

# Propagule size effects across multiple life-history stages in a marine invertebrate

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## Summary

1. Mothers must balance the fecundity costs of increasing propagule size with the fitness benefits of increased propagule performance, and the propagule size-performance relationship is crucial for determining this trade-off.
2. While many studies have examined the propagule size-performance relationship in individual life-history stages, very few have examined the relationship between propagule size and performance across multiple life-history stages despite the ubiquity of complex life cycles.
3. We examined the consistency of the propagule size-performance relationship across several life-history stages in the marine invertebrate *Microcosmus squamiger* (Ascidiacea).
4. Propagule size had effects in some life-history stages and not others: larger propagules hatched sooner as larvae and grew more as juveniles in the field. On the other hand, propagule size had no effect on cell cleavage rates, larval swimming time or post-metamorphic survival in the field.
5. The effects of propagule size on juvenile size were persistent – juveniles that came from larger propagules were still larger than juveniles that came from smaller propagules after 11 weeks in the field.
6. We found no evidence of conflicting selection pressures on propagule size among life-history stages. Rather, in this species at least, the selection on propagule size at both the larval and juvenile stage appeared to favour the production of larger propagules. Nevertheless, the slope of the relationship between propagule size and performance was highly variable among life-history stages.
7. The effects of propagule size across multiple life-history stages are determined by the strength of selection pressures, which can be highly variable in organisms with complex life-cycles.

**Key-words:** fertilization, larval size, propagule performance, post-metamorphic, settlement

## Introduction

The study of propagule size is a central component of life-history theory and has long fascinated evolutionary ecologists (Lack 1947; Bagenal 1969). Propagule size is remarkably variable at all levels of organization – among species, populations, individuals and even within broods (Clarke 1993; Williams 1994; Fox & Czesak 2000; Clark, Ewert & Nelson 2001; Marshall & Keough 2008a). Variation in propagule size can have fundamental consequences for population dynamics, species range expansions, and evolutionary trajectories (Fox, Czesak & Fox 2001; Buckley *et al.* 2003; Benton *et al.* 2005). Interestingly, while propagule size

is a shared trait between mother and propagules, selection acts largely to maximize maternal, rather than propagule fitness (Smith & Fretwell 1974). Mothers are thought to face a trade-off with regard to the size and number of offspring that they can produce – any increase in the size of the propagules that mothers make must be associated with a decrease in the number they can produce (Smith & Fretwell 1974; Giangrande, Geraci & Belmonte 1994). The fecundity cost associated with producing propagules of increased size may be offset by an increase in propagule performance. Selection will favour mothers that optimize the trade-off between propagule size and number, and the major determinant of this optimal balance is the relationship between propagule size and performance (Vance 1973; Smith & Fretwell 1974; Emler & Hoegh-Guldberg 1997; Pechenik 2006).

The propagule size-performance relationship determines the fitness benefits of producing propagules of a particular

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size. When there is a strong relationship between propagule size and performance, selection should favour mothers that produce larger propagules because small increases in propagule investment should yield fitness returns that exceed the associated fecundity costs (Smith & Fretwell 1974; Parker & Begon 1986). Conversely, when there is a weak relationship, mothers will achieve higher fitness by producing smaller, and thus more, propagules. Therefore, mothers should closely track any shifts in the relationship between propagule size and performance. Indeed there are a growing number of studies that demonstrate that shifts in the propagule size-performance relationship result in shifts in the size of the propagules that mothers produce at different scales, from inter-population down to inter-individual variation (Fox, Thakar & Mousseau 1997; Fox 2000; Hendrickx, Maelfait & Lens 2003; Maruyama, Rusuwa & Yuma 2003; Plaistow *et al.* 2007; Russell *et al.* 2007; Marshall & Keough 2008b). The tight coupling between the size of the propagule that mothers produce and the propagule size-performance relationship is perhaps unsurprising in some systems. However, in organisms with complex life-histories, the challenge of optimally provisioning propagules during each life-history stage is far greater, which increases the difficulty for researchers to measure performance at an appropriate point in time.

Most organisms have complex life-cycles whereby propagules pass through multiple life-history stages before reaching adulthood. If propagule size affects each of these life-history stages in different ways, then propagule provisioning becomes problematic. For example, how should mothers provision their propagules if increased propagule size positively influences performance in one life-history stage, but negatively influences a later life-history stage? There is some evidence that propagule size can affect propagule performance in different ways among life-history stages: smaller propagules can be favoured during the larval stage but larger propagules are favoured during the juvenile/adult stage (Kaplan 1992; Einum, Hendry & Fleming 2002; Marshall & Keough 2008a). Given that during different life-history stages propagules can have different trophic requirements, live in different habitats and be subject to different physiological constraints (Moran 1994), it seems inevitable that the relationship between propagule size and performance should vary among life-history stages. If different life-history stages select different optimal propagule sizes, the propagule size that mothers produce may be very different from that which would be predicted based on the relationship between size and performance in one life-history stage alone. Alternatively, selection for different propagule sizes among life-history stages could lead to a bet-hedging strategy (Sutherland 1969; Toonen & Pawlik 2001; Parciak 2002; Koops, Hutchings & Adams 2003) being favoured because mothers are unable to provision propagules optimally as the cumulative propagule size-performance relationship could be highly unpredictable (Marshall, Bonduriansky & Bussière 2008). Thus there is the potential for conflicting selection pressures on optimal propagule size among different life-history stages with interesting consequences, but this potential remains relatively unexplored.

Most studies of propagule size effects are restricted to single life-history stages (Hendry, Day & Cooper 2001; Czesak & Fox 2003; Marshall & Keough 2008a) and most organisms have at least one mobile life-history stage rendering the estimation of the propagule size-performance relationship across the entire life-history problematic. Thus, estimates of the propagule size-performance relationship across multiple life-history stages are extremely rare (but see Einum, Hendry & Fleming 2002; De Block & Stoks 2005). The scarcity of studies that examine propagule size effects means that we currently have little understanding of whether provisioning propagules with complex life-histories is indeed more challenging than provisioning propagules with simple life-histories. Combining the results of several studies, each of which examined a different life-history stage for a single organism, could provide insight into the potential for varying selection on propagule size across life-history stages. However, propagule size effects can vary dramatically among populations and even over time in the same population (Landa 1992; Ouellet & Allard 2002), which limits the insight that can be gained from combining multiple studies. Thus, longitudinal studies of propagule size effects (i.e. studies that examine the effects of propagule size in individuals that are tracked over their life-history) provide the most comprehensive understanding of the overall selection pressure acting on propagule size. Such an approach is precluded by logistical constraints in most systems, so a pragmatic alternative is to examine the effects of propagule size in each life-history stage for different individuals that have been drawn from the same population at the same time.

Here we examine the effects of propagule size across multiple life-history stages in a marine invertebrate, the solitary ascidian *Microcosmus squamiger* (Michaelsen, 1927) (Fig. 1). Marine invertebrates represent an excellent group for the study of propagule size effects for a number of reasons. Firstly, studies show that propagule size affects performance across the entire life-history in this group, from fertilization, through development, larval settlement and post-metamor-



Fig. 1. *Microcosmus squamiger* individuals. Photograph by Prof. Charles L. Griffiths.

phic survival, growth and even reproduction (McLaren 1965; McEdward 1996; Emler & Hoegh-Guldberg 1997; Pechenik, Wendt & Jarrett 1998; Wendt 1998; Moran & Emler 2001; Pechenik & Rice 2001; Pechenik *et al.* 2001; Pechenik, Li & Cochrane 2002; Pechenik 2006; Marshall & Keough 2008a). Secondly, initial studies on individual life-history stages in isolation suggest that selection on propagule size among different life-history stages could indeed be in conflict (Levitan 1996; Marshall, Styan & Keough 2002). Finally, there has been a long standing theoretical interest in the evolution and ecological consequences of propagule size in marine invertebrates, however, field studies remain relatively rare in this group (but see Jarrett & Pechenik 1997; Moran & Emler 2001; Thiyagarajan *et al.* 2007). Here, we focus on a solitary ascidian species which, like most solitary ascidians, is hermaphroditic, produces non-feeding larvae and releases both eggs and sperm into the water column whereupon fertilization occurs externally (Svane & Young 1989). We examined the effect of egg size on the post-fertilization development rate, on the duration of the total planktonic period (hatching time and larval swimming period), and on larval settlement behaviour in the laboratory. Finally, we examined the post-metamorphic survival and growth of juveniles under field conditions, a crucial element when considering propagule size effects (Fox 2000).

## Materials and methods

### COLLECTION SITE

All reproductively mature *M. squamiger* collected for this study were from boulders located at the outer pontoon of the Manly harbour (Brisbane, Queensland, Australia – 27°27'10"S, 153°11'22"E). This location is a sheltered marina protected by a large rocky breakwater. The collections took place during low tide periods between October and December 2006 and between January and March 2009. The individuals were transported in a 20 L insulated container with seawater to the laboratory (travel time ~45 min) where they were placed in a constantly aerated tank (Dimensions: 400 mm × 900 mm × 200 mm; Volume 20 L) with seawater kept at room temperature (ca. 20 °C). All seawater used in the experiments was collected from the field and 0.45 µm filtered before use.

### FERTILIZATION METHODOLOGY

We carried out all fertilizations in the laboratory at room temperature by mixing male and female gametes from four individuals following the standard strip-spawning technique described in Marshall, Styan & Keough (2000). This was done by dissecting the gonads from the mantle of each individual into a Petri dish with 10 mL of filtered sea water. We then gently squashed the gonads and larger pieces of tissue were removed. The gonad extract was poured through a 100 µm filter with filtered seawater into a small beaker, so the eggs were retained inside the filter but the sperm and excess seawater passed through into the beaker. To measure sperm concentration we used a grid haemocytometer (0.0025 mm<sup>2</sup> quadrats) under the stereomicroscope. As maximum fertilization success for *M. squamiger* was found using the highest concentration (Rius, Turon & Marshall 2009), we did all the experiments using the 'dry' sperm concentration (i.e. maximum

concentration attainable, between 10<sup>7</sup> and 10<sup>8</sup> sperm cells/mL) (see Marshall, Styan & Keough 2000). *Microcosmus squamiger* is a simultaneous hermaphrodite; therefore, eggs and sperm from separate individuals were mixed to achieve cross fertilization. Although we know that this species within its introduced range has a 2-year life cycle and a seasonal reproductive cycle (Rius, Pineda & Turon 2009), no studies have investigated the specific characteristics of its early life history stages. We observed *M. squamiger* in laboratory conditions and found that it has a short lived larval stage, most settlement occurring during the first 8 h (i.e. larval swimming time), while metamorphosis normally occurs one day after settlement. We followed post metamorphic individuals (here defined as juveniles) in the field during almost a three month period and by the end of the experiment the individuals could be seen with the naked eye. *M. squamiger* specimens were considered to be adults when they had reached a size of four to five cm (maximal attainable size of this species in the field, M.R. pers. obs.), and when they had well-developed gonads.

### MEASURES OF PROPAGULE SIZE

In order to examine propagule size effects at several stages (from egg to post-metamorphic), we used the most convenient estimate of propagule size according to which aspect of propagule performance we were interested in. We did not follow individuals from eggs all the way through to the post-metamorphic stage as this is logistically very challenging (see below). We took measurements of each developmental stage by taking digital photographs with a camera attached to the stereomicroscope and connected to a computer. We subsequently measured the photographs using Image Pro (v. 5.1.0.12, Media Cybernetics). The egg, larvae, settlers and post-metamorphic forms of *M. squamiger* are irregular in shape so we decided to use the two-dimensional area viewed from above (in all instances, the propagules at different stages were either sitting on or attached to the Petri dish surface). We first determined whether the size of individuals at different life history stages were correlated (i.e. do larger eggs become larger larvae and larger settlers?). To estimate the relationship between egg size and larval size we measured individual eggs, fertilized them *in vitro* as described above, and then placed them into separate Petri dishes (diameter 65 mm, 25 mL of filtered sea water) to allow development to take place. After 14 h, the swimming larvae were individually photographed and measured. Many cleaved eggs did not develop into larvae, which resulted in very few larvae being available for measurement. In the first run we achieved three successful larval measurements, while in the following runs we obtained 22 (second run), six (third run) and four (fourth run) measurements (a total of 35). To determine if larger eggs became larger settlers we separated eggs using a pipette and placed each one in a separate Petri dish with filtered seawater. As biofilmed and rough surfaces have been proven to facilitate larval settlement in ascidians (Wieczorek & Todd 1997), we roughened the Petri dish surface with sand paper and then submerged them in seawater for 24 h. Thus, pre-roughened 65 mm Petri dishes with biofilm and 25 mL of filtered sea water were used in all experiments involving settlement. Twenty-four h after hatching we photographed and measured the juveniles that had settled and metamorphosed (16 in one run and three in another run yielding a total of 19 measurements across two runs). For both our experiments on the egg size-larval size relationship and the egg size-settler size relationship, we used five pools of larvae that were generated from four individuals per pool. Importantly, egg size was correlated with larval size, and egg size was correlated with settler size (see Results).

Once the correlations between egg and larval sizes, and egg and settler sizes were obtained, we examined biologically meaningful correlates of egg size. We started by assessing whether egg size does or does not relate to adult size. Subsequently, we tested how egg size relates to the timing of development (cleavage rate and hatching time), which in turn affects the planktonic period.

#### ADULT-EGG SIZE RELATIONSHIP

We randomly collected 11 adult individuals in the field and measured their maximum body length (to the nearest mm) and wet weight (to the nearest mg). Subsequently, we dissected the individuals and photographed 20 eggs per individual to measure their two dimensional area.

#### EGG SIZE/TIME OF CLEAVAGE RELATIONSHIP

To determine the relationship between egg size and time of cleavage we photo-sequenced fertilized eggs every two minutes. The time was recorded when the 2, 4, 8, and 16 cell stages appeared in each fertilized egg. As we did not know the exact moment of fertilization due to the procedure followed to obtain gametes, we used the time of the first cleavage as the starting time against which we measured the timing of the subsequent stages. We related these times with the egg size measured from the 1st photo taken of the undivided eggs. We did two runs of this experiment, the first one involving 74 complete egg measurements and the second 10 egg measurements.

#### HATCHING TIME VS. LARVAL SIZE

We placed fertilized eggs in a Petri dish with filtered seawater in order to determine the time when the larvae hatched from the eggs. We checked hatching every hour after fertilization. All larvae were removed and placed in a vial with seawater which included a few drops of formalin to preserve them (preservation does not affect estimates of size; unpublished data). Later we measured the size (i.e. larval area) of larvae that had hatched at each time period.

#### MEASUREMENT OF LARVAL SWIMMING TIME

To examine the effects of larval size on larval swimming time, we placed recently hatched larvae individually in separate Petri dishes with filtered seawater to assess larval swimming time. We photographed the larvae and then placed the Petri dishes in complete darkness. We checked for settlement every hour during a 32 h period, tracking a total of 36 larvae.

#### POST-SETTLEMENT PERFORMANCE IN THE FIELD: EFFECT OF SETTLER SIZE ON SURVIVAL AND GROWTH

We placed 20 larvae into a Petri dish (65 mm in diameter, 30 dishes in total) filled with filtered seawater. After 24 h we rinsed them with filtered seawater to remove any unattached larvae, and then the settlers were photographed for measurement. We then marked and numbered their positions using a pencil on the Petri dish surface. Subsequently, we gently made an eight mm hole in the centre of the Petri dishes using a hand drill. We then transported the dishes to the field in seawater in 20 L insulated containers. We attached all Petri dishes to two (500 × 500 mm) Perspex backing plates (15 Petri dishes in each plate) using stainless steel screws. The Petri dish positions were

randomly assigned. We hung the plates from the most external pontoon at Manly harbour at a depth of two metres below the Mean Low Water Spring, facing downwards to reduce the effects of light and sedimentation (following Marshall, Bolton & Keough 2003).

This experiment was run twice. For the first run (30 Petri dishes, 112 recruits), survival was measured weekly after the deployment in the field. To measure survival, we took the dishes out of the water and carefully placed them in an insulated container with seawater directly taken from the location. Immediately after, we started the observations under the stereomicroscope. Once each Petri dish was examined, we brought them back to the insulated container. After all Petri dishes were examined (ca. 30–60 min of observation), they were again attached to the backing plates and returned to the field. From the 3rd week, all Petri dishes were almost entirely covered by other encrusting species, which made direct observation of the settlers in the field impossible. Thus, we brought all the Petri dishes back to the laboratory after the 3rd, 6th and 11th week and estimated settler survival and growth under a stereomicroscope after carefully removing all non-*M. squamiger* species from the dish surface. During the same inspection we took photographs of the settlers for settler size measurements. After the examination, we maintained all Petri dishes in an aerated tank at room temperature overnight. The following day we returned the Petri dishes to the field and placed them on the same backing plates and in the same position. We followed the same methodology in the second run (30 Petri dishes, 93 recruits), which started a week later than the first run. In this case we only measured survival, which was done in the 1st, 2nd, 5th and 10th week.

#### DATA ANALYSIS

To analyse the relationship between egg, larval and settler sizes, adult-egg size relationship, and the influence of propagule size on parameters such as larval swimming time, we used regression when a single run was examined and ANCOVA when we examined multiple runs. In all of these analyses initial propagule size was a continuous predictor and run (where included) was a random factor. The effect of propagule size on time until hatching was analysed using a *t*-test for both hourly measurements (see Results). To examine the effect of settler size on subsequent juvenile size in the field, we used a Repeated-Measures ANCOVA with time as within subject factor and settler size as covariate.

In all of our analyses we found that the interaction between run and settler size was not significant, implying a homogeneous slope of the regression among runs. The interaction term was therefore pooled with the residual error in the final model (Quinn & Keough 2002).

For the effect of settler size on survival in the field, we used Cox's proportional hazard regression, which models a hazard rate as a function of survival time and independent covariates (in our case, settler size). The fit (log-likelihood) of the models with and without the covariates was compared with a chi-square test.

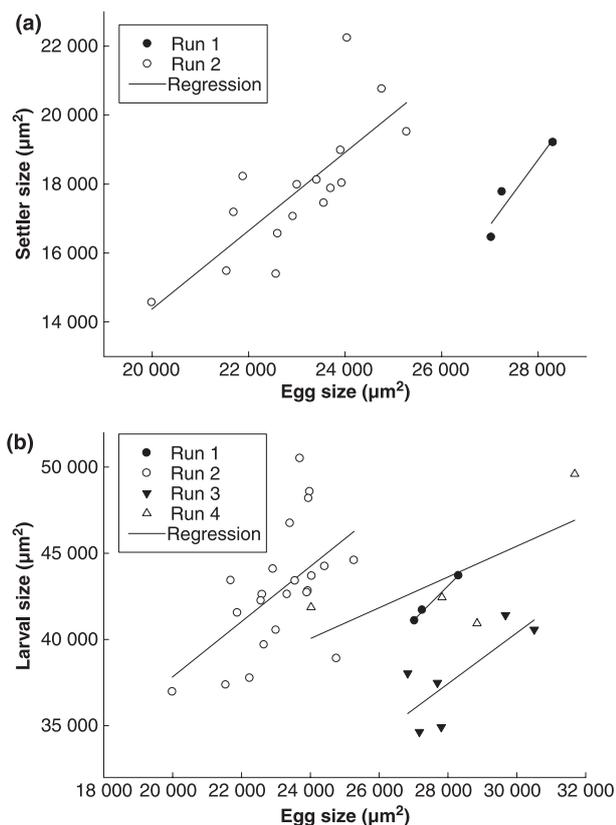
We also measured selection differentials. We calculated standardized selection differentials following Falconer & Mackay (1996) for the effect of propagule size on hatching time, post-metamorphic survival and size at the end of the field experiment. For post-metamorphic growth, we classified juveniles as 'small' or 'large' whenever they fell in the smallest or largest quartile respectively, using these size classes as our two selection groups.

We performed all analyses using the software SYSTAT (v. 11, SPSS Inc., Chicago, IL, USA, 2004).

## Results

### RELATIONSHIPS OF EGG-LARVA-SETTLER SIZES

When we analysed the relationship between estimates of propagule size at different early life-history stages, we found significant effects of both the relationship between egg size and larval size (ANCOVA; Egg size,  $F_{1,30} = 15.46$ ,  $P = 0.0005$ ; Run,  $F_{3,30} = 10.76$ ,  $P = 0.0001$ ) and between egg size and settler size (ANCOVA; Egg size,  $F_{1,16} = 24.3$ ,  $P = 0.0001$ ; Run,  $F_{1,16} = 15.9$ ,  $P = 0.0010$ ). Note that the above models are reduced after testing for homogeneity of slopes. Overall, egg size accounted for 47% and 74% of the variation in larval size and settler size respectively. Importantly, these relationships were consistent among experimental runs (i.e. larger eggs always became larger larvae and settlers) as evidenced by the homogeneity of slopes test (egg size-larvae size  $\times$  Run:  $F_{3,27} = 0.34$ ,  $P = 0.79$ ; egg size-settler size  $\times$  Run:  $F_{1,15} = 0.33$ ,  $P = 0.57$ ). Thus, there was a strong relationship between egg size and larval size [larval size =  $1.29$  (egg size) -  $6543.36$ ,  $R = 0.519$ ], and also a strong relationship between egg size and settler size [settler size =  $1.16$  (egg size) -  $14150.59$ ,  $R = 0.603$ ] (Fig. 2). There were considerable differences among runs, even to the extent that eggs from different runs tended to form discrete non-overlapping size



**Fig. 2.** Relationship between egg size and larval size of *Microcosmus squamiger* (a), and between egg size and settler size (b). Notice that although there was variation among runs, the effect (slope) of both variables in each case was consistent among runs.

classes, which can only be attributed to inter-batch differences in egg size and/or the fact that not all the data were collected the same year.

### ADULT-EGG SIZE RELATIONSHIP

There was no relationship between adult weight or adult length and the size of eggs that individuals produced (wet weight,  $r = 0.172$ ,  $P = 0.614$ ; length,  $r = -0.018$ ,  $P = 0.958$ ).

### EGG SIZE/TIME OF CLEAVAGE RELATIONSHIP

There was no effect of egg size on the time taken for the initial cleavage divisions from 2 to 4 cells, 2 to 8 cells or 2 to 16 cells (ANCOVA; Egg size, all  $P > 0.15$ ; Run,  $F_{1,75} = 0.058$ ,  $P = 0.810$ ;  $F_{1,16} = 2.148$ ,  $P = 0.162$ ;  $F_{1,16} = 17.058$ ,  $P = 0.001$ ; respectively).

### PROPAGULE SIZE EFFECTS ON TIME UNTIL HATCHING

As most of the larvae (*c.* 500) hatched between the 11th and 12th hour, we only measured a subsample of 100 larvae within this period. The following hour, 76 larvae hatched, and on the 14th and 24th hour after fertilization, we found only three larvae each time. As a result, to analyse the effect of hatching time on larval size, we only included the larvae from the 12th ( $n = 100$ , mean larval size =  $45360.77 \mu\text{m}^2$ ,  $\text{SE} = 252.32$ ) and 13th hour ( $n = 76$ , mean larval size =  $41964.24 \mu\text{m}^2$ ,  $\text{SE} = 288.11$ ). Larger larvae (originating from larger eggs) hatched sooner than smaller larvae (*t*-test,  $t = 8.863$ , *d.f.* = 174,  $P < 0.001$ ). The standardised selection differential for the effect of propagule size on hatching time was  $1.133$  ( $\text{SE} = 0.149$ ).

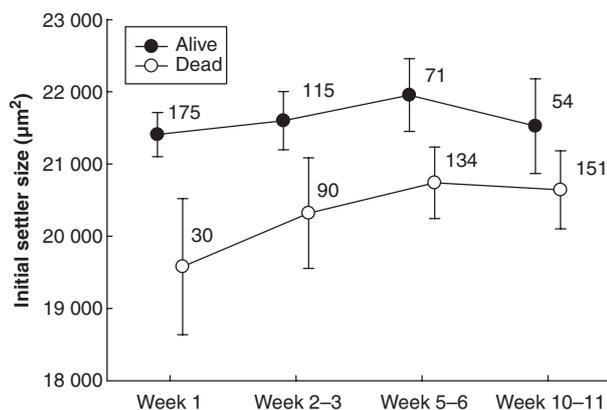
### PROPAGULE SIZE EFFECTS ON LARVAL SWIMMING TIME

We observed that settlement mainly occurred between the 6th and 8th hour, although swimming time spread from the 4th hour up to the 26th hour. There was no relationship between larval size and the time spent swimming before settlement ( $r = -0.116$ ,  $n = 11$ ,  $P = 0.735$ ).

### PROPAGULE SIZE EFFECTS ON POST-METAMORPHIC SURVIVAL AND GROWTH

The experiment started with 205 settlers. Survival decreased gradually throughout the study period with a final figure of *ca.* 40% survival. The effect of initial settler size on survival showed a tendency of greater survival of larger animals that became less marked at later observation times (Fig. 3). Consequently, the overall hazard function (Cox model) showed only marginally significant results ( $\chi^2$  difference between null model and full model =  $3.3072$ , *d.f.* = 1,  $P = 0.0628$ ).

The repeated-measures ANCOVA showed a significant relationship between initial settler size and juvenile size (Table 1)



**Fig. 3.** Mean initial settler size of *Microcosmus squamiger* individuals that survived and died over the study period. Note that we included the individuals from both runs (1st run: measurements 1st, 3rd, 6th and 11th week; 2nd run: 1st, 2nd, 5th and 10th week), and all initial individuals are taken into account to calculate mean sizes for each week. Vertical bars denote standard errors and numbers alongside indicate samples sizes.

**Table 1.** Repeated measures ANCOVA analyzing the settler size as a predictor of juvenile size of *Microcosmus squamiger* in the field throughout the study period. Note that the model was reduced after testing for homogeneity of slopes

Source	d.f.	Mean-Square	F-ratio	P
Between subjects				
Settler size	1	1.6591	7.362	0.0119
Error		0.2253		
Among subjects				
Time	2	15.8071	180.801	<0.0001
Time × Settler size	2	0.0509	0.582	0.5624
Error	50	0.0874		

although this relationship had weakened after 11 weeks in the field (variance explained by settler size was 18%, 39% and 14% for weeks 3, 6 and 11 respectively). At the last observation time, in which the correlation was lower than the two previous observations, settler size was still a good predictor of juvenile size in the field, with a 50% increase in settler size resulting in a 25% increase in juvenile size (Fig. 4).

The standardised selection differential for the effect of post-metamorphic survival was 0.260 (SE = 0.172) and for post-metamorphic size, it was 0.138 (SE = 0.272).

In summary, larger eggs became larger larvae, which developed into larger settlers. Larger propagules hatched sooner as larvae and performed better as juveniles in the field. On the other hand, no effects were found in cleavage rate and larval swimming time.

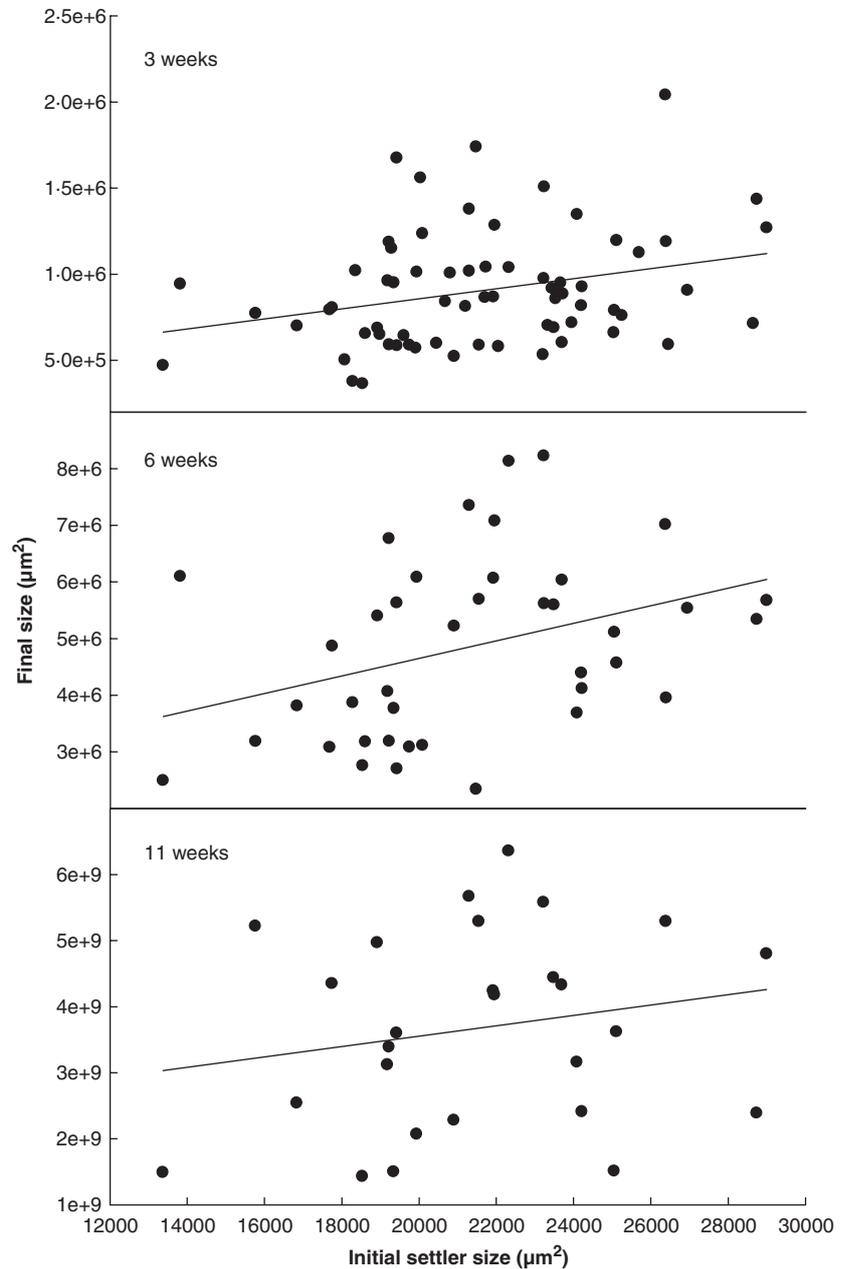
## Discussion

We found mixed effects of propagule size on propagule performance across several life-history stages of *M. squamiger*. The physical effects of propagule size were consistent across early life-history stages: larger eggs became larger larvae and larger settlers. Increasing propagule size affected propagule

performance positively both in the larval phase and the post-metamorphic juvenile phase in the field. While the effects of propagule size on post-metamorphic survival in the field were weak and diminished over time, the effect of propagule size on post-metamorphic growth were remarkably persistent - juveniles that originated as larger settlers were still larger than juveniles that originated as smaller settlers even after more than 10 weeks in the field. Our results suggest that, in this species at least, the relationship between initial propagule size and size of subsequent life-history stages is relatively consistent among the larval and post-metamorphic stages.

## PROPAGULE SIZE EFFECTS ON LARVAL PERFORMANCE

Propagule size affected some larval performance measures but not others. Larvae from larger eggs hatched earlier than larvae from smaller eggs, but propagule size had no effect on the early cleavage rates or the length of the larval swimming period. From a physiological perspective, an association between increased propagule size and faster development is surprising given that larger propagules have smaller surface area to volume ratios and cell cleavage should take longer (Staver & Strathmann 2002). In our case, no relationship between egg size and cleavage rate was observed. Marshall, Styan & Keough (2002) also found that larger propagules hatched into larvae sooner than smaller propagules in the ascidian *Pyura stolonifera*. In some species of ascidian (e.g. *Ciona intestinalis* and *Phallusia obesa*) larger eggs have been reported to take longer to hatch (Marshall & Bolton 2007) whereas in others (e.g. *P. stolonifera* and *M. squamiger*) they hatch earlier. Interestingly, *P. stolonifera* and *M. squamiger* are in the same family, although there are currently too few data to speculate whether the differential effects of propagule size vary according to phylogenetic affinity. Furthermore, the underlying mechanism for the positive effect of propagule size on hatching time in our study requires further exploration - it could be that larger propagules have a higher metabolic rate and develop faster because they have extra resources, although our results show that early cleavage rates do not change with propagule size. It may be that egg composition varies with size in this species and this affects development rate, but this requires further testing. Mortality during the larval phase can be extremely high for many marine invertebrates (Morgan 1995) and any reduction in the length of time spent in this vulnerable phase is likely to have positive effects on fitness. Indeed, one of the first considerations of propagule size evolution in the sea by Vance (1973) focused on the effects of propagule size on the planktonic period. The major benefit associated with increased propagule size in this and later theoretical considerations was a reduction in the planktonic period and thus cumulative mortality (Leviton 1993; Podolsky & Strathmann 1996). In contrast to the effects of propagule size on hatching time, we found no effect of propagule size on larval swimming period prior to settlement. Our findings contrast with studies on other marine invertebrates (including colonial ascidians) whereby larval energetic



**Fig. 4.** Relationship between initial settler size of *Microcosmus squamiger* and juvenile size after 3, 6 and 11 weeks in the field.

reserves strongly affect both larval swimming behaviour and swimming time (Miron *et al.* 2000; Marshall & Keough 2003; Botello & Krug 2006). This has the potential to reduce planktonic mortality and yield higher fitness overall.

#### PROPAGULE SIZE EFFECTS ON POST-METAMORPHIC PERFORMANCE IN THE FIELD

The effects of propagule size in *M. squamiger* extended well beyond metamorphosis in the field, affecting the size of juveniles at least during the first 11 weeks of benthic life (the duration of our experiment). There was also a tendency for juveniles that originated as larger propagules to have a higher survival rate in the field, particularly in the beginning. This is the first time an effect of propagule size on post-metamorphic

performance has been demonstrated for a solitary sessile marine invertebrate over an extend period of time. All the preceding studies of propagule size effects on growth were restricted to colonial marine invertebrates (Marshall, Bolton & Keough 2003; Marshall & Keough 2004). Interestingly, although the effect of propagule size persisted for 11 weeks in the field, the amount of variation in juvenile size that propagule size explained appeared to diminish over time. The mechanism for the effect of propagule size on post-metamorphic size is unclear; larger recruits, by definition, have larger feeding apparatus and may have been able to capture food more effectively (Sherrard & LaBarbera 2005). Alternatively, simple allometric effects may have caused the differences - small initial differences in size may have been magnified as allometric growth occurred.

## PROPAGULE SIZE EFFECTS ACROSS MULTIPLE LIFE-HISTORY STAGES

We were initially interested in examining propagule size effects across life-history stages in this study because we suspected that the direction of selection on propagule size would differ among life-history stages (we suspected that larger propagules would take longer to develop as larvae but would perform better in the field). Surprisingly, we found that propagule size positively affects propagule performance in both life-history stages where an effect was detected. Thus, in this species there were no conflicting selection pressures among the life-history stages that we examined. It seems that the propagule size-performance relationship in the larval phase and the post-metamorphic phase both select for increased propagule size. Thus, based on our results alone, the only balancing selection on propagule size that may counter selection for increased propagule size is the size-fecundity trade-off (Vance 1973; Smith & Fretwell 1974). However, other elements of the life-history that we did not explore may also have had a balancing influence on propagule size selection. We did not examine propagule size effects on fertilization - in some broadcast spawning marine invertebrates including ascidians, larger eggs are more easily fertilized such that in sperm limited environments they have an advantage regarding fertilization (Levitan 1996, 2006; Marshall, Styan & Keough 2002). However, in environments where sperm are in excess, larger eggs are more likely to suffer polyspermy (Levitan 1996; Marshall, Styan & Keough 2002). Thus it would be interesting to examine the performance of differently sized *M. squamiger* eggs under different fertilization environments to determine if there are opposing selection pressures at fertilization to reduce propagule size. In the ascidian *Styela plicata*, mothers avoid this problem by independently varying total egg size (the most important determinant of fertilization success) and maternal energetic investment in each egg (the most important determinant of post-metamorphic performance) via the manipulation of the size of follicle cells surrounding the egg (Crean & Marshall 2008). It may be that egg accessory structures evolved as a means of avoiding potentially conflicting selection pressures at fertilization and beyond, but this requires further testing. Interestingly, when we calculated selection differentials for hatching time, post-metamorphic survival and growth, we found larger values for hatching time, while post-metamorphic survival and growth showed the smallest values. The differences in selection differential among life-history stages are in agreement with other empirical studies but appear to conflict with current theory (Kingsolver *et al.* 2001). While survival in the field will be strongly tied to fitness, hatching time will be less strongly tied to it and so while theory predicts that selection differentials will be greater for traits such as survival compared to hatching time, both our results and the results of meta-analyses find the converse to be true (Kingsolver *et al.* 2001).

Our study shows that the effect of initial propagule size has the potential to affect every one of the different stages across the life history of a particular organism. Overall, in this

species at least, selection on propagule size at both larval and juvenile stages appeared to favour larger propagules. Nevertheless, we eagerly await further studies examining selection on propagule size across the life-history of organisms with complex life-cycles, as we suspect that in many cases selection pressures will be in greater conflict compared to our results here.

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