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Does egg competition occur in marine broadcast-spawners?

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Abstract

When the availability of sperm limits female reproductive success, competition for sperm, may be an important broker of sexual selection. This is because sperm limitation can increase the variance in female reproductive success, resulting in strong selection on females to compete for limited fertilization opportunities. Sperm limitation is probably common in broadcast-spawning marine invertebrates, making these excellent candidates for investigating scramble competition between broods of eggs and its consequences for female reproductive success. Here, we report our findings from a series of experiments that investigate egg competition in the sessile, broadcast-spawning polychaete *Galeolaria caespitosa*. We initially tested whether the order in which eggs encounter sperm affects their fertilization success at two ecologically relevant current regimes. We used a split-clutch—split-ejaculate technique to compare the fertilization success of eggs from individual females that had either first access (competition-free treatment) or second access (egg competition treatment) to a batch of sperm. We found that fertilization success depended on the order in which eggs accessed sperm; eggs that were assigned to the competition-free treatment exhibited significantly higher fertilization rates than those assigned to the egg competition treatment at both current speeds. In subsequent experiments we found that prior exposure of sperm to eggs significantly reduced both the quantity and quality of sperm available to fertilize a second clutch of eggs, resulting in reductions in fertilization success at high and low sperm concentrations. These findings suggest that female traits that increase the likelihood of sperm-egg interactions (e.g. egg size) will respond to selection imposed by egg competition.

Introduction

Our view of sexual selection has been shaped largely by Bateman's (1948) classic work on fruit flies in which he demonstrated a fundamental difference in the reproductive potential of males and females. Bateman's principle states that because sperm are relatively cheap to produce (and therefore abundant), male reproductive success will be limited by the availability of mating partners, while female reproductive success will be limited by the availability of resources needed for reproduction. As a consequence of the disparity in reproductive investment between the sexes, male reproductive success will be highly variable and skewed towards individuals that compete effectively for mating opportunities, while female reproductive success will be more uniformly distributed. A corollary of Bateman's principle, therefore, is that selection for increased fertilization success will act more strongly on males than on females (Trivers, 1972).

The applicability of Bateman's principle to plants and animals that shed gametes externally into the environment has been the subject of debate in recent years (Arnold, 1994; Levitan, 1998a). In such species, parameters such as population density and environmental factors can strongly influence the distribution of sperm and egg concentrations, with important implications for patterns of female reproductive success (Levitan, 2004).

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When sperm from several males are abundant at spawning, sexual selection can proceed in accord with Bateman's principle through sperm competition (Parker, 1998) and (possibly) sperm choice (see discussions by Birkhead, 1998,2000; Pitnick & Brown, 2000). In many species of externally fertilizing fishes, for example, males and females spawn in close proximity in spawning aggregations, resulting in intense sperm competition and high variance in male reproductive success (Petersen & Warner, 1998). By contrast, under sperm limited conditions, sperm competition is likely to be less prevalent and female reproductive success can be extremely variable (Levitan, 2004), resulting in strong selection for increased fertilization success in females as well as males (see Arnold, 1994). An intriguing possibility, suggested by Levitan in several papers (Levitan, 1998a,b,2004), is that under sperm limited conditions; selection may act on female traits (e.g. egg size) that increase their success during egg competition. Despite the intuitive appeal of this suggestion, egg competition has never been investigated in broadcast-spawning animals. Indeed, to our knowledge the only study of egg competition to date focuses on a species of fish exhibiting reversed sex roles (Berglund, 1991).

We suggest that egg competition is a pervasive evolutionary force in sessile and sedentary broadcast-spawning organisms. These systems are frequently viewed as sperm limited (Levitan, 1995; Levitan & Petersen, 1995) and female reproductive success can be highly variable when there are large fluctuations in sperm and egg concentrations due to population density effects (e.g. Levitan, 2004) or hydrodynamic factors such as flow rate and turbulence (Denny & Shibata, 1989). Because sperm very quickly dilute to low concentrations in the dynamic, three-dimensional marine environment, even relatively small distances separating spawning males from females can result in extremely low fertilization rates (Levitan, 1995; Yund, 2000; Marshall, 2002; Marshall *et al.*, 2004a). Indeed, in a wide range of species and environments, the reproductive success of broadcast-spawning females can be strongly limited by the proximity of spawning males, and consequently by the availability of sperm (reviewed by Levitan, 1995; but see Yund, 2000). Marine broadcast-spawners therefore represent a group in which there is likely to be strong selection for increased mating success acting on both sexes (Levitan, 2004).

In this paper we examine the consequences of egg competition for female reproductive success in the tubeworm *G. caespitosa*, a broadcast-spawning polychaete inhabiting the intertidal zone. We reasoned that if eggs compete for fertilizations, the reproductive success of focal females would be reduced in the presence of eggs from competing females (see also Yund & McCartney, 1994). We therefore performed three experiments designed to determine how egg competition influences the reproductive success of focal (competitor) females. In our first experiment we tested whether the order in which eggs access sperm affects fertilization rates. In this experiment we conducted a series of artificial fertilizations under two flow regimes in a small flume. We employed a split-clutch—split-ejaculate design (Marshall *et al.*, 2004b) that compared the fertilization success of individual egg clutches that had either first or second access to a batch of sperm. Our second experiment tested whether the prior exposure of eggs to sperm significantly reduced the number of sperm subsequently available to fertilize a second clutch of (focal female) eggs. We performed this second experiment at two sperm concentrations to simulate conditions of sperm abundance and sperm limitation. Our third experiment tested whether any decline in fertilization rates due to the presence of a competitor female was due to a reduction in the quantity and/or quality of sperm available to fertilize a subsequent batch of eggs.

Materials and methods

General methods

Galeolaria caespitosa is a sessile, filter-feeding polychaete worm that occurs in mixed sex clusters at a wide range of population densities on sheltered and moderately exposed shores in the intertidal zone of Southeastern Australia (Minchinton, 1997). During April/May 2004, reproductively mature *G. caespitosa* adults were collected from pier pilings at Bare Island, Botany Bay and Australia. To collect gametes, individuals were removed from their calcareous tubes and placed in their own 40 mm Petri dish containing 3 mL of filtered seawater. Reproductively mature *G. caespitosa* begin spawning immediately after being removed from their tubes (Kupriyanova & Havenhand, 2002). Spawned gametes were collected with a pipette, maintained at a high sperm concentration to minimize gamete aging (Bolton & Havenhand, 1996) and then used for experiments within 15 min of collection.

Experiment 1 – egg competition and female fertilization success

The aim of this experiment was to determine whether the presence of a batch of unfertilized eggs upstream from a focal batch of eggs affected the fertilization success of the focal batch. We took advantage of the fact that *G. caespitosa* eggs are negatively buoyant and remain on the substrate even under relatively high flow regimes (Marshall, unpublished data). Therefore eggs placed upstream from a focal batch of eggs would remain stationary even in moving water. We used a small, two-lane flume to examine how fertilization success was affected by the order in which eggs accessed sperm at two current speeds: 1 and 5 cm s^{-1} [we used the methods outlined in Yund & Meidel (2003) to determine our current speeds in the

flume]. Although the natural current regimes under which *G. caespitosa* spawn are unknown, the flow rates we used here are likely to be ecologically relevant given that exposed shore intertidal organisms typically spawn during times of low water movement (e.g. Marshall, 2002; Santelices, 2002; Marshall *et al.*, 2004a) and the values here encompass the current speeds that are typical during low water periods in *G. caespitosa* habitats.

The flume was divided in two lanes along its length; each lane had a single seawater input that derived from a single, Y-branched pipe. This design allowed water from the same pipe to simultaneously enter each lane. The seawater pressure was kept constant using a gravity feed, constant head tank. We induced laminar flow in each lane by using a $10 \times 10 \times 10$ cm collimator made of drinking straws (see Yund & Meidel, 2003). The flume was constructed of 1 cm thick acrylic and each flume lane had the internal dimensions of 100 cm (length) $\times$ 10 cm (width) $\times$ 10 cm (depth), and was filled to a depth of 8 cm. After travelling the length of both flume lanes, the water exited the flume through a single overflow pipe (internal radius 2 cm). Initial observations using dye suggested that water flow was laminar across the experimental portion of the flume. Under field conditions, flow will be turbulent rather than laminar but we used laminar flow in this investigation as a first step so that flow rates could be characterized easily.

To conduct the fertilization experiments, we split a focal clutch of *G. caespitosa* eggs in two and placed half of the clutch in each lane of the flume, 55 cm downstream from the collimator. In one lane, another three clutches of eggs were placed 25 cm downstream from the collimator (30 cm upstream from the focal clutch of eggs). Thus there was one lane where sperm exiting the collimator would encounter the nonfocal batches of eggs before encountering the focal batch, and another lane where sperm would only encounter the focal batch. It was crucial for these experiments that identity, concentration and motility levels of sperm were identical between the two lanes. To ensure this was the case, we thoroughly mixed together the ejaculates of 10 males, diluted this solution down to a concentration of 10^7 sperm mL^{-1} (volume: 20 mL) and further split this ejaculate evenly into two vials. We then poured the sperm solutions into each lane of the flume upstream of the collimator. Because this area is where the seawater entered the flume, the sperm solution in each lane was thoroughly mixed by the turbulent flow before entering the collimator. Thus the focal eggs' identity, the sperm source and flow rates were identical between the two lanes, the only difference being the treatments we imposed. We estimate (based on observed fertilization rates) that the eggs in the flume were exposed to a sperm concentration of roughly 10^5 sperm mL^{-1}. Thirty minutes after adding sperm to the flume, all of the focal eggs were collected using a pipette, rinsed with fresh seawater and placed in Petri dishes (1 dish per treatment). Two hours later, per cent fertilization was calculated by, classing 200 eggs as fertilized or unfertilized. Eggs were classed as fertilized if they had begun to cleave and unfertilized if no cleavage was apparent. We conducted 15 separate experimental runs using a different focal female in each run and we randomized the allocation of the two treatments to the different flume lanes.

Statistical analysis of experiment 1

The distribution of data was not significantly different from a normal distribution (Kolmogorov–Smirnov test: $P = 0.708$) and the variances between the two groups were similar. To analyse the effect of current speed and the presence/absence of a competing female's eggs, we used a partly nested design in which experimental run was a random nested factor within current speeds, and current speed and egg competition were fixed factors. Because some of our proportion-fertilized values were close to 0 and 1, we analysed arcsine square root transformed data.

Experiment 2 – effect of sperm mating history on available sperm concentrations

Here we further examine how the 'mating history' of sperm affects their ability to fertilize the eggs of a focal female. To do this we measured (Part A) the number of sperm that were 'removed' during a brief exposure to a clutch of eggs and (Part B) the consequences of sperm-egg exposure for the fertilization rate of a second, focal clutch of eggs. Furthermore, we performed this experiment at a nominally high and low sperm concentration (Marshall & Evans, 2005) to examine whether any observed effect of sperm mating history on sperm availability and fertilization rates was dependent on initial sperm concentrations. Our split clutch design ensured that for each replicate, the only difference between experimental treatments was the mating history of sperm as male identity, sperm age and egg source (female identity) were identical between treatments.

Each male's spawned ejaculate was split and diluted down to two concentrations: nominally high ($1.5 \pm 0.5 \times 10^6$ sperm mL^{-1}) and low ($5 \pm 0.3 \times 10^4$ sperm mL^{-1}). In each case we estimated the sperm concentration using a modified Fuch-Rosenthal haemocytometer (three replicate counts). We then split both sperm concentrations into two groups: one group was exposed to a 1 mL solution of eggs (5000 ± 697 eggs mL^{-1}) for 15 min (hereafter referred to as 'competitor present sperm' to reflect the fact that focal females accessed sperm after the eggs of a competing female) while the other (hereafter referred to as 'competitor absent sperm') was exposed to 1 mL filtered seawater for 15 min. The filtered seawater had previously contained 5000 ± 697 eggs (from the same clutch as those used in the competitor present treatment) but the eggs had been filtered away (this

solution will hereafter be referred to as 'egg water'). We used egg water for the competitor absent treatment because previously it has been shown that in some species, water-soluble components of eggs can increase the activity of sperm (Bolton & Havenhand, 1996). No such effect has been detected for *G. caespitosa* populations in South Australia (Kupriyanova & Havenhand, 2002), but as a conservative measure we exposed competitor present and absent sperm to similar solutions.

After the exposure period, the sperm solutions from both groups were filtered using a 25 μm filter. In both treatment groups the volume of sperm solution was identical, the only difference being that one had been exposed to eggs while the other had been exposed to egg water only. At this stage there were four 1 mL sperm solutions: (1) high concentration competitor absent sperm solution; (2) low concentration competitor absent sperm solution; (3) high concentration competitor present sperm solution and (4) low concentration competitor present sperm solution. For each solution, a small (<0.1 mL) aliquot was taken and sperm concentrations were estimated for each sample using a haemocytometer (Part A). Each solution was then exposed to a 1 mL solution of eggs (5000 ± 439 eggs mL^{-1}) from a split clutch of a single, focal female (Part B). After a 15 min exposure, the sperm solutions were gently filtered off and the eggs were placed in fresh, filtered seawater. The 15 min exposure was chosen because in other studies on *in situ* intertidal spawning events, eggs remained on the surface of spawning individuals for at least 15 min (Marshall, 2002). After a further 2 h we estimated the fertilization rates of eggs in each of the four treatments ($n = 100$ eggs for each treatment). This entire process of pre-exposing sperm to either eggs or seawater and then exposing the different groups of sperm to a second batch of eggs was repeated for five different male ejaculates, using different batches of eggs each time. Thus, we performed a total of $n = 20$ replicate fertilizations – five for each mating history-sperm concentration combination.

The distribution of data was not significantly different from a normal distribution (Kolmogorov–Smirnov test: $P = 0.16$) and more importantly, variances were similar among treatment groups so we analysed our data using parametric statistics. We analysed raw data because the data were not badly skewed and arcsine did not improve the distribution. We initially analysed the data from this experiment with an unreplicated, block design, with male identity included as a random, blocking factor and sperm concentration and sperm treatment as fixed factors. Male identity explained little of the variation and there were no significant interactions between either male identity and sperm concentration ($F_{4,4} = 0.52$, $P = 0.73$) or male identity and sperm treatment ($F_{4,4} = 0.75$, $P = 0.6$). We therefore analysed the data from this experiment using a two-way ANOVA where sperm concentration and sperm treatment were fixed factors.

Experiment 3 – sperm quantity vs. quality effects

The results from the above experiments revealed that the mating history of a batch of sperm strongly affected the subsequent fertilization rate of the focal female's eggs (see Results). Here we determine whether this effect is due simply to a reduction in sperm numbers or whether reductions in sperm quality also contributed to the effect. We tested this by repeating the experiments described above (Experiment 2) but controlling for differences in sperm concentration between the competitor absent and present groups by diluting the competitor absent sperm down to the same concentration as the competitor present sperm (initial concentration of 5×10^5 sperm mL^{-1}). This value was the goal in the two treatments but precise counting performed after the experiment revealed the average (±SE) final sperm concentrations for the two groups to be 4.7 (± 0.3) $\times$ 10^4 sperm mL^{-1} and 5.05 (± 0.45) $\times$ 10^4 sperm mL^{-1} for the competitor absent and present groups, respectively. The slight discrepancy actually resulted in a more conservative design given that the competitor present groups had, on average, a higher sperm concentration. The above design was repeated for 10 different focal females. This design ensured that any observed difference in fertilization rates between the two groups was due to differences in the quality rather than the quantity of sperm available to the focal female. We analysed the resultant data as described above for Experiment 2.

Because the reduction in fertilization success due to using nonvirgin sperm was not due to reductions in sperm number alone (see Results) we examined the motility rates of competitor present and absent sperm. Split ejaculates from 26 males were manipulated as described above and examined for the percentage of motile sperm in a sample. We used a haemocytometer (10 replicate counts per male) and digital recorder attached to a compound microscope to examine the proportion of motile sperm cells in samples taken from competitor present and absent batches of sperm. Sperm were classed as motile if they showed forward motility and displaced >5 μm during the 4 s observation period. Care was taken to examine only sperm that were fully motile and not stuck to the glass of the haemocytometer or coverslip. Sperm samples were left under the microscope for <1 min during these tests. Again, the distribution of the data was not significantly different from a normal distribution (Kolmogorov–Smirnov test: $P = 0.73$). The results were analysed with a paired *t*-test.

Results

Experiment 1: egg competition and female fertilization success

The presence of a competitor female's eggs upstream from those of the focal female strongly reduced the

Table 1 Partly-nested ANOVA of the effect of current speed and egg competition on the fertilization success of a focal female's batch of eggs in *G. caespitosa*.

Source	d.f.	MS	*F*	*P*
Between subjects				
Current speed	1	3.62	60.31	<0.001
Residual	13	0.06		
Within subjects				
Egg competition	1	0.30	14.52	0.002
Current speed × egg competition	1	0.01	0.61	0.448
Residual	13	0.02		

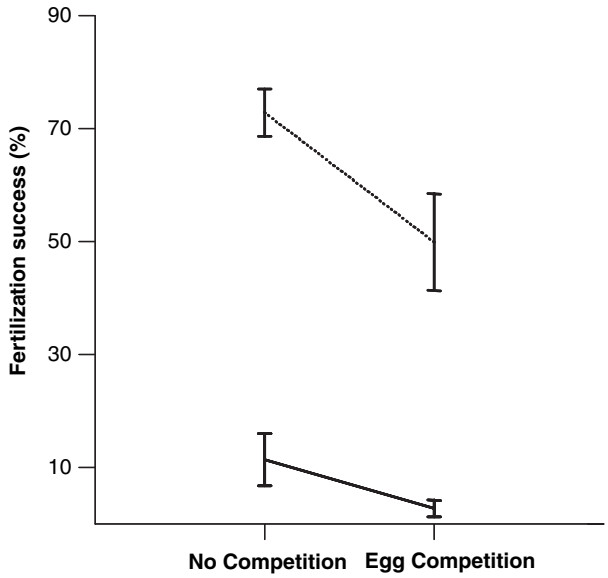

Fig. 1 Effect of egg competition on mean fertilization success (±SE) for *G. caespitosa*. Dashed line represents fertilization success under low (1 cm s^{-1}) current speeds and solid line represents fertilization success under high (5 cm s^{-1}) current speeds.

fertilization success of the focal females at both current speeds (Table 1). Figure 1 slightly underestimates the effect of egg competition because it does not reflect the paired design used in this experiment. On average, there was a twofold reduction in fertilization success of females that accessed sperm second compared to females that accessed sperm first at the lower current speed, and a 1.5-fold reduction for females whose eggs accessed sperm second at the higher current speed.

Experiment 2: effect of sperm mating history on available sperm concentrations

Part A

The exposure of sperm to eggs caused a reduction in the concentration of suspended sperm but the significant interaction between sperm concentration and mating history indicates that this reduction depended on the initial concentration of sperm used for the fertilizations (Table 2). At high sperm concentrations, exposing sperm to eggs greatly reduced the concentration of available sperm, while at low sperm concentrations the reduction was smaller (Fig. 2). In the 'competitor present' treatment, the number of sperm that were removed per egg depended on the original sperm concentration. Many more sperm were removed per egg from the high sperm concentration than the low sperm concentration (approximately 140 vs. 1.5 sperm egg^{-1}, respectively).

Table 2 Effect of egg competition and initial sperm concentration on the concentration of sperm available to a batch of eggs in *G. caespitosa*.

Source	d.f.	MS	*F*	*P*
Initial sperm concentration	1	498	318	<0.001
Egg competition	1	64	41	<0.001
Initial sperm concentration × egg competition	1	62	39	<0.001
Residual	16	1.56		

Part B

The reduced concentration of available sperm due to previous exposure to eggs dramatically reduced the fertilization rate of the focal clutch of eggs. Clutches of eggs that were exposed to competitor absent sperm exhibited much higher fertilization rates than clutches that were exposed to competitor present sperm (Table 3). Interestingly, the reduction in fertilization success was greater at the low sperm concentration than at the high sperm concentration despite the 'removal' of more sperm at the higher concentration (Fig. 3).

Experiment 3: sperm quantity vs. quality effects

The effect of sperm mating history on female fertilization success was not due to a reduction in sperm concentration alone. When competitor present and absent sperm were adjusted to the same concentrations, the success of females with competitor present sperm was still significantly lower than that of eggs exposed to competitor absent sperm (mean fertilization success of competitor absent sperm: 13 ± 1.7%, mean fertilization success of competitor present sperm: 1.6 ± 0.4%; $F_{1,18} = 41.7$, $P < 0.001$).

Competitor present and absent sperm exhibited different percentage motile rates, with competitor absent sperm having a higher percentage of motile sperm than competitor present sperm (mean motility = 38 ± 3.5% and 27 ± 3.3%, respectively; $t = 2.3$, d.f. = 25, $P < 0.05$).

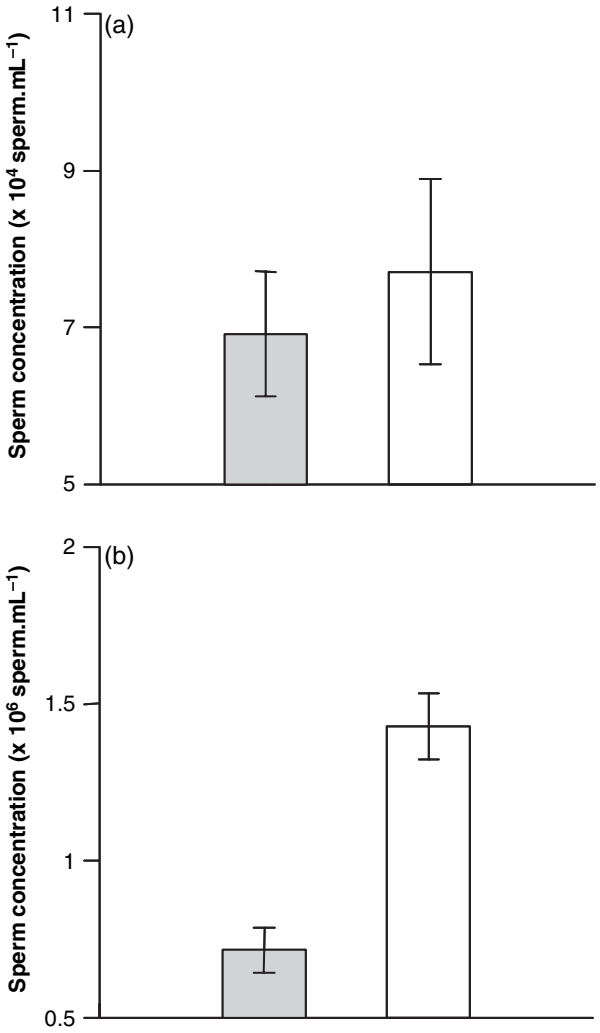

Fig. 2 Effect of egg competition and initial sperm concentration on mean sperm concentration for *G. caespitosa*. Shaded bars represent the mean sperm concentration (±SE) of sperm that that had previously been exposed to eggs where open bars represent the mean (±SE) sperm concentration of sperm that had been exposed to 'egg water' only. Figure 1a shows means for an initially low sperm concentration and Fig. 1b shows means for an initially high sperm concentration.

Table 3 Effect of egg competition and initial sperm concentration on the fertilization success of eggs in *G. caespitosa* (analysis on arcsine transformed data).

Source	d.f.	MS	*F*	*P*
Initial sperm concentration	1	0.359	34	<0.001
Egg competition	1	2.598	247	<0.001
Initial sperm concentration × egg competition	1	0.075	7	0.016
Residual	16	0.011		

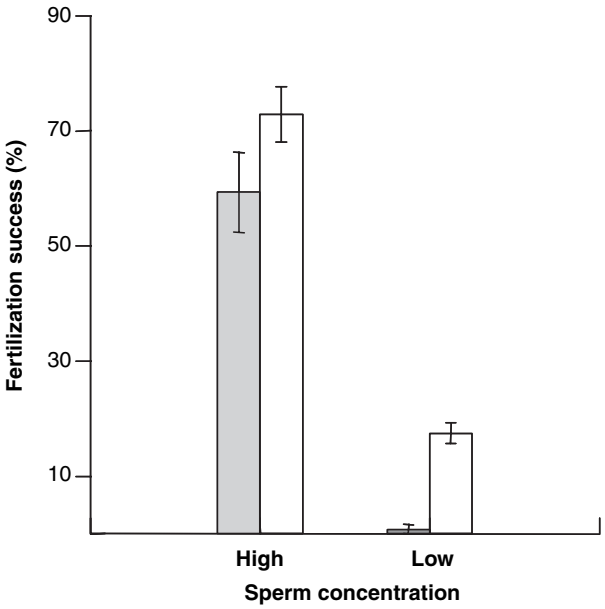

Fig. 3 Effect of egg competition and initial sperm concentration on mean fertilization success of *G. caespitosa* eggs. Shaded bars represent mean fertilization success (±SE) of eggs that accessed sperm that had previously been exposed to another batch of eggs; open bars represent mean fertilization success (±SE) of eggs exposed to sperm that had previously been exposed to 'egg water' only.

Discussion

Levitan (1998b) has argued that egg competition will be apparent when the experimental removal of eggs from competing females increases the fertilization success of a focal female's eggs (see also Yund & McCartney, 1994 for a similar argument pertaining to male-male competition in 'spermcast' organisms). He goes on to argue that the reduction in fertilization rates caused by egg competition will be due to the sperm-depleting effects of egg competition – eggs from rival females will soak up a significant portion of available sperm, making them unavailable for subsequent eggs to encounter. Our first two experiments provide direct experimental support for both of these predictions. We found that egg competition caused a significant reduction in the fertilization success of focal female eggs, and that this effect was due (in part) to the removal of sperm by the competing female's eggs. The paired (split-clutch—split-ejaculate) design employed throughout our experiments ensured that these observed differences in fertilization rates between treatments were not due to sperm aging or male identity effects; in each replicate the sperm samples used for both treatments came from the same male and were treated identically (other than their treatment applications). The dramatic reduction in fertilization rates in the egg competition treatment was partially driven by the removal of a large number of sperm by the competitor's eggs. The number

of sperm that were bound to the these eggs was surprisingly high (~1.5–140 sperm egg^{-1}) and demonstrates that despite the substantial difference in the number of gametes released by males and females, egg competition can rapidly deplete the number of sperm that are available to fertilize eggs. Under natural spawning conditions in marine broadcast-spawners, released eggs can remain on or near females at high concentrations for considerable periods of time (Meidel & Yund, 2001; Marshall, 2002; Yund & Meidel, 2003; Marshall *et al.*, 2004a). Our results suggest that these 'puddles' of eggs have the potential to remove large numbers of sperm before they have the opportunity of fertilizing eggs that are further away.

We also found that in addition to its strong sperm depleting effects, egg competition reduces the average fertilizing efficiency of remaining sperm, suggesting that the most competitive sperm are initially used during initial sperm-egg encounters. This latter finding has important implications for the operation of sexual selection in *G. caespitosa* and possibly other broadcast-spawning invertebrates because it suggests that under a multi-male scenario, sperm competition among rival males may mediate the effects of egg competition on female reproductive success (see also Marshall *et al.*, 2004b). The difference in motility rates between sperm samples (from individual males) in both treatments presumably arose because of the selective removal of highly active sperm during the first sperm-egg encounter (i.e. in the egg competition treatment, Bolton & Havenhand, 1996; Levitan, 1996). If sperm competitive ability differs predictably among males, sexual selection mediated by egg competition may facilitate the selection of sperm from intrinsically 'good' males because the most competitive females will access sperm from the best males (e.g. Yasui, 1997).

Our estimates of sperm motility (the percentage of forwardly motile sperm) suggest that the differences in fertilization rates between the two experimental treatments were due, at least in part, to differences in the percentage of sperm that were motile (but see Kupriyanova & Havenhand, 2002 where no effect of sperm motility on fertilization rates was detected). However, we acknowledge that our measure of 'sperm quality' was relatively crude and did not encompass parameters that are known to influence fertilization success in marine broadcast-spawners. For example, sperm swimming speeds, binding capabilities and sperm head size have all been shown to influence sperm fertilizing capacities in marine broadcast-spawners (Styan, 1998; Palumbi, 1999; Au *et al.*, 2002), including *G. caespitosa* (Kupriyanova & Havenhand, 2002). Nevertheless, our results indicate that the first eggs to contact sperm will remove not only a significant portion of sperm, but also sperm that have the highest fertilization potential. Previously, we have shown that mating-order also affects the quantity and quality (size) of eggs that are available to broadcast-spawning male sea urchins (Marshall *et al.*, 2004b). It appears therefore that in these systems both sexes will be under strong selection to achieve first access to the opposite sex's gametes.

As anticipated, we found that the effects of egg competition on the focal females' fertilization rates were greater at initially low sperm concentrations than at high sperm concentrations. This is presumably because when sperm are limiting the variance in female reproductive success will be high and gametes will compete more intensively for limited fertilization opportunities (Levitan, 2004). However, we did detect a decline in fertilization rates in the egg competition treatment when sperm concentrations were initially high (see Fig. 3), which suggests that the sperm-depleting effects of egg competition may also occur when spawning densities are high (see Levitan, 1998b). Levitan (2004) suggests that, because egg concentration does not affect fertilization success significantly, the number of eggs released by females should not affect fertilization success. We suggest that the effect of one female's eggs on another's fertilization success will depend on the order in which they access sperm.

Our results have interesting implications for the evolution of egg size in sessile broadcast-spawning marine invertebrates. In these species, egg size affects the chances of fertilization because larger eggs present larger 'targets' for sperm (Levitan, 1993; Marshall *et al.*, 2002). Traditionally, the evolution of egg size was thought to be driven by the naturally selective advantage of avoiding sperm limitation. For example, the distribution and abundance of males at spawning has been linked with the evolution of gamete traits in several sea urchin species (Levitan, 2002). Our results suggest that the evolution of egg size may also be influenced by competition from other females for fertilization opportunities. Whenever females compete for fertilization, those that produce larger (or more fertilizable) eggs will be at a selective advantage during the ensuing competition for fertilization, although the benefits of producing large eggs are likely to be traded against the costs of polyspermy (too many sperm rather than too few) when the distribution and abundance of spawning males is very high (Styan, 1998; Franke *et al.*, 2002). Thus, egg competition for limited sperm may select for eggs that are larger than their naturally selected optimum but this will be balanced by the size-number trade-off (Levitan, 1993) and the risk of polyspermy. Furthermore, egg accessory structures and pheromones that enhance fertilization such as jelly coats, which have traditionally been viewed as the products of natural selection (e.g. Farley & Levitan, 2001; Podolsky, 2002,2004), may in fact respond to both natural and sexual selection for increased egg size.

In species that exhibit 'typical' sex roles, evolutionary biologists have largely ignored the possibility that sexual selection can lead to the evolution of female traits that

increase mating success (but see Levitan, 1993,1998a,2004), focusing instead on species that conform to the typical sexual selection paradigm of intrasexual (male) competition and female choice (Bateman, 1948; Trivers, 1972; Arnold, 1994; Andersson, 1994). Our finding, however, that egg competition may strongly influence female reproductive success in *Galeolaria* highlights the possibility that female traits may also respond to sexual selection. Traits such as egg size, the timing of gamete release and spawning aggregation behaviour are known to influence gamete encounter rates in broadcast-spawning marine invertebrates and are therefore likely to evolve in response to intrasexual (female) competition in the same way that a vast array of morphological, physiological, behavioural and ejaculate traits in males have responded to selection imposed by sperm competition (Gomendio & Roldan, 1991; Briskie *et al.*, 1997; Birkhead & Møller, 1998; Ward, 1998; Hosken & Ward, 2001; Simmons, 2001; LaMunyon & Ward, 2002; Gage & Morrow, 2003). We suggest that future research on sexual selection in broadcast-spawning animals should focus on female, as well as male, traits that increase the likelihood of gamete encounters since these are likely to respond to selection imposed by competition for access to gametes.

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