

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

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23 **Running title:** Offspring size across life-history stages

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Abstract

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

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

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

38 4. Offspring size had effects in some life-history stages and not others: larger
39 offspring hatched sooner as larvae and performed better as juveniles in the field. On the
40 other hand, no effects were found in cleavage rate and larval swimming time.

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

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

47 **Keywords:** fertilization, larval size, offspring performance, post-metamorphic,
48 settlement.

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Introduction

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

66 The offspring size-performance relationship determines the fitness benefits of
67 producing offspring of a particular size. When there is a steep relationship between
68 offspring size and performance, selection should favour mothers that produce larger
69 offspring because small increases in offspring investment should yield fitness returns that
70 exceed the associated fecundity costs (Smith *et al.* 1974; Parker & Begon 1986).
71 Conversely, when there is a shallow relationship, mothers will achieve higher fitness by

72 producing smaller offspring. Thus the size of offspring that mothers make should track
73 closely any shifts in the relationship between offspring size and performance. Indeed
74 there are a growing number of studies that demonstrate that shifts in the offspring size-
75 performance relationship result in shifts in the size of offspring that mothers produce at a
76 range of scales, from inter-populations down to inter-individual variation (Fox, Thakar &
77 Mosseau 1997; Fox 2000; Hendrickx, Maelfait & Lens 2003; Maruyama, Rusuwa &
78 Yuma 2003; Plaistow *et al.* 2007; Russell *et al.* 2007; Marshall & Keough 2008b). The
79 tight coupling between the size of offspring that mothers produce and the offspring size-
80 performance relationship is perhaps unsurprising in some systems but those with complex
81 life-histories, the challenge of provisioning offspring optimally is far greater.

82 Most organisms have complex life-cycles whereby offspring pass through
83 multiple life-history stages before reaching adulthood. If offspring size affects each of
84 these life-history stages in different ways, then offspring provisioning becomes
85 problematic. For example, how should mothers provision their offspring if increased
86 offspring size positively influences performance in one life-history stage but negatively
87 influences a later life-history stage? There is some evidence that offspring size can affect
88 offspring performance in different ways among life-history stages: smaller offspring can
89 be favoured during the larval stage but larger offspring are favoured during the
90 juvenile/adult stage (Kaplan 1992; Einum, Hendry & Fleming 2002; Marshall *et al.*
91 2008a). Given that different life-history stages can have different trophic requirements,
92 can live in different habitats and can also be subject to different physiological constraints,
93 it seems inevitable that the relationship between offspring size and performance should
94 vary among life-history stages. If different life-history stages select for different optimal

95 offspring sizes, the offspring size mothers produce may be very different to that which
96 would be predicted based on the relationship in one life-history stage alone.
97 Alternatively, selection for different offspring sizes among life-history stages could lead
98 to a bet-hedging strategy being favoured because mothers are unable to provision
99 offspring optimally as the cumulative offspring size-performance relationship could be
100 highly unpredictable (Marshall, Bonduriansky & Bussiere in press). Thus there is the
101 potential for conflicting selection pressures on optimal offspring size among different
102 life-history stages with interesting consequences but this potential remains relatively
103 unexplored.

104 Most studies of offspring size effects are restricted to single life-history stages.
105 Most organisms have at least one mobile life-history stage rendering the estimation of the
106 offspring size-performance relationship longitudinally across the life-history problematic.
107 Thus, estimates of the offspring size-performance relationship across multiple life-history
108 stages are extremely rare (but see Einum *et al.* 2002). The scarcity of studies that examine
109 offspring size effects longitudinally means that we currently have little understanding of
110 whether provisioning offspring with complex life-histories is indeed more challenging
111 than provisioning offspring with simple life-histories. Combining the results of several
112 studies, each of which examined a different life-history stage for a single organism might
113 give some insight into the potential for varying selection on offspring size across life-
114 history stages. However, offspring size effects can vary dramatically among populations
115 and even over time in the same population (Marshall *et al.* 2008b) and so the insight that
116 can be gained from combining multiple studies is highly limited. Thus, we believe a

141 large rocky breakwater. The collections took place during low tide periods between
142 October and December 2006. The individuals were transported in a 20 litre insulated
143 container with seawater to the lab (travel time ~45 minutes) where they were placed in a
144 constantly aerated tank (20 litres) with locally collected seawater kept at room
145 temperature.

146 *Fertilization methodology*

147 We carried out all fertilizations in the laboratory at room temperature by
148 artificially joining male and female gametes from 4 individuals following Marshall *et al.*
149 (2000). To measure sperm concentration, we used a grid haemocytometer (0.0025 mm²
150 quadrats) under the stereomicroscope. As maximum fertilization success for *M.*
151 *squamiger* was found using the highest concentration (Rius, Turon & Marshall in press),
152 we did all the experiments using the “dry” sperm concentration (between 10⁷ and 10⁸
153 sperm cells/ml) (see Marshall *et al.* 2000).

154 *Measures of offspring size*

155 In order to detect offspring size effects at several stages (from egg to post-
156 metamorphic), we took measurements of each developmental stage by taking digital
157 photographs with a camera attached to the dissecting microscope connected to a
158 computer and subsequently measured the photographs using Image Pro (v. 5.1.0.12,
159 Media Cybernetics). We first asked whether the sizes of individuals at different life
160 history stages were correlated (i.e. do larger eggs become larger larvae? Do larger larvae
161 become larger settlers?). To estimate the relationship between egg size and larval size, we
162 measured individual eggs, fertilised them *in vitro* as described above then placed them
163 into their Petri dishes to allow development to take place. After 14 hours, the swimming

164 larvae were individually photographed and measured. Most of the cleaved eggs did not
165 develop into larvae, which resulted in very few larvae being available for measurement.
166 In the first run, we achieved 6 successful larval measurements, while in the second run
167 only 4 measurements. To determine if larger larvae became larger settlers we separated
168 42 larvae using a pipette and placed each one in separate Petri dishes with filtered
169 seawater. We then photographed and measured the individual larvae as described above.
170 Twenty-four hours after hatching, we photographed and measured those larvae that had
171 settled and metamorphosed ($n = 22$). Importantly, egg size was correlated with larval size
172 (Table 1) and larval size was correlated with settler size ($R^2 = 0.153$, $P = 0.0365$) (Fig. 1).
173 Thus we were able to use the most convenient estimate of offspring size according to
174 which aspect of offspring performance we were interested in (e.g. we could measure
175 settler size alone for examinations of post-metamorphic performance rather than follow
176 individuals from eggs all the way through to settlement. Larger larvae became larger
177 settlers although the relationship between larval size and settler size was weaker than that
178 between egg size and larval size.

179 *Adult-egg size relationship*

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

184 *Egg size/time of cleavage relationship*

185 To determine the relationship between egg size and time of cleavage, we photo-
186 sequenced the fertilized eggs (i.e. showing a clear cleavage) every 2 minutes. The time

187 was recorded when the first 4, 8, 16 cells were cleaved for each fertilized egg. We
188 measured the egg perimeter from the 1st photo taken of the undivided eggs. We did 2 runs
189 of this experiment, the first one involving 74 egg measurements and the second with 10
190 eggs.

191 *Hatching time vs larval size*

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

199 *Settlement experiments*

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

208 *Post-settlement performance in the field: effect of settler size on survival and* 209 *growth*

210 We placed 20 larvae per Petri dish (60 mm, 30 dishes in total) filled with filtered
211 seawater. After 24 hours, we rinsed them with filtered seawater to remove any unattached
212 larvae, and then the settlers were photographed for measurement. We then marked and
213 numbered their positions using a pencil on the Petri dish surface. Subsequently, we gently
214 made an 8 mm hole in the centre of the Petri dish using a hand drill. Immediately, we
215 transported the dishes to the field in seawater in 20 l insulated containers. We attached all
216 Petri dishes to two (500 x 500 mm) Perspex backing plates (15 Petri dishes in each plate)
217 using stainless steel screws. The Petri dish positions were randomly assigned. We hung
218 the plates from the most external pontoon at Manly harbour at a depth of 2 m below the
219 Mean Low Water Spring, facing downwards to reduce the effects of light and
220 sedimentation (following Marshall, Bolton & Keough 2003a).

221 This experiment was run twice. For the first run, the survival was measured
222 weekly after the deployment in the field using a dissecting microscope. From the 3rd
223 week, all petri dishes were almost entirely covered by fouling organisms which made
224 direct observation of the settlers in the field impossible. Thus, we brought back all Petri
225 dishes to the laboratory the 3rd, 6th and 11th week and estimated settler survival and
226 growth under dissecting microscope after carefully removing all non-*M. squamiger*
227 species from the dish surface. During the same inspection, we took photographs of the
228 settlers for settler size measurements. After the examination, we maintained all petri
229 dishes in an aerated tank at room temperature overnight. The following day, we brought
230 back the petri dishes to the field and placed them on the same backing plates and position.
231 We followed the same methodology in the second run, which started a week later than the

232 first run. In this case, we only measured survival, and this was done on the 1st, 2nd, 5th and
233 10th week.

234 *Data analysis*

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

240 For the effect of settler size on survival in the field, we used Cox's proportional
241 hazard regression, which models a hazard rate as a function of survival time and
242 independent covariates (in our case, settler size). The fit (log-likelihood) of the models
243 with and without the covariates was compared with a chi-square test. To examine the
244 effect of settler size on subsequent juvenile size in the field, we used a Repeated-
245 measures ANCOVA where run was a random factor and settler size was a continuous
246 factor. In all of our analyses, we found no effect of Run or an interaction between Run
247 and settler size so both the main effect of Run and the interaction were omitted from the
248 final model (Quinn and Keough 2002). We performed all analyses using the software
249 SYSTAT (v. 11, SPSS Inc., 2004) and STATISTICA (v.6, Statsoft Inc., 2001).

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Results

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Adult-egg size relationship

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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, $r = -0.018$, $P = 0.958$).

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Egg size/time of cleavage relationship

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There was no effect of egg size on the time taken for the eggs to divide (Table 2).

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Offspring size effects on time until hatching

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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).

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Offspring size effects on larval swimming

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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 ($r = -0.116$, $n = 11$, $P = 0.735$).

271

Offspring size effects on post-metamorphic survival and growth

272 The experiment started with 146 settlers. Survival decreased gradually throughout
273 the study period with a final figure of ca. 40% survival. The effect of initial settler size on
274 survival showed a tendency of higher survival in larger animals, a tendency that became
275 less marked at later observation times (Fig. 3). However, the overall regression of size on
276 the hazard function (Cox model) showed only marginally significant results (χ^2 difference
277 between null model and full model = 3.3072, df = 1, P = 0.0628).

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

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282 In summary, larger offspring hatched sooner as larvae and performed better as
283 juveniles in the field. On the other hand, no effects were found in cleavage rate and larval
284 swimming time (Table 4).

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Discussion

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289 We found effects of offspring size on offspring performance in several life-history
290 stages of *Microcosmus squamiger*. Increasing offspring size affected offspring
291 performance positively both in the larval phase and the post-metamorphic juvenile phase
292 in the field. While the effects of offspring size on post-metamorphic survival in the field
293 were weak and diminished over time, the effect of offspring size on post-metamorphic
294 growth were remarkably persistent - juveniles that originated as larger settlers were still
295 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

296 performance is relatively constant among the larval and post-metamorphic life-history
297 stages.

298 *Offspring size effects on larval performance*

299 Offspring size had mixed effects on larval performance. Larvae from larger eggs
300 hatched approximately 10% earlier than larvae from smaller eggs but offspring size had
301 no effect on the early cleavage rates or the length of the larval swimming period.
302 Mortality during the larval phase can be extremely high for many marine invertebrates
303 (Morgan 1995) and any reduction in the length of time spent in this vulnerable phase is
304 likely to have positive effects on fitness. Indeed, one of the first considerations of
305 offspring size evolution in the sea by Vance (1973) focused on the effects of offspring
306 size on planktonic period and the major benefit associated with increased offspring size in
307 this and later theoretical considerations was a reduction in the planktonic period and thus
308 cumulative mortality (Levitan 1993; Podolsky & Strathmann 1996). However, Vance
309 (1973) explicitly partitioned the effects of offspring size pre-feeding period (denoted as l)
310 and the feeding period (p) and predicted that increases in offspring size should *increase*
311 the length of l and *decrease* the length of p . For non-feeding larvae, only l is relevant and
312 previous studies supported Vance's assumption that increasing offspring size lengthened
313 the development time (l) of non-feeding larvae (Staver & Strathmann 2002; Marshall &
314 Bolton 2007). In our study, we found the opposite effect - larger offspring developed into
315 larvae sooner than smaller offspring.

316 From a physiological perspective, an association between faster development and
317 increased offspring size is surprising given that larger offspring have smaller surface area
318 to volume ratios and cell cleavage should take longer (Staver *et al.* 2002). Marshall *et al.*

319 (2002) also found that larger offspring hatched into larvae sooner than smaller offspring
320 in the ascidian *Pyura stolonifera*. Thus in some species of ascidian (e.g. *Ciona*
321 *intestinalis*, *Phallusia obesa*) larger eggs take longer to hatch (Marshall *et al.* 2007)
322 whereas in others (e.g. *P. stolonifera* and *M. squamiger*) smaller eggs take longer to
323 hatch. Interestingly, *P. stolonifera* and *M. squamiger* are in the same family but there are
324 currently too few data to speculate whether the differential effects of offspring size vary
325 according to phylogenetic affinity. Furthermore, the underlying mechanism for the
326 positive effect of offspring size on hatching time in our study requires further exploration
327 - it could be that larger offspring have a higher metabolic rate and develop faster because
328 of they have extra resources but our results show that early cleavage rates do not change
329 with offspring size. It may be that egg composition varies with size in this species and
330 this affects development rate but this requires further testing. Regardless of the
331 underlying cause, larger eggs hatch into larvae sooner than smaller eggs and this is likely
332 to reduce planktonic mortality and yield higher fitness overall.

333 In contrast to the effects of offspring size on hatching time, we found no effect of
334 offspring size on larval swimming period prior to settlement - larval size did not affect
335 the maximum time spent swimming before settling. Our findings contrast with studies on
336 other marine invertebrates (including colonial ascidians) whereby larval energetic
337 reserves strongly affect both swimming behaviour and settlement time (Miron *et al.* 2000;
338 Marshall & Keough 2003b; Botello & Krug 2006). Nevertheless, our study suggests that
339 larvae from different size eggs will differ slightly in their dispersal potential - larger
340 larvae likely to disperse less far than smaller larvae because they hatch and become
341 competent to metamorphose sooner. Thus, in marine invertebrates with non-feeding

342 larvae, there appear to be two mechanisms by which offspring size can affect larval
343 dispersal, first by affecting when they hatch (as in this study) and second by determining
344 the length of the larval period prior to settlement.

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

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

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

361 We were initially interested in examining offspring size effects longitudinally in this
362 study because we suspected that the direction of selection on offspring size would differ
363 among life-history stages (we suspected that larger offspring would take longer to
364 develop as larvae but would perform better in the field). We were surprised to find that

365 offspring size positively affects offspring performance in both life-history stages where
366 an effect was detected and thus, in this species, there were no conflicting selection
367 pressures among the life-history stages that we examined. It seems that the offspring size-
368 performance relationship in the larval phase and the post-metamorphic phase both select
369 for increased offspring size. Thus, based on our results alone, the only balancing selection
370 on offspring size that may counter selection for increased offspring size is the size
371 fecundity trade-off (Vance 1973). However, other elements of the life-history that we did
372 not explore may also have had a balancing influence on offspring size selection. We did
373 not examine offspring size effects on fertilisation - in some broadcast spawning marine
374 invertebrates including ascidians, larger eggs are more easily fertilised and so in sperm
375 limiting environments, they have an advantage at fertilisation (Levitan 1996; Marshall *et*
376 *al.* 2002). However, in environments where sperm are in excess, larger eggs can be more
377 likely to suffer polyspermy (Levitan 1996; Marshall *et al.* 2002). Thus it would be
378 interesting to examine the performance of different sized *M. squamiger* eggs under
379 different fertilisation environments to determine if there is ever a countering selection
380 pressures at fertilisation to reduce offspring size. Interestingly, in the ascidian *Styela*
381 *plicata*, mothers avoid this problem by independently varying total egg target size
382 (important for fertilisation) and ovicell size (important for post-fertilisation performance)
383 via the manipulation of the size of follicle cells surrounding the egg (Crean & Marshall
384 2008). It may be that egg accessory structures evolved as a means of avoiding potentially
385 conflicting selection pressures at fertilisation and beyond but this requires further testing.
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For Peer Review

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References

- 412 Bagenal, T.B. (1969) Relationship between egg size and fry survival in brown trout
413 *Salmo trutta* L. *Journal of Fish Biology* **1**, 349-353.
- 414 Benton, T.G., Plaistow, S.J., Beckerman, A.P., Lapsley, C.T. & Littlejohns, S. (2005)
415 Changes in maternal investment in eggs can affect population dynamics.
416 *Proceedings of the Royal Society B-Biological Sciences* **272**, 1351-1356.
- 417 Botello, G. & Krug, P.J. (2006) Desperate larvae revisited: age, energy and experience
418 affect sensitivity to settlement cues in larvae of the gastropod *Alderia* sp. *Marine*
419 *Ecology Progress Series* **312**, 149-159.
- 420 Buckley, Y.M., Downey, P., Fowler, S.V., Hill, R., Memmot, J., Norambuena, H.,
421 Pitcairn, M., Shaw, R., Sheppard, A.W., Winks, C., Wittenberg, R. & Rees, M.
422 (2003) Are invasives bigger? A global study of seed size variation in two invasive
423 shrubs. *Ecology* **84**, 1434-1440.
- 424 Clarke, A. (1993) Reproductive Trade-Offs in Caridean Shrimps. *Functional Ecology* **7**,
425 411-419.
- 426 Crean, A.J. & Marshall, D.J. (2008) Gamete plasticity in a broadcast spawning marine
427 invertebrate. *Proceedings of the National Academy of Sciences of the United*
428 *States of America* **105**, 13508-13513.
- 429 Einum, S., Hendry, A.P. & Fleming, I.A. (2002) Egg-size evolution in aquatic
430 environments: does oxygen availability constrain size? *Proceedings of the Royal*
431 *Society of London Series B-Biological Sciences* **269**, 2325-2330.
- 432 Fox, C.W. (2000) Natural selection on seed-beetle egg size in nature and the laboratory:
433 Variation among environments. *Ecology* **81**, 3029-3035.
- 434 Fox, C.W. & Czesak, M.E. (2000) Evolutionary ecology of progeny size in arthropods.
435 *Annual Review of Entomology* **45**, 341-369.
- 436 Fox, C.W., Czesak, M.E. & Fox, R.W. (2001) Consequences of plant resistance for
437 herbivore survivorship, growth, and selection on egg size. *Ecology* **82**, 2790-2804.
- 438 Fox, C.W., Thakar, M.S. & Mosseau, T.A. (1997) Egg size plasticity in a seed beetle: An
439 adaptive maternal effect. *American Naturalist* **149**, 149-163.
- 440 Hendrickx, F., Maelfait, J.P. & Lens, L. (2003) Relationship between fluctuating
441 asymmetry and fitness within and between stressed and unstressed populations of
442 the wolf spider *Pirata piraticus*. *Journal of Evolutionary Biology* **16**, 1270-1279.
- 443 Kaplan, R.H. (1992) Greater maternal investment can decrease offspring survival in the
444 frog *Bombina orientalis*. *Ecology* **73**, 280-288.
- 445 Lack, D. (1947) The significance of clutch size. *Ibis* **89**, 302-352.
- 446 Levitan, D.R. (1993) The importance of sperm limitation to the evolution of egg size in
447 marine invertebrates. *American Naturalist* **141**, 517-536.
- 448 Levitan, D.R. (1996) Predicting optimal and unique egg size in free spawning marine
449 invertebrates. *American Naturalist* **148**, 174-188.
- 450 Marshall, D.J. & Bolton, T.F. (2007) Effects of egg size on the development time of non-
451 feeding larvae. *Biological Bulletin* **212**, 6-11.
- 452 Marshall, D.J., Bolton, T.F. & Keough, M.J. (2003a) Offspring size affects the post-
453 metamorphic performance of a colonial marine invertebrate. *Ecology* **84**, 3131-
454 3137.

- 455 Marshall, D.J., Bonduriansky, R. & Bussiere, L.F. (in press) Offspring size variation
456 within broods as a bet-hedging strategy in unpredictable environments. *Ecology*.
- 457 Marshall, D.J. & Keough, M.J. (2003b) Variation in the dispersal potential of non-
458 feeding invertebrate larvae: the desperate larva hypothesis and larval size. *Marine*
459 *Ecology Progress Series* **255**, 145-153.
- 460 Marshall, D.J. & Keough, M.J. (2004) Variable effects of larval size on post-
461 metamorphic performance in the field. *Marine Ecology Progress Series* **279**, 73-
462 80.
- 463 Marshall, D.J. & Keough, M.J. (2008a) The evolutionary ecology of offspring size in
464 marine invertebrates. *Advances in Marine Biology* **53**, 1-60.
- 465 Marshall, D.J. & Keough, M.J. (2008b) The relationship between offspring size and
466 performance in the sea. *American Naturalist* **171**, 214-224.
- 467 Marshall, D.J., Styan, C.A. & Keough, M.J. (2000) Intraspecific co-variation between
468 egg and body size affects fertilization kinetics of free-spawning marine
469 invertebrates. *Marine Ecology Progress Series* **195**, 305-309.
- 470 Marshall, D.J., Styan, C.A. & Keough, M.J. (2002) Sperm environment affects offspring
471 quality in broadcast spawning marine invertebrates. *Ecology Letters* **5**, 173-176.
- 472 Maruyama, A., Rusuwa, B. & Yuma, M. (2003) Interpopulational egg-size variation of a
473 landlocked *Rhinogobius* goby related to the risk of larval starvation.
474 *Environmental Biology of Fishes* **67**, 223-230.
- 475 Miron, G., Walters, L.J., Tremblay, R. & Bourget, E. (2000) Physiological condition and
476 barnacle larval behavior: a preliminary look at the relationship between
477 TAG/DNA ratio and larval substratum exploration in *Balanus amphitrite*. *Marine*
478 *Ecology Progress Series* **198**, 303-310.
- 479 Morgan, S.G. (1995) Life and Death in the plankton: larval mortality and adaptation.
480 *Ecology of Marine Invertebrate Larvae* (ed L. McEdward), pp. 279-322. CRC
481 Press Inc., Boce Raton.
- 482 Parker, G.A. & Begon, M. (1986) Optimal Egg Size and Clutch Size - Effects of
483 Environment and Maternal Phenotype. *American Naturalist* **128**, 573-592.
- 484 Plaistow, S.J., St Clair, J.J.H., J., G. & Benton, T.G. (2007) How to put all your eggs in
485 one basket: empirical patterns of offspring provisioning through out a mother's
486 lifetime. *American Naturalist* **170**, 520-529.
- 487 Podolsky, R.D. & Strathmann, R.R. (1996) Evolution of egg size in free spawners:
488 consequences of the fertilisation-fecundity trade-off. *American Naturalist* **148**,
489 160-173.
- 490 Rius, M., Turon, X. & Marshall, D.J. (in press) Non-lethal effects of an invasive species
491 in the marine environment - the importance of early life-history stages. *Oecologia*.
- 492 Russell, A.F., Langmore, N.E., Cockburn, A., Astheimer, L.B. & Kilner, R.M. (2007)
493 Reduced egg investment can conceal helper effects in cooperatively breeding
494 birds. *Science* **317**, 941-944.
- 495 Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of
496 offspring. *American Naturalist* **108**, 499-506.
- 497 Staver, J.M. & Strathmann, R.R. (2002) Evolution of fast development of planktonic
498 embryos to early swimming. *Biological Bulletin* **203**, 58-69.
- 499 Vance, R.R. (1973) On reproductive strategies in marine benthic invertebrates. *American*
500 *Naturalist* **107**, 339-352.

501 Wiczorek, S.K. & Todd, C.D. (1997) Inhibition and facilitation of bryozoan and
502 ascidian settlement by natural multi-species biofilms: effects of film age and the
503 roles of active and passive larval attachment. *Marine Biology* **128**, 463-473.
504 Williams, M.D. (1994) Intraspecific variation in egg size and egg composition in birds:
505 effects on offspring fitness. *Biological Reviews* **68**, 38-59.
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547 **Tables and figures legends**

548

549 Table 1. ANCOVA test of egg size on larval size of *Microcosmus squamiger*.

550 Note that the model is reduced after testing for homogeneity of slopes.

551 Table 2. ANCOVA tests assessing the effect of egg size of *Microcosmus*

552 *squamiger* on the time taken for cell eggs to divide. Note that the models are reduced

553 after testing for homogeneity of slopes.

554 Table 3. Repeated measures ANCOVA analyzing the settler size as a predictor of

555 juvenile size of *Microcosmus squamiger* in the field throughout the study period. Note

556 that the model was reduced after testing for homogeneity of slopes.

557 Table 4. Summary table of the effects found for each of the life-history stages

558 studied in *Microcosmus squamiger*.

559 Figure 1. Relationship between larval size and settler size of *Microcosmus*

560 *squamiger*. The trend line refers to the linear regression indicated in the text.

561 Figure 2. Mean larval size of *Microcosmus squamiger* at 12 and 13 hours after

562 fertilization. Vertical bars denote standard errors.

563 Figure 3. Mean initial settler size of *Microcosmus squamiger* of the individuals

564 that survived and died over the study period. Vertical bars denote standard errors.

565 Figure 4. Relationship between initial settler size of *Microcosmus squamiger* and

566 juvenile size after 3, 6 and 11 weeks in the field.

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570 **Tables and figures**

571

572 Table 1.

Source	df	Mean-Square	F-ratio	P
RUN	1	8.7953E ⁺⁰⁷	14.735	0.006
EGGSIZE	1	4.4833E ⁺⁰⁷	7.511	0.0145
Error	7	5969036.185		

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606 Table 2.

2 to 4 cells / Source	df	Mean-Square	F-ratio	P
RUN	1	0.637	0.058	0.810
EGGSIZE	1	3.657	0.333	0.566
Error	75	10.990		
2 to 8 cells / Source	df	Mean-Square	F-ratio	P
RUN	1	0.576	2.148	0.162
EGGSIZE	1	2.342	0.902	0.356
Error	16	0.596		
2 to 16 cells / Source	df	Mean-Square	F-ratio	P
RUN	1	91.993	17.058	0.001
EGGSIZE	1	10.655	1.976	0.179
Error	16	5.393		

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621 Table 3.

Source	df	Mean-Square	F-ratio	P
<i>Between subjects</i>				
Settler size	1	1.6591	7.3626	0.0119
<i>Among Subjects</i>				
Time	2	15.8071	180.8015	<0.0001
Time x Settler size	2	0.0509	0.5823	0.5624
Error	50	0.0874		

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641 Table 4.

Life-history stage	Effect?	Effect of offspring size on fitness
<i>Cleavage rate</i>	No	N/A
<i>Hatching time</i>	Yes	Positive
<i>Swimming time</i>	No	N/A
<i>Post-metamorphic survival</i>	Yes	N/A
<i>Post-metamorphic growth</i>	Yes	Positive

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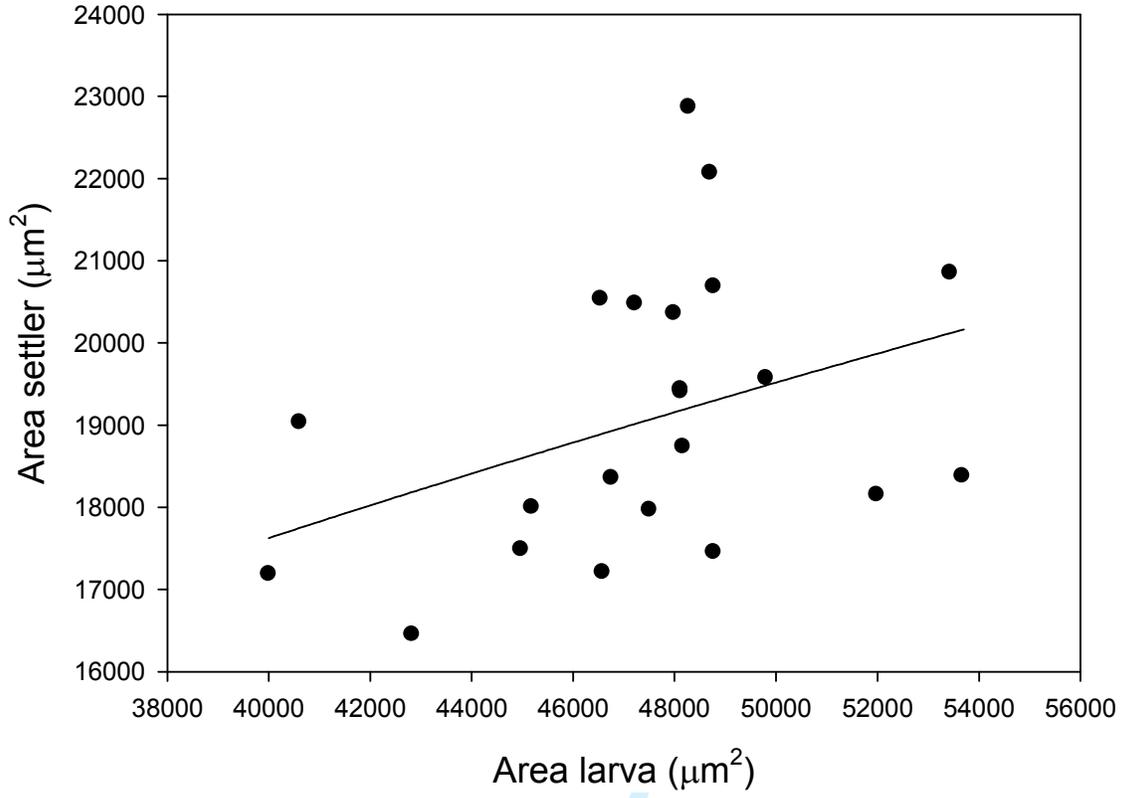
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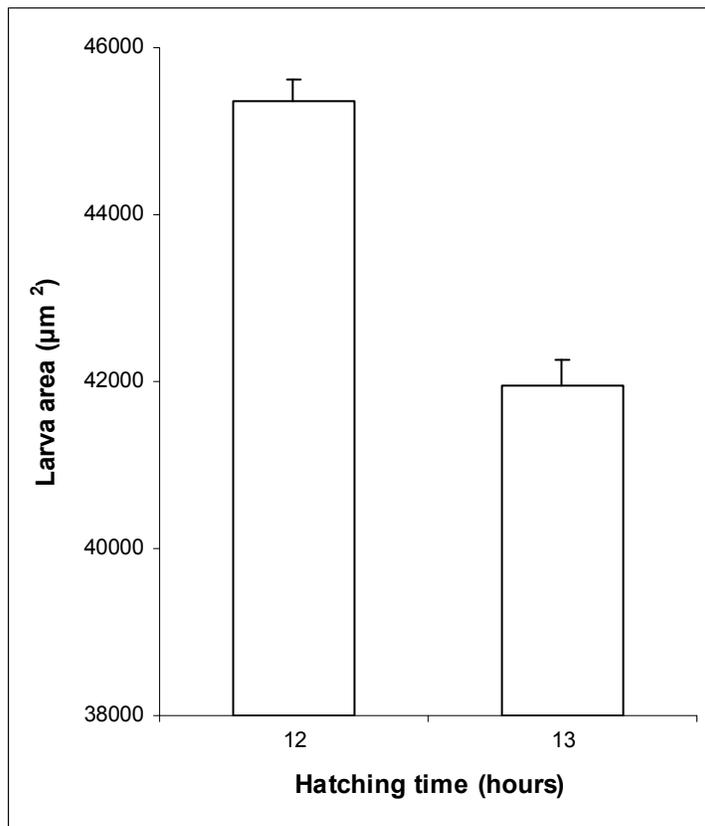
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657 Figure 1



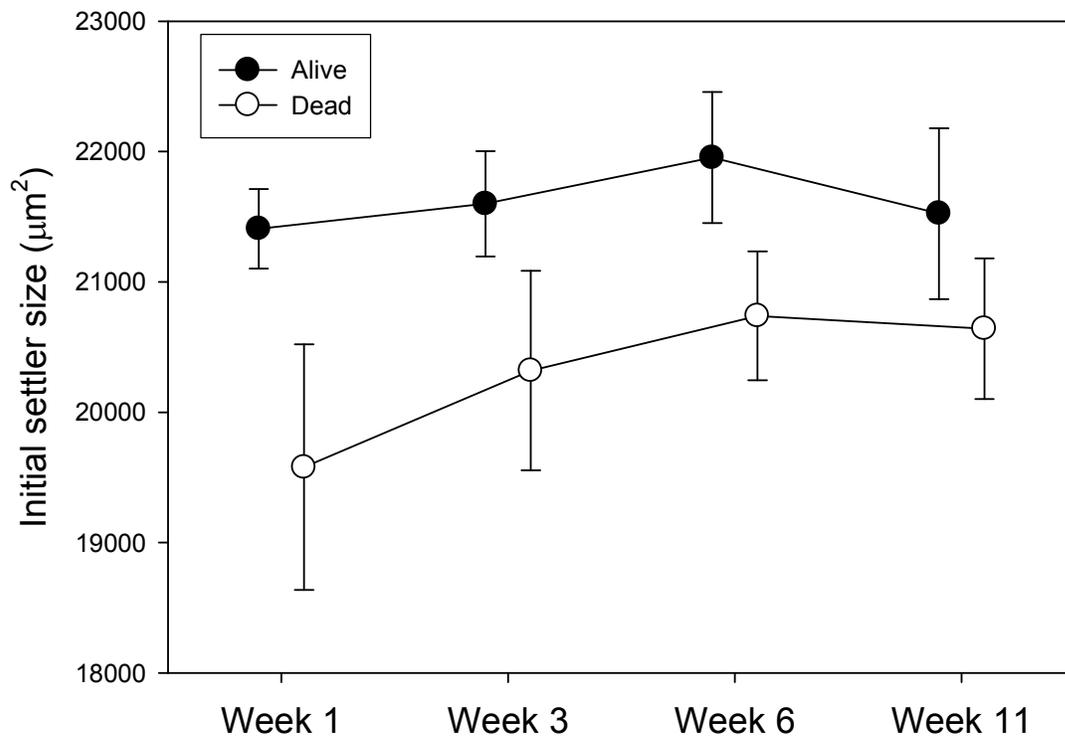
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680 Figure 2
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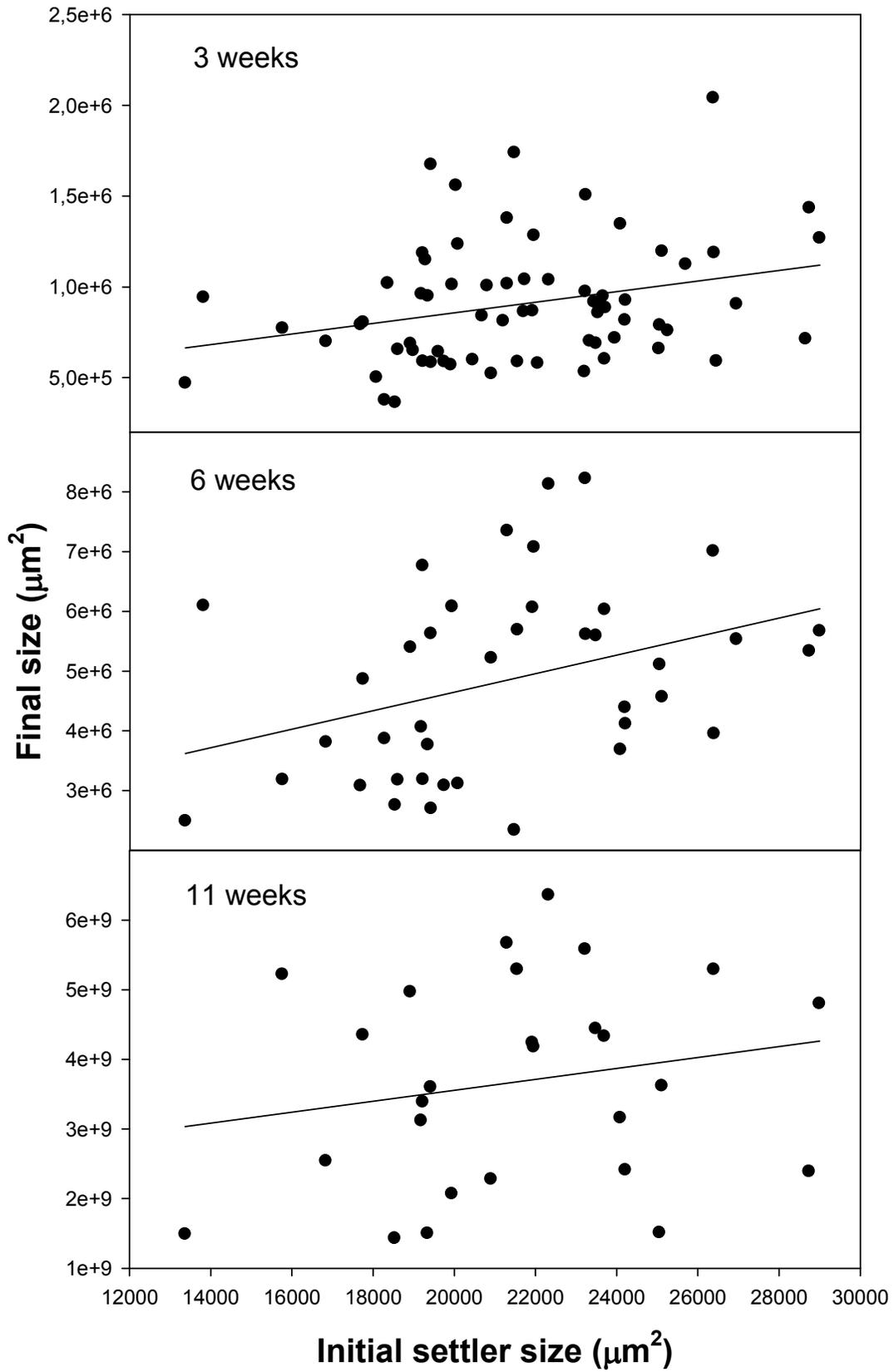
704 Figure 3
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Review

728 Figure 4



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