1   **Offspring size variation within broods as a bet-hedging strategy in**

2   **unpredictable environments**

3

4

5   Dustin J. Marshall[1], Russell Bonduriansky[2] & Luc F. Bussière[2,3,4]

6

7

8   *[1]School of Integrative Biology/Centre for Marine Studies, The University of Queensland, 4072, Qld, Australia;*

9   *[2]Evolution & Ecology Research Centre and School of Biological, Earth & Environmental Sciences, University*

10 *of New South Wales, 2052, NSW, Australia;*

11 *[3] Zoologisches Museum der Universität Zürich, Winterthurerstrasse 190, CH 8057, Zürich, Switzerland*

12 *[4] Current address: School of Biological and Environmental Sciences, University of Stirling, FK9 4LA, Stirling,*

13 *United Kingdom*

14

15   d.marshall1@uq.edu.au

16   r.bonduriansky@unsw.edu.au

17   luc.bussiere@stir.ac.uk

18

19

22

23

Abstract

24   Offspring size is strikingly variable within species. Although theory can account for variation

25   in offspring size among mothers, an adaptive explanation for variation *within* individual

26   broods has proven elusive. Theoretical considerations of this problem assume that producing

27   offspring that are too small results in reduced offspring viability, but producing offspring that

28   are too large (for that environment) results only in a lost opportunity for increased fecundity.

29   However, logic and recent evidence suggest that offspring above a certain size will also have

30   lower fitness, such that mothers face fitness penalties on either side of an optimum. Although

31   theory assuming intermediate optima has been developed for other diversification traits, the

32   implications of this idea for selection on intra-brood variance in offspring size have not been

33   explored theoretically. Here we model the fitness of mothers producing offspring of uniform

34   vs. variable size in unpredictably variable environments and compare these two strategies

35   under a variety of conditions. Our model predicts that producing variably sized offspring

36   results in higher mean maternal fitness and less variation in fitness among generations when

37   there is a maximum and minimum viable offspring size, and many mothers under- or over-

38   estimate this optimum. This effect is especially strong when the viable offspring size range is

39   narrow relative to the range of environmental variation. To determine whether this prediction

40   is consistent with empirical evidence, we compare within- and among-mother variation in

41   offspring size for 5 phyla of marine invertebrates with different developmental modes

42   corresponding to contrasting levels of environmental predictability. Our comparative analysis

43   reveals that in the developmental mode in which mothers are unlikely to anticipate the

44   relationship between offspring size and performance, size-variation within mothers exceeds

45   variation among mothers, but the converse is true when optimal offspring size is likely to be

46   more predictable. Together, our results support the hypothesis that variation in offspring size

47   within broods can reflect an adaptive strategy for dealing with unpredictably variable

48   environments. We suggest that when there is a minimum and a maximum viable offspring

49   size and the environment is unpredictable, selection will act on both the mean *and* variance

50   of offspring size.

51

52

Introduction

52

53   For over 50 years, biologists have sought to understand the remarkable variation in

54 offspring size among species (Lack 1947, Bagenal, 1969) and the study of offspring size has

55 become an important branch of life-history research (Stearns 1992). The resources available

56 to mothers are finite, so they can produce either many small or fewer, large offspring. Larger

57 offspring typically have higher fitness than smaller offspring, thereby offsetting any decrease

58 in fecundity. Smith and Fretwell (1974) produced one of the first theoretical examinations of

59 how mothers optimally balance the size and number of offspring they produce. Their classic

60 study has formed the basis for most theory on offspring size/number trade-offs, and most

61 models published since share a number of features with their original work. These models

62 typically derive the best maternal allocation strategy assuming a trade-off between offspring

63 size and number and a positive correlation between offspring fitness and offspring size (e.g.

64 Vance 1973, Smith and Fretwell 1974, Sargent et al. 1987). The general prediction from this

65 work is that under constant environmental conditions (and thus a constant offspring size-

66 fitness relationship), a single offspring size will be optimal. However in nature, offspring

67 sizes are extremely variable within populations and offspring size can be correlated with a

68 number of maternal factors such as body size or nutrition (Turner and Lawrence 1977).

69 While the earliest models could not account for such variation, more recent models

70 incorporating the effects of maternal phenotype on the natal environment successfully predict

71 the observed variation in offspring sizes among different mothers (e.g. Parker and Begon

72 1986, Hendry et al. 2001, Sakai and Harada 2001). Empirical evidence supports these

73 models. For example, in species of fish where the maternal phenotype can affect the

74 offspring size-fitness relationship, offspring size variation within populations is relatively

75   high (Einum and Fleming 2002). Similarly, Fox et al. (1997) showed that when mothers can

76   detect the likely environment of their offspring, they adjust the size of their offspring

77   accordingly suggesting that inter-individual variation in offspring size represents an adaptive

78   maternal effect.

79   Although theory can now account for variation in offspring size among mothers,

80   explaining variation in the size of offspring from the same mother remains problematic.

81   Offspring size shows remarkable variation within individual broods in many taxa (Turner and

82   Lawrence 1977, Williams 1994, Fox and Czesak, 2000, Kudo 2001, Marshall et al. 2003).

83   There are numerous verbal arguments for producing offspring that vary in size (e.g. Capinera

84   1979, Crump 1981, Lips 2001). In such considerations, within-brood variation is viewed as a

85   form of bet-hedging in which females ensure that at least a few offspring approach the

86   optimum in some unpredictable future environment (Koops et al. 2003). However, the few

87   formal models of within brood offspring size variation do not support such an adaptive

88   explanation for this variation (McGinley et al. 1987, Einum and Fleming 2004b, but see

89   Geritz [1995] for a rare exception using a game theoretic approach). In most instances,

90   producing a single offspring size within each brood is predicted to be optimal (note that

91   Vance [1973] and similar models predict two optima but one of these is simply a product of

92   infinitely small offspring retaining some fitness – an unrealistic situation). In the few cases

93   where producing variably-sized offspring within broods is favored in these models, it is

94   under restrictive and improbable conditions. For example, McGinley et al. (1987) found that

95   producing offspring of variable size was only advantageous when mothers could strictly

96   control the dispersal of their offspring. Similarly, Einum and Fleming (2004) found that

97   within-brood offspring size variation (described as diversified bet-hedging) was a less

98   effective strategy than producing very large offspring (described as conservative bet-

99   hedging) for coping with environmental uncertainty. Consequently, within-brood variation in

100   offspring size is increasingly viewed as a product of physiological constraints that prevent

101   mothers from producing offspring of identical size, rather than as an adaptive strategy (Fox

102   and Czesak 2000, Einum and Fleming 2004b). In their review of offspring size effects in

103   insects, Fox and Czesak (2000, p. 358) concluded that '…some authors have suggested that

104   at least some of the variation within families is an adaptive response to living in a variable

105   environment. At this time however, there are few experimental studies and too little

106   theoretical work to generalize." Thus, despite the intuitive appeal of intra-clutch variation in

107   offspring size as a mechanism for coping with environmental heterogeneity, theoretical

108   support for the concept remains elusive.

109   While offspring size theory has struggled to account for within-brood variation in

110   offspring size, parallel developments in the more general theory of bet-hedging have long

111   predicted a selection advantage for producing offspring with variable phenotypes. Cohen's

112   (1966) classic model and others since have shown that when the environment varies

113   unpredictably, mothers should produce offspring with a range of phenotypes either in a single

114   reproductive bout (Gillespie 1977, Bull 1987, Simons and Johnston 2006) or across multiple

115   reproductive bouts (Cooper and Kaplan 1982). Thus we face the puzzling situation where

116   general theory predicts a selection advantage for variation in offspring traits but specific

117   theory for offspring size typically does not.

118   The nature of optimality models may be partly responsible for the prevalence of

119   theory showing that within-brood offspring size variation is not adaptive. Most optimality

120   models use highly asymmetrical offspring fitness functions whereby, as offspring size

121   increases, offspring fitness increases with diminishing returns, or levels off at a constant

122   maximum value (Smith and Fretwell 1974, McGinley et al. 1987, Einum and Fleming 2000,

123   2004b). In contrast, more general models of bet-hedging assume a symmetrical, curvilinear

124   relationship between offspring phenotype and fitness such that there are fitness penalties at

125   each end of the offspring phenotype continuum (e.g. Cohen 1966, Cooper and Kaplan 1982).

126   We suggest that offspring size models using an asymmetrical function are unrealistic and

127   underestimate the benefits of a diversified bet-hedging strategy in unpredictable

128   environments while overestimating the benefits of a conservative bet-hedging strategy (i.e.

129   producing offspring of a constant, large size).

130   The use of a Smith-Fretwell fitness function is problematic because it assumes that

131   above a certain size, larger offspring (including infinitely large offspring) have equal fitness.

132   Therefore, the only fitness cost to mothers of producing large offspring is a reduction in

133   fecundity. In nature, however, offspring exceeding a certain size will have lower fitness due

134   to physiological or anatomical constraints (Strathmann and Chaffee 1984, Congdon and

135   Gibbons 1987, Kaplan 1992, Strathmann 1995, Bernardo 1996), increased predation risk

136   (Dibattista et al. 2007) or an increased risk of polyspermy (Styan 1998, Marshall et al. 2002).

137   Thus offspring are likely to suffer a direct fitness cost if they are too small *or too large* for

138   their environment. If the environment varies unpredictably, then a conservative bet-hedging

139   strategy (i.e. simply producing larger offspring) is unlikely to insulate mothers from this

140   unpredictability because excessively large offspring will suffer reduced fitness. Furthermore,

141   with direct fitness costs on both sides of an optimum, the benefits of producing variably sized

142   offspring may be increased in unpredictable conditions. The benefits of within brood

143   offspring size variation have not been modeled under the assumption that both very small and

144   very large offspring suffer reduced fitness.

145   Here, we compare the fitness of mothers employing contrasting reproductive

146   strategies: (1) an 'invariant' strategy where all offspring (or eggs) within a brood are of equal

147   size, and (2) a 'variable' strategy where offspring within broods vary in size. Like previous

148   authors, we found that this problem precludes a tractable analytic solution, and opted for a

149   simulation approach (Einum and Fleming 2004a). However, our approach differs from

150   previous studies in that we use a fitness function that penalizes offspring that are too large as

151   well as those that are too small for that environment. Recent empirical work has shown that

152   optimal offspring sizes can vary 2-fold over very small spatial scales within similar habitats

153   (Marshall et al. 2006, Marshall and Keough 2006), so the chances of mothers producing the

154   'wrong' offspring size for any particular environment are probably high. Therefore, we

155   varied the probability that mothers will produce offspring of a size that is not optimal for that

156   environment (i.e., make "errors"), and examined the relative fitness of the variable and

157   invariant reproductive strategies. Our simulation analysis suggested that, when mothers are

158   likely to make large errors with regard to optimal offspring size and viable offspring sizes are

159   bounded by a minimum and a maximum, within brood variation in offspring size increases

160   maternal fitness. We then examined whether species that were less able to predict the natal

161   environment produced more variably sized broods through a comparative analysis of

162   offspring size variation among marine invertebrate species that differ in their capacity to

163   predict the environment of their offspring (i.e. direct developers without dispersive young

164   *versus* indirect developers with highly dispersive young). A direct test of the predictions of

165   our model requires verifying the presumed causal link between a maximum viable egg size

166   and selection on the variable strategy – a challenging problem. Our comparative analysis

167   does not constitute such a direct test. Nonetheless, the comparative analysis enables us to

168   determine whether the empirical evidence is consistent with our model's predictions.

169

170   **Materials and Methods**

171   *Model*

172   Following traditional offspring size models (e.g. Smith and Fretwell 1974), we

173   assume that there is a function linking egg fitness to egg size (m), a minimum viable egg size

174   (m_{min}), and a trade-off between the mean size of the eggs that a female produces and the

175   number of eggs that she can produce (Smith and Fretwell 1974). However, unlike previous

176   authors, we also assume that egg fitness decreases when egg size exceeds an optimum, so

177   that there is a maximum viable egg size (m_{max}). Thus, the fitness of an individual egg, $\phi(m)$,

178   is zero when egg size is less than m_{min} or greater than m_{max}. In the viable range between m_{min}

179   and m_{max}, egg fitness is given by

180

$$\phi(m) = \frac{\left(1 - \dfrac{m_{min}}{m}\right)\left(1 - \dfrac{m}{m_{max}}\right)}{m}, \tag{1}$$

181

182

183   where m is the size of an individual egg. This represents a convex function relating egg

184   fitness to egg size (see Appendix A: Fig. 1).

185   We assume that all females have an equal quantity of resources, R, to invest in

186   reproduction, and that there is a trade-off between the mean size of a female's eggs (brood

9

187   mean, μ), and the number of eggs that she can produce. Thus, the number of eggs, $N(\mu)$,

188   produced by a female with a brood-mean egg size μ is

189
$$N(\mu) = R\left(\frac{M}{\mu}\right), \tag{2}$$

190

191   where M is the mean egg size in the population (equal to the optimum egg size and the

192   expected value of the distribution of μ; see below). Consequently, females whose mean egg

193   size (μ) is smaller than the population average (i.e., $\mu < M$) produce more eggs than the

194   population average egg number (i.e., $N(\mu) > N(M)$), whereas females whose mean egg size is

195   greater than the population average (i.e., $\mu > M$) produce fewer eggs than the population

196   average (i.e., $N(\mu) < N(M)$), such that total reproductive output (the sum of the sizes of all

197   eggs produced by a female) is equal for all females.

198   The fitness of a particular mother, W, is thus equal to the summed fitnesses of all her

199   eggs,

200

201
$$W = \sum_{}^{N(\mu)} \phi(m), \tag{3}$$

202

203   where m is the size of a particular egg produced by a female with mean egg size μ.

204   To determine whether the variable strategy could yield higher fitness than the

205   invariant strategy in unpredictably variable environments, we simulated different degrees of

206   environmental variability, and examined their consequences for the relative fitnesses (i.e.,

207   recruitment rates) of two populations of 500 females, one population pursuing an invariant

208   reproductive strategy (no variation in egg size within broods), and the other population

209   pursuing a variable reproductive strategy (within-brood variation in egg size). We used

210   populations, rather than a single individual, to represent each strategy within each generation

211   because this approach seems more biologically relevant, corresponding, for example, to a

212   situation where clonal populations of marine invertebrates or plants pursue contrasting

213   reproductive strategies. Simulating populations also greatly diminishes the probability of

214   genotype extinction, enabling us to analyse variation in strategy fitness within and across

215   generations as a continuous variable. Environmental variability (CV_E) was a proxy for

216   mothers' abilities to predict the optimum brood-mean egg size: on average, the magnitude of

217   maternal "errors" (i.e., deviations from the optimum) increased with increasing

218   environmental variability. Note that we manipulated mean egg size for a population of

219   females rather than manipulating environmental parameters. This approach is functionally

220   equivalent to modeling environmental variation because the distribution of mismatches

221   between maternal mean egg size and environment are the same in each case. Our approach

222   has the advantage of allowing manipulation of variation in the mean distance from an

223   optimum independently of changes in minimum and maximum viable egg size. In each

224   simulation (representing a generation or reproductive bout), a brood-mean egg size, μ, was

225   generated randomly for each of 500 mothers. The distribution of μ was log-normal, and its

226   expected value, M, always corresponded to the optimum egg size (i.e. the egg size that

227   maximizes maternal fitness). This assumes that the population mean is centered at the fitness

228   optimum, but that individuals within the population may over- or underestimate the optimum

229   egg size. The coefficient of variation of this distribution, CV_E, represented environmental

230   variability. We examined six degrees of environmental variability: CV_E = 0.3, 0.6, 1.0, 1.15,

231   1.3, 2.1. The biological significance of these values of CV_E can be appreciated by inspecting

232 their consequences for reproductive failure rate and among-generation variation in fitness

233 (see Results and Appendix A), which can be compared directly with parameters measured in

234 real populations. Indeed empirical studies strongly suggest that reproductive failure rates due

235 to producing offspring of the 'wrong' size can be even higher than those generated in our

236 simulations (Marshall and Keough 2007).

237 For each of the 500 brood-mean egg sizes, μ, we then randomly generated $N(\mu)$

238 individual egg sizes, m, distributed normally about μ with standard deviation σ. Although

239 some eggs could thus have negative sizes, we assume that egg size is measured on an

240 arbitrary scale, and negative sizes simply yield zero fitness because they are less than m_{min}.

241 For a given female with brood-mean egg size μ, maternal fitness was then calculated in two

242 ways. First, maternal fitness under the invariant strategy, W_{inv}, was calculated based on $N(\mu)$

243 eggs of size μ. Second, maternal fitness under the variable strategy, W_{var}, was calculated

244 based on $N(\mu)$ eggs of different sizes, m. The arithmetic mean fitness of each strategy within

245 each generation (or reproductive bout) was then calculated from the fitnesses of the 500

246 females, and the relative fitness of the variable strategy ('Fitness differential', ΔW) within a

247 generation was calculated as

248

249 $$\Delta W = \frac{\left(\sum W_{var} - \sum W_{inv}\right)}{\sum W_{inv}}, \qquad (4)$$

250

251 where $\sum W_{var}$ and $\sum W_{inv}$ are the summed fitnesses of the 500 females based on the variable

252 and invariant strategies. Thus, $\Delta W < 0$ indicates higher fitness for the invariant strategy,

253 whereas $\Delta W > 0$ indicates higher fitness for the variable strategy within a generation or

254 reproductive bout.

255   To examine how the magnitude of within brood variance in egg size affects maternal

256   fitness, we simulated different coefficients of within brood variation, CV_B (defined as σ / μ).

257   We examined values of CV_B ranging from 0.01 to 0.5 because these values encompass those

258   observed in nature (Marshall and Keough 2007, Kohn and Perron, 1994). The value of σ was

259   adjusted for each female so as to maintain a constant CV_B despite variation in μ.

260   To investigate how the shape of the function relating egg fitness to egg size affects

261   the performance of the variable strategy, we simulated every combination of CV_E and CV_B

262   for three different egg fitness functions varying 16-fold in the width of the viable egg size

263   range (see Fig. 2). For each parameter combination, we conducted 500 simulations, with

264   different random distributions of μ and m generated in each simulation, and tested the null

265   hypothesis $\Delta W = 0$ by t-test. Where ΔW was not significantly different from zero, we

266   conducted up to 2000 additional simulations. Appendix A: Figure 1 illustrates the

267   performance of females under the variable and invariant strategy for several parameter

268   values. We checked our model for systematic bias by setting CV_B to very small values, and

269   confirming that ΔW approaches zero as CV_B approaches zero.

270   As an additional index of relative performance, we compared the coefficients of

271   variation of $\sum W_{var}$ and $\sum W_{inv}$ over multiple simulations, assuming that the strategy exhibiting

272   less variation in mean fitness across generations enjoys a long-term advantage (Roff, 1992).

273   Simulations were conducted in Mathcad Plus 6.0 Professional Edition (© MathSoft, Inc.,

274   Cambridge, Mass., U.S.A.).

275

276   *Comparative Analysis*

277　　　　　Marine invertebrates are an ideal group to examine offspring size variation across

278　　　species because they are taxonomically diverse with a wide range of offspring dispersal

279　　　modes. Many marine invertebrate lineages exhibit a range of developmental modes,

280　　　indicating repeated, independent evolution of different developmental modes (Hart et al.

281　　　2003) and multiple dispersal modes are present within some populations of the same species

282　　　(Krug 1998). Importantly, offspring size consistently has fitness consequences in marine

283　　　invertebrates and can be important at each life-history stage, sometimes in a conflicting

284　　　manner (Hart 1995, Levitan 1996, Moran and Emlet 2001, Marshall and Keough 2003,

285　　　Marshall et al. 2003).

286　　　　　Marine invertebrates can be divided into distinct developmental groups: direct

287　　　development, indirect development with no feeding, and indirect development with feeding.

288　　　Direct developers (D) typically produce relatively large offspring that either emerge from the

289　　　adult as a fully developed juvenile or emerge from an egg capsule as a juvenile. There is

290　　　extremely little dispersal during development and fertilisation in usually internal. Non-

291　　　feeding, indirect developers (NF) typically produce 'medium 'sized offspring that are

292　　　released from the adult as eggs (in the case of external fertilisers) or larvae (in the case of

293　　　internal fertilisers or brooders). The larvae typically have no feeding structures, relying on

294　　　maternal provisioning, and spend minutes to a few days in the plankton before encountering

295　　　a suitable habitat whereupon they metamorphose and begin adult life (Havenhand 1995).

296　　　Feeding, indirect developers (F) produce small eggs that are either externally or internally

297　　　fertilised and spend days to months feeding in the plankton. They may go through several

298　　　larval stages while in the plankton before metamorphosing and becoming an adult. Thus

299   there is a clear ranking in the dispersal capabilities of D, NF and F offspring with D being the

300   least dispersive and F being the most dispersive.

301   There are clear differences in the ability of mothers with directly and indirectly

302   developing offspring to predict the relationship between offspring size and overall

303   performance (and thus the optimal offspring size that should be produced). Because

304   indirectly developing offspring are far more dispersive and can pass through multiple life-

305   history stages: i) the natal habitat (defined as the habitat in which offspring become

306   independent from maternal nutrition sources and begin to feed) is unlikely to be assessed by

307   mothers, ii) the natal habitat is unlikely to be affected by maternal phenotype and iii) there

308   can be conflicting selection pressures on offspring size among stages (Marshall et al. 2002).

309   These three factors all suggest that mothers with F offspring have a lower chance of

310   optimally provisioning individual offspring. In contrast, mothers with D offspring should be

311   better able to predict their offspring's environment. Because directly developing offspring are

312   released into the maternal environment there is at least the potential for mothers to assess the

313   environmental conditions and adaptively adjust the size of their offspring (e.g. Fox et al.

314   1997, Einum and Fleming 2002). Moreover, because direct developing offspring are

315   relatively weak dispersers, sibling competition and maternal phenotype are more likely to

316   affect the quality of the natal environment (i.e., essentially make it more predictable, Parker

317   and Begon 1986, Hendry et al. 2001). Finally, there is less potential for conflicting selection

318   pressures on offspring size in direct developers because they have fewer life-history stages

319   making the relationship between offspring size and performance more likely to be

320   predictable. If environmental unpredictability favours the production of variably sized

321   offspring, then we should see higher levels of variation *within broods* of F species than D

322   species. Similarly, if the offspring environment of D species is more predictable, we should

323   see more variation *among mothers* in D species than F species. It is important to note that for

324   all the developmental groups, we expect there to be a minimum and maximum offspring size

325   that will be viable although the underlying causes of the upper constraints will probably

326   differ among the developmental modes. For example, physiological constraints probably

327   limit the maximum offspring size in direct developers but polyspermy effects limit offspring

328   size in broadcast spawners (Marshall and Keough 2007). Thus, while our theoretical analysis

329   considered the effects of environmental predictability on fitness under the assumption that

330   offspring can suffer fitness costs if they exceed an optimum size, our comparative analysis

331   examined the relationship between environmental predictability and components of variation

332   in offspring size in order to test our model's predictions. To determine whether the empirical

333   evidence was consistent with the predictions arising from our simulations, we compiled data

334   on variation in offspring size among and within marine invertebrate mothers from the

335   available literature and from unpublished data of our own (see Table 1). For more detailed

336   methods on our comparative analysis, see Appendix A.

337

Results

339   *Model*

340   Our simulations suggest that, in unpredictably variable environments, mothers

341   producing offspring of variable size within each brood (variable strategy) have higher mean

342   fitness within generations, as well as lower variance in fitness across generations, than

343   mothers producing offspring of uniform size (invariant strategy). This is because the

344   production of offspring of different sizes insures that, for some females whose brood-mean

345   egg size falls outside the viable egg size range, some offspring are nonetheless able to

346   survive.

347   Within generations, the invariant strategy yields higher mean fitness when a large

348   proportion of females are able to produce mean egg sizes near the optimum, since all the

349   eggs produced by invariant strategy females have high fitness. However, when many females

350   produce mean egg sizes that deviate substantially from the optimum, the variable strategy

351   tends to do better on average because fewer females suffer total reproductive failure (see

352   below), and many females produce some eggs of near-optimum size, even if their brood-

353   mean egg size deviates from the optimum. When environmental variability is moderate (e.g.,

354    $CV_E = 0.6$), a small degree of within brood variability in egg size (e.g., $CV_B = 0.01$)

355   nonetheless results in significantly higher mean maternal fitness than an invariant strategy,

356   although higher levels of within-brood variability are disadvantageous (Fig. 1; Appendix B:

357   Table 1). For example, egg fitness function 'c' in Fig. 1 yields a significant advantage for the

358   variable strategy (with $CV_B = 0.01$) at $CV_E = 0.6$, which corresponds to a reproductive failure

359   rate (i.e., probability of having no surviving offspring) of 24% for invariant strategy females.

360   When environmental variability is high (e.g., $CV_E \geq 1.3$, resulting in reproductive failure for

361   > 50% of invariant strategy females), maternal fitness increases with the degree of within-

362   brood variability (CV_B). At intermediate levels of environmental variation (e.g., $CV_E = 1.0$ to

363   1.15), a complex transition occurs where selection on CV_B appears to be disruptive.

364   We investigated how the strength of stabilizing selection on egg size affects the

365   relative advantage of the variable strategy by examining three egg fitness functions differing

366   16-fold in the viable egg size range. The narrowest egg fitness function (function 'a' in Fig.

367   1), representing the strongest stabilizing selection on egg size, results in the greatest

368   advantage for the variable strategy in variable environments (Appendix C: Fig. 2). However,

369   the difference between the two wider egg fitness functions (functions 'b' and 'c' in Fig. 1) is

370   less pronounced. This is because, given the trade-off between egg size and number, females

371   with large brood-mean egg sizes have few offspring and, thus, little influence on mean

372   fitness.

373   In addition, the variable strategy always results in lower variation in fitness across

374   generations (Fig. 2; Appendix B: Table 2), and this represents an additional advantage of the

375   variable strategy (see Roff 1992 and Discussion).

376   The fitness advantages of the variable reproductive strategy, both in terms of higher

377   mean fitness within generations and lower coefficients of variation in fitness across

378   generations, partly reflects lower rates of complete reproductive failure (Appendix C: Fig. 3).

379   For females pursuing the variable egg size strategy, reproductive failure rate declines with

380   increasing within-brood variability (CV_B) (Appendix B: Table 3) and, even with the smallest

381   degree of within-brood variability ($CV_B = 0.01$), the variable strategy yields a significantly

382   lower reproductive failure rate than the invariant strategy (Sign test: $N = 12500$ simulation

383   runs, $Z = 111.66$, $P < 0.0001$). Reduced rates of reproductive failure thus moderate the effects

384   of environmental unpredictability.

385

386   *Comparative analysis*

387   The comparative data are consistent with the hypothesis that within-brood offspring

388   size variation reflects an adaptive maternal bet-hedging strategy in unpredictable

389   environments. The principle source of variation in offspring size strongly depends on the

390   development type of that species (Table 2). For direct developers (where mothers have the

391   greatest ability to predict offspring environment), most of the variation in offspring size is at

392   the among-mother level (Fig. 3) whereas for indirect developers with feeding larvae (where

393   mothers have little ability to predict offspring environment), most of the variation is at the

394   within-mother level (Fig. 3). The indirect developers with non-feeding larvae are

395   intermediate to the two other groups, with equal levels of variation within and among

396   mothers. This pattern is maintained regardless of the taxonomic level at which it is tested

397   (Scale of variation × Development type interaction, family level: $F_{2,18} = 7.05$, $P = 0.0055$;

398   order level: $F_{2,15} = 6.11$, $P = 0.0011$; class level: $F_{2,8} = 6.11$, $P = 0.0245$; Fig. 3). Examining

399   within-mother variation in offspring size alone, there is a significant difference among

400   development types ($F_{2,22} = 5.54$, $P = 0.011$) and within-mother variation is highest in indirect

401   developers with feeding larvae (Dunnett's test: F vs. NF: $P < 0.001$; F vs. D: $P = 0.04$).

402

Discussion

404   We found that when environments are unpredictable (such that mothers are likely to

405   produce offspring of a mean size that deviates from the optimal size) and offspring fitness is

406   maximized at an intermediate size, mothers producing a range of offspring sizes within a

407   brood (variable strategy) are likely to have higher fitness than mothers producing offspring of

408   identical size within broods (invariant strategy). Typically, the benefit of producing variably

409   sized offspring is assumed to be a reduction in among-generation variance in fitness (Seger

410   and Brockman 1987, Simons and Johnston 1997, Lips 2001, Laaksonen 2004), and our

411   analysis supports this view. This represents an advantage for the variable strategy because

412   genotypes pursuing such a strategy will face a lower risk of extinction resulting from low

413   population size in some generations, and will have a higher net growth rate over multiple

414   generations (i.e. they will have a higher *geometric* mean fitness: for a detailed description of

415   why geometric mean fitness may be more important for selection, see Roff 1992 and Orr

416   2007). This reduction in among-generation variation in fitness (yielding a higher geometric

417   mean) forms the basis of bet-hedging theory: bet-hedgers don't necessarily do best all the

418   time, but they perform most consistently and are therefore favored by selection (Cohen 1966,

419   Roff 1992). However, our simulations show that mean fitness *within* generations is also

420   higher for mothers that produce offspring of variable size when the environment is

421   sufficiently variable (i.e. a higher *arithmetic* mean fitness) – a novel finding. Together, these

422   findings represent a large potential fitness advantage for the variable strategy in some

423   environments.

424   The finding that a variable strategy can attain higher fitness within generations

425   reflects the fact that the variable strategy outperforms the invariant strategy when brood-

426   mean egg size is displaced from the egg size optimum. Thus, the net relative fitness of the

427   variable strategy within generations reflects the average displacement of brood-mean egg

428   size from the egg size optimum, and a net advantage for the variable strategy can arise if the

429   mode of the distribution of brood-mean egg sizes is displaced from the egg size optimum. In

430   our simulations, the expected value of the brood-mean egg size distribution always coincides

431   with the optimum egg size, but the mode of the distribution is to the left of the egg size

432   optimum (as a consequence of the shape of the log-normal distribution), yielding a net

433   advantage within generations for the variable strategy under some parameter combinations.

434   We suggest that, in natural populations, the mode of the distribution of offspring size may

435 often be shifted to the left of the optimum as a result of resource limitation, or classic bet-

436 hedging whereby genotypes that produce a greater number of small offspring may

437 outperform those that produce fewer, larger offspring when optimum egg size is difficult to

438 predict.

439   Our findings provide some of the first compelling theoretical support for the idea that

440 the production of heterogeneous broods, so often observed in nature, can reflect an adaptive

441 reproductive strategy that yields higher fitness within generations (see Kudo [2001] for an

442 interesting empirical example). Our results suggest that whenever there is a minimum and a

443 maximum viable offspring size, there are two ecological conditions under which producing

444 offspring of variable size may be favored: highly unpredictable environments and narrow

445 fitness functions. There is good evidence suggesting that optimal offspring sizes can vary

446 considerably, even over small spatial and temporal scales due to a range of physical (e.g.

447 desiccation stress on either side of a surge channel; Moran and Emlet 2001) and biological

448 factors (Bervan and Chadra 1988, Marshall et al. 2006), but what conditions are likely to

449 result in narrow fitness functions? Obviously constraints on maximum size will result in

450 narrowed fitness functions (Strathmann and Chaffee 1984, Congdon and Gibbons 1987,

451 Strathmann 1995). But there are factors other than functional constraints that penalize

452 offspring that are too large. For example, in the frog *Bombina orientalis*, tadpoles from larger

453 eggs can have lower performance than tadpoles from smaller eggs (Kaplan 1992). For

454 marine broadcast spawners, there is likely to be a narrow range of offspring sizes that results

455 in the optimal fertilization of eggs because fertilisation is egg size-dependent. Smaller eggs

456 face sperm limitation and larger eggs may suffer from polyspermy (Marshall et al. 2002). In

457 species with non-feeding larvae, egg size is *positively* correlated with development time and

458   as such, larger eggs will remain in the plankton for longer (reviewed in Marshall and Keough

459   2007). Given that mortality rates in the plankton can be very high, increasing offspring size

460   in species with non-feeding larvae may reduce rather than increase offspring fitness. As a

461   result of such factors, only a narrow range of offspring sizes may be viable. In species such

462   as these, we suggest that even a small level of environmental unpredictability may result in

463   variable strategies being favored (for a more detailed review of the negative effects of

464   increasing offspring size see Bernardo 1996). We should note that we do not suggest that *all*

465   within brood variation in offspring size is necessarily adaptive, merely that there is an

466   adaptive element to this variation when environments are unpredictably variable and

467   production of offspring that are too large or too small results in direct fitness costs. Our

468   results are robust to a 16-fold difference in the viable egg size range. However, it would be

469   interesting to investigate the consequences of altering the shape of the egg fitness function in

470   a variety of other ways (e.g., disruptive selection on egg size).

471   The theoretical finding that within-brood variation in egg size can represent an

472   adaptive form of diversified bet-hedging in highly unpredictable environments accords with

473   comparative empirical evidence, which shows that within-brood variation in offspring size is

474   relatively low in species where mothers have greater opportunity to anticipate the

475   relationship between offspring size and performance (i.e., direct developers), but high in

476   species where this relationship is likely to be unpredictable (i.e., indirect developers with

477   feeding larvae). Based on the key assumption that offspring fitness is maximized at an

478   intermediate size, our theoretical analysis suggests that the observed relationship between

479   environmental predictability and within-brood variation in offspring size in marine

480   invertebrates reflects adaptive variation in reproductive strategies. The converse pattern was

481   observed for variation among mothers. The high level of among-mother variation in

482   offspring size for direct developers is predicted to occur only if mothers can adjust the size of

483   their offspring according to local conditions. If mothers can produce offspring that are close

484   to the optimal size, then fitness will be higher under an invariant strategy. While adaptive

485   plasticity in offspring size has been demonstrated in insects (Fox et al. 1997), we know of no

486   study that has demonstrated this in marine direct developers.

487   Previous studies have examined variation in offspring size in more or less predictable

488   environments but they did not partition variation into among- and within-mother levels

489   (Poulin and Hamilton 2000, Einum and Fleming 2002, Dziminski and Alford 2005). Our

490   findings in both the simulations and the comparative analysis highlight the fact that selection

491   will act very differently on these two sources of variation, suggesting that they should be

492   considered separately. Environmental unpredictability should select for increased among-

493   mother variation for species that can predict the environment that their offspring will

494   encounter, whereas it should select for increased within-brood variation for species that

495   cannot predict the conditions that their offspring are likely to experience.

496   Both non-feeding and feeding indirect developers had much lower levels of among-

497   mother variation in offspring size than direct developers, but only the indirect developers

498   with feeding larvae had high levels of within-brood variation. This accords with our model's

499   predictions: most indirect developers with feeding larvae spend weeks to months in the

500   plankton, passing through multiple life-history stages, and disperse to habitats far removed

501   from the maternal habitat. The different developmental modes differed not only in their

502   dispersal but also the number of life-history stages at which offspring size could affect

503   performance. For example, fertilization was external in most of the indirect developers and

504   other studies have shown that egg size can affect fertilization kinetics in external fertilizers

505   (Levitan 1996, Marshall et al. 2002). Thus, we would expect that the relationship between

506   offspring size and performance would be least predictable in this group and, accordingly,

507   high levels of offspring size variation within-broods should be favored. It should be noted

508   that the species within the different developmental modes vary across a range of different

509   life-history traits, all of which have the potential to affect offspring size variation. For

510   example, all of the direct developing species have a mobile adult stage but many of the

511   indirect developers were sessile as adults. Thus we cannot rule out other factors that may also

512   affect the differences in offspring size variation observed in this study.

513   An alternative explanation for the high levels of variation in offspring size *among*

514   mothers in direct developers is that maternal phenotype and the natal environment are linked.

515   Larger mothers typically produce more offspring and, in weakly dispersing species, this may

516   result in higher levels of sibling competition. McGinley et al. (1987) suggest that larger

517   mothers may therefore provision their offspring with more resources (i.e. make them larger)

518   to deal with the increased levels of sibling competition, thus producing a correlation between

519   maternal and offspring size. In many marine invertebrates, offspring size is correlated with

520   maternal size (Marshall et al. 2000, Marshall and Keough 2003) but it is, as yet, unclear

521   whether this relationship is more common in direct developers.

522   Overall, our theoretical and comparative analyses support the view that within-brood

523   variation in offspring size, like mean offspring size, is under selection. When environment is

524   unpredictably variable, and the range of viable offspring sizes is relatively narrow, selection

525   is likely to favor production of offspring of variable sizes within broods. In contrast, stable

526   environments and a large range of viable offspring sizes will generally favor a constant

527 offspring size within broods. One component that theoretical considerations of offspring size,

528 including our own, fail to incorporate is the physiological cost of producing offspring of

529 uniform size. We agree with Fox and Czesak (2000)'s suggestion that at least some of the

530 within-brood variation in offspring size may reflect the cost associated with the production of

531 uniformly sized offspring, or physiological constraints against uniform offspring size.

532 Nevertheless, it is clear that systematic differences in the level of within-brood variation

533 occur among organisms with different life-history strategies and we suggest that these

534 differences may be adaptive.

535

Acknowledgements

545

Literature Cited

547 Bagenal, T. B. 1969. Relationship between egg size and fry survival in brown trout *Salmo*

548   *trutta* L. *Journal of Fish Biology* **1**:349-353.

549   Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size:

550   Patterns models, quality of evidence and interpretations. *American Zoologist* **36**:216-

551   236.

552   Bervan, K. A., and B. G. Chadra. 1988. The relationship among egg size, density and food

553   level on larval development in the wood frog (*Rana sylvatica*). *Oecologia* **75**:67-72.

554   Bull, J. J. 1987. Evolution of phenotypic variance. *Evolution* **41**:303-315.

555   Capinera, J. L. 1979. Qualitative variation in plants and insects - effect of propagule size on

556   ecological plasticity. *American Naturalist* **114**:350-361.

557   Cohen, D. 1966. Optimizing reproduction in a randomly varying environment. *Journal of*

558   *Theoretical Biology* **12**:119-129.

559   Collin, R. 2000. Sex change, reproduction, and development of *Crepidula adunca* and

560   *Crepidula lingulata* (Gastropoda: Calyptraeidae). *Veliger* **43**:24-33.

561   Congdon, J. D., and J. W. Gibbons. 1987. Morphological constraint on egg size - a challenge

562   to optimal egg size theory. *Proceedings of the National Academy of Sciences of the*

563   *United States of America* **84**:4145-4147.

564   Cooper, W. S., and R. H. Kaplan. 1982. Adaptive "coin-flipping": A decision-theoretic

565   examination of natural selection for random individual variation. *Journal of*

566   *Theoretical Biology* **94**:135-151.

567   Crump, M. L. 1981. Variation in propagule size as a function of environmental uncertainty

568   for tree frogs. *American Naturalist* **117**:724-737.

569   Dibattista, J. D., K. A. Feldheim, S. H. Gruber, and A. P. Hendry. 2007. When bigger is not

570   better: selection against large size, high condition and fast growth in juvenile lemon

571   sharks. *Journal of Evolutionary Biology* **20**:201-212.

572 Dziminski, M. A., and R. A. Alford. 2005. Patterns and fitness consequences of intraclutch

573   variation in egg provisioning in tropical Australian frogs. *Oecologia* 146:98-109.

574 Einum, S., and I. A. Fleming. 2000. Highly fecund mothers sacrifice offspring survival to

575   maximize fitness. *Nature* **405**:565-567.

576 Einum, S., and I. A. Fleming. 2002. Does within-population variation in fish egg size reflect

577   maternal influences on optimal values. *American Naturalist* **160**:756-765.

578 Einum, S., and I. A. Fleming. 2004a. Does within-population variation in egg size reduce

579   intraspecific competition in Atlantic Salmon, *Salmo salar*? *Functional Ecology*

580   **18**:110-115.

581 Einum, S., and I. A. Fleming. 2004b. Environmental unpredictability and offspring size:

582   conservative versus diversified bet-hedging. *Evolutionary Ecology Research* **6**:443-

583   455.

584 Emlet, R. B. 1986. Facultative planktotrophy in the tropical echinoid *Clypaster rosaceus*

585   (Linnaeus) and a comparison with obligate planktotrophy in *Clypaster subdepressus*

586   (Gray) (Clypasteroida: Echinoidea). Journal of Experimental Marine Biology

587   Ecology **95**:183-202.

588 Fox, C. W., Thakar, M. S. and Mosseau, T. A. (1997). Egg size plasticity in a seed beetle: An

589   adaptive maternal effect. *American Naturalist* **149:** 149-163.

590 Fox, C. W., and M. E. Czesak. 2000. Evolutionary ecology of progeny size in arthropods.

591   *Annual Review of Entomology* 45:341-369.

592 Geritz, S. A. H. 1995. Evolutionarily stable seed polymorphism and small-scale spatial

593   variation in seedling density. *American Naturalist* **146**:685-707.

594   Gillespie, J. H. 1977. Natural selection for variances in offspring numbers: a new

595   evolutionary principle. *American Naturalist* **111**:1010-1014.

596   Hadfield, M. G., and M. F. Strathmann. 1996. Variability, flexibility and plasticity in life

597   histories of marine invertebrates. *Oceanologica acta* **19** 323-334.

598   Hart, M. W., M. Byrne, and S. L. Johnson. 2003. *Patiriella pseudoexigua* (Asteroidea :

599   Asterinidae): a cryptic species complex revealed by molecular and embryological

600   analyses. *Journal of the Marine Biological Association of the United Kingdom*

601   **83**:1109-1116.

602   Harvey, P. H., and M. D. Pagel. 1991, The comparative method in evolutionary biology,

603   Oxford University Press.

604   Havenhand, J. N. 1995. Evolutionary ecology of larval types. Pages 79-122 *in* L. R.

605   McEdward editor. Ecology of marine invertebrate larvae. CRC, Boca Raton.

606   Hendry, A. P., T. Day, and A. B. Cooper. 2001. Optimal size and number of propagules:

607   Allowance for discrete stages and effects of maternal size on reproductive output and

608   offspring fitness. *American Naturalist* **157**:387-407.

609   Kaplan, R. H. 1992. Greater maternal investment can decrease offspring survival in the frog

610   *Bombina orientalis*. *Ecology* **73**:280-288.

611   Kohn, A. J., and F. E. Perron. 1994. Life-history and biogeography patterns in *Conus*.

612   Clarendon Press, Oxford.

613   Krug, P. J. 1998. Poecilogony in an estuarine opisthobranch: planktotrophy, lecithotrophy,

614   and mixed clutches in a population of the ascoglossan *Alderia modesta*. *Marine*

615   *Biology* **132**:483-494.

616 Kudo, S. 2001. Intraclutch egg-size variation in acanthosomatid bugs: adaptive allocation of

617   maternal investment. *Oikos* **92**:208-214.

618 Laaksonen, T. 2004. Hatching asynchrony as a bet-hedging strategy - an offspring diversity

619   hypothesis. *Oikos* **104**:616-620.

620 Lack, D. 1947. The significance of clutch size. *Ibis* **89**:302-352.

621 Levitan, D. R. 1993. The importance of sperm limitation to the evolution of egg size in

622   marine invertebrates. *American Naturalist* **141**:517-536.

623 Levitan, D. R. 1996. Effects of gamete traits on fertilisation in the sea and the evolution of

624   sexual dimorphism. *Nature* **382**: 153-155.

625 Levitan, D. R. 2000. Optimal egg size in marine invertebrates: theory and phylogenetic

626   analysis of the critical relationship between egg size and development time in

627   echinoids. *American Naturalist* **156**:175-192.

628 Lips, K. R. 2001. Reproductive trade-offs and bet-hedging in *Hyla calypsa*, a Neotropical

629   treefrog. *Oecologia* **128**:509-518.

630 Lloyde, D. G. 1987. Selection of offspring size at independence and other size-versus-

631   number strategies. *American Naturalist* **129**:800-817.

632 Marshall, D. J., T. F. Bolton, and M. J. Keough. 2003. Offspring size affects the post-

633   metamorphic performance of a colonial marine invertebrate. *Ecology* **84**:3131-3137.

634 Marshall, D. J., M. J. Keough 2007. Evolutionary ecology of offspring size in marine

635   invertebrates. *Advances in Marine Biology* **53:** 1-60.

636 Marshall, D.J., C. N. Cook, and R. B. Emlet. 2006. Offspring size effects mediate

637   competition in a colonial marine invertebrate. *Ecology* **87**: 214-225.

638   Marshall, D. J., and M. J. Keough. 2003. Sources of variation in larval quality for free-

639   spawning marine invertebrates: Egg size and the local sperm environment.

640   *Invertebrate Reproduction and Development* **44**:63-70.

641   Marshall, D. J., C. A. Styan, and M. J. Keough. 2000. Intraspecific co-variation between egg

642   and body size affects fertilisation kinetics of free-spawning marine invertebrates.

643   *Marine Ecology Progress Series* **195**:305-309.

644   Marshall, D. J., C. A. Styan, and M. J. Keough. 2002. Sperm environment affects offspring

645   quality in broadcast spawning marine invertebrates. *Ecology Letters* **5**:173-176.

646   McGinley, M. A., D. H. Temme, and M. A. Geber. 1987. Parental investment in offspring in

647   variable environments: theoretical and empirical considerations. *American Naturalist*

648   **130**:370-398.

649   Moran, A. L., and R. B. Emlet. 2001. Offspring size and performance in variable

650   environments: field studies on a marine snail. *Ecology* **82**:1597-1612.

651   Orr, H., A. 2007. Absolute fitness, relative fitness and utility. *Evolution* **61**: 2997-3000.

652   Parker, G. A., and M. Begon. 1986. Optimal egg size and clutch size - effects of environment

653   and maternal phenotype. *American Naturalist* **128**:573-592.

654   Podolsky, R., D., and R. Strathmann, R. 1996. Evolution of egg size in free spawners:

655   consequences of the fertilisation-fecundity trade-off. *American Naturalist* **148**:160-

656   173.

657   Podolsky, R. D. 2002. Fertilization ecology of egg coats: physical versus chemical

658   contributions to fertilization success of free-spawned eggs. *Journal of Experimental*

659   *Biology* **205**:1657-1668.

660   Poulin, R., and W. J. Hamilton. 2000. Egg size variation as a function of environmental

661   variability in parasitic trematodes. *Canadian Journal of Zoology-Revue Canadienne*

662   *De Zoologie* **78**:564-569.

663   Roff, D. A. 1992. The evolution of life histories. Chapman & Hall,, New York.

664   Sakai, S., and Y. Harada. 2001. Why do large mothers produce large offspring? Theory and a

665   test. *American Naturalist* **157**:348-359.

666   Sargent, R. C., P. D. Taylor, and M. R. Gross. 1987. Parental care and the evolution of egg

667   size in fishes. *American Naturalist* **129**:32-46.

668   Seger, J., and H. J. Brockman. 1987. What is bet-hedging? *Oxford Surveys in Evolutionary*

669   *Biology* 182-211.

670   Simons, A. M., and M. O. Johnston. 1997. Developmental instability as a bet-hedging

671   strategy. *Oikos* **80**:401-406.

672   Simons, A. M., and M. O. Johnston. 2006. Environmental and genetic sources of

673   diversification in the timing of seed germination: Implications for the evolution of bet

674   hedging. *Evolution* **60**:2280-2292.

675   Smith, C. C., and S. D. Fretwell. 1974. The optimal balance between size and number of

676   offspring. *American Naturalist* **108**:499-506.

677   Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.

678   Strathmann, R. R. 1995. Peculiar constraints on life histories imposed by protective or

679   nutritive devices for embryos. *American Zoologist* **35**:426-433.

680   Strathmann, R. R., and C. Chaffee. 1984. Constraints on egg masses. II. Effect of spacing ,

681   size and number of eggs on ventiliation of masses of embryos in jelly, adherent

682   groups or thin walled capsules. *Journal of Experimental Marine Biology and Ecology*

683   **84**:85-93.

684   Styan, C. A. 1998. Polyspermy, egg size and the fertilization kinetics of free-spawning

685   marine invertebrates. *American Naturalist* **152**:290-297.

686   Turner, R., and J. M. Lawrence. 1977. Volume and composition of echinoderm eggs:

687   implications for the use of egg size in life-history models, Pages 25-40 *in* S. E.

688   Stancyk, ed., *Reproductive ecology of marine invertebrates*. Columbia, University of

689   South Carolina Press

690   Vance, R., R. 1973. On reproductive strategies in marine benthic invertebrates. *American*

691   *Naturalist* **107**:339-352.

692   Williams, M. D. 1994. Intraspecific variation in egg size and egg composition in birds:

693   effects on offspring fitness. *Biological Review* **68**:38-59.