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Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained

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Fertilization success may be severely limited in marine invertebrates that spawn both male and female gametes. In a diverse group of aquatic organisms only sperm are released, with sperm–egg fusion occurring at the mother. Here, we report fertilization kinetics data for two such 'brooding' or 'spermcast' species—representing each major clade of the animal kingdom. High levels of fertilization were achieved at sperm concentrations of two or three orders of magnitude lower than is common with broadcast spawning species. At a concentration of 100 sperm ml^{-1}, fertilization rates of a bryozoan and colonial ascidian were near maximum, whereas most broadcast spawners would have displayed near complete reproductive failure. A further experiment looked at the rate of uptake of sperm under natural conditions. Results suggested that sperm released at *ca*. 0.9 m from an acting female could be collected at a rate of 3–12 times greater than the minimum required simply to avoid sperm limitation. Thus, evolutionary pressures on gametic and other reproductive characteristics of many species that release sperm but retain eggs may be quite different from those of broadcast spawners and may confer on the former an enhanced scope for sperm competition and female choice.

Keywords: fertilization kinetics; free spawning; *Diplosoma listerianum*; *Celleporella hyalina*

1. INTRODUCTION

It is well known that internal fertilization following copulatory mating generally involves massive wastage of sperm. Very appreciable sperm concentrations are also required to ensure the external fertilization of an egg spawned into the sea. Rapid dilution in turbulent flow and restricted sperm longevity may severely limit mating distances in marine invertebrates that release both sets of gametes, promoting behaviour such as aggregated or synchronized spawning (Levitan & Petersen 1995). Fertilization success, which can commonly vary between 0% and 100%, depends on whether dispersing clouds of eggs and sperm happen to intersect during a crucial interval immediately following release (but see Meidel & Yund 2001). The threat of such sperm limitation may have important consequences for patterns of reproductive behaviour and investment, sexual dimorphism, gamete attributes and development (Levitan 1996).

A diverse group of aquatic organisms mate in a third way, dispersing only male gametes that subsequently fertilize eggs that have been retained rather than spawned. The terms 'brooders' or 'free spawners' have been applied to these organisms but both are somewhat imprecise (see electronic Appendix A available on The Royal Society's Publications Web site). In the absence of a truly accepted and acceptable name we have suggested 'spermcast' organisms (Pemberton *et al.* 2003) and will use that term here. Spermcast mating occurs in a phylogenetically diverse range of taxa (see electronic Appendix B). The group encompasses a range of reproductive characteristics from the simple collision of a non-flagellated male gamete with a naked egg held on the surface of the mother, to complex uptake and reconcentration of swimming spermatozoa by a female with sperm storage before true internal fertilization and embryonic brooding. We focus on two of the latter type of spermcast organisms, the bryozoan *Celleporella hyalina* (L.) (Protostomia) and the colonial ascidian *Diplosoma listerianum* Milne Edwards (Deuterostomia). It is important to note that both species are actively pumping suspension feeders, and would thus be expected to have relatively greater potential for efficient sperm capture. Caution should be exercised in extrapolating our results to every spermcast species, especially the 'simpler' forms that may lack active sperm collection and storage.

There has been a tendency in the literature not to dwell on spermcast species or group them together with broadcast spawners when considering the dynamics of released gametes. In the past decade, zoologists have started to entertain the idea that fundamental life-history differences may follow from the retention of eggs. Previous suggestions that spermcast species possess fertilization dynamics that differ from those of broadcast spawning taxa have been based more on well thought-out arguments than direct evidence (Bishop 1998; Yund 2000). Here, we report the first quantitative data that allow direct comparisons between the reproductive strategies. We suggest that some spermcast species may be able to gather sufficient sperm from low ambient concentrations to avoid fecundity being limited by sperm supply. An estimate of the rate of sperm uptake under natural conditions suggests that certain spermcast organisms may experience moderate sperm competition, occupying a novel intermediate ground between broadcast spawners (where conflicting sperm limitation and polyspermy jeopardize reproductive success, e.g. Marshall 2002) and copulatory species (where the insemination of very many sperm and accessory substances threatens to swamp the female system).

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2. MATERIAL AND METHODS

The basic biology and laboratory culture of *Diplosoma listerianum* and *Celleporella hyalina* have been described in detail elsewhere (Bishop *et al.* 2001; Manríquez *et al.* 2001). Summaries of these as well as detailed methods for the experiments outlined here are given in electronic Appendix C.

(a) *Experiment 1: fertilization kinetics in two spermcast species*

Replicate virgin acting-female sub-colonies were placed in serial dilutions of a sperm suspension for a period in excess of the maximum longevity of released sperm. All resulting brooded late-stage larvae were counted.

(b) *Experiment 2: rate of sperm capture by* Diplosoma listerianum *in wild conditions*

Replicate sub-colonies from three genetic individuals were placed within the wild population at Queen Anne's Battery Marina, Plymouth, UK. The outer wall of the marina was the closest colonized structure to the acting females at a distance of 0.9 ± 0.1 m. Ramets were removed at predetermined intervals and returned to the laboratory. Fertilizations were scored by the presence of brooded tailed larvae 22 and 29 days after the start of the exposures.

3. RESULTS

(a) *Experiment 1*

Figure 1*a* plots the fertilization curves of the two spermcast species and compares them with that of a representative broadcast spawner. A 100% fertilization value for the broadcast spawner is where all *in vitro* spawned eggs undergo early embryonic development. In the bryozoan and ascidian, as vitellogenesis itself is triggered by compatible sperm (Bishop *et al.* 2000*a*) and fertilization is internal, fertilization values are derived from the numbers of brooded larvae that result from the *in vivo* crosses. Data for the bryozoan are relative to the highest observed fecundity within each of six acting female clones (coincident data points at 0% and 100% are slightly offset). Data for the ascidian are relative to the highest observed fecundity in each of four male + female clonal combinations, which was in a three-successive-matings treatment (not plotted, as the effective sperm concentration is not comparable; see electronic Appendix C) in three cases. In figure 1*b*, these data have been combined with results from a literature review to facilitate comparisons with multiple broadcast spawning species. Electronic Appendix D tabulates these data along with the commonly quoted concentration of 50% maximum fertilization. Both spermcast species appear able to use highly dilute sperm to achieve significant rates of fertilization. High levels of fertilization were achieved at sperm concentrations of two or three orders of magnitude lower than is common with broadcast spawning species.

(b) *Experiment 2*

Figure 2 shows the rate of compatible allosperm accumulation by colonies of *D. listerianum* placed within a natural population. As laboratory animals grow rapidly in the wild and exposure times differed, acting females varied in somatic size upon return to the laboratory. Larger ramets can produce more progeny. To achieve meaningful comparisons across exposure times, fecundity is expressed as the number of first-ovulation brooded larvae per zooid. Differences in sexual maturity of the rapidly growing animals are likely to account for much of the interclone variation in fecundity as well as some of the intraclone 'noise'. The asymptote shows the time required

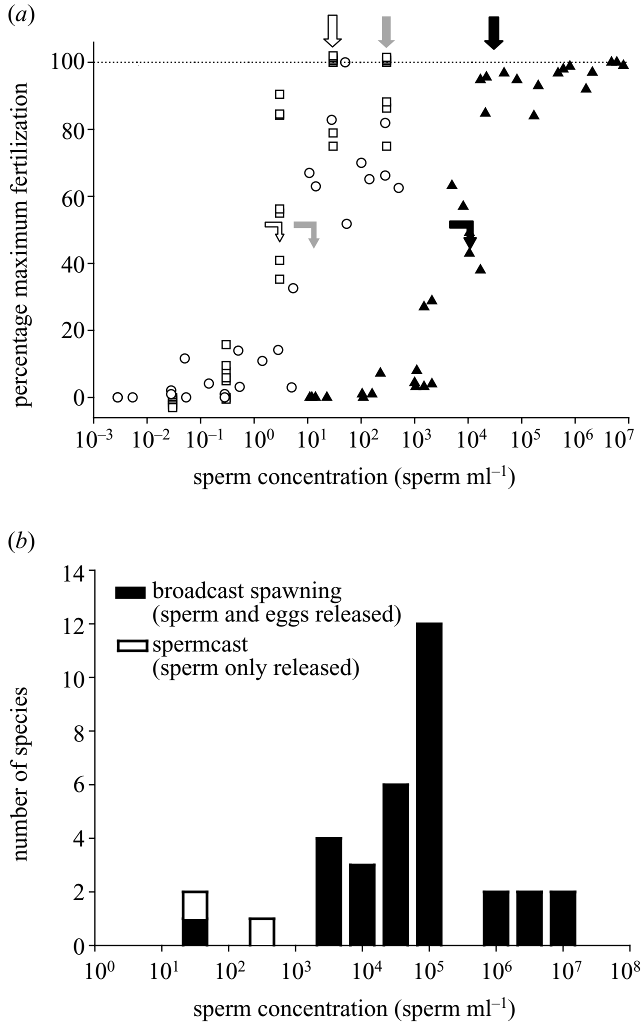

Figure 1. (*a*) Fertilization success at a variety of sperm concentrations in a representative broadcast spawner (triangles; data are for the purple sea urchin *Strongylocentrotus purpuratus*, from Levitan (1993) with permission), compared with two spermcast species (sperm only released): a bryozoan (squares) and a colonial ascidian (circles). Straight arrows indicate the concentration at which sperm become limiting, bent arrows show the concentration of 50% maximum fertilization (black arrows, urchin; white arrows, bryozoan; grey arrows, colonial ascidian). (*b*) Comparison across a range of species of the concentration at which sperm become limiting (as represented by the straight arrows in figure 1*a*). For sources see table 2 in electronic Appendix D.

for all mature zooids to capture sufficient sperm to produce at least one larva. The data for clone T (figure 2*b*) are unclear, the regression not reaching a plateau during the experiment but with maximum recorded fecundity at 60 hours.

4. DISCUSSION

Comparisons across a range of broadcast spawning taxa of the sperm concentration required for successful fertilization show these two spermcast species to be at the extreme lower end of the distribution (experiment 1). It is interesting that such high efficiency appears to be shared with only one broadcast spawner, the crown of thorns starfish, a species recognized as having exceptional fertiliz-

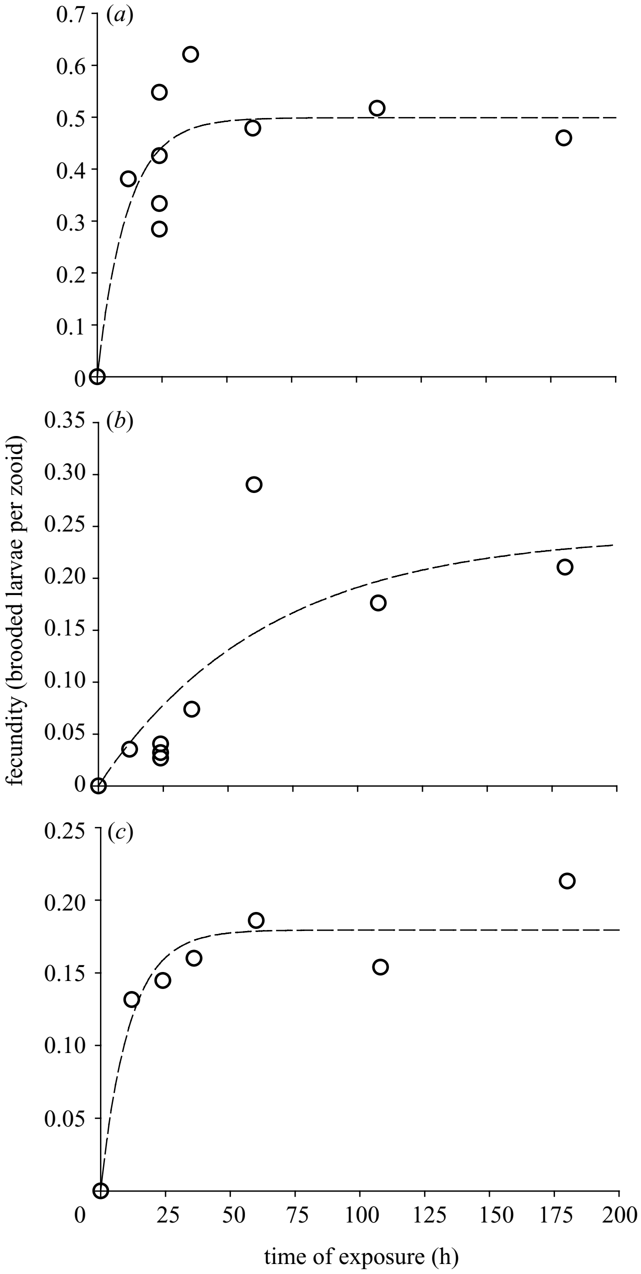

Figure 2. The change in fecundity of *Diplosoma listerianum* placed for different time intervals within a natural population. Equation of curve: Fecundity = $Q(1 - \exp(-(a \times \text{time})/Q))$, where Q is the maximum fecundity, i.e. height of plateau, and a is the initial slope. (*a*) Clone V: $R^2 = 0.74$, $Q = 0.4988$; $a = 0.0433$; (*b*) clone T: $R^2 = 0.67$, $Q = 0.2430$; $a = 0.0038$; and (*c*) clone W: $R^2 = 0.91$, $Q = 0.1794$; $a = 0.0161$.

ation dynamics that may partly explain its capacity for population outbreaks (Benzie & Dixon 1994).

Data from experiment 2 showed that sperm can be readily gathered in the wild. As previous work on a different spermcast ascidian (*Botryllus schlosseri*; Grosberg 1991) and a red alga (*Gracilaria gracilis*; Engel *et al.* 1999) suggested that spermcast fertilizations decrease rapidly beyond 0.5 and 1 m, respectively, from a source colony, our new data (separation of 0.9 ± 0.1 m) probably provide a conservative estimate of sperm capture rates. At least in the laboratory, a given zooid ovulates a single ovum at intervals of 7–12 days (Ryland & Bishop 1990). If sperm uptake is linear then, in the conditions of the field exposures, a zooid would collect somewhere in the region of 3–12 times more sperm than the minimum required simply to avoid sperm limitation. This pattern fits in with the findings of Burighel & Martinucci (1994*a*,*b*), who observed multiple spermatozoa in the female tract of wild *D. listerianum* zooids. Largely freed from the constraints of sperm limitation surrounding external fertilization but without the massive insemination of copulating species, the mating systems of many spermcast species may be shaped by a novel balance of selective forces (see also electronic Appendices E and F).

The results presented here indicating efficient collection of male gametes support previous suggestions that sperm would be gathered and stored from multiple males. The competitive interactions of sperm in these sorts of organisms are largely unknown, although the complexity of the female tract (Burighel & Martinucci 1994*a*,*b*), sperm precedence (Bishop *et al.* 2000*b*, 2001) and potential sperm ageing effects (Pemberton *et al.* 2003) have been documented in *D. listerianum*. Evidence of cryptic female 'control' over paternity in spermcast ascidians has been previously documented in terms of sexual compatibility (Scofield *et al.* 1982; Bishop *et al.* 1996) and frequency dependence of mate choice (Pemberton *et al.* 2003). A lack of control over the identity of the sperm donor is not unique to spermcast marine species, being shared with land plants receiving pollen from wind or pollinator vectors where knowledge of non-random fertilization, especially genetic complementarity and sexual compatibility, is much more advanced (de Nettancourt 2001). Precise theoretical justification for polyandry in copulatory animals remains unresolved (reviewed in Jennions & Petrie 2000). It is interesting that spermcast species may represent a relatively uncluttered terrain for the study of indirect (in other words, genetic) benefits conferred by polyandry in animals, since most of the costs (e.g. time and energy, predation, female injury, reduced paternal care, transfer of disease) and many of the non-genetic benefits found in copulating species can be largely discounted (Bishop & Pemberton 1997). What is certain is that an adequate sperm supply is a prerequisite for the evolution of female choosiness. Our results suggest that such a sperm supply may indeed be available.

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