Competence is seldom directly linked to the development of a morphological structure (e.g. Pechenik & Heyman 1987, Eyster & Pechenik 1988; but see Kriegstein 1977) and the timing of the attainment of competence varies considerably within species (Gibson 1995), even among siblings within broods (e.g. Jackson et al. 2005, Smith et al. 2008).

If larvae gain fitness benefits by delaying metamorphosis, why do larvae often respond to settlement cues and metamorphose prior to developing adult structures (e.g. Davis 1994, Yazaki 1995, Mercier et al. 2000, Takahashi et al. 2002, this study)? One possibility is that laboratory settlement assays may be a poor approximation of the way in which larvae encounter settlement cues in nature, exposing them to unnaturally high and stable concentrations of chemical cues and minimal water movement (Koehl & Hadfield 2010). Whilst concentrations of histamine used in this study were equivalent to concentrations measured in natural habitats (Swanson et al. 2006), our understanding of how such compounds vary in time and space at the level of larvae is limited (Koehl & Hadfield 2010). It is possible that earlier stage larvae would be rarely ever exposed to the high concentrations of chemical cues used in our settlement assays, as they are more typical of benthic surfaces than the open ocean.

Alternatively, early settlement may be maladaptive, but preserved because of genetic linkages among traits across life stages (Moran 1994). However, selection can work independently on different life stages of marine invertebrates, so it might be expected that

traits should not be maintained if they are generally maladaptive (Aguirre et al. 2014). This leaves the possibility that larval mortality may be so great in natural systems that, despite the costs, early settlement for at least some of a larval cohort may be a good bet-hedging strategy. Estimates of larval mortality rates from predation are often extremely high (90 to 100% per day) (Young & Chia 1987, Rumrill 1990, Vaughn & Allen 2010; but see White et al. 2014). Marine organisms are generally unable to predict the conditions experienced by dispersing offspring (reviewed by Pechenik 1999). A range of larval phenotypes that respond to settlement cues differently may increase the probability of survival in a range of possible conditions ('sweepstakes reproductive success' hypothesis; Hedgecock & Pudovkin 2011), similar to the way in which intra-brood variation in egg size increases maternal fitness in unpredictable environments (Marshall et al. 2008). Supporting this, the rate at which larvae develop the ability to respond to settlement cues is (in part) determined genetically (Jackson et al. 2005, Degnan & Degnan 2010) and via maternal effects, such as variation in egg size within broods (Marshall & Steinberg 2014).

The fate and the behaviour of larvae is strongly influenced by the environment encountered during the larval phase. Our experiments were done under optimal conditions with larvae that were fed liberal amounts of a highly nutritious diet, which may be more common in nature than is generally appreciated (Wolfe et al. 2015). However, in a food-limited environment there may be no benefits of an extended larval phase. Like lecithotrophic larvae (Pechenik et al. 1998, Marshall & Keough 2003), planktotrophic larvae in low-food environments may 'choose' to settle as soon as the option is available (Knight-Jones 1953). There appears to be a well-developed mechanism that enables larvae to make this 'choice'. NO signalling inhibits metamorphosis in larvae that are capable of responding to settlement cues (Froggett & Leise 1999, Bishop & Brandhorst 2001, Bishop et al. 2001, Pechenik et al. 2002, 2007, Bishop & Brandhorst 2003, Romero et al. 2013). NO production is closely linked to nitrogen metabolism (Hobbs et al. 1994) and is therefore thought to be regulated by the nutritional status of larvae (Romero et al. 2013).

Larvae avoid costs of settling too early

We also found clear evidence to support our second hypothesis, that larvae actively avoid the costs of settlement early in their development. The response of larvae to settlement cues is thought to be concentration-dependent (e.g. Fitt et al. 1990, Steinberg et al. 2001, Swanson et al. 2012). Larvae in the water column can detect low concentrations of waterborne chemicals that, if they signal suitable habitat, induce larvae to stop swimming and initiate searching behaviour on the benthos. Metamorphosis is triggered in these larvae if a high concentration of the settlement cue is detected (Steinberg et al. 2001). We found that for both species exposed to low concentrations of ecologically relevant settlement cues and seawater from adult habitat, advanced larvae that possessed adult structures commenced searching behaviour by moving towards the substrate. However, there was no response by early larvae that did not possess adult structures when exposed to these cues. Similarly, Coon et al. (1990) found settlement behaviour and metamorphosis of oyster larvae *Crassostrea gigas* in response to L-3,4-dihydroxyphenylalanine and epinephrine corresponded with the development of lateral eyespots. Maintaining normal swimming behaviour when exposed to low concentrations of settlement cues in the water column may prevent larvae without adult structures from being exposed to high concentrations of settlement cues near the substrate that initiate metamorphosis. Although not tested here, it would be interesting to explore how nutritionally compromised but poorly developed larvae respond to low concentrations of settlement cues.

By investigating the fitness consequences of development during dispersal, we found the formation of adult structures allows larvae to trade-off search costs without reducing specificity for optimal habitat. This study further highlights that 'metamorphosis is not a new beginning' (Pechenik et al. 1998); larval experience can profoundly determine post-metamorphic success. In marine systems, larval development may be one of many life history characteristics that influence fitness during dispersal (Elkin & Marshall 2007). Only by incorporating these characteristics into general models of habitat selection will there be greater understanding about how fitness consequences drive habitat selection and, in turn, influence population structure and community dynamics.

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LITERATURE CITED

Aguirre JD, Blows MW, Marshall DJ (2014) The genetic covariance between life cycle stages separated by metamorphosis. Proc R Soc B 281:20141091

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Arnold SJ, Wassersug RJ (1978) Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. Ecology 59: 1014–1022

Bishop CD, Brandhorst BP (2001) NO/cGMP signaling and HSP90 activity represses metamorphosis in the sea urchin *Lytechinus pictus.* Biol Bull 201:394–404

Bishop CD, Brandhorst BP (2003) On nitric oxide signaling, metamorphosis, and the evolution of biphasic life cycles. Evol Dev 5:542–550

Bishop CD, Bates WR, Brandhorst BP (2001) Regulation of metamorphosis in ascidians involves NO/cGMP signaling and HSP90. J Exp Zool 289:374–384

Bishop CD, Huggett MJ, Heyland A, Hodin J, Brandhorst BP (2006) Interspecific variation in metamorphic competence in marine invertebrates: the significance for comparative investigations into the timing of metamorphosis. Integr Comp Biol 46:662–682

Byrne M, Andrew N (2013) *Centrostephanus rodgersii.* In: Lawrence JM (ed) Sea urchins: biology and ecology. Elsevier, Croydon, p 243–256

Byrne M, Prowse TAA, Sewell MA, Dworjanyn S, Williamson JE, Vaitilingon D (2008) Maternal provisioning for larvae and larval provisioning for juveniles in the toxopneustid sea urchin *Tripneustes gratilla.* Mar Biol 155:473–482

Byrne M, Ho M, Wong E, Soars NA and others (2011) Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. Proc R Soc B 278: 2376–2383

Cameron RA, Hinegardner RT (1974) Initiation of metamorphosis in laboratory cultured sea urchins. Biol Bull 146: 335–342

Chia FS, Burke RD (1978) Echinoderm metamorphosis: fate of larval structures. In: Chia FS, Rice ME (eds) Settlement and metamorphosis of marine invertebrates. Elsevier, North Holland, NY, p 219–234

Coon SL, Fitt WK, Bonar DB (1990) Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas.* Mar Biol 106:379–387

Davis M (1994) Short-term competence in larvae of queen conch *Strombus gigas*: shifts in behavior, morphology and metamorphic response. Mar Ecol Prog Ser 104: 101–108

Degnan SM, Degnan BM (2010) The initiation of metamorphosis as an ancient polyphenic trait and its role in metazoan life-cycle evolution. Philos Trans R Soc Lond B 365: 641–651

Downie JR, Bryce R, Smith J (2004) Metamorphic duration: an under-studied variable in frog life histories. Biol J Linn Soc 83:261–272

Doyle RW (1975) Settlement of planktonic larvae: a theory of habitat selection in varying environments. Am Nat 109: 113–126

Dworjanyn SA, Pirozzi I (2008) Induction of settlement in the sea urchin *Tripneustes gratilla* by macroalgae, biofilms and conspecifics: a role for bacteria? Aquaculture 274: 268–274

Elkin C, Marshall DJ (2007) Desperate larvae: influence of deferred costs and habitat requirements on habitat selection. Mar Ecol Prog Ser 335:143–153

Emlet RB, Hoegh-Guldberg O (1997) Effects of egg size on postlarval performance: experimental evidence from a sea urchin. Evolution 51:141–152

Emlet RB, Sadro SS (2006) Linking stages of life history: how larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). Integr Comp Biol 46:334–346

Eyster LS, Pechenik JA (1988) Attachment of *Mytilus edulis* L. larvae on algal and byssal filaments is enhanced by water agitation. J Exp Mar Biol Ecol 114:99–110

Fitt WK, Coon SL, Walch M, Weiner RM, Colwell RR, Bonar DB (1990) Settlement behaviour and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. Mar Biol 106:389–394

Froggett SJ, Leise EM (1999) Metamorphosis in the marine snail *Ilyanassa obsoleta*, yes or NO? Biol Bull 196:57–62

Gibson G (1995) Why be choosy? Temporal changes in larval sensitivity to several naturally-occurring metamorphic inducers in the opisthobranch *Haminaea callidegenita.* J Exp Mar Biol Ecol 194:9–24

Gosselin P, Jangoux M (1998) From competent larva to exotrophic juvenile: a morphofunctional study of the perimetamorphic period of *Paracentrotus lividus* (Echinodermata, Echinoida). Zoomorphology 118:31–43

Grunbaum D, Strathmann RR (2003) Form, performance and trade-offs in swimming and stability of armed larvae. J Mar Res 61:659–691

Hadfield MG (2000) Why and how marine invertebrate larvae metamorphose so fast. Semin Cell Dev Biol 11: 437–443

Hadfield MG, Strathmann MF (1996) Variability, flexibility and plasticity in life histories of marine invertebrates. Oceanol Acta 19:323–334

Hedgecock D, Pudovkin AI (2011) Sweepstakes reproductive success in highly fecund marine fish and shellfish: a review and commentary. Bull Mar Sci 87:971–1002

Highsmith RC, Emlet RB (1986) Delayed metamorphosis: effect on growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroida). Bull Mar Sci 39:347–361

Hinegardner RT (1969) Growth and development of the laboratory cultured sea urchin. Biol Bull 137:465–475

Hobbs AJ, Fukuto JM, Ignarro LJ (1994) Formation of free nitric oxide from L-arginine by nitric oxide synthase: direct enhancement of generation by superoxide dismutase. Proc Natl Acad Sci USA 91:10992–10996

Hodin J, Ferner MC, Ng G, Lowe CJ, Gaylord B (2015) Rethinking competence in marine life cycles: ontogenetic changes in the settlement response of sand dollar larvae exposed to turbulence. R Soc Open Sci 2:150114

Huey RB (1991) Physiological consequences of habitat selection. Am Nat 137:S91–S115

Huk T, Kuhne B (1999) Substrate selection by *Carabus clatratus* (Coleoptera, Carabidae) and its consequences for offspring development. Oecologia 121:348–354

Jackson DJ, Ellemor N, Degnan BM (2005) Correlating gene expression with larval competence, and the effect of age and parentage on metamorphosis in the tropical abalone *Haliotis asinina.* Mar Biol 147:681–697

Jaenike J, Holt RD (1991) Genetic variation for habitat preference: evidence and explanations. Am Nat 137:S67–S90

Knight-Jones EW (1953) Decreased discrimination during setting after prolonged planktonic life in larvae of *Spiror-*

bis borealis (Serpulidae). J Mar Biol Assoc UK 32: 337–345

Koehl MAR, Hadfield MG (2010) Hydrodynamics of larval settlement from a larva's point of view. Integr Comp Biol 50:539–551

Kriegstein AR (1977) Stages in the post-hatching development of *Aplysia californica.* J Exp Zool 199:275–288

Lamare MD, Barker MF (1999) *In situ* estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). Mar Ecol Prog Ser 180:197–211

Leis JM, Hay AC, Gaither MR (2011) Swimming ability and its rapid decrease at settlement in wrasse larvae (Teleostei: Labridae). Mar Biol 158:1239–1246

Ling SD (2008) Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. Oecologia 156:883–894

Ling SD, Johnson CR, Ridgway K, Hobday AJ, Haddon M (2009) Climate-driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. Glob Change Biol 15:719–731

Marshall DJ, Keough MJ (2003) Variation in the dispersal potential of non-feeding invertebrate larvae: the desperate larva hypothesis and larval size. Mar Ecol Prog Ser 255:145–153

Marshall DJ, Steinberg PD (2014) Larval size and age affect colonization in a marine invertebrate. J Exp Biol 217: 3981–3987

Marshall DJ, Bolton TF, Keough MJ (2003) Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. Ecology 84:3131–3137

Marshall DJ, Bonduriansky R, Bussière LF (2008) Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. Ecology 89:2506–2517

McEdward LR, Qian PY (2001) Effects of the duration and timing of starvation during larval life on the metamorphosis and initial juvenile size of the polychaete *Hydroides elegans* (Haswell). J Exp Mar Biol Ecol 261:185–197

Mercier A, Battaglene SC, Hamel JF (2000) Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. J Exp Mar Biol Ecol 249:89–110

Miller SE (1993) Larval period and its influence on post-larval life history: comparison of lecithotrophy and facultative planktotrophy in the aeolid nudibranch *Phestilla sibogae.* Mar Biol 117:635–645

Miller BA, Emlet RB (1999) Development of newly metamorphosed juvenile sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*): morphology, the effects of temperature and larval food ration, and a method for determining age. J Exp Mar Biol Ecol 235:67–90

Moran NA (1994) Adaptation and constraint in the complex life cycles of animals. Annu Rev Ecol Syst 25:573–600

Mos B, Cowden KL, Nielsen SJ, Dworjanyn SA (2011) Do cues matter? Highly inductive settlement cues don't ensure high post-settlement survival in sea urchin aquaculture. PLOS ONE 6:e28054

Moss GA, Tong LJ (1992) Effect of stage of larval development on the settlement of the abalone, *Haliotis iris.* NZ J Mar Freshw Res 26:69–73

Munday PL (2001) Fitness consequences of habitat use and competition among coral-dwelling fishes. Oecologia 128: 585–593

Olson RR, Cameron JL, Young CM (1993) Larval development (with observations on spawning) of the pencil urchin *Phyllacanthus imperialis*: a new intermediate larval form? Biol Bull 185:77–85

Pechenik JA (1987) Environmental influences on larval survival and development. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of marine invertebrates, Book IX. Blackwell Scientific, Palo Alto, CA, p 551–608

Pechenik JA (1999) On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Mar Ecol Prog Ser 177:269–297

Pechenik JA, Eyster LS (1989) Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. Biol Bull 176: 14–24

Pechenik JA, Heyman WD (1987) Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). J Exp Mar Biol Ecol 112:27–38

Pechenik JA, Rice ME (2001) Influence of delayed metamorphosis on postsettlement survival and growth in the sipunculan *Apionsoma misakianum.* Invertebr Biol 120: 50–57

Pechenik JA, Rittschof D, Schmidt AR (1993) Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*. Mar Biol 115:287–294

Pechenik JA, Estrella MS, Hammer K (1996) Food limitation stimulates metamorphosis of competent larvae and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*. Mar Biol 127:267–275

Pechenik JA, Wendt DE, Jarrett JN (1998) Metamorphosis is not a new beginning; larval experience influences juvenile performance. Bioscience 48:901–910

Pechenik JA, Li W, Cochrane DE (2002) Timing is everything: the effects of putative dopamine antagonists on metamorphosis vary with larval age and experimental duration in the prosobranch gastropod *Crepidula fornicata.* Biol Bull 202:137–147

Pechenik JA, Cochrane DE, Li W, West ET, Pires A, Leppo M (2007) Nitric oxide inhibits metamorphosis in larvae of *Crepidula fornicata*, the slippershell snail. Biol Bull 213: 160–171

Qian PY, Pechenik JA (1998) Effects of larval starvation and delayed metamorphosis on juvenile survival and growth of the tube-dwelling polychaete *Hydroides elegans* (Haswell). J Exp Mar Biol Ecol 227:169–185

Qian PY, McEdward LR, Chia FS (1990) Effects of delayed settlement on survival, growth, and reproduction in the spionid polychaete, *Polydora ligni.* Invertebr Reprod Dev 18:147–152

Rahman MA, Yusoff FM, Arshad A, Uehara T (2014) Effects of delayed metamorphosis on larval survival, metamorphosis, and juvenile performance of four closely related species of tropical sea urchins (genus *Echinometra*). ScientificWorldJournal 2014:918028

Rodriguez SR, Ojeda FP, Inestrosa NC (1993) Settlement of benthic marine invertebrates. Mar Ecol Prog Ser 97: 193–207

Romero MR, Phuong MA, Bishop C, Krug PJ (2013) Nitric oxide signaling differentially affects habitat choice by two larval morphs of the sea slug *Alderia willowi*: mechanistic insight into evolutionary transitions in dispersal strategies. J Exp Biol 216:1114–1125

Rumrill SS (1990) Natural mortality of marine invertebrate larvae. Ophelia 32:163–198

Sewell MA, Cameron MJ, McArdle BH (2004) Developmental plasticity in larval development in the echinometrid sea urchin *Evechinus chloroticus* with varying food ration. J Exp Mar Biol Ecol 309:219–237

Smith MM, Smith LC, Cameron RA, Urry LA (2008) The larval stages of the sea urchin, *Strongylocentrotus purpuratus.* J Morphol 269:713–733

Stamps JA, Krishnan VV, Reid ML (2005) Search costs and habitat selection by dispersers. Ecology 86:510–518

Steinberg PD, de Nys R, Kjelleberg S (2001) Chemical mediation of surface colonisation. In: McClintock JB, Baker BJ (eds) Marine chemical ecology. CRC Press, Boca Raton, FL, p 355–387

Strathmann RR (1981) The role of spines in preventing structural damage to echinoid tests. Paleobiology 7:400–406

Strathmann R, Fenaux L, Strathmann M (1992) Heterochronic developmental plasticity in larval sea urchins and its implications for evolution of nonfeeding larvae. Evolution 46:972–986

Sutherby J, Giardini JL, Nguyen J, Wessel G, Leguia M, Heyland A (2012) Histamine is a modulator of metamorphic competence in *Strongylocentrotus purpuratus* (Echinodermata: Echinoidea). BMC Dev Biol 12:14

Swanson RL, de Nys R, Huggett MJ, Green JK, Steinberg PD (2006) *In situ* quantification of a natural settlement cue and recruitment of the Australian sea urchin *Holopneustes purpurascens.* Mar Ecol Prog Ser 314:1–14

Swanson R, Byrne M, Prowse T, Mos B, Dworjanyn S, Steinberg P (2012) Dissolved histamine: a potential habitat marker promoting settlement and metamorphosis in sea urchin larvae. Mar Biol 159:915–925

Takahashi Y, Itoh K, Ishii M, Suzuki M, Itabashi Y (2002) Induction of larval settlement and metamorphosis of the sea urchin *Strongylocentrotus intermedius* by glycoglycerolipids from the green alga *Ulvella lens.* Mar Biol 140: 763–771

Takami H, Kawamura T, Yamashita Y (2002) Effects of delayed metamorphosis on larval competence, and postlarval survival and growth of abalone *Haliotis discus hannai.* Aquaculture 213:311–322

Vaitilingon D, Morgan R, Grosjean P, Gosselin P, Jangoux M (2001) Effects of delayed metamorphosis and food rations on the perimetamorphic events in the echinoid *Paracentrotus lividus* (Lamarck, 1816) (Echinodermata). J Exp Mar Biol Ecol 262:41–60

Valentine J, Edgar G (2010) Impacts of a population outbreak of the urchin *Tripneustes gratilla* amongst Lord Howe Island coral communities. Coral Reefs 29:399–410

Vaughn D, Allen JD (2010) The peril of the plankton. Integr Comp Biol 50:552–570

Ward SA (1987) Optimal habitat selection in time-limited dispersers. Am Nat 129:568–579

White JW, Morgan SG, Fisher JL (2014) Planktonic larval mortality rates are lower than widely expected. Ecology 95:3344–3353

Whitham TG (1980) The theory of habitat selection: examined and extended using *Pemphigus* aphids. Am Nat 115:449–466

Wolfe K, Dworjanyn SA, Byrne M (2013) Thermal and pH/pCO_2 fluctuations in the intertidal habitat of *Heliocidaris erythrogramma*: effects on post-metamorphic juveniles. Cah Biol Mar 54:657–666

Wolfe K, Graba-Landry A, Dworjanyn SA, Byrne M (2015) Larval starvation to satiation: influence of nutrient regime on the success of *Acanthaster planci.* PLOS ONE 10:e0122010

Yazaki I (1995) Quantitative analysis of metamorphosis induced by L-glutamine in embryos of the sea urchin, *Hemicentrotus pulcherrimus.* Zoolog Sci 12:105–112

Yazaki I (2002) Mechanisms of sea urchin metamorphosis: stimuli and responses. In: Yokota Y, Matranga V, Smolenicka Z (eds) The sea urchin: from basic biology to aquaculture. A. A. Balkema Publishers, Lisse, p 51–71

Young CM, Chia FS (1987) Abundance and distribution of pelagic larvae as influenced by predation, behavior and hydrographic factors. In: Giese AC, Pearse JS, Pearse VB (eds) Reproduction of marine invertebrates. Blackwell Scientific, Palo Alto, CA, p 385–463

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