© 2014. Published by The Company of Biologists Ltd | The Journal of Experimental Biology (2014) 217, 3981-3987 doi:10.1242/jeb.111781

RESEARCH ARTICLE

# Larval size and age affect colonization in a marine invertebrate

**Dustin J. Marshall[1,*] and Peter D. Steinberg[2]**

## ABSTRACT

The relationship between offspring size and performance determines the optimal trade-off between producing many small offspring or fewer large offspring and the existence of this relationship has become a central tenet of life-history theory. For organisms with multiple life-history stages, the relationship between offspring size and performance is determined by the effects of offspring size in each life-history stage. Marine invertebrates have long been a model system for examining the evolutionary ecology of offspring size, and whilst offspring size effects have been found in several life-history stages, the crucial stage of colonization has received less attention. We examined the effect of offspring size on the settlement response of sea-urchin larvae (*Heliocidaris erythrogramma*) to preferred and less preferred host plants, how these effects changed over the larval period and estimated the success of juveniles in the field on preferred and less-preferred host plants. We found that smaller larvae became competent to respond to preferred host plant cues sooner than larger larvae but larger larvae rejected less-preferred host plants for longer than smaller larvae. Overall, smaller *H. erythrogramma* larvae are likely to have less dispersal potential and are more likely to settle in less-preferred habitats whereas larger larvae appear to have an obligately longer dispersal period but settle in preferred habitats. Our results suggest that marine invertebrates that produce non-feeding larvae may have the potential to affect the dispersal of their offspring in previously unanticipated ways and that offspring size is subject to a complex web of selection across life-history stages.

**KEY WORDS: Egg size, Maternal effects, Bet-hedging, Size–number trade-off**

## INTRODUCTION

A central tenet of life history theory is that mothers face a trade-off between the number and size of offspring they produce. Thus mothers must balance the benefit of making larger, fitter offspring with the cost of making fewer offspring. Crucial to this balance is the relationship between offspring size and performance, and for more than 50 years, ecologists have examined this relationship in an attempt to understand the selection pressures acting on mothers (Bagenal, 1969; Lack, 1947; Stearns, 1992). The principal tools for visualizing these selection pressures have been optimality models that include an offspring size to number trade-off and non-linear offspring size fitness relationships (Marshall et al., 2006; Sargent et al., 1987; Smith and Fretwell, 1974; Vance, 1973). Traditionally, the relationship between offspring size and performance has been examined at a single life-history stage only (Fox, 2000; Marshall et al., 2003; Marshall and Morgan, 2011; Moran and Emlet, 2001; Williams, 1994), but more recent studies recognize that offspring size can affect multiple life-history stages (Hendry et al., 2001; Marshall and Morgan, 2011). Clearly, the overall offspring-size–performance fitness relationship will be a product of offspring-size-based performance at each stage. This greatly complicates our view of the selection pressures acting on offspring size: for example, examining the relationship between offspring size and performance in a single life-history stage could lead to misleading conclusions regarding optimal offspring size. If we hope to understand the evolution of offspring size, we need to determine which life-history stages are affected by offspring-size effects and how these effects propagate throughout the life history.

Benthic marine invertebrates have long been the subject of interest with regard to offspring size (Thorson, 1950) and one of the first models examining 'optimal' offspring size was based on this group (Vance, 1973). Since Vance's model, later models have become more complex, reflecting the fact that offspring provisioning affects multiple life-history stages. For example, larger eggs are more easily fertilized in free-spawners because they provide a larger 'target' for sperm (Levitan, 1996; Marshall et al., 2002). In species with feeding larvae, larger offspring can have lower mortality in the plankton (Hart, 1995) and more recently, larger offspring have been shown to have greater survival, growth and reproduction after metamorphosis (Marshall et al., 2003; Marshall et al., 2006; Moran and Emlet, 2001). Despite the recent, intense interest in offspring-size effects in marine invertebrates (Levitan, 2000; Levitan, 2002; McEdward and Miner, 2003; Moran and Emlet, 2001; Podolsky and Strathmann, 1996; Podolsky, 2001), one crucial phase of the life-history stage has largely been overlooked – colonization of habitat following larval dispersal (Burgess et al., 2013). Importantly, there is a clear, but largely untested mechanism for offspring size to affect colonization in marine invertebrates.

Locating and colonizing a suitable surface is a fundamental event for benthic marine invertebrates. In organisms with a sessile or sedentary adult stage, colonization 'choices' can determine post-metamorphic success and given the typically high rates of mortality shortly after settlement, this stage is likely to exert strong selection pressure (Hunt and Sheibling, 1997; Keough and Downes, 1982; Raimondi and Keough, 1990). Dispersing larvae are faced with an array of potential settlement sites that will vary widely in their suitability for survival and subsequent growth and the consequences of settlement can be particularly dramatic when juvenile and adult stages have stringent habitat requirements (e.g. Krug and Zimmer, 2000; Williamson et al., 2004). Accordingly, many species have evolved to respond to highly specific colonization cues in order to maximize their chances of colonizing a suitable habitat (Steinberg et al., 2001). In some cases, a clear ranking of preferred colonization habitats exists for a species, which reflects relative performance of the colonizer in that habitat (Williamson et al., 2004). However, as non-feeding larvae age, they deplete their energetic reserves and in many species, they can become less discriminating with regards to settlement cues (Botello and Krug, 2006; Swanson et al., 2007). The loss of specificity of settlement cues in older larvae has been called

[1]School of Biological Sciences, Monash University, Melbourne, VIC 3800, Australia. [2]School of Biological, Earth & Environmental Sciences, University of New South Wales, Sydney, 2052 NSW, Australia.

*Author for correspondence (dustin.marshall@monash.edu)

**Received 30 July 2014; Accepted 11 September 2014**

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111781

the 'desperate-larva hypothesis' (Toonen and Pawlik, 2001) where older larvae are viewed as being close to the energetic minimum necessary to succeed after metamorphosis and will therefore settle spontaneously. The desperate-larva hypothesis is essentially an energetic argument and it suggests that larval energy reserves will affect the onset of a loss of specificity. Generally, conspecifics from larger eggs have higher energetic reserves than smaller conspecifics (Clarke, 1993; McEdward and Chia, 1991; Moran and Emlet, 2001) and so we would predict that smaller offspring would become 'desperate' sooner. While initial indications support this prediction in bryozoans (Burgess et al., 2013; Marshall and Keough, 2003b), the effect of offspring size on the crucial stage of colonization remains largely unexplored.

Given the importance of colonization for fitness and the potential for offspring size to affect colonization choices, we examined how offspring size affected the colonization choices of the sea urchin *Heliocidaris erythrogramma* Troschel 1872. As an adult, this urchin is found throughout beds of the kelp *Ecklonia radiata* (Agardh 1848) upon which it feeds and also on areas of shallow sandstone reef where kelp is absent (known as urchin barrens). In contrast to adults, recruits in the field are predominantly found on the erect coralline alga *Amphiroa anceps* (Decaisne 1842) and in the laboratory, the larvae strongly prefer to settle in the presence of this alga (Huggett et al., 2006). *E. radiata* induces some metamorphosis and some larvae metamorphose in the absence of any known cue, but it appears that habitat preferences of recruits and adults in this species are quite different (Huggett et al., 2006). We first tested how larval age and offspring size affected colonization choices. We then estimated the consequences of newly metamorphosed individuals colonizing the preferred and less-preferred host plants in the field.

## RESULTS

### Experiment 1. Effect of larval age and host plant

*Heliocidaris erythrogramma* larvae showed consistently higher metamorphosis when exposed to *A. anceps* throughout the assay period and generally, across all settlement cues, metamorphosis increased to 100% as larvae aged (Table 1). Sterile seawater and *E. radiata* initially induced very little metamorphosis (< 10%) but as larvae aged, this increased to ~50% (Fig. 1).

### Experiment 2a. Effect of larval size in young larvae

Smaller larvae were much more likely to metamorphose in response to the preferred settlement cue than larger larvae (likelihood test from logistic regression: $\chi^2$=4.3, d.f.=1, $P$=0.039). The average size of larvae that metamorphosed on day 3 was 10% smaller than the size of larvae that metamorphosed on day 4 (Fig. 2). All of the larvae that failed to metamorphose 3 days after fertilization initiated metamorphosis 1 day later.

**Table 1. Repeated measures ANOVA of effect of larval age and plant host on the metamorphosis of *Heliocidaris erythrogramma* larvae**

| Source | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| Within subjects | | | | |
| Host plant | 2 | 1.35 | 24.4 | **<0.001** |
| Error | 15 | 0.06 | | |
| Among subjects | | | | |
| Larval age | 3 | 0.90 | 24.9 | **0.000** |
| Larval age × host plant | 6 | 0.13 | 3.7 | **0.004** |
| Error | 45 | 0.04 | | |

Significant *P*-values are shown in bold.

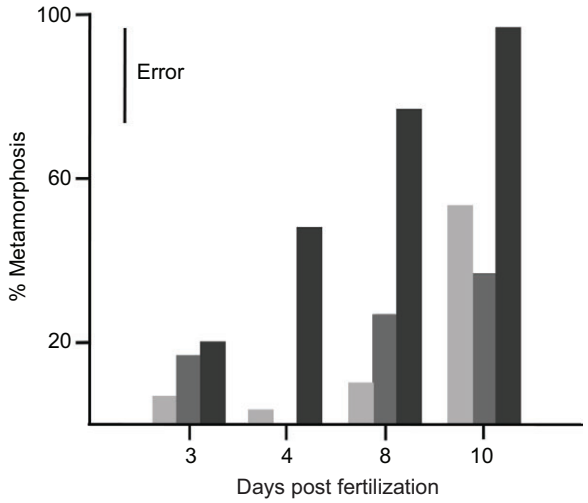


**Fig. 1. Effect of larval age and plant host on percentage metamorphosis of *Heliocidaris erythrogramma*.** Error bar represents the mean square error from the within-subjects error term of the relevant repeated measures ANOVA and is the appropriate error by which to judge changes over time (Quinn and Keough, 2002). Each bar represents metamorphosis in response to a different host plant: black bars, metamorphosis in response to *Amphiroa anceps*; dark grey bars, metamorphosis in response to *Ecklonia radiata*; light grey bars, metamorphosis in response to sterile seawater.

### Experiment 2b. Effect of larval size in old larvae

Larvae that settled in response to *E. radiata* were on average much smaller than larvae that settled in response to *A. anceps*. Evidence for an interaction between 'mother' and 'host plant' was not strong (Table 2) but, across all mothers, the average size of larvae that metamorphosed in response to the preferred host plant was greater than those that metamorphosed in response to the less-preferred host (Table 2; Fig. 3).

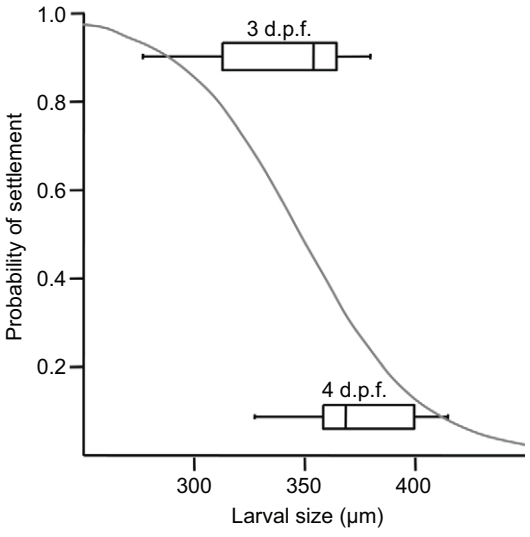


**Fig. 2. Effect of larval size on the probability of metamorphosis of *Heliocidaris erythrogramma* larvae.** The top box-plot represents the size distribution of larvae that metamorphosed 3 days post fertilization (d.p.f.) and the bottom box-plot represents the size distribution that metamorphosed 4 d.p.f. The line represents the predicted probability of metamorphosis as calculated from logistic regression.

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111781

**Table 2. Mixed model ANOVA on the size of *Heliocidaris erythrogramma* larvae that metamorphosed in response to a preferred or a less-preferred host plant**

| Source | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| Host plant | 1 | 13,152 | 9.10 | **0.039** |
| Mother | 4 | 428 | 0.66 | 0.623 |
| Host plant × mother | 4 | 1446 | 2.24 | 0.089 |
| Error | 30 | 647 | | |

Model also includes the effect of mother on the size of larvae that settled; settlement assay was performed on eight-day-old larvae.
Significant *P*-values are shown in bold.

### Experiment 3. Do the host-plant choices of large and small larvae differ over time?

In 4-day-old larvae, the host-plant choices of large and small larvae were similar: exposure to *A. anceps* resulted in the highest proportions of metamorphosis and sterile sea water had the lowest (Fig. 4). However, as larvae aged, differences in the response of larger and smaller larvae became apparent (Table 3, Fig. 4). Smaller larvae settled in *E. radiata* at much higher levels than larger larvae, whereas settlement levels in *A. anceps* and sterile seawater remained roughly the same between the two groups.

### Experiment 4. Consequences of host-plant choices

Settled juveniles were much more likely to be retained in the field jars after 2 days in the field in the presence of *A. anceps* than in the presence of either no host plant or *E. radiata* (Fig. 5). Planned comparisons showed no difference in the number of juveniles retained in the *E. radiata* and the blank field jars, but when these two treatments were pooled, there was a strong difference between *A. anceps* and the pooled group (Table 4).

## DISCUSSION

Offspring size in *H. erythrogramma* affects a number of aspects of the crucial life-history stage of colonization. This study is the first to our knowledge for any organism that has shown offspring-size effects on the timing of metamorphic competence and the

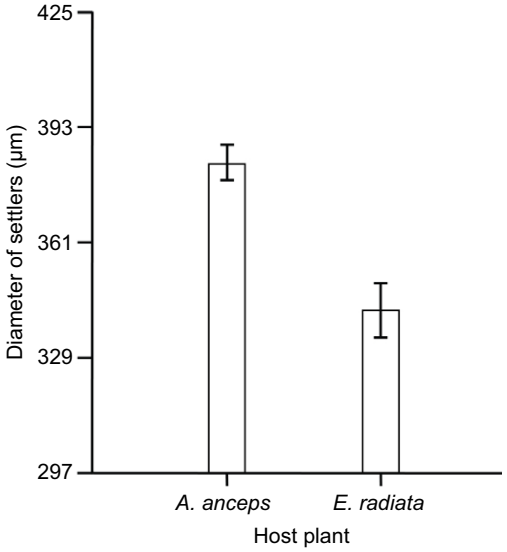


**Fig. 3. Mean size of metamorphosed *Heliocidaris erythrogramma* juveniles that metamorphosed in response to host plant.** Preferred plant host is *A. anceps* and less-preferred host is *E. radiata*. Error bars represent s.e.

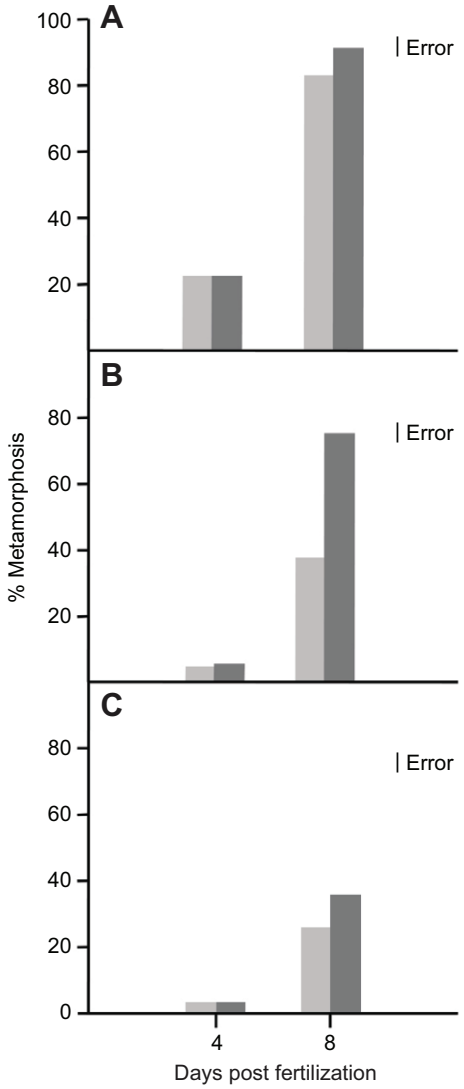


**Fig. 4. Response of *Heliocidaris erythrogramma* larvae to host plants.** (A) *A. anceps*. (B) *E. radiata*. (C) Sterile seawater. Error bars represent the mean square error from the relevant repeated-measures ANOVA and is the appropriate error by which to judge changes over time (Quinn and Keough, 2002). Light grey bars indicate mean settlement of large (from >380 µm eggs) larvae and dark grey bars indicate mean settlement of small (from <380 µm eggs) larvae.

colonization choices of young and old larvae – all of which are likely to have significant consequences for fitness. Overall, our study highlights the pervasive nature of offspring-size effects in marine invertebrates and the necessity of incorporating the effects of offspring size on colonization in the theoretical consideration of offspring size.

*Heliocidaris erythrogramma* larvae showed a difference in their response to *A. anceps* at colonization in comparison with their responses to *E. radiata* or sterile seawater, and this ranking appears to reflect subsequent success in the field. In our field experiment, we found that metamorphs were twice as likely to remain in the field jars containing *A. anceps* than in jars that contained *E. radiata*. We cannot rule out the possibility that settlers that left our field jars survived well in other habitats, but we believe that this movement out of the cover of the field jars would have resulted in much higher mortality from predators or resuspension into the plankton. However, despite the benefits of settling in *A. anceps*, larvae began

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111781

**Table 3. Repeated measures ANOVA on the effect of larval size and larval age on the proportion of metamorphosis of *Heliocidaris erythrogramma* in response to different potential host plants**

| Source | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| Within subjects | | | | |
| Larval size | 1 | 0.003 | 0.073 | 0.789 |
| Host plant | 2 | 1.923 | 46.58 | **<0.001** |
| Larval size × host plant | 2 | 0.282 | 6.825 | **0.002** |
| Error | 54 | 0.041 | | |
| Among subjects | | | | |
| Larval age | 1 | 6.193 | 170.4 | **0.000** |
| Larval age × larval size | 1 | 0.228 | 6.265 | **0.015** |
| Larval age × host plant | 2 | 0.498 | 13.70 | **0.000** |
| Age × size × host plant | 2 | 0.166 | 4.576 | **0.015** |
| Error | 54 | 0.036 | | |

Host plants were *Ecklonia radiata* and *Amphiroa anceps*.
Significant *P*-values are shown in bold.

**Table 4. ANOVA and planned contrasts on the effect of host plant on the number of juvenile *Heliocidaris erythrogramma* retained in the field**

| Source | d.f. | MS | *F* | *P* |
|---|---|---|---|---|
| Host plant | 2 | 2.927 | 3.948 | **0.044** |
| Planned contrasts | | | | |
| *Eck.* versus blank | 1 | 0.476 | 0.642 | 0.436 |
| *Amph.* versus (*Eck.* + blank) | 1 | 5.853 | 7.893 | **0.014** |
| Error | 14 | 0.741 | | |

Experiment was carried out for 2 days at Bare Island, Sydney, Australia.
Significant *P*-values are shown in bold.
*Eck.*, *Ecklonia radiata*; *Amph.*, *Amphiroa anceps*.

to metamorphose in response to *E. radiata* and eventually, in response to no cue at all. Because larval swimming is energetically costly in *H. erythrogramma* (Hoegh-Guldberg and Emlet, 1997) it appears that non-feeding larvae trade-off the benefits of settling with some energetic reserves for post-metamorphic performance with the benefits of settling in a poor-quality habitat. This species adds to a growing list of marine invertebrates that exhibit decreasing selectivity with regards to settlement cues as they age (Bishop et al., 2006; Elkin and Marshall, 2007) and emphasizes the dynamic nature of larval settlement behaviour.

The effect of larval size on the onset of indiscriminate settlement may be due to a range of factors. First, larger larvae have more energetic reserves and, given the importance of energetic reserves for post-metamorphic performance in *H. erythrogramma* (Emlet and Hoegh-Guldberg, 1997), large larvae may be more able to 'afford' to delay for longer. Larger larvae also have a lower surface area to volume ratio and so the relative costs of swimming may be lower in larger larvae compared with smaller larvae (Wendt, 2000). Regardless of the ultimate causes, larger larvae are much more likely to reject lower-ranked host-plant cues than smaller larvae as they age.

Whilst larger larvae appear to be more likely to reject lower-quality habitats, there are some environments where the production

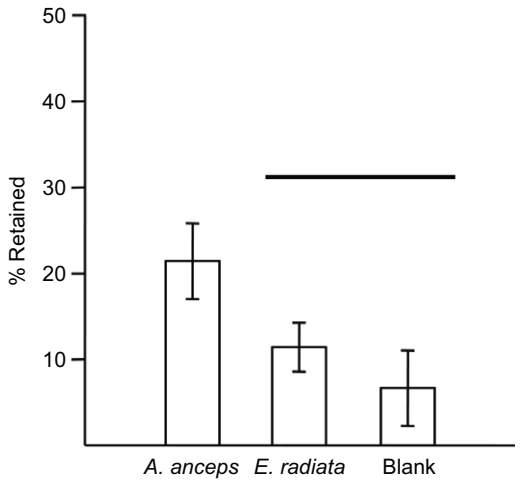


**Fig. 5. Effect of different host-plant substrates on *H. erythrogramma* settlement.** The mean proportion (± s.e.) of settlers retrieved after 2 days in the field is shown. Bar indicates no significant difference in *post hoc* tests. 'Blank' field jars contained no plants.

of smaller larvae may carry fitness benefits. We found that smaller larvae are able to react to the high-ranked host-plant cues a day earlier than larger larvae. It should be noted that the onset of metamorphic competence between large and small larvae may be somewhat overestimated in our study because we only tested larvae every 24 hours. However, clearly, smaller larvae can react sooner than larger larvae to cues for high-quality habitat. The reasons for smaller offspring being able to react to cues sooner are unclear, but interspecific comparisons on marine invertebrates show that species with larger eggs tend to have slower initial cell-cycle durations than species with smaller eggs (Staver and Strathmann, 2002). Regardless, the effects of offspring size on the onset of metamorphic competence and the onset of indiscriminate settlement behaviour is likely to result in large and small offspring having very different dispersal profiles.

## Implications for dispersal

Assuming that a longer planktonic duration means a greater capacity for dispersal, larger *H. erythrogramma* larvae are likely to disperse farther than small larvae as a result of them taking longer to become metamorphically competent and remaining more discriminate with respect to host-plant cues for longer. This has some interesting consequences for the way in which we view dispersal in marine invertebrates with non-feeding larvae. Typically, larvae for a particular species are viewed (and modeled) as being relatively homogenous in their dispersal potential (Black et al., 1991; Connolly and Roughgarden, 1999; Connolly, 1999). Our data suggest that mothers that produce larger offspring will produce much more dispersive offspring than mothers that produce smaller offspring. Thus, the significant variation in offspring size we observed among mothers will result in differences in the dispersal profiles of the young of those mothers. It will be interesting to determine whether *H. erythrogramma* mothers that exist in poor-quality habitats produce larger offspring than mothers in low-quality habitats and this increases the likelihood of those young dispersing to a better place (Krug, 1998; Krug, 2001). Given that offspring size also varies significantly within broods, mothers are effectively producing offspring with a range of dispersal profiles. Interestingly, McGinley et al. (McGinley et al., 1987) suggested that the production of offspring of variable size was only adaptive when mothers could direct their young of different sizes into appropriate habitats, a condition they believed was unlikely. However, our results suggest that there is a clear mechanism for 'directed' dispersal of different sized young into different habitats, although actual settlement outcomes depend on the size and spacing of settlement habitats (Burgess et al., 2013). When high-quality habitats are small and widely spaced, larvae will become desperate to settle overall but larger offspring will be more likely to go into

The Journal of Experimental Biology (2014) doi:10.1242/jeb.111781

good-quality habitats and smaller offspring will be more likely to go into poorer-quality habitats. However, when high-quality habitats are closely spaced, smaller larvae are more likely to be able to settle sooner. Given the levels of variation in offspring size observed within broods (Isomura and Nishimura, 2001; Marshall et al., 2008; Marshall and Keough, 2003a; McEdward and Chia, 1991; Turner and Lawrence, 1977), the minimum dispersal potential of lecithotrophic larvae is likely to be polymorphic within a brood. Polymorphic dispersal profiles have been argued to be adaptive for mothers, ensuring that offspring are spread throughout a range of habitats, ameliorating risk and mitigating intraspecific competition (Raimondi and Keough, 1990; Strathmann, 1974). Similarly, Laaksonen (Laaksonen, 2004) argues that hatching asynchrony is an adaptive strategy in birds that maximizes parental fitness by reducing variation in the success of their offspring (i.e. bet-hedging). It remains unclear whether variation in egg size within broods is an adaptive or bet-hedging trait in marine invertebrates (Einum and Fleming, 2002; Marshall et al., 2008) or merely an inevitable consequence of egg production (Ramirez-Llodra, 2002; Strathmann, 1995). Nevertheless, our results suggest that any factors that reduce the size of offspring that are produced (e.g. pollution, maternal diet, depth) (Bertram and Strathmann, 1998; Cox and Ward, 2002; Meidel et al., 1999) will not only result in offspring that perform poorly after metamorphosis, but will also result in previously unanticipated changes in the dispersal profiles of the offspring.

From an ecological standpoint, the effects of offspring size on colonization have some interesting implications. The strength of links between different populations has typically been viewed as a product of the number individuals that are exchanged. Our results suggest that the size and/or quality of individuals will also affect the strength of these links because larger larvae are more likely to make 'good' colonization choices and thus are more likely to survive. From an evolutionary standpoint, our results suggest that offspring size is subject to a complex web of selection, and will be affected not only by post-metamorphic conditions (Emlet and Hoegh-Guldberg, 1997) but also by the availability and type of colonization habitat (Burgess et al., 2013).

## MATERIALS AND METHODS

### Production, maintenance and measurement of larvae

Adult *Heliocidaris erythrogramma* were collected regularly from Bare Island, Botany Bay (33° 59′ S, 151° 13′ W) in New South Wales, Australia, throughout the reproductive season (October–March). To collect gametes, we injected males and females with 5 ml of 0.5 M KCl, which usually induced spawning within minutes. We collected gametes from adults within 3 days of collection from the field. Eggs were fertilized using standard protocols (Marshall et al., 2004) with sperm pooled from at least three different males used as a sperm solution in each case. To measure eggs, larvae or metamorphosed juveniles, we placed them under a dissecting microscope in a small Petri dish containing filtered seawater and captured images using a digital camera attached to a microscope. Later, we used ImagePro V.4 to digitally measure the largest diameter (for eggs or metamorphosed juveniles) or longest larval length as described previously (Marshall et al., 2004). It should be noted that egg size, larval size and metamorphosed juvenile size are strongly correlated in this species (Marshall et al., 2004).

To maintain larvae from different mothers, larvae were kept in sterile 1 litre glass beakers (at least two replicate beakers per mother) in 0.45-µm-filtered, autoclaved seawater with constant air bubbling through the jar. Larvae were kept at a density of ~100 larvae $l^{-1}$. The beakers were maintained in a constant temperature room at 20°C throughout the assays and 50% water changes were made every 2 days. In all cases, the number of replicate settlement jars that were used (see below) reflects the number of culture beakers that were used for each mother.

### Settlement assays

We conducted settlement assays by placing 10 larvae in a settlement jar (70 ml, polyethylene specimen jar) filled with 30 ml of freshly collected 0.45-µm-filtered seawater, and depending on the treatment, a small piece (2 $cm^2$) of the host plant. Again, the number of settlement jars reflected the number of culture beakers and as such was the unit of replication throughout the analysis. The erect coralline alga *A. anceps* has been shown to be a strong metamorphosis inducer of *H. erythrogramma*, while the brown alga *E. radiata* is a moderate (typically 20–40% metamorphosis) inducer (Huggett et al., 2006). Incubating larvae in still, filtered seawater results in a very small (<10%) proportion of larvae initiating metamorphosis in the absence of host-plant cues (Huggett et al., 2006). The algae were collected from Bare Island on the morning of the settlement assays and washed briefly in 0.45-µm-filtered seawater before being cut into pieces. Larvae were exposed to the settlement cues during the settlement assays for 6 hours at 20°C in a constant temperature room before being checked for metamorphosis. We classed larvae as 'metamorphosed' if they had produced tube feet and had begun to change shape substantially. All assessments of metamorphosis were done blind with regard to the treatment classification.

### Experiments

#### 1. Do responses to host plants change as larvae age?

To examine whether larvae remain constant in their response to potential algal hosts, we exposed larvae from the same batches but of differing ages to various settlement cues. Larvae were produced from three mothers and sired with a pooled sperm solution. There were two replicate settlement jars for each mother and treatment combination. We examined the settlement responses of larvae 3, 4, 8 and 10 days after fertilization (d.p.f.). Pilot studies showed that very few larvae are capable of metamorphosis at 3 d.p.f. and after 10 d.p.f. most larvae exhibit 'spontaneous' metamorphosis in the culture vessels. At each assay time, we removed larvae from the culture beakers and placed 10 into each settlement jar with *E. radiata*, *A. anceps* or just sterile seawater.

#### 2a. Do smaller larvae react to settlement cues sooner than larger larvae?

To examine whether larval size affected the ability of young (3 d.p.f.) larvae to settle in response to a preferred settlement cue, we compared the size of larvae that settled in response to *A. anceps* with larvae that did not settle. We first measured the size of each larva and then exposed individual larvae (i.e. each larva was in its own jar) from three different mothers (five larvae per mother) to the settlement inducer as described above. Some larvae did not metamorphose at 3 d.p.f. and we exposed these larvae to *A. anceps* the next day (4 d.p.f.). All of these settled on their second exposure.

#### 2b. Does larval size affect the settlement of 'old' larvae in response to a preferred or less-preferred inducer?

We were interested whether, like old larvae, larger larvae were 'choosier' with respect to plant hosts than smaller larvae. To test this, we exposed larvae from different mothers to the preferred plant host (*A. anceps*) or the less-preferred plant host (*E. radiata*). We then measured the size of newly metamorphosed settlers that settled in response to each of the host plants. We used larvae from five different mothers and had 2–6 replicates per plant host × mother combination, resulting in 40 replicates in total.

#### 3. Do the host-plant responses of large and small larvae differ over time?

Our results in this experiment suggested that smaller larvae are able to react to preferred settlement cues sooner than larger larvae and larger larvae remained choosier with regard to host plants for longer than smaller larvae (see Results). To test this explicitly, we collected eggs from 12 different females (two experimental runs using six females in each), measured 50 eggs from each female and classified each female's clutch as containing either 'large' (391±1.3 µm) or 'small' (372±0.7 µm) eggs (these diameters translate into a difference of ~15% in volume). These size classes were based on the observed median of the population (380 µm diam.). After fertilization we split the developing eggs from each clutch into multiple culture beakers and allowed them to develop for 4 days. At 4 d.p.f., we removed larvae from the beakers and exposed them to either sterile seawater, *E. radiata* or *A. anceps* and assayed metamorphosis as described above. We

repeated this process at 8 d.p.f. with new larvae from the culture beakers and again assayed metamorphosis in response to the three different cues.

#### 4. Post-metamorphic consequences of responses to different host plants

To estimate the consequences of metamorphosing in response to the preferred (*A. anceps*) or less-preferred (*E. radiata*) host plant, we transplanted settlers into the field on various substrates and measured how many were retained after 2 days in the field. We cultured larvae from five mothers as described above, pooled the cultures and induced metamorphosis at 5 d.p.f. using pieces of *A. anceps* as an inducer. We used *A. anceps* as an inducer rather than the species that the settlers would be deployed into the field on because this would have resulted in systematic differences in the size of settlers among the treatments (see Results). Thus all of the larvae had been exposed to *A. anceps* previously, but were then incubated in 'blank' beakers for a further 2 days. We then placed the metamorphosed individuals into a 5 l beaker containing filtered seawater and left them there for 2 days to completely develop into juveniles. At 7 d.p.f. we placed 10 randomly selected juveniles in each of 17 'field jars'. The field deployment jars consisted of a 120 ml clear, polyethylene jar with a 20-mm-wide bulldog clip affixed to the inside of the jar with clear silicone. Before placing the larvae into the field jars, each jar was filled to the brim with 0.45-µm-filtered seawater. Of the 17 jars, seven also had a small piece of *A. anceps* placed into the bulldog clip and seven had a small piece of *E. radiata* placed in the bulldog clip leaving three 'blank' field jars that only contained a bulldog clip. We sealed the jars with their lids, placed the jars into insulated aquaria and transported them to the field site at Bare Island (a 20 min drive). To deploy the field jars into the field, we had affixed small (200×200 mm) stainless steel frames to the sandstone seabed with Dynabolts (3 m depth). The frames were haphazardly spread across a relatively flat bed of *A. anceps* that had *E. radiata* scattered through it. Using SCUBA, we attached the sealed field jars to the subtidal frames with cable ties and then left the jars undisturbed for 10 min so that any dislodged juveniles in the jars could re-attach to a surface. We then carefully removed the lids of each field jar, taking care to ensure that all 10 juveniles were present when we left each jar. We returned to the field after 2 days, resealed the jars with their lids and then retrieved the jars to the laboratory. We chose 2 days because this length of time meant that we could confidently assign the juveniles as 'experimentally deployed juveniles' rather than newly metamorphosed juveniles that had recruited from the plankton (there was no settlement of new juveniles in any of the field jars). The pieces of algae from each treatment remained in place throughout the field deployment. To assess the number of juveniles that were left in each jar, we poured the water from each through a 100 µm plankton mesh and examined the inside surfaces of the jars and the pieces of algae under a dissecting microscope (×40 mag.).

### Data analysis

For experiment 1, we used a repeated-measures ANOVA where larval age and host plant were fixed factors. For experiment 2a we compared the sizes of larvae that had settled with those that had not, we used logistic regression where metamorphosis at 3 or 4 d.p.f. was the binary response variable and larval size was a continuous predictor variable. We had first included 'mother' as a factor in a logistic ANCOVA but there was no significant interaction between mother and settlement response ($P$=0.98), and mother explained little variation so it was omitted from the final model, leaving a simple logistic regression. For experiment 2b, we analysed the data used a mixed-model ANOVA where host plant was a fixed factor, mother was a random factor and proportion metamorphosed was the response variable.

For experiment 3, we used a repeated-measures ANOVA to analyse these data where larval size and host plant were fixed factors and settlement rate at 4 and 8 d.p.f. were the response variables. We first included 'run' as a random blocking factor, but there were no interactions between this factor and the treatments of interest (run × larval size × host plant × larval age: $F_{2,48}$=0.517; run × larval size × host plant: $F_{2,48}$=1.12; run × larval size: $F_{1,48}$=0.751) and because it explained little or no variation (run: $F_{1,48}$=0.009), it was omitted from the final model. For experiment 4, we used ANOVA and planned comparisons to test the effect of host plant on juvenile retention. We *a priori* expected that more juveniles would be retained on *A. anceps* than *E. radiata* so we first tested whether there was a difference between the blank jars and those containing *E. radiate*; we found none, so we pooled these and then tested this pooled group against *A. anceps*.

#### Acknowledgements

We are grateful to Megan Huggett and Rebecca Swanson for assistance in the field, and Hayley Cameron and several reviewers for helpful comments on the manuscript.

#### Competing interests

The authors declare no competing financial interests.

#### Author contributions

D.J.M. and P.D.S. designed the experiment, D.J.M. performed the experiment and analysed the data. D.J.M. and P.D.S. wrote and drafted the manuscript.

#### Funding

This research was supported by Australian Research Council Discovery grants to D.J.M.

#### References

**Bagenal, T. B.** (1969). Relationship between egg size and fry survival in brown trout *Salmo trutta* L. *J. Fish Biol.* **1**, 349-353.
**Bertram, D. F. and Strathmann, R. R.** (1998). Effect of maternal and larval nutrition on growth and form of planktotrophic larvae. *Ecology* **79**, 315-327.
**Bishop, C. D., Huggett, M. J., Heyland, A., Hodin, J. and Brandhorst, B. P.** (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.
**Black, K. P., Moran, P. J. and Hammond, L. S.** (1991). Numerical models show coral reefs can be self-seeding. *Mar. Ecol. Prog. Ser.* **74**, 1-11.
**Botello, G. and Krug, P. J.** (2006). Desperate larvae revisited: age, energy and experience affect sensitivity to settlement cues in larvae of the gastropod Alderia sp. *Mar. Ecol. Prog. Ser.* **312**, 149-159.
**Burgess, S. C., Bode, M. and Marshall, D. J.** (2013). Costs of dispersal alter optimal offspring size in patchy habitats: combining theory and data for a marine invertebrate. *Funct. Ecol.* **27**, 757-765.
**Clarke, A.** (1993). Egg size and egg composition in polar shrimps (Caridea: Decapoda). *J. Exp. Mar. Biol. Ecol.* **168**, 189-203.
**Connolly, S. R. and Roughgarden, J.** (1999). Theory of marine communities: competition, predation, and recruitment-dependent interaction strength. *Ecol. Monogr.* **69**, 277-296.
**Cox, E. F. and Ward, S.** (2002). Impact of elevated ammonium on reproduction in two Hawaiian scleractinian corals with different life history patterns. *Mar. Pollut. Bull.* **44**, 1230-1235.
**Einum, S. and Fleming, I. A.** (2002). Does within-population variation in fish egg size reflect maternal influences on optimal values? *Am. Nat.* **160**, 756-765.
**Elkin, C. M. and Marshall, D. J.** (2007). Desperate larvae: the influence of deferred costs and habitat requirements on habitat selection. *Mar. Ecol. Prog. Ser.* **335**, 143-153.
**Emlet, R. A. and Hoegh-Guldberg, O.** (1997). Effect of egg size on post-larval performance: experimental evidence from a sea urchin. *Evolution* **51**, 141-152.
**Fox, C. W.** (2000). Natural selection on seed-beetle egg size in nature and the laboratory: variation among environments. *Ecology* **81**, 3029-3035.
**Hart, M. W.** (1995). What are the costs of small egg size for marine invertebrate with feeding planktonic larvae. *Am. Nat.* **146**, 415-426.
**Hendry, A. P., Day, T. and Cooper, A. B.** (2001). Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *Am. Nat.* **157**, 387-407.
**Hoegh-Guldberg, O. and Emlet, R. B.** (1997). Energy use during the development of a lecithotrophic and a planktotrophic echinoid. *Biol. Bull.* **192**, 27-40.
**Huggett, M. J., Williamson, J. E., de Nys, R., Kjelleberg, S. and Steinberg, P. D.** (2006). Larval settlement of the common Australian sea urchin *Heliocidaris erythrogramma* in response to bacteria from the surface of coralline algae. *Oecologia* **149**, 604-619.
**Hunt, H. L. and Sheibling, R. E.** (1997). Role of post-settlement mortality in recruitment of benthic marine invertebrates. *Mar. Ecol. Prog. Ser.* **155**, 269-301.
**Isomura, N. and Nishimura, M.** (2001). Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals. *Coral Reefs* **20**, 309-315.
**Keough, M. J. and Downes, B. J.** (1982). Recruitment of marine invertebrates: the role of active larval choices and early mortality. *Oecologia* **54**, 348-352.
**Krug, P. J.** (1998). Poecilogony in an estuarine opisthobranch: planktotrophy, lecithotrophy, and mixed clutches in a population of the ascoglossan *Alderia modesta*. *Mar. Biol.* **132**, 483-494.
**Krug, P. J.** (2001). Bet-hedging dispersal strategy of a specialist marine herbivore: a settlement dimorphism among sibling larvae of *Alderia modesta*. *Mar. Ecol. Prog. Ser.* **213**, 177-192.
**Krug, P. J. and Zimmer, R. K.** (2000). Developmental dimorphism and expression of chemosensory-mediated behavior: habitat selection by a specialist marine herbivore. *J. Exp. Biol.* **203**, 1741-1754.
**Laaksonen, T.** (2004). Hatching asynchrony as a bet-hedging strategy – an offspring diversity hypothesis. *Oikos* **104**, 616-620.
**Lack, D.** (1947). The significance of clutch size. *Ibis* **89**, 302-352.

**Levitan, D. R.** (1996). Effects of gamete traits on fertilisation in the sea and the evolution of sexual dimorphism. *Nature* **382**, 153-155.
**Levitan, D. R.** (2000). Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. *Am. Nat.* **156**, 175-192.
**Levitan, D. R.** (2002). Density-dependent selection on gamete traits in three congeneric sea urchins. *Ecology* **83**, 464-479.
**Marshall, D. J. and Keough, M. J.** (2003a). Sources of variation in larval quality for free-spawning marine invertebrates: Egg size and the local sperm environment. *Invertebr. Reprod. Dev.* **44**, 63-70.
**Marshall, D. J. and Keough, M. J.** (2003b). 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, D. J. and Morgan, S. G.** (2011). Ecological and evolutionary consequences of linked life-history stages in the sea. *Curr. Biol.* **21**, R718-R725.
**Marshall, D. J., Styan, C. A. and Keough, M. J.** (2002). Sperm environment affects offspring quality in broadcast spawning marine invertebrates. *Ecol. Lett.* **5**, 173-176.
**Marshall, D. J., Bolton, T. F. and Keough, M. J.** (2003). Offspring size affects the post-metamorphic performance of a colonial marine invertebrate. *Ecology* **84**, 3131-3137.
**Marshall, D. J., Steinberg, P. D. and Evans, J. P.** (2004). The early sperm gets the good egg: mating order effects in free spawners. *Proc. Biol. Sci.* **271**, 1585-1589.
**Marshall, D. J., Cook, C. N. and Emlet, R. B.** (2006). Offspring size effects mediate competitive interactions in a colonial marine invertebrate. *Ecology* **87**, 214-225.
**Marshall, D. J., Bonduriansky, R. and Bussière, L. F.** (2008). Offspring size variation within broods as a bet-hedging strategy in unpredictable environments. *Ecology* **89**, 2506-2517.
**McEdward, L. R. and Chia, F. S.** (1991). Size and energy content of eggs from echinoderms with pelagic lecithotrophic development. *J. Exp. Mar. Biol. Ecol.* **147**, 95-102.
**McEdward, L. R. and Miner, B. G.** (2003). Fecundity-time models of reproductive strategies in marine benthic invertebrates: fitness differences under fluctuating environmental conditions. *Mar. Ecol. Prog. Ser.* **256**, 111-121.
**McGinley, M. A., Temme, D. H. and Geber, M. A.** (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. *Am. Nat.* **130**, 370-398.
**Meidel, K., Scheibling, R. E. and Metaxas, A.** (1999). Relative importance of parental and larval nutrition on the larval development and metamorphosis of the sea urchin *Strongylocentrotus droebachiensis*. *J. Exp. Mar. Biol. Ecol.* **240**, 161-178.
**Moran, A. L. and Emlet, R. B.** (2001). Offspring size and performance in variable environments: field studies on a marine snail. *Ecology* **82**, 1597-1612.
**Podolsky, R. D.** (2001). Evolution of egg target size: an analysis of selection on correlated characters. *Evolution* **55**, 2470-2478.
**Podolsky, R. D. and Strathmann, R. R.** (1996). Evolution of egg size in free spawners: consequences of the fertilisation-fecundity trade-off. *Am. Nat.* **148**, 160-173.
**Quinn, G. P. and Keough, M. J.** (2002). *Experimental design and data analysis for biologists*. Cambridge, UK: Cambridge University Press.
**Raimondi, P. T. and Keough, M. J.** (1990). Behavioural variability in marine larvae. *Aust. J. Ecol.* **15**, 427-437.
**Ramirez-Llodra, E. R.** (2002). Fecundity and life-history strategies in marine invertebrates. In *Advances in Marine Biology*, Vol. 43 (ed. C. M. Young), pp. 88-172. San Diego, CA; London: Academic Press.
**Sargent, R. C., Taylor, P. D. and Gross, M. R.** (1987). Parental care and the evolution of egg size in fishes. *Am. Nat.* **129**, 32-46.
**Smith, C. C. and Fretwell, S. D.** (1974). The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499-506.
**Staver, J. M. and Strathmann, R. R.** (2002). Evolution of fast development of planktonic embryos to early swimming. *Biol. Bull.* **203**, 58-69.
**Stearns, S. C.** (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.
**Steinberg, P. D., de Nys, R. and Kjelleberg, S.** (2001). Chemical mediation of surface colonisation. In *Marine Chemical Ecology* (J. B. McClintock and Bill J. Baker), pp. 255-388. Boca Raton, FL: CRC Press.
**Strathmann, R. R.** (1974). The spread of sibling larvae of sedentary marine invertebrates. *Am. Nat.* **108**, 29-44.
**Strathmann, R. R.** (1995). Peculiar constraints on life histories imposed by protective or nutritive devices for embryos. *Am. Zool.* **35**, 426-433.
**Swanson, R. L., Marshall, D. J. and Steinberg, P. D.** (2007). Larval desperation and histamine: how simple responses can lead to complex changes in larval behaviour. *J. Exp. Biol.* **210**, 3228-3235.
**Thorson, G.** (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev. Camb. Philos. Soc.* **25**, 1-45.
**Toonen, R. J. and Pawlik, J. R.** (2001). Settlement of the gregarious tube worm *Hydroides dianthus* (Polychaeta: Serpulidae). II. Testing the desperate larva hypothesis. *Mar. Ecol. Prog. Ser.* **224**, 115-131.
**Turner, R. and Lawrence, J. M.** (1977). Volume and composition of echinoderm eggs: implications for the use of egg size in life-history models. In *Reproductive Ecology of Marine Invertebrates* (ed. S. E. Stancyk), pp. 25-40. Columbia, SC: University of South Carolina Press.
**Vance, R. R.** (1973). On reproductive strategies in marine benthic invertebrates. *Am. Nat.* **107**, 339-352.
**Wendt, D. E.** (2000). Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). *Biol. Bull.* **198**, 346-356.
**Williams, T. D.** (1994). Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. *Biol. Rev. Camb. Philos. Soc.* **69**, 35-59.
**Williamson, J. E., Carson, D. G., de Nys, R. and Steinberg, P. D.** (2004). Demographic consequences of an ontogenetic shift by a sea urchin in response to host plant chemistry. *Ecology* **85**, 1355-1371.