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

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26   **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.

49   **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

117   longitudinal study offspring size effects in a single population is the most informative

118   approach.

119   Here we examine the effects of offspring size across multiple life-history stages in

120   a marine invertebrate, the solitary ascidian *Microcosmus squamiger*. Marine invertebrates

121   represent an excellent group for the study of offspring size effects across life-history

122   stages for a number of reasons. First, studies show that offspring size affects offspring

123   performance across the entire life-history in this group, from fertilisation, through

124   development, larval settlement and post-metamorphic survival, growth and even

125   reproduction (Marshall *et al.* 2008a). Second, initial studies on individual life-history

126   stages in isolation suggest that selection on offspring size among different life-history

127   stage could indeed be in conflict (Levitan 1996; Marshall, Styan & Keough 2002).

128   Finally, there has been long standing theoretic interest in the evolution and ecological

129   consequences of offspring size in marine invertebrates but field studies remain relatively

130   rare in this group. We examined the effect of offspring size on post-fertilisation

131   development rate and larval settlement behaviour in the laboratory and we then examined

132   post-metamorphic survival and growth of juveniles under field conditions, a crucial

133   element when considering offspring size effects (Fox 2000).

134

135   **Materials and Methods**

136
137   *Collection site*

138   All reproductively mature *Microcosmus squamiger* collected for this study were

139   from boulders located at the outer pontoon of the Manly harbour (Brisbane, Queensland,

140   Australia - 27º27'10"S, 153º11'22"E). This location is a sheltered marina protected by 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 mm^2

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 10^7 and 10^8

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).

250

251   **Results**

252   *Adult-egg size relationship*

253   There was no relationship between either adult weight nor adult length and the

254   size of eggs that individuals produced (wet weight, $r = 0.1717$, P = 0.6137; length, $r = -$

255   0.018, P = 0.958).

256   *Egg size/time of cleavage relationship*

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

258   *Offspring size effects on time until hatching*

259   As most of the larvae (approximately 500) hatched between the 11th and 12th hour,

260   we only measured a subsample of 100 larvae within this hour period. The following hour,

261   76 larvae hatched, and at both the 14th and 24th hour after fertilization, we found only 3

262   larvae each time. As a result, to analyse the effect of larval size on hatching time, we only

263   included the larvae from the 12th (n = 100) and 13th hour (n = 76). Larger larvae (and thus

264   larvae from larger eggs) hatched sooner than smaller larvae (t-test, t = 8.863, df = 174, P

265   < 0.001) (Fig. 2).

266   ***Offspring size effects on larval swimming***

267   We observed that settlement mainly occurred between the 6th and 8th hours,

268   although swimming time spread from 4 hours up to 26 hours. There was no relationship

269   between larval size and the time spent swimming before settlement ($r = -0.116$, n = 11, P

270   = 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).

281

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).

285

286   **Discussion**
287
288   We found effects of offspring size on offspring performance in several life-history

289   stages of *Microcosmus squamiger*. Increasing offspring size affected offspring

290   performance positively both in the larval phase and the post-metamorphic juvenile phase

291   in the field. While the effects of offspring size on post-metamorphic survival in the field

292   were weak and diminished over time, the effect of offspring size on post-metamorphic

293   growth were remarkably persistent - juveniles that originated as larger settlers were still

294   larger than juveniles that originated as smaller settlers even after 10 weeks in the field.

295   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.

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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547   **Tables and figures legends**

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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
Between subjects				
Settler size	1	1.6591	7.3626	0.0119
Among Subjects				
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
Cleavage rate	No	N/A
Hatching time	Yes	Positive
Swimming time	No	N/A
Post-metamorphic survival	Yes	N/A
Post-metamorphic growth	Yes	Positive

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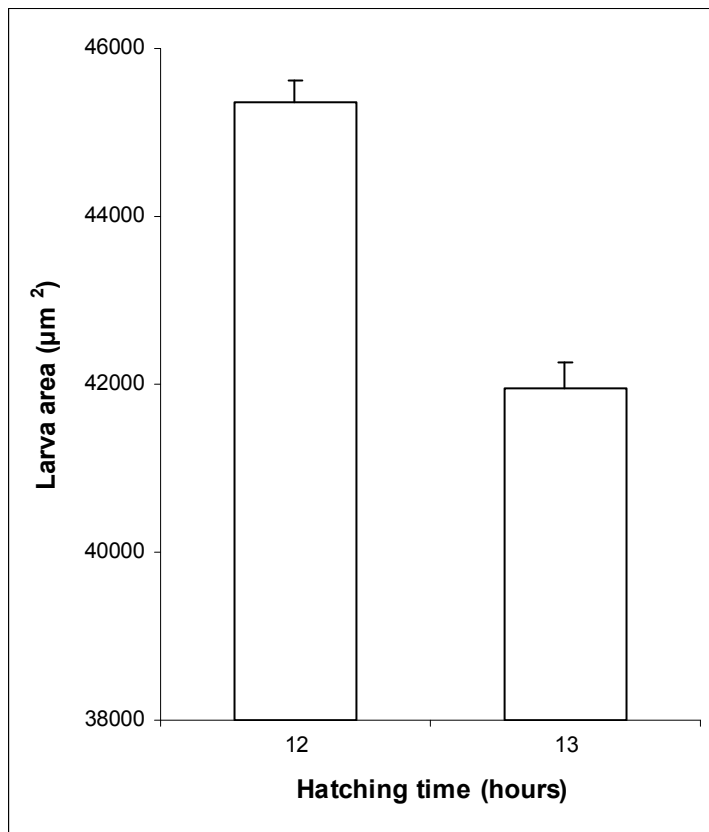
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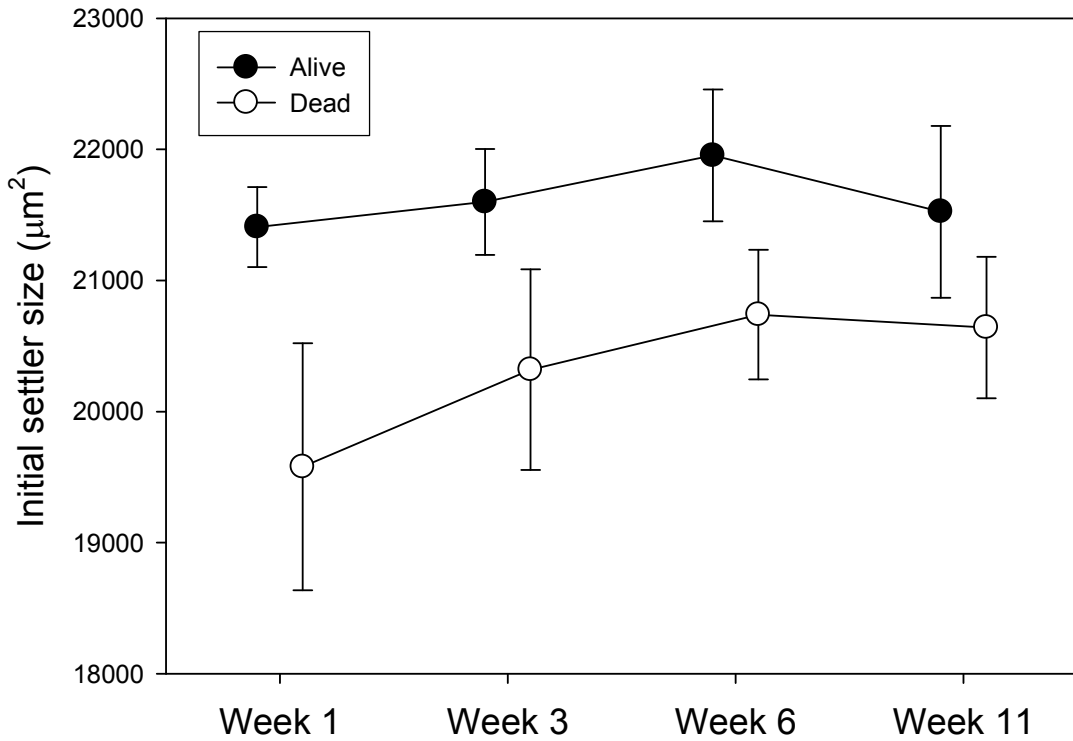
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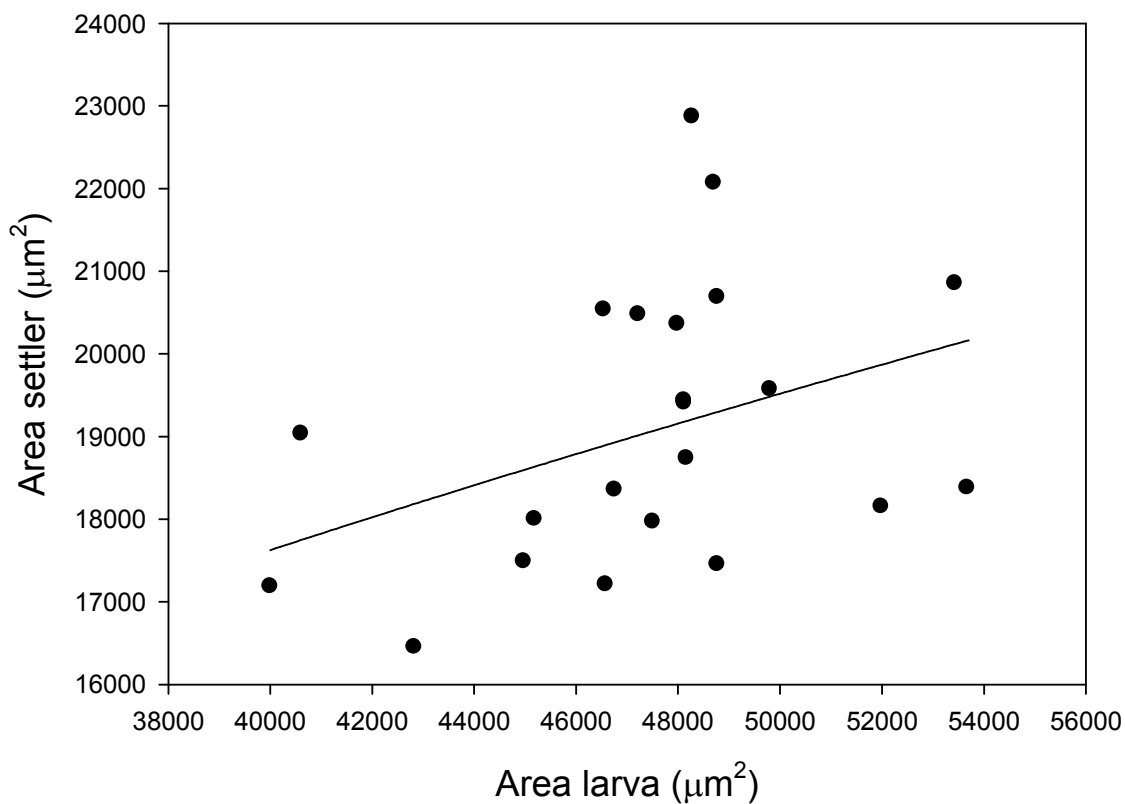
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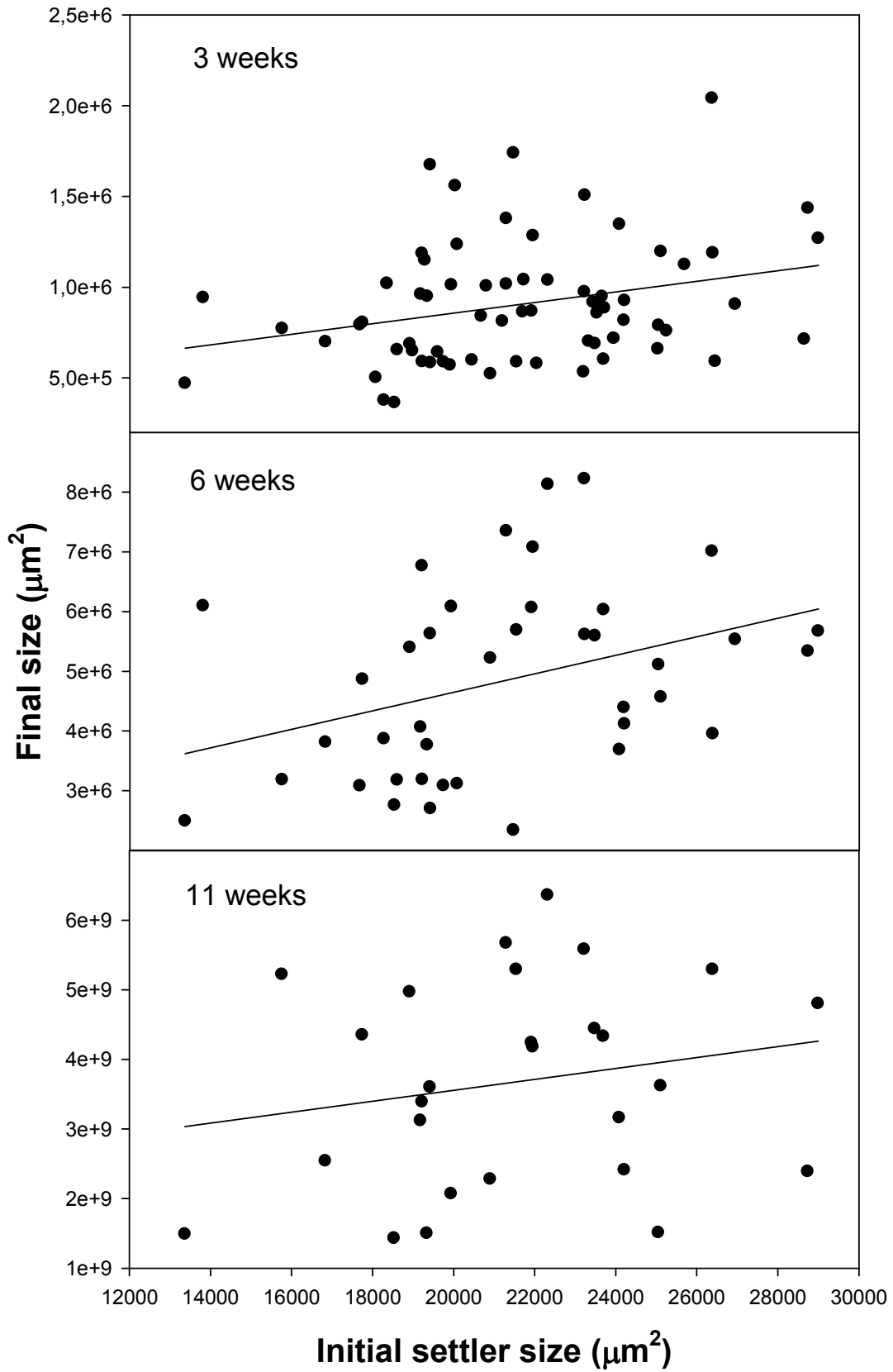689
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704   Figure 3
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728　　Figure 4