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Fertilization success in *Galeolaria caespitosa* (Polychaeta: Serpulidae): gamete characteristics, role of sperm dilution, gamete age, and contact time

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SUMMARY: Variability in gamete traits and the factors affecting fertilization success were studied in a common gregarious broadcast-spawning serpulid polychaete *Galeolaria caespitosa*. Variability of egg size and sperm velocity were not related to the adult size. High sperm concentrations (10^7-10^8 sperm. ml^{-1}) were required to achieve fertilization rates of 60-80%. The gamete contact time required to achieve high fertilization rates (70-80%) in *G. caespitosa* did not exceed 5 minutes of gamete exposure given sufficiently high sperm concentrations. This suggests that attachment of sperm to the egg takes place very rapidly, and a short-term exposure to highly concentrated sperm is a common feature of fertilization ecology of this gregarious species. A sharp decline in fertilization rates at high sperm concentrations usually attributable to polyspermy was not observed. Sperm were motile for up to 6 hours after activation; however, swimming velocity and fertilization success decreased after 2 hours. Eggs of *G. caespitosa* were fertilizable up to 10 hours after spawning, but the number of embryos resulting from fertilizations by fresh sperm decreased after 2 hours. The gamete traits of *G. caespitosa* appear to have evolved to enable this sessile organism to reproduce under conditions of high population density and increased risk of polyspermy.

Keywords: *Galeolaria caespitosa*, Serpulidae, Polychaeta, egg size, gamete longevity, sperm behaviour, fertilization success, gamete concentration and age.

RESUMEN: EXITO DE LA FERTILIZACIÓN EN *GALEOLARIA CAESPITOSA* (POLYCHAETA: SERPULIDAE): CARACTERÍSTICAS DE LOS GAMETOS, PAPEL DE LA CONCENTRACIÓN DEL ESPERMA, EDAD DE LOS GAMETOS Y TIEMPO DE CONTACTO. – La variabilidad de los gametos y los factores que afectan el éxito en la fertilización han sido estudiados en una especie gregaria común, el serpúlido *Galeolaria caespitosa*. La variabilidad encontrada para el tamaño de los huevos y la velocidad del esperma no estuvo relacionada con el tamaño de los adultos. Elevadas concentraciones de esperma (10^7-10^8 esperma ml^{-1}) se necesitaron para alcanzar ratios de fertilización del 60-80%. El tiempo de contacto de los gametos que se necesitó para alcanzar elevados ratios de fertilización (70-80%) en *G. Caespitosa* no excedió los 5 minutos si se daban suficientes concentraciones de esperma. Ello sugiere que la adhesión del esperma a los huevos se realiza muy rápidamente y una pequeña exposición en cantidades de esperma suficiente es algo común en esta especie gregaria de poliqueto. Un rápido declive de los ratios de fertilización cuando hay grandes concentraciones de esperma atribuible a poliespermia no fue observada. El esperma se mantuvo con movilidad 6 horas después de su activación, sin embargo, la velocidad de desplazamiento y el éxito de la fertilización decrecieron pasadas las 2 primeras horas. Huevos de *G. caespitosa* fueron fecundados 10 horas después de la puesta pero el número de embriones obtenido de la fertilización decreció después de las primeras 2 horas. Los gametos de *G. caespitosa* parecen haber evolucionado para proporcionar a este organismo sésil la capacidad de reproducirse en condiciones de elevada densidad poblacional y riesgo elevado de poliespermia.

Palabras clave: *Galeolaria caespitosa*, Serpulidae, Polychaeta, tamaño de los huevos, longevidad de gametos, conducta espermática, éxito de la fertilización, concentración de gametos y edad.

INTRODUCTION

Many marine invertebrates reproduce by releasing eggs and sperm into the water column where fertilization and subsequent development occur. In such free-spawning organisms the ambient sperm concentration into which spawned eggs are released has been long recognised as a key factor affecting fertilization success (e.g. Brown and Knouse, 1973; Vogel *et al*., 1982; Pennington, 1985; Levitan *et al*., 1991; Benzie and Dixon, 1994; Levy and Couturier, 1996). Because fertilization success can be limited by sperm availability, the selection exerted by sperm limitation results in adaptations aimed to reduce sperm dilution. Such adaptations are reflected in field distribution, density, and behaviour of reproductively active individuals, and their gamete attributes.

Mobile organisms are able to form mating aggregations and often show a high degree of spawning synchrony (e.g. Babcock and Mundy, 1992). In sessile organisms, in addition to synchronous spawning (e.g. Coma and Lasker, 1997), such adaptations may include evolution of sperm storage (Bishop and Ryland, 1991; Rouse, 1996) and accumulation of dilute sperm from water (Bishop, 1998). The aggregated field distribution is one of the most important adaptations to ensure high rates of fertilization in sessile marine invertebrates (e.g. Levitan, 1991; Levitan *et al*., 1992), but there are costs associated with high population densities. Crowding may adversely affect growth and/or gamete production because of food limitation (e.g. Levitan, 1989). Eggs of gregarious species may encounter too much sperm, resulting in polyspermy, which is lethal to the embryos of most taxa. Therefore, one might expect that fertilization ecology of gregarious sessile species should show the adaptations to maximise reproductive success under conditions of high density and increased risk of polyspermy (Levitan, 1998a, b).

Most investigations of fertilization success in marine invertebrates have concentrated on echinoderms and commercial bivalve molluscs, and only recently, a few studies on polychaete fertilization ecology have appeared in the literature (Thomas, 1994; Williams *et al*., 1997; Pernet, 1999; Williams and Bentley, 2002; Kupriyanova and Havenhand, 2002). The only fertilization curve describing the influence of sperm concentration on fertilization success in a serpulid polychaete is known for *Hydroides elegans* (Pechenik and Qian, 1998).

The present laboratory study examines fertilization success in the gregarious serpulid polychaete *Galeolaria caespitosa* Lamarck. This common Australian endemic species is found in dense aggregations in the mid-littoral zone from Western Australia, around Southern Australia to southern Queensland. Natural spawning of *G. caespitosa* in the field has never been described and *G. caespitosa* adults have never been observed to spawn spontaneously in a marine aquarium. However, large amounts of eggs or sperm are released immediately by adults whose tubes have been broken or have been disturbed mechanically (Kupriyanova and Havenhand, 2002). Most adults within *G. caespitosa* aggregations have abdomens swollen with gametes throughout the year, but within any given aggregation of *G. caespitosa* at any time of the year there is a small proportion of worms that appear to have spawned. These spent individuals had compressed abdomens with only a small number of fertilizable gametes (pers. obs.; Bolton, 1999). This observation suggests that *G. caespitosa* may spawn continuously throughout the year along the coast of South Australia.

An earlier work (Kupriyanova and Havenhand, 2002) demonstrated that natural intraspecific variability of sperm swimming behaviour and gamete specific combining abilities affect fertilization rates of *G. caespitosa*. The present paper determines how fertilization varies with sperm concentration and the time of egg-sperm contact, and examines egg and sperm longevity in this species. In some marine invertebrates (Ito, 1997; Marshall *et al*., 2000, 2002) egg size varies with maternal size. Therefore, this study also examined the existence of such dependence in *G. caespitosa*.

MATERIAL AND METHODS

Collection of animals and gamete extraction

G. caespitosa worms were collected routinely at low tides from the pilings of Glenelg jetty, South Australia. The animals were held in recirculating marine aquaria for no more than two weeks. Aggregations of *G. caespitosa* were broken apart and individuals were carefully removed from their tubes with fine forceps. Forceps were rinsed in 0.5M KCl to immobilise any adherent sperm (Bolton and Havenhand, 1996) after contact with an animal.

Sperm and eggs were extracted according to the methodology detailed in Kupriyanova and Havenhand (2002). The concentrated sperm of individual males was held in a refrigerator at 4°C and the eggs released from individual females were suspended in 100 ml beakers in filtered seawater (FSW) in a constant temperature cabinet at 21°C prior to experimentation. Because temperature may affect fertilization success and gamete aging rate (Kupriyanova and Havenhand, in press.), all experiments were conducted in a constant temperature room at 21°C.

Effect of adult body sizes on gamete characteristics

Females of *G. caespitosa* released eggs varying in diameter from 26 μm to 68 μm and from opaque to bright orange in colour, but only bright orange eggs with diameters exceeding 53 μm were fertilizable (pers. obs.; Bolton 1999). Eggs of 20 females were extracted and rinsed through a 53 μm mesh sieve. Fifty eggs of each female were measured using an ocular micrometer under an Olympus BH-2 compound microscope.

Sperm were extracted from 40 males and sperm movement was recorded with a video camera mounted on an Olympus BH-2 compound microscope using differential-interference contrast microscopy. At a later date sperm velocities were determined using a VP110 Motion Analysis System (*Motion Analysis Corp, Santa Rosa, CA, USA*) according to Kupriyanova and Havenhand (2002). After gamete extraction, both adult males and females were preserved in 70% ethanol, blot-dried on a filter paper and weighed without the tube.

Effect of sperm concentration and gamete contact time on fertilization success

A sub-sample (10 ml) of concentrated refrigerated sperm was suspended in 10 ml of 0.5 M KCl to stop flagellar movement. Two drops of the diluted sperm solution were placed on a haemocytometer and the number of sperm counted. These data were used to calculate the appropriate aliquot of the original concentrated sperm to provide a stock solution with concentration of 10^8 sperm ml^{-1} after dilution with FSW. The stock solution was used for serial dilutions to provide sperm solutions with final concentrations of 10^8, 10^7, 10^6, 10^5, and 10^4 sperm ml^{-1}. Serial dilutions were prepared immediately prior to the fertilization experiments. Three replicates of each dilution were used in each experiment.

Freshly extracted eggs (concentration adjusted to 500 egg ml^{-1}) were used in fertilization experiments conducted with three periods of gamete contact time (1, 5, and 10 minutes) and five levels of sperm concentration (10^8, 10^7, 10^6, 10^5, and 10^4 sperm ml^{-1}) according to the technique detailed in Kupriyanova and Havenhand (2002). To reduce the effect of parent-specific variations of fertilizing capacities (Kupriyanova and Havenhand, 2002), eggs collected from 5 females and sperm collected from 5 males were pooled and used in each trial. Five trials were conducted. The fertilized eggs were left for 2 hours and fertilization success was determined by counting at least 300 eggs under the compound microscope and recording the proportion of eggs undergoing normal cleavage. Both fertilized and unfertilized eggs used in each trial were left overnight in the constant temperature cabinet and their development was observed the next day after each experiment. In each experiment, a batch of eggs was not artificially fertilized as a control of sperm contamination at the time of gamete extraction.

Sperm longevity

Sperm longevity was determined as the time the sperm remained motile and as the time sperm maintained their ability to fertilize freshly extracted eggs. To determine how sperm age affected sperm velocity, concentrated sperm of 5 males was diluted to the concentration of 10^7 sperm ml^{-1} in 5 individual 10-ml test tubes and kept at 21°C throughout the experiment. The movement of the diluted sperm of each individual male was recorded as described above at the intervals of 0.5, 1, 2, 4, 6, 8 and 12 hours after activation.

To determine how long activated sperm maintained their fertilizing ability, sperm pooled from 5 males (concentration adjusted to 10^7 sperm ml^{-1}) was used to fertilize eggs (concentration adjusted to 500 egg ml^{-1}) freshly extracted from 5 females every 2 hours for a total of 12 hours (2, 4, 6, 8, 10, and 12 hours after activation). Five trials (three replicates of each) were conducted.

Egg longevity

Eggs collected from 5 females (concentration adjusted to 500 egg ml^{-1}) were pooled and kept at

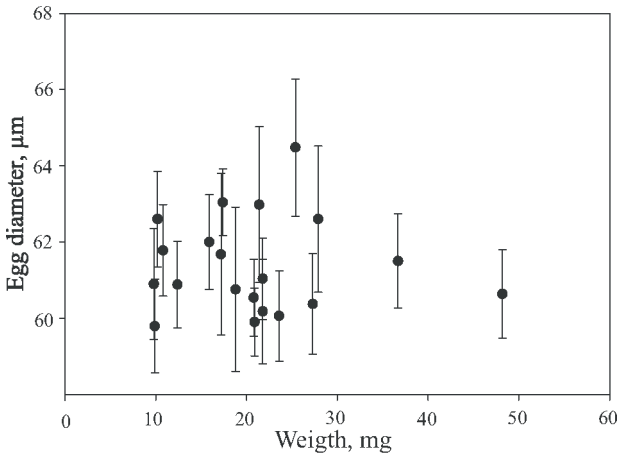

FIG. 1. – Relationship between female wet body weight (without the tube) and mean egg diameters. Error bars are standard deviations (SD), 50 eggs per female measured.

21°C in 100-ml beakers for 12 hours. These eggs were fertilized with pooled sperm of 5 males (concentration adjusted to 10^7 sperm ml^{-1}) freshly extracted every 2 hours (2, 4, 6, 8, 10, and 12 hours after female spawning). Three trials (three replicates of each) were conducted.

RESULTS

Effect of adult body sizes on gamete characteristics

The observed variability of mature (>53 µm) eggs in *G. caespitosa* (average egg diameters range from 59.8 to 64.5 μm, mean 61.29 ± SD 1.25) was not related to female size ($r^2 = 0.0005$, $F = 0.09$, $P = 0.93$, Fig. 1). Also there was no relationship between male body size and sperm swimming velocity (average sperm swimming velocity range from 55.38 to 140 μm s^{-1}, mean 107.31 ± SD 22.04, $r^2 = 0.142$, $F = 6.636$, $P = 0.0138$, Fig. 2).

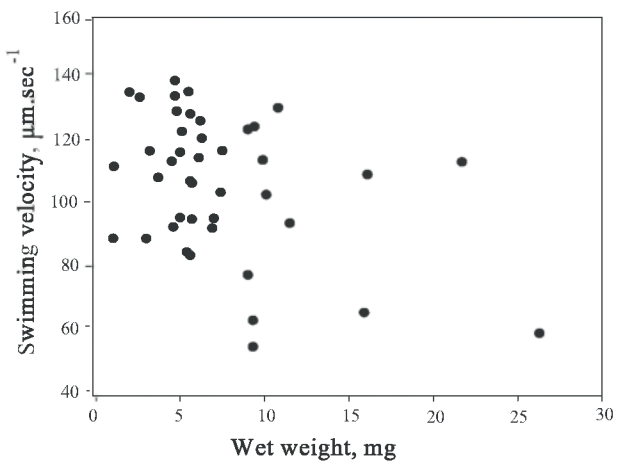

FIG. 2. – Relationship between adult body size (wet weight without the tube) and sperm swimming velocity in *Galeolaria caespitosa*.

Effect of sperm concentration and contact time on fertilization success

Of the five trials conducted, the results of two were discarded because of sperm contamination as revealed by the controls (in one 2% and in the other 3.6% of control eggs were fertilized). In the other trials, the proportion of fertilized eggs in *G. caespitosa* increased with increased sperm concentration when freshly spawned gametes were used and egg concentrations were held constant (Fig. 3). Sperm concentrations required to achieve high fertilizations rates (60-80%) were 10^7-10^8 sperm ml^{-1}. Levels of fertilization >50% were achieved only at sperm concentrations >10^6 sperm ml^{-1}, whereas at lower concentrations, fertilization rate dropped rapidly. Only <10% of eggs were fertilized at sperm concentrations of 10^5 sperm ml^{-1} and practically no fertilizations were observed at concentrations below 10^5 sperm ml^{-1}.

At the concentration of 10^6 sperm ml^{-1} fertilization rates increased from 33% (±SD 3.1) to 46.4% (± SD 3.65) when the contact time increased from 1 minute to 5 minutes and to 51.4% (±SD 4.5) when contact time was 10 minutes. At the concentration of 10^7 sperm ml^{-1}, 1 minute and 5 minutes of gamete contact resulted in average fertilization rates of 46.4% (±SD 8.5) and 66.4% (±SD 8.2) respectively, but further increase from 5 minutes to 10 minutes resulted in only slightly higher average fertilization success (67.6 ±SD 8.2) in this species (Fig. 3).

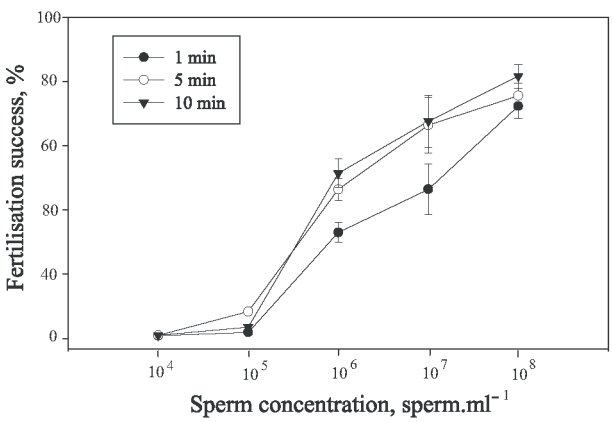

FIG. 3. – Effect of sperm concentration and gamete contact time on fertilization success in *Galeolaria caespitosa*.

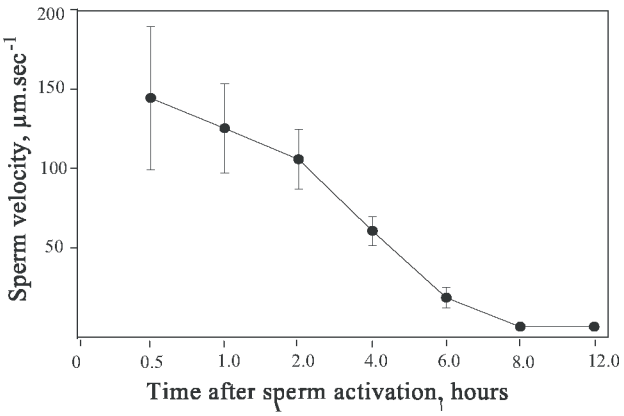

FIG. 4. – The effect of sperm aging on sperm swimming velocity in *Galeolaria caespitosa*. Each point represents the average value obtained from three separate experiments conducted with 5 individual males. Error bars are SD.

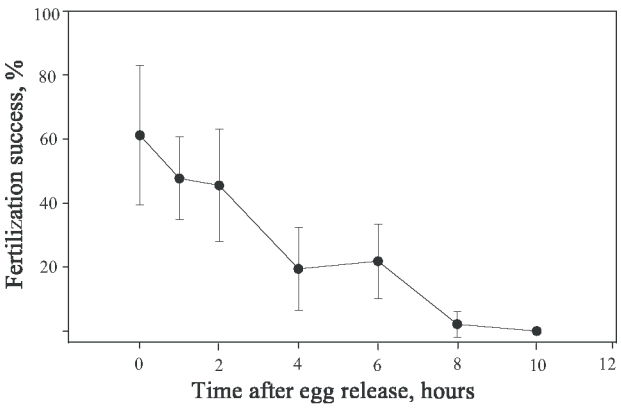

FIG. 6. – The effect of egg age on fertilization success in *Galeolaria caespitosa*. Each point represents the average value obtained from three separate experiments in which eggs were pooled from 5 females and sperm was pooled from 5 males. Error bars are SD.

When sperm concentration was 10^8 sperm ml^{-1}, fertilization rates were 72.3 (±SD 4.0), 76.5 (±SD 3.9), and 81.6 (±SD 3.7) at 1, 5, and 10 minutes of gamete contact, respectively. None of the concentration/contact time combinations resulted in 100% fertilization success rate.

Gamete longevity

Sperm of *G. caespitosa* retained at least some motility for up to 6 hours after activation, but the sperm velocity steadily decreased with sperm age. The highest average swimming velocity of 144.15 (± SD 45.24) $\mu m\ s^{-1}$ for fresh *G. caespitosa* sperm dropped to 105.62 (± SD 118.76) $\mu m\ s^{-1}$ (Fig. 4) after two hours and to 60.49 (± SD 9.14) $\mu m\ s^{-1}$ 4 hours after sperm activation.

The fertilization success also correspondingly declined with sperm aging in *G. caespitosa*. The average percentage of developing embryos resulted from mixing of fresh gametes was 66.42% (±SD 9.94) and it dropped to 40.15% (± SD 5.13) after 2 hours and to 16.42% (±SD 4.81) after 4 hours of sperm activation (Fig. 5) when fresh eggs were used. No eggs were fertilized by sperm aged for 6 hours and more.

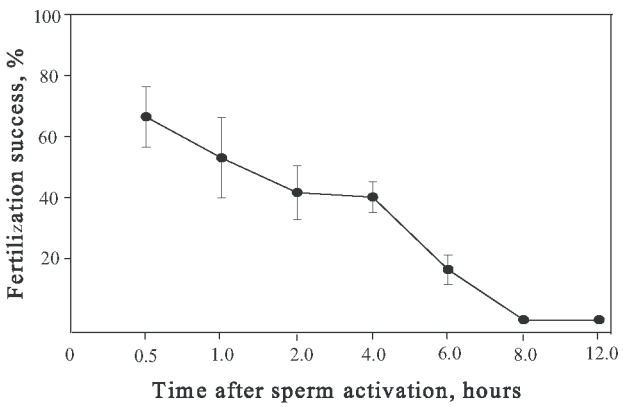

FIG. 5. – The effect of sperm aging on fertilization success in *Galeolaria caespitosa*. Each point represents the average value obtained from five separate experiments in which eggs were pooled from 5 females and sperm was pooled from 5 males. Error bars are SD.

Fertilization rates of *G. caespitosa* also decreased with the age of oocytes (Fig. 6). Although some eggs of *G. caespitosa* (<5%) were still fertilizable by fresh sperm 10 hours after spawning, the average fertilization success of 61.18% resulting from fertilizations by fresh sperm decreased to 45.5% 2 hours and to 21.8% 6 hours after egg extraction (Fig. 6). Preliminary qualitative observations also showed that fertilization of aged eggs (4 hours and more after spawning) resulted in a large number of abnormally developing trochophores.

DISCUSSION

The results of this study demonstrate that neither variability in egg size nor in sperm velocity were related to the adult body size in *G. caespitosa*. In this polychaete, increased investment in gametes affects the number of eggs produced, but not the egg characteristics such as size. In some marine invertebrates egg size does increase with increased maternal size (opisthobranchs: Ito, 1997; ascidians: Marshall *et al.*, 2000) and such a relationship may have important consequences for sperm concentrations required for successful fertilizations.

Some models suggest that the optimal egg size evolved to optimise fertilization success (Levitan, 1993; Styan, 1998). Levitan (1993) hypothesised

that sperm limitation should lead to the evolutionary increase in egg size. Alternatively, a polyspermy-adjusted model of Styan (1998) predicts that, at high sperm concentrations, smaller eggs may be evolutionarily advantageous, therefore the risk of polyspermy may at least in part explain the retention of small eggs in some free-spawners. The small egg size and high sperm density (see below) needed to achieve high fertilization levels in *G. caespitosa* are consistent with the predictions of the Styan (1998) model.

Sperm concentration had a significant effect on fertilization rate in *G. caespitosa*, which is not surprising since fertilization success in free-spawning invertebrates has been invariably reported to be highly dependent on sperm concentration (e.g. Pennington, 1985, Levitan *et al.*, 1991: echinoids; Benzie and Dixon, 1994: asteroids; Gruffydd and Beaumount, 1970, Sprung and Bayne, 1984, Andre and Lindegarth, 1995, Styan and Butler, 2000, Powell *et al.*, 2001: bivalves; Babcock and Keesing, 1999: gastropods; Williams *et al.*, 1997, Williams and Bentley, 2002: polychaetes; Brown and Knouse, 1973: chelicerates; Yund, 1990: hydroids; Oliver and Babcock, 1992: corals).

However, fertilization dynamics of *G. caespitosa* showed some unusual features. First, high sperm concentration was required to achieve fertilization rates ≈80% in this species. In most free-spawning invertebrates, the optimal sperm concentration is in the range 10^3-10^6 sperm ml^{-1}. For example, in the mussel *Mytilis edulis* such a concentration is 10^4 (Sprung and Bayne, 1984) or 10^6 sperm ml^{-1} (Levy and Couturier, 1996). Abalones *Haliotis laevigata* and *H. tuberculata* have highest fertilization rates at sperm concentrations of 10^4-10^6 sperm ml^{-1} (Babcock and Keesing, 1999; Baker and Tyler, 2001). In scallops *Challis* (*Equichlamys*) *bifrons* and *C. asperrima* maximum fertilization occurred at sperm concentration of 10^5 sperm ml^{-1} (Styan and Butler, 2000). Fertilization rates of corals reach a maximum at sperm concentrations of 10^5-10^6 sperm ml^{-1} (Oliver and Babcock, 1992). At concentrations of 10^5 -10^3 sperm ml^{-1} fertilization rates were over 90% for the starfish *Acanthaster planci* (Benzie and Dixon, 1994). Fertilization rates for *Strongylocentrotus franciscanus* were around 100% when sperm concentration was 10^5 sperm ml^{-1} (Levitan *et al.*, 1991) and over 50% of the eggs of *S. droebachiensis* were fertilized in sperm suspensions $>10^3$ (Pennington, 1985).

In the present study the optimal concentrations in *G. caespitosa* were in the range of 10^7-10^8 sperm ml^{-1}, which is at least an order of magnitude greater than those reported for most of the invertebrates studied. Similar rates were only reported for the soft-shelled clam *Laternula elliptica* ($>10^7$ sperm ml^{-1}) and for the limpet *Nacella concinna* (10^6-10^8 sperm ml^{-1}). Like *G. caespitosa*, these molluscs exhibited extremely low fertilization rates at concentrations $\leq 10^6$ sperm ml^{-1} (Powell *et al.*, 2001).

At the high sperm concentrations such as those needed for successful fertilizations in *G. caespitosa*, many marine invertebrates show a sharp decline in fertilization rates (Oliver and Babcock, 1992; Sprung and Bayne, 1984; Clavier, 1992; Desrosiers *et al.*, 1996; Styan and Butler, 2000) which is taken as indicative of polyspermy (Styan, 1998). Various polyspermy-preventing mechanisms are found in free-spawning marine organisms (Jaffe and Gould, 1985) and some levels of polyspermy can be encountered even under sperm-limited conditions (e.g. Franke *et al.*, 2002). The way a fertilization curve changes with increasing concentration may depend on the polyspermy block activation time and on how fertilization success is scored. Styan (1998) suggested that when presence of fertilization membranes rather than normal cleavages were used as a measure of fertilization success, zygote production might have been overestimated due to inclusion of polyspermic eggs. In the present study only normally cleaving zygotes were scored, so observed lack of decline in fertilization success with increased concentration suggests that time required to induce polyspermy blocks is very short for *G. caespitosa* eggs. In sessile animals living in dense aggregations that are likely to routinely face high sperm concentrations, the evolution of effective and fast-acting polyspermy blocks is clearly advantageous.

A decline in fertilization success at high sperm concentrations may also be attributable to oxygen depletion by gametes resulting in death of embryos (Powell *et al.*, 2001). This is likely to occur when high concentration of gametes is maintained for a reasonably long time (i.e. in small isolated tide pools) and should not be observed if dilution of gametes is rapid (i.e. in high-energy wave-swept environments). In laboratory experiments, the effect of oxygen depletion may contribute to the effect of polyspermy if fertilized eggs are left to develop in concentrated sperm (e.g. Styan and Butler, 2000).

In this study, gamete contact time required to achieve high fertilization rates in *G. caespitosa* was very short, approximately 5 minutes of gamete exposure given sufficiently high sperm concentrations. In comparison, in *Haliotis tuberculata* maximum fertilization success was reported after 30 minutes of sperm-egg contact (Baker and Tyler, 2001). This suggests that attachment of sperm to the egg takes place rapidly, and a short-term exposure to highly concentrated sperm is a common feature of fertilization ecology of this gregarious species. Experimental methodology adopted in this study when eggs were rinsed of sperm after a limited exposure simulated rapid gamete dilution in the field, eliminating the possibility of oxygen depletion.

The gamete longevity experiments in *G. caespitosa* supported observations from other studies that there is a limited post-spawning period in which the oocyte can be successfully fertilized. The reported gamete longevity in free-spawning organisms varies from a few hours to a few days. For example, eggs of *S. droebachiensis* are fertilizable for 24-72 hours (Pennington, 1985; Meidel and Yund, 2001) and those of the starfish *Asterias rubens* lose their fertilization capacity after 24 hours (Williams and Bentley, 2002). Oocytes of the polychaetes *Arenicola marina* and *Nereis virens*, and the ascidian *Ascidia mentula* remain fertilizable for >96 hours (Williams and Bentley, 2002; Havenhand, 1991). A sperm longevity of <20 minutes is reported for *Strongylocentrotus droebachiensis* (Pennington, 1985) and the longevity is 2.5 hours for another sea urchin, *S. fransiscanus* (Levitan *et al.*, 1991). In the ascidians *Ciona intestinalis* and *Ascidiella aspersa* sperm activity lasts for >12 hours (Bolton and Havenhand, 1996). Sperm are capable of fertilizing fresh ova for 65 hours in *Nacella concinna* and for >90 hours in *Laternula elliptica* (Powell *et al.*, 2001), the longest reported sperm longevity. However, sperm longevity may not be directly comparable for different species because it can be extended when sperm is kept at higher concentration (e.g. Chia and Bickel, 1983; Baker and Tyler, 2001) and/or at lower temperatures (Sprung and Bayne, 1984; Kupriyanova and Havenhand, 2005) and can be reduced after contact with egg-conditioned water (Bolton and Havenhand, 1996; Williams and Bentley, 2002).

Eggs are typically reported to have a higher longevity than sperm (e.g. Pennington, 1985; Benzie and Dixon, 1994) and the results of the present study fit this rule. However, although eggs of *G. caespitosa* are still fertilizable for up to 10 hours and sperm retain some activity and minimal fertilizing ability up to 6 hours after activation, the post-spawning period optimal for fertilization is much shorter. Gamete aging starts to negatively affect fertilization early and within 2 hours the proportion of fertilized oocytes drops below 50%. Similarly, in *Asterias rubens* fertilization success does not fall to zero until 24 hours after spawning, but high fertilization success is observed for the first 4 hours only (Williams and Bentley, 2002).

The oocyte age not only affects fertilization rates, but delayed fertilization is also reported to decrease larval production. Although oocytes of the ascidian *Ascidia mentula* could be fertilized up to 144 hours after spawning, normal larvae were obtained from ova fertilized within 96 hours of spawning (Havenhand, 1991). In another ascidian *Botryllus schlosseri* (Stewart-Savage *et al.*, 2001) viable larvae are only produced when fertilization occurs within 19 hours although >50% of eggs can be fertilized within 38-49 hours. Similarly, eggs of the polychaete *Nereis virens* can be fertilized for 96 hours after spawning, but almost all zygotes fertilized 48 hours after spawning develop abnormally (Williams and Bentley, 2002). The preliminary qualitative observations from this study indicate that fertilization of *G. caespitosa* eggs 4 hours after spawning results in a high proportion of abnormally developing trochophores, even though early cleavage seems to proceed normally.

The role of gamete longevity on fertilization levels in the field is believed to depend on fertilization strategy adopted by a species (Williams and Bentley, 2002). When dilution of gametes below fertilizable concentrations occurs well before the viable life of gametes has expired, gamete longevity may be unimportant (Pennington, 1985; Denny and Shibata, 1989; Levitan *et al.*, 1991). However, in species with various adaptations to reduce sperm gamete dilution, gamete longevity is likely to be an important component of fertilization success (Yund, 2000). When gamete interaction in the field occurs hours after spawning, as, for example, in the infaunal polychaete *Arenicola marina*, the high gamete longevity can be crucial (Williams and Bentley, 2002). In *G. caespitosa* relatively fast gamete aging and early offset of developmental abnormalities related to the oocyte age indicate that during natural spawnings gamete longevity does not play an important ecological role.

In summary, the results of this study suggest that fertilization strategy of *G. caespitosa* is affected by its highly aggregated intertidal field distribution. This is consistent with Levitan's (2002) hypothesis that distribution of organisms co-evolve with gamete traits to maximise reproductive success. Field distribution and fertilization strategy of *G. caespitosa* appear to present a compromise between conflicting risks of sperm limitation and polyspermy. High population density ensures that this species is unlikely to encounter the problem of sperm limitation. At the same time, gamete traits of *G. caespitosa* enable them to perform efficiently under conditions of high sperm density and increased risk of polyspermy. Such attributes may include small egg size and high sperm concentration required to achieve high fertilization rates coupled with fast egg-sperm bonding and activation of the polyspermy-block mechanisms.

An intriguing question not addressed by this study is what factors and gamete attributes ensure efficient fertilizations in sessile organisms, particularly serpulid polychaetes, found in low-density populations. To address this question, future studies could compare the fertilization strategy of *G. caespitosa* with that of *G. hystrix*, a closely-related sympatric subtidal serpulid found in populations of relatively low density. A comparative study of these two closely related species would provide an insight into the mechanisms for enhanced fertilization success at greater distances in the field.

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