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REPORT

Sperm environment affects offspring quality in broadcast spawning marine invertebrates

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Abstract

The provisioning of offspring can have far-reaching consequences for later life in a wide range of organisms and generally this provisioning is thought to be under maternal influence or control. In experiments with a broadcast-spawning ascidian, we found that the size of offspring was determined by egg size and the abundance of sperm present during fertilization. Larger eggs were fertilized at low sperm concentrations, whilst smaller eggs were successfully fertilized at high sperm concentrations. These differences in fertilized egg size resulted in differences in the development rate, hatching success and mean size of the subsequent larvae. Our results suggest that, in contrast to females that reproduce by other mating systems, free-spawning mothers lack some control over the provisioning of offspring. Furthermore, because males can alter the sperm environment, they can exert paternal (non-genetic) control over key offspring characteristics.

Keywords

Ascidians, egg size, fertilization dynamics, maternal effects, *Pyura stolonifera*.

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INTRODUCTION

Maternal provisioning can have far-reaching effects on the survival and growth of progeny for a wide variety of taxa (Bernado 1996; Azevedo *et al.* 1997). The degree of provisioning can be highly plastic and depend on maternal size, age or nutrition (Bernado 1996; Rossiter 1996). In some organisms offspring provisioning appears to be highly regulated by mothers to increase either their own (e.g. birds: Cunningham & Russell 2000; fish: Einum & Fleming 2000) or their offspring's fitness (e.g. insects: Fox *et al.* 1997; fish: Kerrigan 1997). Recently, there has been much debate on the factors determining optimal egg size/provisioning for free-spawning marine organisms (Podolsky & Strathmann 1996; Levitan 2000). In addition to providing eggs with sufficient resources for development, these mothers face the added challenge of ensuring that their eggs are fertilized (Levitan 2000).

For free-spawning organisms, successful fertilization of eggs can be a difficult process. Fertilization can be limited by the low availability of sperm (Levitan & Petersen 1995) but sperm limitation is not ubiquitous (Yund 2000) and lethal polyspermy can occur in field conditions (Brawley 1992). Fertilization success also depends on the size of eggs, because this determines the target size for sperm to contact (Levitan 1996; Marshall *et al.* 2000). Consequently, when sperm are scarce, larger eggs are more likely to be fertilized because they are more likely to be found by searching sperm (Levitan 1996). Conversely, a fertilization model predicts that when sperm are abundant, larger eggs are more likely to become polyspermic because they have a greater chance of contacting multiple, coincident sperm (Styan 1998). Egg size can vary quite substantially within species and within individual broods of free-spawners (Levitan 2000; Marshall *et al.* 2000). We hypothesized that this variation in egg size would interact with sperm abundance to produce zygotes that varied in size, according to the sperm concentration. We were also interested in whether this variation in zygote size resulted in differences in any larval characteristics.

EGG SIZE AND SPERM CONCENTRATION

We tested our hypothesis by conducting two sets of laboratory experiments with the intertidal, solitary ascidian, *Pyura stolonifera* (Heller). In the first experiment, we demonstrated that the relative fertilization success of large or small eggs within broods was conditional on sperm concentration. Eggs were stripped from eight ripe *P. stolonifera* in January 1999 (for method see Marshall *et al.* 2000). Eggs from each individual were divided into three batches and placed in Petri dishes, with one batch exposed to a high sperm concentration (10^4 sperm μL^{-1}), one to a low sperm concentration (10 sperm μL^{-1}), and one batch to no sperm at all (see Marshall *et al.* 2000 for relative fertilization rates at

these sperm concentrations). The sperm used to fertilize eggs from each mother was stripped from a different father. After leaving eggs to develop signs of successful fertilization, the diameters of 100 randomly sampled eggs that were not undergoing cleavage were then measured from each egg batch. We could not directly measure the size of eggs that had been fertilized because cleaving eggs change shape almost immediately, but we could measure the size of eggs that remained once developing eggs were excluded. In ascidians, polyspermic eggs rarely divide (Lambert & Lambert 1981) and during the course of this study no (putatively) polyspermic eggs were seen to develop. Therefore we could not distinguish between unfertilized and polyspermic eggs; regardless, neither were going to develop into larvae. We compared average non-developing egg size for the sperm-exposed batches with the average size of unexposed eggs in the associated control batches, using paired *t*-tests, for both the high sperm and low sperm concentrations.

Exposed to a low sperm concentration, predominantly larger eggs within a brood were fertilized (Fig. 1), and the average size of the remaining eggs was significantly lower than the unexposed eggs ($t = 3.98$, d.f. $= 7$, $P = 0.005$). Levitan (1996) observed similar results when he exposed echinoderm eggs to low sperm concentrations in the field. Exposed to a higher sperm concentration, only smaller eggs developed into larvae (Fig. 1) and the average size of eggs that remained was significantly larger than the unexposed

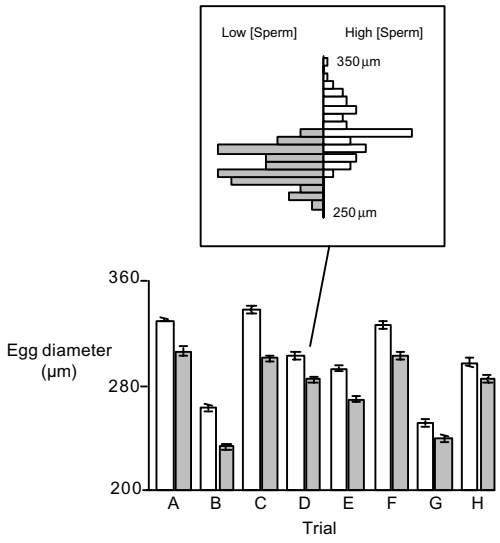

Figure 1 Mean (± SE) diameter of non-developing *Pyura stolonifera* eggs following exposure to either a high (shaded bars) or a low (white bars) sperm concentration for 30 min. Each pair of bars represents one specific mating. Inset: an example of the difference in size frequency distributions of non-developing eggs in a single trial.

eggs ($t = 5.74$, d.f. $= 7$, $P = 0.001$). These larger eggs that remained are unlikely to be eggs that merely increased in size after contact with sperm as (putatively) polyspermic eggs show no increase in size in *P. stolonifera* (D. Marshall, unpubl. data).

Variable egg size may simply be an unavoidable consequence of maternal nutrition and oocyte development (Jaeckle 1995), or may represent a strategy allowing mothers to cope with uncertain sperm environments. Whatever the cause of this variation, it results in size-selective fertilization within broods. Furthermore this difference in zygote size was reflected in the size of the resultant larvae.

SPERM CONCENTRATION AND OFFSPRING TRAITS

In a second experiment, groups of larvae, with shared parentage and reared under identical conditions, differed in several key traits as a result of the sperm environment in which they were fertilized. Paired batches of large or small eggs were differentially fertilized as above, in either low (10 sperm μL^{-1}) or high (10^4 sperm μL^{-1}) sperm concentrations. The paired batches were placed in three replicate larval culture pots per batch in a flow-through sea-water system. This was repeated for six unique, randomly assigned, mother–father matings. Starting 16 h after fertilization, eggs/larvae were subsampled without replacement from culture pots every 30 min, for 9 h, to estimate development success (percentage hatched into larvae), developmental rate and, once hatched, how long larval activity persisted. Ascidian larvae begin swimming vigorously whenever a shadow is passed over them; this response has been termed a shadow response (Young & Chia 1985). We estimated how long larvae remained active post-fertilization by assessing the proportion of 60 sampled larvae from each pot that could be induced to show a shadow response at each time, in each batch. Plots of the proportion of eggs that hatched and larvae that were inactive through time were constructed for each batch. Polynomial and logistic models were fitted for each plot, and the times until 50% of larvae hatched and until 50% of larvae had stopped responding were calculated. We estimated the size of larvae 24 h after fertilization, by measuring the largest transverse width of the tadpole head and by measuring the (tail) length between the start of the head region and the tip of the tail, averaging across 40 larvae in each pot (repeated for only four unique mother–father matings). For all larval traits, the difference between fertilization in high and low sperm concentrations across matings was compared using paired *t*-tests on the means of replicate pots within batches.

The pattern illustrated in Fig. 2 was repeated in each *P. stolonifera* mating we conducted. Larvae that developed from eggs exposed to a high sperm concentration (i.e. from smaller eggs) took longer to hatch ($t = 3.2$, d.f. $= 5$,

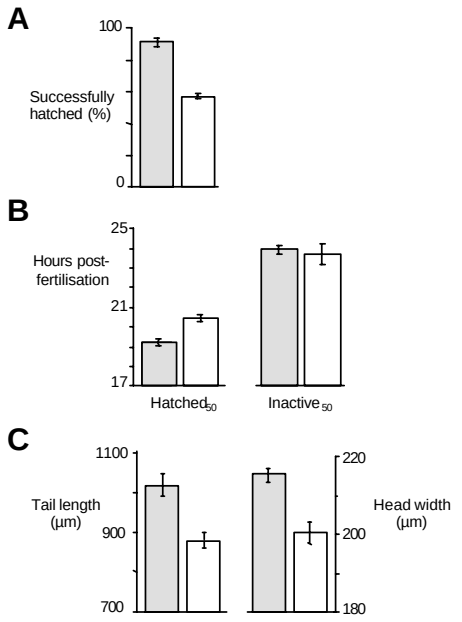

Figure 2 An example (from one mating with 3 replicates) of the differences in offspring traits that result from altering the abundance of sperm during fertilization in *Pyura stolonifera*. Mean (± SE of 3 replicates within matings) traits of offspring fertilized in a low sperm concentration (10^1 sperm μL^{-1}) are represented by shaded bars, those of offspring fertilized in a high concentration (10^4 sperm μL^{-1}) are shown in white. A. Percentage of cleaved eggs that hatched into swimming tadpole larvae. B. Time taken for half of the embryos to hatch, and the time until half of the larvae stopped swimming. C. Mean tail length and head width of tadpole larvae.

$P = 0.024$; mean difference ± SE = 0.47 h ± 0.12) and were smaller, with shorter tails ($t = 6.28$, d.f. = 3, $P = 0.008$; mean difference ± SE = 53.33 μm ± 6.40) and narrower heads ($t = 3.33$, d.f. = 3, $P = 0.044$; mean difference ± SE = 10.84 μm ± 4.49) than larvae developed from eggs exposed to low sperm concentration. A greater proportion of embryos that developed from eggs exposed to a low sperm concentration hatched into larvae than eggs that were fertilized in a high sperm concentration ($t = 2.84$, d.f. = 5, $P = 0.036$; mean difference ± SE = 15.92% ± 4.09). We could not detect a difference in the time until 50% of larvae ceased responding to shadows ($t = 1.06$, d.f. = 5, $P = 0.334$; mean difference ± SE = 0.17 h ± 0.16). Given that eggs fertilized in high sperm concentrations took longer to hatch, this suggests that they also spent less time as active larvae. In species with non-feeding larvae such as *P. stolonifera*, development rate, larval size and larval endurance are likely to affect dispersal, planktonic survival and the likelihood of successful settlement (Svane & Young 1988). These stages are likely to be of high evolutionary importance because this is a time of high mortality and consequently, large selective pressures (Morgan 1995).

As in other mating systems, offspring provisioning in free-spawners is initially determined by maternal influences. However, the size distribution of eggs that get fertilized is directly determined by the surrounding sperm concentration. Our experiments suggest that the effects of this size-selective fertilization are not trivial and affect key larval characteristics. Sperm concentrations in the marine environment can vary greatly and unpredictably depending on a wide range of biological and hydrodynamic factors (Levitan 1995). Our experiments represent only two points on this continuum of potential sperm concentrations, but a fertilization model predicts size-dependent fertilization of eggs at any sperm concentration. It seems unlikely, then, that free-spawning mothers can completely control the final size distribution of eggs that are fertilized; they can only produce eggs of a given size range and can not control which of these eggs are fertilized. However, it is possible that by controlling the release of sperm, males can exert some paternal (non-genetic) control over key offspring characteristics. These represent previously unrecognized and potentially important consequences of reproducing by freely spawned gametes.

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REFERENCES

Azevedo, R.B.R., French, V. & Partridge, L. (1997). Life-history consequences of egg size in *Drosophila melanogaster*. *Am. Nat.*, 150, 250–282.

Bernado, J. (1996). The particular maternal effect of propagule size, especially egg size: Patterns models, quality of evidence and interpretations. *Am. Zool.*, 36, 216–236.

Brawley, S.H. (1992). Fertilisation in natural populations of the dioecious brown alga *Fucus ceranoides* and the importance of the polyspermy block. *Mar. Biol.*, 113, 145–157.

Cunningham, E.J.A. & Russell, A.F. (2000). Egg investment is influenced by male attractiveness in the mallard. *Nature*, 404, 74–77.

Einum, S. & Fleming, I.A. (2000). Highly fecund mothers sacrifice offspring survival to maximise fitness. *Nature*, 405, 565–567.

Fox, C.W., Thakar, M.S. & Mosseau, T.A. (1997). Egg size plasticity in a seed beetle: An adaptive maternal effect. *Am. Nat.*, 149, 149–163.

Jaeckle, W.B. (1995). Variation in the size, energy content and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: *Ecology of Marine Invertebrate*

Larvae ed. McEdward, L. CRC Press, Boca Raton, FL, pp. 49–78.

Kerrigan, B.A. (1997). Variability in larval development of the tropical reef fish *Pomacentrus amboinensis* (Pomacentridae): the parental legacy. *Mar. Biol.*, 127, 395–402.

Lambert, C.C. & Lambert, G. (1981). Formation of the block to polyspermy in ascidian eggs: Time course, ion requirements and the role of the accessory cells. *J. Exp. Zool.*, 217, 291–295.

Levitan, D.R. (1995). The ecology of fertilisation in free-spawning invertebrates. In: *Ecology of Marine Invertebrate Larvae* ed. McEdward, L. CRC Press, Boca Raton, FL, pp. 125–152.

Levitan, D.R. (1996). Effects of gamete traits on fertilisation in the sea and the evolution of sexual dimorphism. *Nature*, 382, 153–155.

Levitan, D.R. (2000). Optimal egg size in marine invertebrates: theory and phylogenetic analysis of the critical relationship between egg size and development time in echinoids. *Am. Nat.*, 156, 175–192.

Levitan, D.R. & Petersen, C. (1995). Sperm limitation in the sea. *Trends Ecol. Evol.*, 10, 228–231.

Marshall, D.J., Styan, C.A. & Keough, M.J. (2000). Intraspecific co-variation between egg and body size affects fertilisation kinetics in free-spawning invertebrates. *Mar. Ecol. Progr. Series*, 195, 305–309.

Morgan, S.G. (1995). Life and Death in the plankton: larval mortality and adaptation. In: *Ecology of Marine Invertebrate Larvae* ed. McEdward, L. CRC Press, Boca Raton, FL, pp. 279–322.

Podolsky, R.D. & Strathmann, R.R. (1996). Evolution of egg size in free spawners: consequences of the fertilisation–fecundity tradeoff. *Am. Nat.*, 148, 160–173.

Rossiter, M.C. (1996). Incidence and consequences of inherited environmental effects. *Ann. Rev. Ecol. Syst*, 27, 451–476.

Styan, C.A. (1998). Polyspermy, egg size and the fertilisation kinetics of free-spawning marine invertebrates. *Am. Nat.*, 152, 290–297.

Svane, I. & Young, C.M. (1988). The ecology and behavior of ascidian larvae. *Ann. Rev. Oceanogr. Mar. Biol.*, 27, 45–90.

Young, C.M. & Chia, F.-S. (1985). An experimental test of shadow response function in ascidian tadpoles. *J. Exp. Mar. Biol. Ecol.*, 85, 165–175.

Yund, P.O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends Ecol. Evol.*, 15, 10–13.

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