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

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Running title: Offspring size across life-history stages
Abstract

1. Mothers must balance the fecundity costs of increasing offspring size with the fitness benefits of increased offspring performance and the offspring size-performance is crucial for determining this trade-off.

2. While many studies have examined the offspring size-performance relationship in individual life-history stages, very few have examined the relationship between offspring size and performance across multiple life-history stages despite the ubiquity of complex life cycles and the challenge they represent to provisioning.

3. We examined the offspring size-performance relationship across several life-history stages in the marine invertebrate Microcosmus squamiger (Asciidacea) and specifically asked whether selection on offspring size was consistent across the life-history.

4. Offspring size had effects in some life-history stages and not others: larger offspring 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.

5. The effects of offspring size on juvenile size were remarkably persistent - juveniles that came from larger offspring were still larger than juveniles that came from smaller offspring after 11 weeks in the field.

6. We found no evidence of conflicting selection pressures on offspring size among life-history stages, rather, in this species at least, selection on offspring size at both the larval and juvenile stage appeared to favour larger offspring.

Keywords: fertilization, larval size, offspring performance, post-metamorphic, settlement.
Introduction

The study of offspring size is a central component of life-history theory and has long fascinated evolutionary ecologists (Lack 1947; Bagenal 1969). Offspring size is remarkably variable at all levels of organisation - among species, populations, individuals and even within broods (Clarke 1993; Williams 1994; Fox & Czesak 2000; Marshall & Keough 2008a). Variation in offspring size can have fundamental consequence for population dynamics, species range expansions and evolutionary trajectories (Fox, Czesak & Fox 2001; Buckley et al. 2003; Benton et al. 2005) Interestingly, while offspring size is a shared trait between mother and offspring, selection acts largely to maximise maternal, rather than offspring fitness (Smith & Fretwell 1974). Mothers are thought of face a trade-off with regard to the size and number of offspring that they can produce - any increase in the size of offspring that mothers make must be associated with a decrease in the number they can produce (Smith et al. 1974). The fecundity cost associated with producing offspring of increased size is offset by an increase in offspring performance. Selection will favour mothers that optimise the trade-off between offspring size and number and the major determinant of this optimal balance is the relationship between offspring size and performance (Smith et al. 1974).

The offspring size-performance relationship determines the fitness benefits of producing offspring of a particular size. When there is a steep relationship between offspring size and performance, selection should favour mothers that produce larger offspring because small increases in offspring investment should yield fitness returns that exceed the associated fecundity costs (Smith et al. 1974; Parker & Begon 1986). Conversely, when there is a shallow relationship, mothers will achieve higher fitness by
producing smaller offspring. Thus the size of offspring that mothers make should track closely any shifts in the relationship between offspring size and performance. Indeed there are a growing number of studies that demonstrate that shifts in the offspring size-performance relationship result in shifts in the size of offspring that mothers produce at a range of scales, from inter-populations down to inter-individual variation (Fox, Thakar & Mosseau 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 offspring that mothers produce and the offspring size-performance relationship is perhaps unsurprising in some systems but those with complex life-histories, the challenge of provisioning offspring optimally is far greater.

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

Most studies of offspring size effects are restricted to single life-history stages. Most organisms have at least one mobile life-history stage rendering the estimation of the
offspring size-performance relationship longitudinally across the life-history problematic.
Thus, estimates of the offspring size-performance relationship across multiple life-history
stages are extremely rare (but see Einum et al. 2002). The scarcity of studies that examine
offspring size effects longitudinally means that we currently have little understanding of
whether provisioning offspring with complex life-histories is indeed more challenging
than provisioning offspring with simple life-histories. Combining the results of several
studies, each of which examined a different life-history stage for a single organism might
give some insight into the potential for varying selection on offspring size across life-
history stages. However, offspring size effects can vary dramatically among populations
and even over time in the same population (Marshall et al. 2008b) and so the insight that
can be gained from combining multiple studies is highly limited. Thus, we believe a
longitudinal study offspring size effects in a single population is the most informative approach. Here we examine the effects of offspring size across multiple life-history stages in a marine invertebrate, the solitary ascidian *Microcosmus squamiger*. Marine invertebrates represent an excellent group for the study of offspring size effects across life-history stages for a number of reasons. First, studies show that offspring size affects offspring performance across the entire life-history in this group, from fertilisation, through development, larval settlement and post-metamorphic survival, growth and even reproduction (Marshall et al. 2008a). Second, initial studies on individual life-history stages in isolation suggest that selection on offspring size among different life-history stage could indeed be in conflict (Levitan 1996; Marshall, Styan & Keough 2002). Finally, there has been long standing theoretic interest in the evolution and ecological consequences of offspring size in marine invertebrates but field studies remain relatively rare in this group. We examined the effect of offspring size on post-fertilisation development rate and larval settlement behaviour in the laboratory and we then examined post-metamorphic survival and growth of juveniles under field conditions, a crucial element when considering offspring size effects (Fox 2000).

**Materials and Methods**

**Collection site**

All reproductively mature *Microcosmus 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. The individuals were transported in a 20 litre insulated container with seawater to the lab (travel time ~45 minutes) where they were placed in a constantly aerated tank (20 litres) with locally collected seawater kept at room temperature.

**Fertilization methodology**

We carried out all fertilizations in the laboratory at room temperature by artificially joining male and female gametes from 4 individuals following Marshall *et al.* (2000). To measure sperm concentration, we used a grid haemocytometer (0.0025 mm² quadrats) under the stereomicroscope. As maximum fertilization success for *M. squamiger* was found using the highest concentration (Rius, Turon & Marshall in press), we did all the experiments using the “dry” sperm concentration (between $10^7$ and $10^8$ sperm cells/ml) (see Marshall *et al.* 2000).

**Measures of offspring size**

In order to detect offspring size effects at several stages (from egg to post-metamorphic), we took measurements of each developmental stage by taking digital photographs with a camera attached to the dissecting microscope connected to a computer and subsequently measured the photographs using Image Pro (v. 5.1.0.12, Media Cybernetics). We first asked whether the sizes of individuals at different life history stages were correlated (i.e. do larger eggs become larger larvae? Do larger larvae become larger settlers?). To estimate the relationship between egg size and larval size, we measured individual eggs, fertilised them *in vitro* as described above then placed them into their Petri dishes to allow development to take place. After 14 hours, the swimming
labeled larvae were individually photographed and measured. Most of the cleaved eggs did not develop into larvae, which resulted in very few larvae being available for measurement. In the first run, we achieved 6 successful larval measurements, while in the second run only 4 measurements. To determine if larger larvae became larger settlers we separated 42 larvae using a pipette and placed each one in separate Petri dishes with filtered seawater. We then photographed and measured the individual larvae as described above. Twenty-four hours after hatching, we photographed and measured those larvae that had settled and metamorphosed (n = 22). Importantly, egg size was correlated with larval size (Table 1) and larval size was correlated with settler size (R² = 0.153, P = 0.0365) (Fig. 1). Thus we were able to use the most convenient estimate of offspring size according to which aspect of offspring performance we were interested in (e.g. we could measure settler size alone for examinations of post-metamorphic performance rather than follow individuals from eggs all the way through to settlement. Larger larvae became larger settlers although the relationship between larval size and settler size was weaker than that between egg size and larval size.

_Adult-egg size relationship_

We randomly collected 11 adult individuals in the field and measured them using the maximum longitude of the tunic (to the nearest mm) and the wet weight (to the nearest mg). Subsequently, we dissected the individuals and we photographed 20 eggs per individual to measure their perimeter.

_Egg size/time of cleavage relationship_

To determine the relationship between egg size and time of cleavage, we photo-sequenced the fertilized eggs (i.e. showing a clear cleavage) every 2 minutes. The time
was recorded when the first 4, 8, 16 cells were cleaved for each fertilized egg. We measured the egg perimeter from the 1st photo taken of the undivided eggs. We did 2 runs of this experiment, the first one involving 74 egg measurements and the second with 10 eggs.

**Hatching time vs larval size**

In order to determine the time when the larvae hatched from the eggs, we placed fertilized eggs in a petri dish with filtered seawater. We checked hatching every hour after the fertilization. All hatched larvae within each hour were removed and placed in a vial with seawater including a few drops of formalin to preserve them (preservation does not affect estimates of size; unpubl. data). Later we measured the larvae that had hatched at each time period and we measured the external body perimeter of each larva as our estimate of size.

**Settlement experiments**

To examine the effects of larval size on larval settlement time, as biofilm has been proven to facilitate larval settlement in ascidians (Wieczorek & Todd 1997), we used Petri dishes (60 mm) with biofilm and pre-roughened surface in all settlement experiments. We roughened the Petri dish surface with sand paper and then submerged in seawater for 24 hours. We placed individual hatched larvae in separate Petri dishes with filtered seawater to assess larval swimming time. We photographed the larvae and we placed the Petri dishes to complete darkness. We checked for settlement every hour during a 32 hour period. We tracked a total of 36 larvae.

**Post-settlement performance in the field: effect of settler size on survival and growth**
We placed 20 larvae per Petri dish (60 mm, 30 dishes in total) filled with filtered seawater. After 24 hours, 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 8 mm hole in the centre of the Petri dish using a hand drill. Immediately, we transported the dishes to the field in seawater in 20 l insulated containers. We attached all Petri dishes to two (500 x 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 2 m below the Mean Low Water Spring, facing downwards to reduce the effects of light and sedimentation (following Marshall, Bolton & Keough 2003a).

This experiment was run twice. For the first run, the survival was measured weekly after the deployment in the field using a dissecting microscope. From the 3rd week, all petri dishes were almost entirely covered by fouling organisms which made direct observation of the settlers in the field impossible. Thus, we brought back all Petri dishes to the laboratory the 3rd, 6th and 11th week and estimated settler survival and growth under dissecting microscope 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 brought back the petri dishes to the field and placed them on the same backing plates and position.

We followed the same methodology in the second run, which started a week later than the
first run. In this case, we only measured survival, and this was done on the 1st, 2nd, 5th and 10th week.

Data analysis

To analyse the influence of offspring size on different development parameters such as egg hatching time and larval swimming time, we used regression and t-test when a single run was examined and ANCOVA when we examined multiple runs. In all of these analyses, offspring size was a continuous predictor and run (where included) was a random factor.

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. To examine the effect of settler size on subsequent juvenile size in the field, we used a Repeated-measures ANCOVA where run was a random factor and settler size was a continuous factor. In all of our analyses, we found no effect of Run or an interaction between Run and settler size so both the main effect of Run and the interaction were omitted from the final model (Quinn and Keough 2002). We performed all analyses using the software SYSTAT (v. 11, SPSS Inc., 2004) and STATISTICA (v.6, Statsoft Inc., 2001).
Results

Adult-egg size relationship

There was no relationship between either adult weight nor adult length and the size of eggs that individuals produced (wet weight, \( r = 0.1717, P = 0.6137 \); 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 eggs to divide (Table 2).

Offspring size effects on time until hatching

As most of the larvae (approximately 500) hatched between the 11th and 12th hour, we only measured a subsample of 100 larvae within this hour period. The following hour, 76 larvae hatched, and at both the 14th and 24th hour after fertilization, we found only 3 larvae each time. As a result, to analyse the effect of larval size on hatching time, we only included the larvae from the 12th (n = 100) and 13th hour (n = 76). Larger larvae (and thus larvae from larger eggs) hatched sooner than smaller larvae (t-test, \( t = 8.863, df = 174, P < 0.001 \)) (Fig. 2).

Offspring size effects on larval swimming

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

Offspring size effects on post-metamorphic survival and growth
The experiment started with 146 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 higher survival in larger animals, a tendency that became
less marked at later observation times (Fig. 3). However, the overall regression of size on
the hazard function (Cox model) showed only marginally significant results ($\chi^2$ difference
between null model and full model = 3.3072, df = 1, P = 0.0628).

Settler size was a good predictor of juvenile size in the field throughout our study
period (Table 3) with a 50% increase in settler size resulting in a 25% increase in juvenile
size (Fig. 4).

In summary, larger offspring 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 (Table 4).

Discussion

We found effects of offspring size on offspring performance in several life-history
stages of Microcosmus squamiger. Increasing offspring size affected offspring
performance positively both in the larval phase and the post-metamorphic juvenile phase
in the field. While the effects of offspring size on post-metamorphic survival in the field
were weak and diminished over time, the effect of offspring 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 10 weeks in the field.
Our results suggest that in this species at least, the relationship between offspring size and
performance is relatively constant among the larval and post-metamorphic life-history stages.

**Offspring size effects on larval performance**

Offspring size had mixed effects on larval performance. Larvae from larger eggs hatched approximately 10% earlier than larvae from smaller eggs but offspring size had no effect on the early cleavage rates or the length of the larval swimming period.

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 offspring size evolution in the sea by Vance (1973) focused on the effects of offspring size on planktonic period and the major benefit associated with increased offspring size in this and later theoretical considerations was a reduction in the planktonic period and thus cumulative mortality (Levitan 1993; Podolsky & Strathmann 1996). However, Vance (1973) explicitly partitioned the effects of offspring size pre-feeding period (denoted as $l$) and the feeding period ($p$) and predicted that increases in offspring size should increase the length of $l$ and decrease the length of $p$. For non-feeding larvae, only $l$ is relevant and previous studies supported Vance’s assumption that increasing offspring size lengthened the development time ($l$) of non-feeding larvae (Staver & Strathmann 2002; Marshall & Bolton 2007). In our study, we found the opposite effect - larger offspring developed into larvae sooner than smaller offspring.

From a physiological perspective, an association between faster development and increased offspring size is surprising given that larger offspring have smaller surface area to volume ratios and cell cleavage should take longer (Staver et al. 2002). Marshall et al.
(2002) also found that larger offspring hatched into larvae sooner than smaller offspring in the ascidian Pyura stolonifera. Thus in some species of ascidian (e.g. Ciona intestinalis, Phallusia obesa) larger eggs take longer to hatch (Marshall et al. 2007) whereas in others (e.g. P. stolonifera and M. squamiger) smaller eggs take longer to hatch. Interestingly, P. stolonifera and M. squamiger are in the same family but there are currently too few data to speculate whether the differential effects of offspring size vary according to phylogenetic affinity. Furthermore, the underlying mechanism for the positive effect of offspring size on hatching time in our study requires further exploration - it could be that larger offspring have a higher metabolic rate and develop faster because of they have extra resources but our results show that early cleavage rates do not change with offspring size. It may be that egg composition varies with size in this species and this affects development rate but this requires further testing. Regardless of the underlying cause, larger eggs hatch into larvae sooner than smaller eggs and this is likely to reduce planktonic mortality and yield higher fitness overall.

In contrast to the effects of offspring size on hatching time, we found no effect of offspring size on larval swimming period prior to settlement - larval size did not affect the maximum time spent swimming before settling. Our findings contrast with studies on other marine invertebrates (including colonial ascidians) whereby larval energetic reserves strongly affect both swimming behaviour and settlement time (Miron et al. 2000; Marshall & Keough 2003b; Botello & Krug 2006). Nevertheless, our study suggests that larvae from different size eggs will differ slightly in their dispersal potential - larger larvae likely to disperse less far than smaller larvae because they hatch and become competent to metamorphose sooner. Thus, in marine invertebrates with non-feeding
larvae, there appear to be two mechanisms by which offspring size can affect larval
dispersal, first by affecting when they hatch (as in this study) and second by determining
the length of the larval period prior to settlement.

**Offspring size effects on post-metamorphic performance in the field**

The effects of offspring size in *M. squamiger* extended well beyond metamorphosis in
the field affecting the size of juveniles after almost three months in the field. There was
also a tendency for juveniles that originated as larger offspring to have higher survival in
the field, particularly initially, but this effect was not significant. This is the first time an
effect of offspring size on post-metamorphic has been demonstrated for a solitary sessile
marine invertebrate, all of the preceding studies of offspring size effects on growth have
been restricted to colonial marine invertebrates (Marshall *et al.* 2003a; Marshall &
Keough 2004). Interestingly, although the effect of offspring size persisted for 11 weeks
in the field, the amount of variation in juvenile size that offspring size explained appeared
to diminish over time. The mechanism for the effect of offspring size on post-
metamorphic size is unclear, larger settlers, by definition, had larger feeding apparatuses
and may have been able to capture food more effectively. Alternatively, simple allometric
effects may have driven the differences - small initial differences in size may have been
magnified as allometric growth occurred.

**Offspring size effects across multiple life-history stages**

We were initially interested in examining offspring size effects longitudinally in this
study because we suspected that the direction of selection on offspring size would differ
among life-history stages (we suspected that larger offspring would take longer to
develop as larvae but would perform better in the field). We were surprised to find that
offspring size positively affects offspring performance in both life-history stages where an effect was detected and thus, in this species, there were no conflicting selection pressures among the life-history stages that we examined. It seems that the offspring size-performance relationship in the larval phase and the post-metamorphic phase both select for increased offspring size. Thus, based on our results alone, the only balancing selection on offspring size that may counter selection for increased offspring size is the size fecundity trade-off (Vance 1973). However, other elements of the life-history that we did not explore may also have had a balancing influence on offspring size selection. We did not examine offspring size effects on fertilisation - in some broadcast spawning marine invertebrates including ascidians, larger eggs are more easily fertilised and so in sperm limiting environments, they have an advantage at fertilisation (Levitan 1996; Marshall et al. 2002). However, in environments where sperm are in excess, larger eggs can be more likely to suffer polyspermy (Levitan 1996; Marshall et al. 2002). Thus it would be interesting to examine the performance of different sized *M. squamiger* eggs under different fertilisation environments to determine if there is ever a countering selection pressures at fertilisation to reduce offspring size. Interestingly, in the ascidian *Styela plicata*, mothers avoid this problem by independently varying total egg target size (important for fertilisation) and ovicell size (important for post-fertilisation 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 fertilisation and beyond but this requires further testing.
Acknowledgements: We are grateful to B. Galletly for assistance both in the field and the laboratory. Funding was obtained from project DPO6666147 of the Australian Research Council to DJM, the project CTM2007-66635 of the Spanish Government to XT and a travel grant to MR from the Spanish “Ministerio de Educación y Ciencia”. This work was carried out in accordance with the laws of Australia.


Tables and figures legends

Table 1. ANCOVA test of egg size on larval size of *Microcosmus squamiger*. Note that the model is reduced after testing for homogeneity of slopes.

Table 2. ANCOVA tests assessing the effect of egg size of *Microcosmus squamiger* on the time taken for cell eggs to divide. Note that the models are reduced after testing for homogeneity of slopes.

Table 3. 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.

Table 4. Summary table of the effects found for each of the life-history stages studied in *Microcosmus squamiger*.

Figure 1. Relationship between larval size and settler size of *Microcosmus squamiger*. The trend line refers to the linear regression indicated in the text.

Figure 2. Mean larval size of *Microcosmus squamiger* at 12 and 13 hours after fertilization. Vertical bars denote standard errors.

Figure 3. Mean initial settler size of *Microcosmus squamiger* of the individuals that survived and died over the study period. Vertical bars denote standard errors.

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

Table 1.

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<td>Positive</td>
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</tbody>
</table>
Figure 1

![Graph showing the relationship between area larva ($\mu m^2$) and area settler ($\mu m^2$).]
Figure 2

![Graph showing hatching time vs larva area (µm²)]
Figure 3

![Graph showing Initial settler size (µm²) over Week 1, Week 3, Week 6, and Week 11. The graph compares Alive and Dead settler sizes with error bars.]

- Alive
- Dead

Initial settler size (µm²)

Week 1 | Week 3 | Week 6 | Week 11
---|---|---|---
18000 | 19000 | 20000 | 21000
22000 | 23000 | 22000 | 21000
Figure 4

3 weeks

Final size (µm²) vs. Initial settler size (µm²)

6 weeks

11 weeks

Functional Ecology