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SPERM SWIMMING SPEEDS IN THE EASTERN OYSTER *CRASSOSTREA VIRGINICA* (GMELIN, 1791)

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ABSTRACT Oysters, like the vast majority of sessile marine invertebrates, shed sperm and eggs into the water column where fertilization subsequently occurs. The fate of the gametes depends on their passive movements at various scales in a high-viscosity environment, the longevity of the sperm's ability to affect oriented movement, the rate of sperm movement toward the egg target, and the ability of sperm to effect fertilization. Oyster sperm swim in a helical pattern with a mean forward progression velocity of 0.057 ± 0.010 mm/sec (SE; $n = 25$) with the 95 percentile range extending from 0.036–0.078 mm/sec, a value comparable with that reported for echinoderm sperm.

KEY WORDS: sperm swimming speed, oyster, *Crassostrea virginica*

INTRODUCTION

Oysters of the genus *Crassostrea* release eggs and sperm into the water column where fertilization subsequently occurs. Fertilization efficiency (proportion of the eggs fertilized) is dependent on the synchrony of gamete release by the contributing parents, their proximity to one another, local mixing and dilution effects at various scales of the overlaying water, half-life of viability of the gametes, probability of encounter and fertilization of the individual gametes, and more. Farley (2002) provides a brief history of the interest in the fertilization process dating back to the early work of Lillie (1915), Mortensen (1938), and Thorson (1950), and proceeding to the more recent development of fertilization models by Vogel et al. (1982) and Denny et al. (Denny 1988, Denny & Shibata 1989, Denny et al. 1992). For marine species with free release of gametes into the water column, the major historical focus has been on echinoderms, including the early works of Lillie (1915) and Rothschild and Swann (1951), and more recently the work of Levitan et al. (Levitan 1991, Levitan et al. 1991, Levitan et al. 1992, Sewell & Levitan 1992, Levitan 1993, Levitan & Young 1995, Levitan 1996a, Levitan 1996b, Levitan 1998, Levitan 2000). We can find no comparable data for oysters.

As part of a larger study of the fertilization kinetics of oysters of the genus *Crassostrea*, noted for their aggregative settlement and subsequent close proximity during the adult spawning events, we sought to describe sperm swimming speeds.

MATERIALS AND METHODS

Newly released sperm and eggs were obtained from the Virginia Institute of Marine Science oyster hatchery at Gloucester Point, Virginia. Sperm were maintained in filtered seawater at the temperature (24°C) and salinity (20) of origin throughout the experiment.

Sperm suspensions were introduced to one end of an optically flat glass capillary tube with an internal dimension of 600 μ m depth (vertical dimension), a width of 3.5 mm (flat, north–south as viewed in the microscope oculars), and a length of 8 cm. Egg suspension was added to the other end of the capillary tube and

the contents observed on the stage of a Zeiss IM35 inverted microscope using bright field illumination with a 25 \times or 40 \times objective. The observed material was recorded on VHS videotape using a Dage low-light video camera. The video signal was passed through a time/date stamp recorder to allow superimposition of date, time, and a stop watch feature onto each video “frame” to the nearest 0.01 sec.

The depth of the capillary tube was critical to obtaining swimming speeds that have minimal wall effect from an adjacent surface. Viscous forces, already high for swimming sperm (described later), are enhanced in close proximity to the capillary walls, so we measured swimming speeds in the center of the tube. Despite the fact that the objectives chosen have wide numerical apertures, the depth of field of observation was limited to less than the 600- μ m capillary depth. For video recording, the depth of field under observation was set by moving the focus up and down serially to record the positions equivalent to the inner top and inner bottom surface of the capillary, then the focus was fixed at a midway point.

Recordings were made of active swimming sperm within the capillary. The proximity of eggs, given that both sperm and egg share the same limited water mass within the capillary, was assumed to ensure swimming typical of that in nature during a spawning event. A typical recording included hundreds, if not thousands, of sperm over the time course of observation. Oyster eggs do not swim. Farley (2002) argues that echinoderm eggs exhibit Brownian motion. This may well be the case for oyster eggs, although their movement within the capillary in this experiment, given their diameter of approximately 50 μ m, is limited by viscous forces. For this reason, no attempts were made to record egg movements in the current experiment. Oyster sperm are described in Eble and Scro (1996) as having a head of approximately 2–3 μ m and a flagella tail of up to 40 μ m in length. The nature of flagellar movement results in the sperm swimming forward in a helical pattern. Thus, several dimensions can be used to describe the swimming, including absolute velocity of the sperm, which includes the helical pattern of progression, forward progression as a lesser value that describes a linear value along to the axis of the helix, the diameter of the helix, and the pitch of the helix. Although the helical swimming pattern results in a slower forward progression than a straight-line velocity equal to the absolute velocity, the helical pattern confers added

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elements of stability in control of the direction of movement (not unlike a bullet emerging as a spinning projectile from the rifled barrel of a gun). For the current experiment, the focus was on recording the forward progression velocity of the sperm. Note that the sperm helices progress in straight lines in general, and thus the diameter of the helix is critical to the overall forward movement in setting the volume searched by the sperm in seeking an egg target. A helix diameter that is too small does not optimize the search area. A diameter that is too large forms the pattern of a “hollow tube” and may again fail to optimize search volume.

The video records were replayed and the following criterion applied for records to be included in the final analysis: all sperm should exhibit continual swimming throughout the examination period (typically 0.6–1.0 sec at an equivalent of 30 recording periods). Depending on the objective used, the recording field viewed on the replay monitor was approximately 200 μm across.

RESULTS

After rejecting values not meeting the prerequisites of observation, a total of 25 records were considered acceptable. They are illustrated from lowest to highest values in Figure 1. Inclusion of all data points results in a mean forward progression velocity of 0.057 ± 0.010 mm/sec (SE), with the 95 percentile range extending from 0.036–0.078 mm/sec. One value exceeds the remainder by a considerable margin, although no rationale is immediately evident to exclude the data point. Even considering this as spurious, the corresponding values are reduced as follows: mean forward progression velocity of 0.050 ± 0.007 mm/sec (SE), with the 95 percentile range extending from 0.035–0.064 mm/sec. These values are of the same order of magnitude as that reported for echinoderm sperm—0.027 mm/sec—by Farley (2002). The Reynolds number for an oyster sperm swimming at the mean velocity measured in this experiment is 1.21×10^{-4} , indicating a large dominance of viscous forces over inertial forces. For comparison, this velocity is roughly equivalent to pushing a baseball through Crisco shortening at 3 mm/sec. The density (ρ) of Crisco shortening ≈ 0.8 g/cm³ (data source:

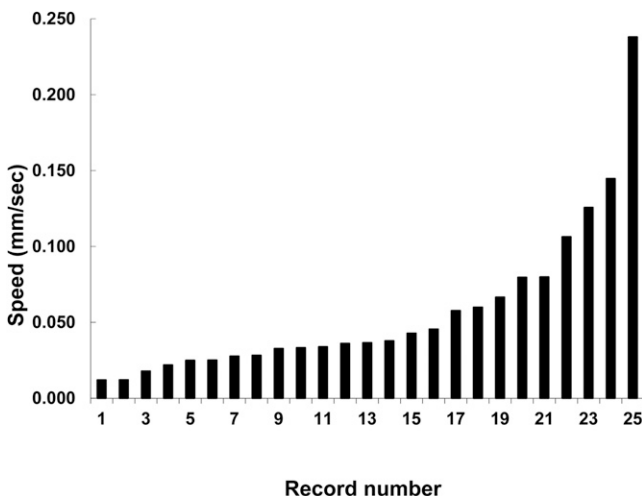


Figure 1. Distribution of individual records of oyster sperm swimming speed.

http://www.scholarchemistry.com/msds/Crisco_Shortening_235.70.pdf); the dynamic viscosity (μ) of Crisco ranges from $1.0\text{--}2.0 \times 10^3$ kg/m/sec (data source: <http://www.research-equipment.com/research-equipment.html>); and the diameter of a regulation baseball is 7.5 cm (data source: MLB.com, Official Rules).

DISCUSSION

The similarity of mean forward progression velocity for oyster and echinoderm sperm is not unexpected given similarities in size and the viscous environment encountered in both situations.

Consideration of the fertilization process in an essentially infinite volume of seawater can be viewed in both absolute concentrations of gametes (numbers per unit volume) for which very large values are commonplace, or in terms of relative volumes (volume of gametes relative to the volume of water in which they are suspended). In the latter instance, even very high absolute concentrations can result in extraordinarily low relative volumes that describe more accurately the dilemma of an individual sperm seeking to fertilize a single target egg. A simple spreadsheet calculation illustrates this challenge. Consider a single point source spawning event in an overlaying body of water above a flat surface. Assume that the eggs disperse into a perfect hemisphere, the origin of which is the point of discharge from the originating organism. The concentration of the eggs thus declines as the volume of the hemisphere increases. Further assume, for this example, that distribution within the hemisphere is uniform. Figure 2 illustrates such a facsimile.

The volume of the hemisphere increases by the cube of the radius (Fig. 2A). If 1 million eggs are released, the egg concentration (measured in numbers per milliliter) as a function of hemisphere radius is shown in Figure 2B. Given an assumed egg diameter of 50 μm , the total volume of the eggs per milliliter of seawater as a function of radius hemisphere is given in Figure 2C. Assuming that the eggs are distributed uniformly in space, the distance between adjacent eggs can be described by the radius of the sphere occupied by a single egg. This radius, less the radius of the egg itself, sets the maximum distance a sperm would need to swim to encounter an egg, assuming that the helical swimming pattern of the egg actually “hits the target” of a the individual egg. (Note that that this calculation also provides an estimate of mean swim distance in the case of randomly distributed eggs and sperm when there is a 1:1 ratio of sperm to egg; under the more plausible condition of sperm to egg ratios 2–4 orders of magnitude higher, mean swim distance would decrease, but maximum swim distance in the simplified scenario we have created would remain the same.) This distance can be expressed in micrometers or body length equivalents of single sperm assuming a 40- μm length, and is shown in relation to the hemisphere radius in Figure 2D and 2E, respectively. Alternatively, assuming a mean forward progression velocity of 0.057 ± 0.010 mm/sec, the time for a sperm to swim this distance can also be estimated (Fig. 2F).

Of note in Figure 2 is the rapidly decreasing value in the volume of eggs relative to the volume of water (Fig. 2C), and the corresponding increasing value in maximum swim distances and times for sperm (Fig. 2D–F). Given the assumed inability (or at least, yet to be demonstrated ability) of sperm to use complex search behavior for egg targets (presumably, they just swim), the probability of encounter with an egg, even

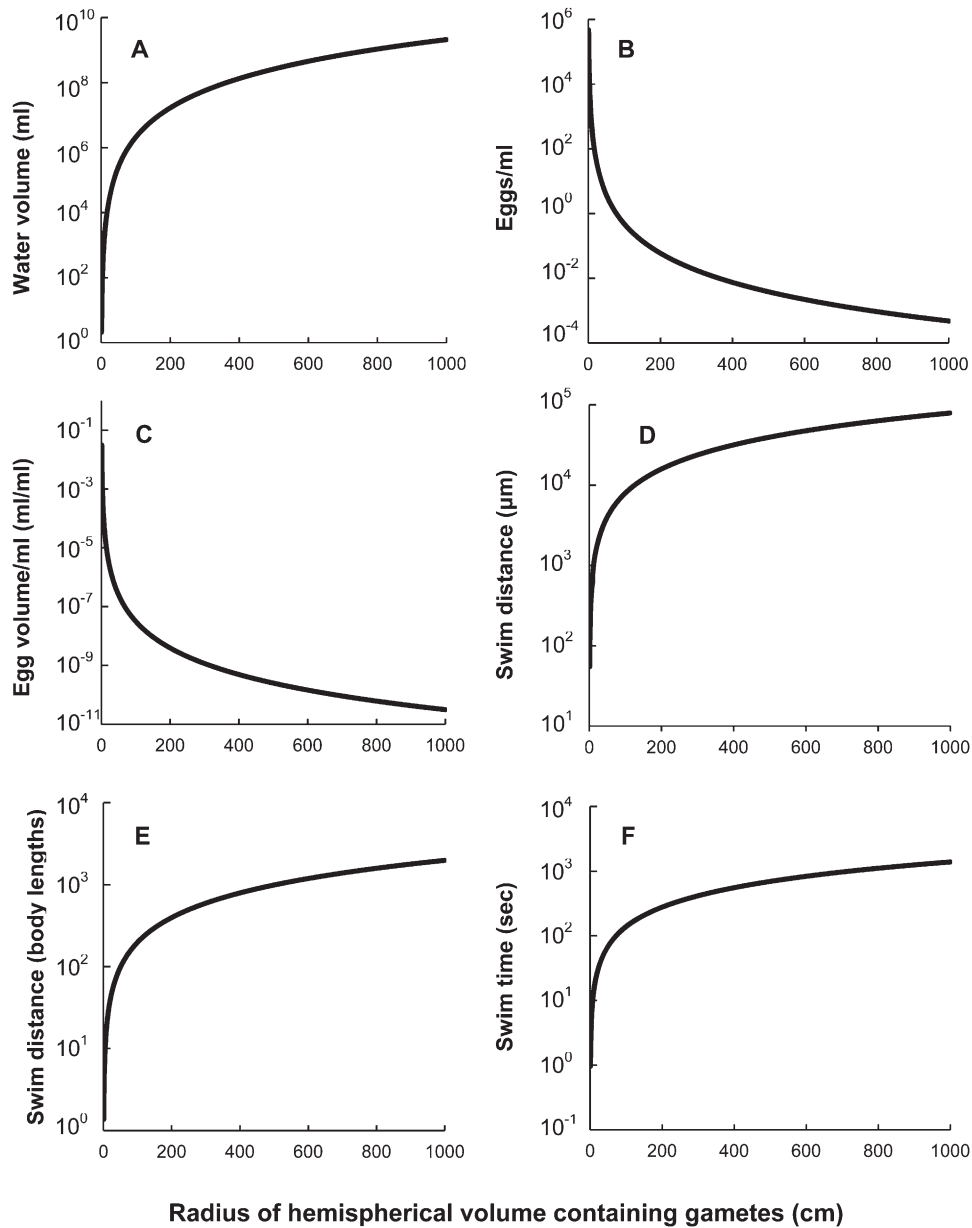


Figure 2. (A–F) Examination of sperm concentration in a half sphere of increasing radius with a loading of 1,000,000 eggs with an individual diameter of 50 μm . Estimates are developed for the relationship between the radius of this hemisphere and water volume (A); egg concentration (B); egg volume per milliliter (C); maximum swimming distance to accomplish fertilization, assuming uniform sperm concentration in microns (D) and sperm body lengths (E); and estimated maximum swimming time at a mean swimming velocity (F).

at high absolute egg concentrations, is modest at best. At hemisphere radii in excess of 10–20 cm, these modest probabilities are countered only by a consideration of extraordinary numbers of sperm moving along random, oriented swimming paths in the vicinity of the dispersed eggs.

Of particular note in the context of Figure 2 is the dilution of even extraordinary absolute numbers of eggs at hemisphere radii in excess of 50–100 cm. Even with modest water movement, this can occur in but a few minutes, yet sperm viability is known from hatchery operations to be on the order of tens of minutes to more than 1 h, which is greater than that typically observed for echinoderms (again, the records in Farley (2002) suggest a maximum of 20–30 min). This time frame of endurance

by oyster sperm appears somewhat incompatible with the scenario we have constructed in which gamete concentrations are presumed to be reduced to very low levels in a matter of minutes. This “incompatibility” raises 2 points of speculation: could the facsimile presented here be a very poor representation of diffusion and turbulence at the times of spawning in sedentary invertebrates, and do oyster sperm swim continually? Sundby et al. (1994) have suggested that very small-scale turbulence can increase and sustain close proximity of cod larvae to their prey organisms, thus creating a feeding environment where mean prey densities do not correctly portray actual feeding concentrations within the turbulence-induced feeding “aggregations.” Could small-scale turbulence create a similar

scenario with maintenance of sperm and eggs in aggregations with half-lives such that sperm-egg encounter rates are increased? Similarly, the significance of apparent extended half-life of sperm raises the question of continuous versus discontinuous swimming. Answering either of these questions is beyond the scope of this investigation. The salient point here is that there remains much that we do not know about the biological and physical

dynamics that affect fertilization in oysters and other free-spawning marine invertebrates.

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