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Sperm release strategies in marine broadcast spawners: the costs of releasing sperm quickly

Dustin J. Marshall[1,*] and Toby F. Bolton[2]

[1]*School of Integrative Biology/Centre for Marine Studies, University of Queensland, 4072, QLD, Australia and*
[2]*Lincoln Marine Science Centre, School of Biological Sciences, Flinders University, 5606, SA, Australia*

*Author for correspondence (e-mail: d.marshall1@uq.edu.au)

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Summary

When under competition for fertilisations, males are thought to increase their reproductive success by releasing as many sperm as possible into the reproductive arena and in many species, this prediction holds. For marine invertebrates, which utilise the ancestral strategy of broadcast spawning eggs and sperm, however, it appears that males tend to release their sperm more slowly than females release their eggs. Marine invertebrate eggs typically have a relatively slow permanent block to polyspermy (whereby eggs become impermeable to further sperm attachment), and for several minutes after fertilisation, sperm can continue to attach to a fertilised egg. We hypothesised that releasing sperm slowly minimises the 'wastage' of sperm on already fertilised eggs. We simulated different sperm release rates in a flume using the broadcast spawning polychaete, *Galeolaria caespitosa*. Sperm release rates strongly affected overall fertilisation success: higher release rates resulted in lower fertilisation rates. Laboratory studies confirmed that the 'permanent' block to polyspermy in *G. caespitosa* took less than a minute to form but this lag was sufficient to result in some sperm wastage. Thus upstream, fertilised eggs that have not formed a permanent block to polyspermy can remove sperm from the pool that would otherwise fertilise downstream sibling eggs. We suggest that while electrical blocks to polyspermy evolved in response to excess sperm, permanent blocks to polyspermy could have evolved in response to sperm limitation (insufficient sperm).

Key words: fertilisation, polyspermy, sperm competition.

Introduction

The classic view of sexual selection is that males compete for the fertilisation of a female's eggs (Bateman, 1948). When males face significant competition for fertilisations from other males, established sperm competition theory predicts that males should release as many sperm as possible (Parker, 1990; Parker, 1993; Parker, 1998; Parker and Ball, 2005). Indeed across many organisms, in species where sperm competition is more likely, males have relatively larger testes (Byrne et al., 2002; Stockley et al., 1997). The relationship between sperm competition and male release strategies is driven by the numerical basis of sperm competition: if a male achieves numerical superiority at fertilisation, it is predicted that he will sire the highest proportion of eggs (see references above). Whilst the dynamics of sperm release have been well studied for some organisms, marine broadcast spawning (the most common form of reproduction in the sea) has received relatively little attention. This is despite broadcast spawners representing the ancestral mode of reproduction and their repeated use as a basis for sperm competition theory (Ball and Parker, 1996; Williams et al., 2005), species recognition (Panhuis et al., 2006) and the evolution of anisogamy [sperm and eggs of different sizes (Levitan, 1996; Levitan and Ferrell, 2006)].

Sperm competition is probably intense for most broadcast spawners. Sperm limitation (incomplete fertilisation success due to insufficient sperm) may be common in the marine environment (Levitan and Petersen, 1995; Yund, 2000), but males rarely, if ever, gain exclusive access to a batch of eggs and indeed initial studies suggest that broadcast spawning is highly polygamous (Brockmann et al., 1994; Levitan, 2004; Levitan, 2005a). Most observations of spawning events show that multiple individuals spawn, sometimes within a dense aggregation, and therefore multiple ejaculates of sperm will be competing for a limited pool of eggs (Hardege and Bentley, 1997; Lamare and Stewart, 1998; Marshall, 2002; Marshall et al., 2004). Spawning times can be restricted to narrow environmental windows (Babcock et al., 1986; Marshall, 2002) and as such, males are probably unable to 'wait' for periods of less intense sperm competition (Fuller, 1998). Consequently, much of the theory on sperm competition would predict that in the presence of many competing ejaculates, broadcast spawning males should release all their sperm as quickly as possible over a short time scale (Ball and Parker, 1996; Stockley et al., 1996).

Current observations of sperm release rates in broadcast spawners do not support the prediction that broadcast spawning males should release sperm quickly (Table 1). For a number of species, males tend to release their gametes more slowly than females (Table 1). Furthermore, some species release their

Table 1. *Summary table of the relative length of spawning periods in male and female broadcast spawners*

Study	Species	Spawning behaviour
(Selvakumaraswamy and Byrne, 2000)	*Ophionereis shayeri*	♂: >1 h ♀: ~10 s
(Miller, 2005)	*Oikopleura dioica*	♂: 0.25–0.9 h ♀: ~5 s
(McEuen, 1988)	*Psolus chitonoides*	♂: release for up to 7.5 h ♀: release up to 1.5 h
(McEuen, 1988)	*Cucumaria miniata*	♂: release for 3–6 h ♀: release for 0.75–4 h
(McEuen, 1988)	*Eupentacta quinquesemita*	♂: release for 1 h ♀: release for 1–1.75 h
(McEuen, 1988)	*Molpadia intermedia*	♂: release for 0.5–3 h ♀: release for 3–4 s
(Hamel and Mercier, 1995)	*Leptasteria polaris*	♂: release for 1 h ♀: release for 0.6 h
(Levitan, 2002)	*Strongylocentrotus* spp.	♂: release for longer than ♀
(Hardege and Bartels-Hardege, 1995)	*Perinereis nuntia* var. *brevicirrus*	♂: release for longer than ♀
(Hardege and Bentley, 1997)	*Arenicola marina*	♂: release over several days

Precise data on maximum spawning periods of each sex have been supplied where possible, but in the absence of quantitative data, statements regarding the relative length of spawning period have also been included.

sperm in a viscous matrix that further reduces the advection of sperm from the site of spawning such that sperm slowly wisps away (Marshall, 2002; Marshall et al., 2004; Thomas, 1994). At higher temporal scales, males repeatedly spawn over successive days rather than release all their sperm in a single event (Hardege and Bentley, 1997). Finally, larger males do not release more sperm than smaller males in any one spawning event (Hardege and Bentley, 1997; Styan and Butler, 2003). All of these behaviours appear to be contrary to the predictions of traditional theory concerning sperm competition (Williams et al., 2005). Why do broadcast spawning males release sperm slowly when achieving numerical superiority with sperm should carry a fitness benefit?

The kinetics of fertilisation in broadcast spawners may account for the sperm release strategies of broadcast spawning males. In a number of different marine invertebrate taxa, a few seconds after an egg is fertilised by a sperm, a ‘fast’ electrical block is formed that prevents additional sperm from fusing with the egg (Gould and Stephano, 2003; Wong and Wessel, 2006). This first block prevents polyspermy (a lethal condition in marine invertebrate eggs) but does not prevent sperm from attaching to the egg. Within minutes of fertilisation a second ‘slow’ or permanent block then forms, which prevents the attachment of subsequent sperm to outside of the egg (Gould and Stephano, 2003). The mechanisms for this change in the attachment properties of the egg vary among taxa but can involve the retraction of microvilli, the expansion of a fertilisation envelope, or the release of cortical granules that modify the surface of the vitteline coat (Gould and Stephano, 2003). Because we will discuss the speed of onset of these blocks and use of terms such as ‘fast, slow blocks’ would be confusing, for clarity, we will refer to these two blocks as ‘electrical’ and ‘permanent’, respectively, throughout the manuscript.

Given the time taken for fertilised eggs to form the permanent block to polyspermy, a temporal window exists in which sperm can attach to the egg surface without achieving fertilisation, and as such eggs can act as ‘sperm sinks’, removing sperm from the pool without resulting in further fertilisations. A recent study showed that a single egg could remove up to 140 sperm (Marshall and Evans, 2005b), and therefore eggs that have not formed slow polyspermy blocks could represent a significant drain on the sperm pool. Here, we test the hypothesis that faster sperm release rates result in a higher proportion of sperm being wasted whereas slower release speeds allow sufficient time for permanent polyspermy blocks to form. Simultaneously, we explore the potential selection pressures that may have led to the evolution of permanent polyspermy blocks: why do females have a permanent polyspermy block if the electrical block is sufficient to prevent polyspermy? If we hope to understand the effect of permanent polyspermy blocks on the benefits of releasing sperm quickly or slowly, then we need direct measures of the fertilisation success of males that release sperm at different rates under hydrodynamic conditions that are as realistic as possible. We used flume experiments to simulate the fast and slow release of sperm and compared fertilisation rates across two groups of eggs on the broadcast spawning marine invertebrate, *Galeolaria caespitosa*. We used two groups of eggs because we predicted that any upstream ‘wastage’ of eggs generated by a fast release speed would result in decreased fertilisation success downstream. We found that sperm release rates had dramatic consequences for male fertilisation success and so we further investigated the time course requirements for the formation of polyspermy blocks.

Materials and methods

Study site and species

Galeolaria caespitosa Savigny 1818 is a sessile polychaete worm that occurs at a wide range of densities in the intertidal zone of sheltered and exposed areas of south-eastern Australia. The sexes are separate and females release their gametes as a viscous matrix that persists under low flow conditions. For the flume experiments, we collected individuals from the intertidal regions of pilings on Port Lincoln Pier, South Australia. For the laboratory experiments, we collected individuals from pier pilings at Bare Island, Botany Bay, New South Wales. To collect gametes, we used standard methods outlined elsewhere (Marshall and Evans, 2005a; Marshall and Evans, 2005b).

The sperm of *G. caespitosa* are active immediately upon release (D.J.M. and T.F.B., personal observation) and remain viable and achieve high rates of fertilisation for up to 3 h after release (Ross and Bidwell, 2001). It is important to note that in *G. caespitosa*, exposure to eggs or seawater that has contained eggs ('egg water') does not activate or change the motility of sperm in this species (Kupriyanova and Havenhand, 2002).

Flume experiment

The aim of this experiment was to examine the effect of male release speed on subsequent fertilisation success across two batches of eggs, one batch downstream from the other. The internal dimensions of the flume were 1500 mm long×300 mm wide, filled with water to a depth of 80 mm. These dimensions do not preclude wall effects (Nowell and Jumars, 1987), but given that we were principally interested in comparing differences among runs within the same flume, these effects are unlikely to affect the outcome of our study. We induced laminar flow in each lane by using a 300 mm×300 mm×200 mm collimator made of drinking straws (see Yund and Meidel, 2003). Filtered seawater pumped from Boston Bay was first pumped to a 20-litre, constant head tank and then gravity fed into the flume. After travelling the length of the flume, the water exited the flume over a wall 80 mm high (the flume was non-recirculating). Initial observations using dye suggested that water flow was laminar across the experimental arena. The current speed within the flume was kept at a constant 100 mm s^{-1} [current speed estimated as described elsewhere (Yund and Meidel, 2003)]. At the head of the flume, the sperm (concentration: $\sim 1\times 10^{8}$ sperm ml^{-1}) from 3–4 males was released from a 60 ml syringe. To simulate fast release of sperm, two short bursts of 10 ml each were released within 10 s. To simulate the slow release of sperm, 12 short bursts were released over 120 s. Thus in both treatments, a total of 20 ml of sperm was released. Care was taken to ensure that we used the same rate of plunger depression across the treatments, so that the sperm in one treatment did not experience different shear forces to those in the other (Mead and Denny, 1995), but the duration of release differed between the two treatments. Downstream from the release points were two batches of eggs, each consisting of eggs spawned from 3–5 females. The proximal batch of eggs was 200 mm from the sperm release point and the distal batch was 800 mm (both batches were placed in the longitudinal centre of the flume). To place the eggs into the flume, we used a syringe to gently release the eggs onto the surface of the flume. *Galeolaria caespitosa* eggs are negatively buoyant and remained in a small 'clump' even while water was running through the flume. We allowed the eggs in both batches to accumulate fertilisations and then 10 min after sperm release, the eggs were collected using pipettes and placed into 70 ml polyethylene specimen jars, where they were allowed to develop for a further 2 h before fertilisation was assessed as in (Marshall and Evans, 2005a). We alternated fast release and slow release runs (10 of each) and conducted at least two runs on any given day. Between each run we drained the flume and rinsed it with freshwater before refilling.

Laboratory experiments

Our flume experiments indicated that sperm that is released over a longer period achieves greater fertilisation success in the distal batch of eggs than sperm that is released over a shorter period (see Results). Previous work on *G. caespitosa* has shown that unfertilised eggs deplete sperm solutions having a ratio of more than one sperm per egg (Marshall and Evans, 2005b). We conducted laboratory trials to determine if eggs that had recently been fertilised had the same sperm depleting effect as unfertilised eggs. To do this we conducted two experiments. The first compared the effects of unfertilised eggs and eggs that had been fertilised (for 1, 10 or 30 min) on the abundance of sperm after 5 min. Our protocol was similar to that described previously (Marshall and Evans, 2005b) but included additional treatments and some modifications. We first collected a batch of eggs from three females and split the eggs into four groups. The eggs in each were diluted in filtered seawater to a final concentration of 1000 eggs ml^{-1}. The first group of eggs was exposed to a high concentration ($1.5\pm 0.5\times 10^{6}$ sperm ml^{-1}) of (non-focal) sperm from three males for a period of 1 min before being thoroughly rinsed in filtered seawater to remove any sperm that had not bound to the eggs (the eggs were retained on a 25 μm plankton mesh filter). We then set this group aside for 10 min. 9 min later, the second group was exposed as above and set aside for only 1 min. Thus two pre-exposed groups of eggs were the same age but differed in the length of time for which they had been allowed to develop after exposure to the non-focal sperm. The third group of eggs was not exposed to sperm but was rinsed and filtered as in the other groups. We then took all three batches of eggs (in a 2 ml solution) and exposed them to 1 ml of sperm from a focal male for 15 min. A fourth vial containing only 2 ml seawater was also included as a control. The concentration of sperm was first diluted to a concentration of ($1.5\pm 0.5\times 10^{6}$ sperm ml^{-1}). Thus, the focal sperm from a single male was split into four groups and was exposed to (i) unfertilised eggs, (ii) eggs that had been fertilised only 1 min earlier, (iii) eggs that had been fertilised 10 min earlier, (iv) just seawater. For all of our experiments, the concentration of non-focal sperm was sufficient to result in >95% fertilisation success in the eggs. We then filtered the eggs from the solution (the control was treated identically) and estimated the concentration of focal sperm that remained in solution. We also exposed the focal sperm to additional 'fresh' eggs to assess their fertilisation capacity, but logistical constraints resulted in us being unable to expose sperm from the '1 min' treatment to eggs. Consequently, we repeated the above experiments but had only two treatments: a control, where

sperm were exposed to seawater only, and the ‘1 min’ treatment group of eggs. We then examined the subsequent fertilisation success of the focal sperm with fresh eggs.

Statistical analyses

We analysed the results of our flume experiment using a two-factor ANOVA where sperm-release speed and egg position were both fixed factors. To analyse the results of our laboratory experiment we used a mixed-model, two-factor ANOVA where focal male identity was a random factor and egg treatment was a fixed factor. We first ran a mixed model ANOVA including male identity and treatment; however, there was no interaction between male identity and treatment ($F_{20,76}$=0.79, P=0.71) and so it was removed from the final model (Quinn and Keough, 2002). To further examine the differences between levels of the egg treatment, we used incremental planned comparisons (Quinn and Keough, 2002) and pooled levels that were not significantly different from each other.

Results

Flume and laboratory experiments

The speeds at which sperm were released strongly affected the overall fertilisation success that those sperm achieved, with slow-released sperm achieving a much higher rate of fertilisation than fast-released sperm (Fig. 1). This difference was due to a strong interaction between sperm release speed and egg position (Table 2). The release rate of sperm did not affect the fertilisation rate of the nearest eggs; both release rates achieved about ~75% fertilisation success. However, release rate strongly affected the fertilisation rate of the eggs that were further downstream with the slow release rate resulting in much higher fertilisation rates than the fast release rate.

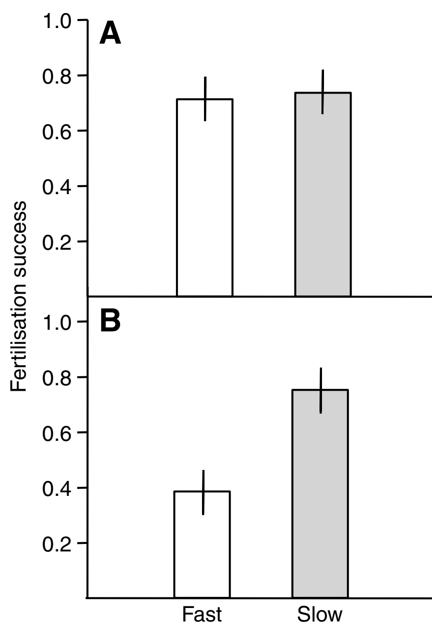

Fig. 1. Fertilisation success (mean ± s.e.m.) of simulated fast (open bars) or slow (shaded bars) sperm release rates across two groups of *Galeolaria caespitosa* eggs in a flume. (A) Fertilisation success of the upstream group of eggs; (B) fertilisation success in the downstream group of eggs.

Table 2. *Effect of the release rate of sperm on subsequent fertilisation success across eggs that were near and far from the point of sperm release in flume experiments on Galeolaria caespitosa*

Source	d.f.	Mean square	F-ratio	P
Egg position	1	0.266	4.12	0.050
Release speed	1	0.382	5.93	**0.020**
Interaction	1	0.291	4.51	**0.041**
Error	36	0.064		

Significant values are shown in bold type.

Exposure of sperm to unfertilised eggs caused an almost 50% reduction in sperm concentration relative to the control (Table 3; Fig. 2). However, exposure to eggs that had been previously exposed to sperm had less of an effect on focal sperm concentrations. Eggs that been exposed to sperm only 1 min before exposure to the focal sperm had no significant effect on the subsequent focal sperm concentration. Subsequent fertilisation rates of eggs fertilised with pre-exposed sperm reflected this pattern. Sperm that had been exposed to unfertilised eggs achieved much lower fertilisation rates than sperm that had been exposed to fertilised eggs or no eggs at all (Fig. 2; $F_{2,12}$=23.93, P<0.001; Tukey’s pairwise comparisons: Control=10 min>unfertilised). Similarly, sperm that had been

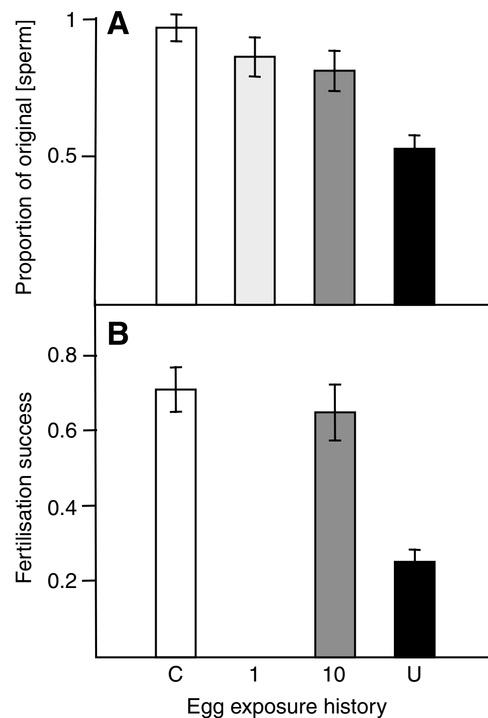

Fig. 2. Effect of prior exposure of sperm to unfertilised or recently (1–10 min post exposure) fertilised eggs on (A) sperm concentration and (B) subsequent fertilisation success in *Galeolaria caespitosa*. All values are means ± s.e.m.; open bars indicate the control (C), lightly shaded bars indicate exposure to recently fertilised eggs (1 and 10 min post fertilisation in A and 10 min post-fertilisation only in B), and black bars indicate exposure to unfertilised eggs (U).

Table 3. *Effect of pre-exposure of sperm to unfertilised or recently fertilised eggs on the subsequent concentration of sperm available for further fertilisations*

Source	d.f.	Mean square	*F*-ratio	*P*
Sperm concentration				
Treatment	4	0.614	10.217	**<0.001**
Male	5	0.402	6.692	**<0.001**
Error	96	0.060		
Planned comparisons				
10 *vs* 30				0.250
10+30 *vs* 1				0.959
10+30+1 *vs* C				0.111
10+30+1+C *vs* U				**<0.001**

Note that the model is reduced after testing for a non-significant interaction (see text for details). C, control; U, unfertilised.

Significant values are shown in bold type.

exposed to recently (1 min) exposed eggs had similar fertilisation rates to sperm that been exposed to no eggs at all (t=0.161, d.f.=10, P=0.875).

Discussion

The rate at which sperm were released strongly affected fertilisation success in *Galeolaria caespitosa*. Total fertilisation success achieved by the slow-released ejaculate (SRE) was higher than the total fertilisation success achieved by fast-released ejaculate (FRE). The difference in fertilisation success between the two release strategies was principally due the SRE achieving much higher rates of fertilisation at the second group of eggs. It appears that under the FRE strategy, many sperm are being removed from the available pool by the first group of eggs, leaving fewer for the downstream group. When the sperm were released slowly, the upstream group of eggs had sufficient time to form permanent blocks to polyspermy and therefore far more sperm from SRE were able to access the downstream group of eggs. It is difficult to imagine an alternative explanation for the differences in total fertilisation success that we observed. The FRE was released in two separate discharges whereas the SRE was released in twelve. It could be argued that despite the same volumes being released, the more numerous discharges in SRE treatment might have decreased the chances of any eggs being 'missed' by sperm, and this may have affected the results. However, we think this is unlikely for several reasons. First, the sperm were well mixed upstream of the collimator such that there were no individual 'wisps' of sperm passing over the eggs. Second, any effect of different number of discharges between the SRE and FRE treatments should have resulted in equal chances of 'missing' both the upstream and downstream eggs (flow was close to laminar in the flume after the collimator), but we only observed a decrease in the downstream batch of eggs. Thus we are confident that the decrease in the fertilisation downstream eggs in the FRE treatment is due to increased rates of depletion of sperm by the upstream eggs when sperm are released quickly. This result supports a previous study that demonstrated that the presence of upstream eggs can reduce the fertilisation success of downstream eggs (Marshall and Evans, 2005b), but shows that the level of depletion depends on the release rate of males.

Despite the strong research effort devoted to theoretical considerations of sperm release strategies in external fertilisers (Parker and Ball, 2005; Parker, 1982; Parker, 2000; Parker et al., 1996; Parker et al., 1997; Parker and Begon, 1986; Parker and Begon, 1993; Williams et al., 2005), studies that empirically examine the effects of different male release rates (over small temporal scales) on subsequent fertilisation success are rare. Levitan (Levitan, 2005b) showed strong pre-emption effects in the field with males that access eggs first having higher fertilisation. Levitan also predicted that releasing sperm slowly over time may be advantageous when sperm competition is low because it may increase the likelihood of sperm encountering eggs (Levitan, 2005b). Our present results provide further support for this prediction, as releasing sperm slowly will also decrease sperm 'wastage' on nearby females. We believe the next step in exploring what sperm release strategies should be favoured by broadcast spawning males is to use game theoretic models, previously developed for a 'generalised external fertiliser' (e.g. Ball and Parker, 1996), but with assumptions that are more relevant to broadcast spawners.

The relative benefits of releasing sperm quickly or slowly are likely to depend on a number of biological factors. The time taken for fertilised eggs to form the slow block to polyspermy (i.e. cease being sperm sinks) will be the most important factor, with slower blocks favouring slow sperm release rates and faster blocks favouring faster release rates. Currently, there are too few examinations of permanent polyspermy blocks to generalise but at least for some species, it appears that slower release rates are likely to be favoured. Our results are probably most relevant for species in which spawned eggs are negatively buoyant and tend to remain on the substratum during fertilisation. It is in these species that sperm are most likely to travel over several groups of eggs. Whilst this is a common mode of reproduction in broadcast spawners (Marshall, 2002; Marshall et al., 2004; Meidel and Yund, 2001; Yund and Meidel, 2003), other species such as scleractinian corals release positively buoyant gametes that are likely to disperse in clouds along with any released sperm (Babcock et al., 1986). In such situations, there is still a 'leading edge' of eggs that encounter the sperm first and may rob downstream eggs of sufficient sperm, but the cloud of gametes will be more diffuse. We can only speculate as to whether sperm release rates will have similar effects in species such as these and look forward to future studies.

The likelihood of achieving additional fertilisations downstream from the nearest female will also strongly affect the relative benefits of a fast or slow sperm release strategy. If males have a small chance of fertilising females downstream (either because of sperm dilution effects or pre-emption by other males) then there will be few benefits of spawning slowly as males can afford to 'waste' all their sperm on the first female. It would be interesting to determine if the rate at which sperm are released is facultative, adjusted by spawning males according to local conditions. While females have been shown to adjust their spawning behaviour according to the proximity of spawning males (Marshall et al., 2004), we know of no similar study on males adjusting their release rates in broadcast spawners. This is despite clear evidence that spawners communicate

information regarding reproduction prior to spawning events (Hamel and Mercier, 1997; Hardege and Bentley, 1997). Male–male competition is likely to be crucial in determining the costs and benefits of different release strategies in broadcast spawners. Assuming male fertilisation success operates on a raffle principle in broadcast spawners, then males with the highest concentration of sperm present should secure the most fertilisations (Parker et al., 1996). Thus if there are many females spawning locally, then a fast release rate might still be favoured because the reduction in downstream fertilisations becomes less important. However, there are two key issues that could further affect sperm release strategies: assortative mating and polyspermy. Several studies now suggest that fertilisation does not operate according to a raffle principle, rather different combinations of sperm and eggs are more compatible with each other than others (Palumbi, 1999; Evans and Marshall, 2005; Marshall and Evans, 2005a; Levitan and Ferrell, 2006). Initial evidence suggests that these differences in compatibility could affect the outcome of sperm competition in broadcast spawners (Levitan and Ferrell, 2006) and this is likely to affect sperm release strategies in broadcast spawners. Polyspermy (whereby multiple sperm enter the eggs before a fast block is formed) will also affect male release strategies (Bode and Marshall, 2007). Whilst releasing more sperm should increase a male's local reproductive success, if other males do the same, overall fertilisation success will decrease due to polyspermy (Bode and Marshall, 2007). Thus there are multiple factors that will affect what is the 'best' sperm release strategy, but regardless of these factors, our study suggests that males that release all of their sperm quickly are not always going to achieve the highest reproductive success overall.

Our results have some counter-intuitive implications for our understanding of the evolution of permanent polyspermy blocks. Our flume experiments were used to examine the effect of sperm release rates on male fertilisation success. We could have used the same experimental design to examine how the time for a permanent polyspermy block to form will affect female fertilisation success. To explain: changing the sperm release rate whilst the permanent polyspermy block speed stayed constant, from a fertilisation kinetics perspective, is identical to holding the sperm release rate constant and changing onset speed of the permanent polyspermy block (Fig. 3). In both instances, the total number of sperm that pass over the eggs before a permanent polyspermy block forms is the only thing that varies. Thus a fast sperm release rate simulates a slower permanent block and *vice versa*. From the perspective of female fertilisation success, therefore, our flume results show that when sperm concentrations limit fertilisation, eggs with faster acting permanent polyspermy blocks should have higher fertilisation overall. This is because, even in a single clutch of eggs, there will be upstream eggs that access sperm before downstream eggs and effectively 'rob' their (potential) siblings of fertilisations. Any decrease in the time taken for eggs to become impervious to sperm should reduce the number of sperm that are wasted by the upstream eggs, thereby enhancing fertilisation success downstream. However, it should be noted that this prediction will only hold if the duration of sperm release exceeds the time taken for the permanent polyspermy block to form, a condition that seems likely (Table 1). Overall then faster permanent polyspermy blocks should enhance fertilisation under sperm limiting conditions. Polyspermy blocks have been cited as evidence that sperm excess conditions are common in natural populations (Yund, 2000). We agree, but suggest that the two types of polyspermy blocks and their selection pressures should be distinguished. Electrical polyspermy blocks may have evolved in response to sperm excess but permanent polyspermy blocks may have evolved in response to sperm limiting conditions. Note that we do not suggest that sperm limitation is the sole selective pressure that led to the evolution of permanent blocks; other changes associated with the induction of

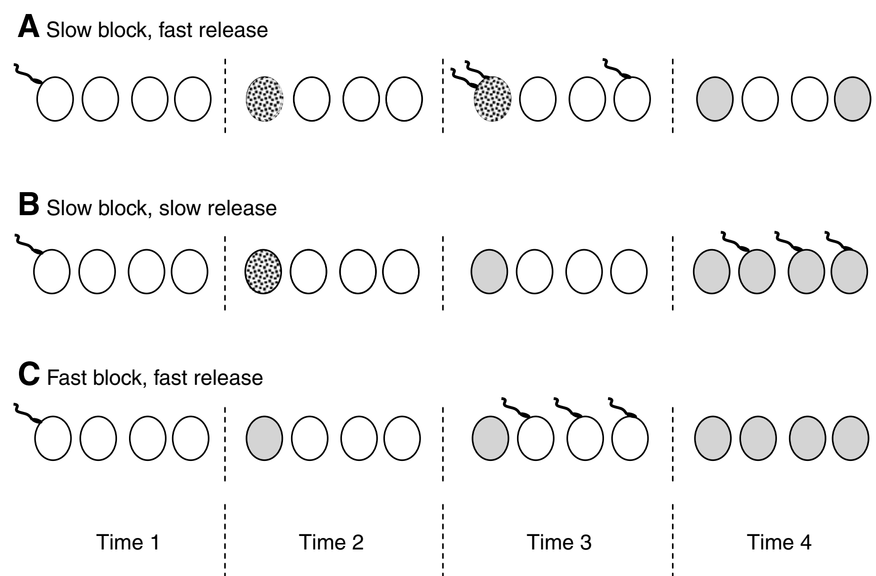

Fig. 3. Diagram illustrating the consequences of different sperm release rates and permanent polyspermy block speeds on subsequent fertilisation success. Open circles represent unfertilised eggs; shaded circles represent fertilised eggs that have produced a permanent block to polyspermy; stippled circles represent fertilised eggs that have not yet formed a permanent block to polyspermy. (A) The consequences of a slow permanent polyspermy block combined with a fast sperm release rate. When additional sperm arrive at Time 3, the upstream, newly fertilised eggs have not yet formed a permanent polyspermy block and so sperm at time 3 are 'wasted' on an already fertilised egg. (B) Consequences of a slower sperm release rate. Sperm arriving at eggs at Time 4 are not wasted on the upstream egg because sufficient time has passed for a permanent polyspermy block to form and overall success is higher. (C) Consequences of a (hypothetical) faster permanent polyspermy block, by the time sperm arrive at Time 3, the upstream egg is already impervious to further sperm attachment so no sperm is wasted. Overall then, changing the time until the permanent polyspermy block forms or changing the release rate of sperm has an effect on fertilisation success.

permanent blocks may also be important (e.g. egg hardening may protect from physical stress and pathogens).

Our laboratory results suggest that in *Galeolaria caespitosa*, eggs become impervious to sperm attachment within 1 min of fertilisation. Direct observations of eggs and sperm further support this suggestion, with unfertilised eggs having multiple sperm attached whilst recently fertilised eggs do not (Fig. 4). Studies examining blocks to polyspermy in polychaetes are rare, but this time course seems faster than for other species (Eckberg and Anderson, 1985). Similarly, sperm attachment can continue for up to 5 min after fertilisation in other taxa (Gould and Stephano, 2003; Wong and Wessel, 2006). The density of adult *Galeolaria caespitosa* varies dramatically in the field and so it is difficult to predict the typical sperm environment for eggs of this species, but a recent manipulative study suggests that concentrations in the field will be limiting (Hollows et al., 2007). That the onset of the permanent polyspermy block is rapid further supports the notion that sperm are limiting in the field and there is strong selection pressure to reduce the number of sperm that are 'wasted' by fertilised eggs.

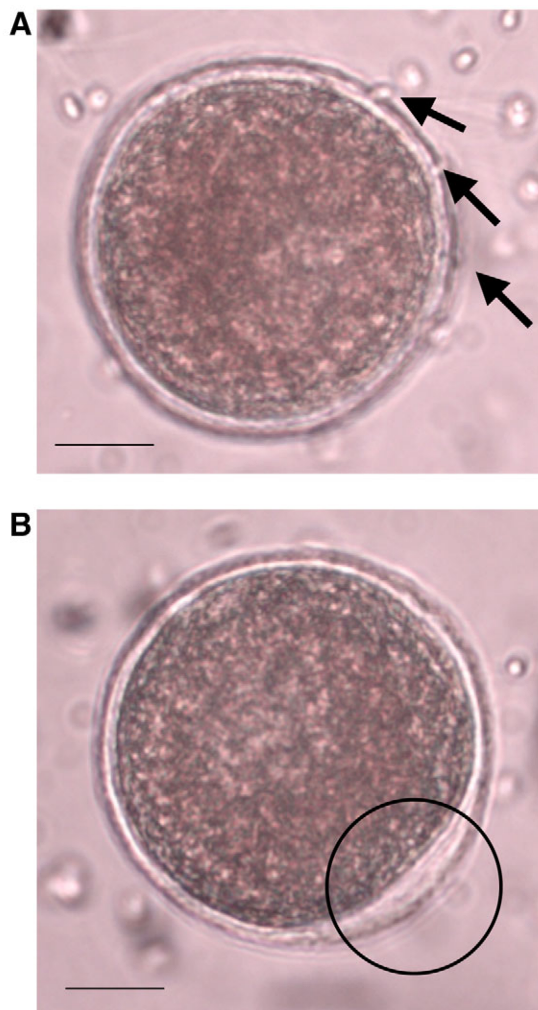

Fig. 4. Light micrograph of (A) unfertilised and (B) recently (within a few minutes) fertilised *Galeolaria caespitosa* eggs. Arrows in A indicate sperm that have attached to the outer region of the egg. The circle in B highlights the raised fertilisation 'cone' where the sperm has entered the egg. Scale bars, 25 μm.

A recent review (Wedell et al., 2002) challenged the notion that sperm are a cheap commodity for males such that '*the word excess has no meaning for males*', highlighting a number of instances of male 'prudence' with regards to sperm release. Our results further support this challenge, whereby males that release sperm slowly will waste fewer sperm than males that release quickly. The scarce data on male release rates in the wild further support the notion that males are 'prudent' with regards to sperm release. We suggest that in the ancestral mode of reproduction, broadcast spawning, sperm release strategies represent a compromise by which males compete for fertilisations as is the traditional view, but also minimise sperm wastage.

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