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Reproductive success in large populations: empirical measures and theoretical predictions of fertilization in the sea biscuit *Clypeaster rosaceus*

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Abstract

For organisms that free-spawn gametes into the environment, sperm limitation can be a major determinant of reproductive success. Previous tests of sperm limitation have been restricted to very small experimental populations. Here we test and then use a fertilization model to explore sperm limitation in large populations. Predictions of the fertilization model are compared with measures of dye diffusion and in situ fertilization of the sea biscuit *Clypeaster rosaceus* (Linnaeus). The model could not be rejected in either test. Then this model was used to simulate large-scale spawning events in a natural population of *C. rosaceus*. The results of our simulations indicate that both population size and population density are important to fertilization over a very large range (2 to over 250000 individuals), but we also found an important interaction between population size and density. The importance of high density was great in small populations but negligible in large populations. This result may provide insight into why aggregation during spawning is not universally seen in nature. Overall, results indicate that sperm limitation can both constrain reproductive success and mediate social behaviors in a wide range in population sizes.

Keywords: Allee effect; *Clypeaster rosaceus*; Echinoderm; Fertilization success; Reproductive success; Sperm dispersal; Sperm limitation; Water flow

1. Introduction

When organisms release gametes into the environment, reproductive success is

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in part, a function of fertilization success. Sperm and pollen can disperse so quickly that fertilization becomes unlikely short distances from a spawning male (Handel, 1976; Willson, 1983; Pennington, 1985; Denny & Shibata, 1989; Grosberg, 1991; Okubo & Levin, 1989; Yund, 1990; Levitan, 1991; Levitan et al., 1992; Babcock et al., 1994). The available evidence suggests that for wind-pollinated (Augspurger, 1980; Willson, 1983) and animal-pollinated (Schemske et al., 1978; Bierzychudek, 1981) plants, free-spawning marine invertebrates (Pennington, 1985; Yund, 1990; Levitan, 1991, 1993; Brazeau & Lasker, 1992; Levitan et al., 1992; Oliver & Babcock, 1992; Sewell & Levitan, 1992; Babcock et al., 1994), and fish (Petersen, 1991; Petersen et al., 1992), fertilization limitation can severely constrain reproductive success. In every case in which fertilization has been investigated in free-spawning animals, sperm have been shown to be limiting (Levitan, 1993, 1995; Levitan & Petersen, 1995).

The frequency with which ova/ovules are fertilized depends in part on the distribution and abundance of spawning conspecifics (Willson, 1983; Levitan et al., 1992). Experimental studies of free-spawning invertebrates demonstrate that fertilization success (the percent of eggs a female releases that are fertilized) increases with population size and degree of aggregation (Pennington, 1985; Levitan, 1991; Levitan et al., 1992). Although these studies were conducted over realistic ranges of population density (1–32/m^2), population size was quite small (1–8 males). Extrapolating to predict levels of fertilization in populations larger than a few individuals is difficult, because fertilization success is so sensitive to small changes in population size (Levitan et al., 1992).

Unfortunately, examining sperm limitation in larger marine populations has been problematic. Conducting large-scale experiments that involve inducing hundreds to thousands of individuals to spawn is logistically difficult (Levitan et al., 1992), and natural spawning events are rarely seen and even more rarely by a scientist with the proper equipment to collect data on fertilization (Babcock et al., 1992; Sewell & Levitan, 1992). An alternate approach is to use theoretical models to simulate large spawning events.

Denny and his colleagues (Denny, 1988; Denny & Shibata, 1989; Denny et al., 1992) have developed equations to estimate sperm concentration and fertilization rates, as a function of distance from a spawning male, based on theoretical predictions of sperm diffusion in turbulent flow. They used these models to predict rates of fertilization on high-energy, wave-swept shores and suggest that fertilization can be either very low (Denny & Shibata, 1989) or very high (Denny et al., 1992), depending on the assumptions of the model and the nature of water movement. That predictions can be swung to such extremes by changes in either the parameter values or the assumptions suggests that it would be prudent to test these models over relevant environmental conditions before using them in a large-scale simulation.

In the present paper, we (1) develop a model of fertilization that incorporates both the physical environment and the biological attributes of gametes (Denny considered only the former, but see Levitan, 1993, for species-specific variation in fertilization rates), (2) test the assumptions of this model over a small spatial scale

with measures of particle diffusion and fertilization of eggs, and then (3) use the model in a simulation to predict the degree of sperm limitation and fertilization success over a larger spatial scale in a natural population of the echinoid *Clypeaster rosaceus* (Linnaeus). The results of our simulation suggest that sperm can be limiting in even large populations and therefore that increases in population size can have a positive effect on reproductive success and population growth (the Allee effect, Allee, 1931) over a wide range of population sizes in free-spawning organisms.

2. Methods

2.1. Modelling fertilization

To predict levels of fertilization in nature, we must understand both the biological characteristics of fertilization and the physical environment in which fertilization occurs. In the laboratory, and dependent on the species, fertilization becomes unlikely when sperm are diluted below a concentration of between 0.1 and 10.0 sperm per μl (Lillie, 1915; Rothschild & Swann, 1951; Hultin & Hagstrom, 1956; Brown & Knouse, 1973; Vogel et al., 1982; Pennington, 1985; Levitan et al., 1991; Levitan, 1993). At these or higher sperm concentrations, where fertilization is possible, an egg would be millimeters or less from a spermatozoan. Because sperm travel at approximately 0.1 mm/s (Gray, 1955; Levitan et al. 1991; Levitan, 1993) for minutes to hours (depending on species-specific characters and sperm concentration, Levitan, 1993), sperm swimming ability (projectile velocity), along with egg cross-sectional area (target size) and the receptiveness of the egg to fertilization, influences the rate of fertilization in the laboratory (Vogel et al., 1982; Levitan et al., 1991; Levitan, 1993). These factors along with sperm concentration (egg concentration has little effect on rates of fertilization, Lillie, 1915; Levitan et al., 1991) determine rates of fertilization.

Because sperm cannot out-swim most water currents, the transport, dilution, and mixing of gametes in the field is largely a function of water movement (Denny, 1988). Thus, the information gained from the laboratory is useful only against the background of how water (1) transports and mixes gametes released from different individuals and (2) behaves at the very small spatial scales at which fertilization occurs. Advective currents can transport gametes from one location to another, whereas turbulent eddies can stretch and fold parcels of water, separating or joining gamete plumes. The length, longevity, and distribution of eddies changes as a function of the turbulence of the water (Mitchell et al., 1985; Okubo, 1988; Vincent & Meneguzzi, 1991). As turbulence decreases, the length of minimum eddies (Kolmogorov eddies) increases, the velocity of particles within these eddies decreases, and the distances between eddies increases (Okubo, 1988; Vincent & Meneguzzi, 1991). Minimum eddy lengths can range from 0.1 cm in highly turbulent water to 3.5 cm in calm seas (Mitchell et al., 1985; Mann & Lazier, 1991). In highly turbulent water, a 0.1-cm eddy, with a maximum internal

velocity of 0.1 cm/s, may last only a second and be 0.9 cm away from other eddies. In contrast, in calm water, a 3.5-cm eddy, with a maximum internal velocity of only 0.003 cm/s, can last 20 min and be 20 cm from other eddies (Mitchell et al., 1985; Vincent & Meneguzzi, 1991, A. Okubo, pers. comm.). The spaces between eddies are regions of temporary calm (Okubo, 1988), where the morphology and behavior of gametes might greatly influence fertilization rates.

Laboratory work indicates that most fertilization occurs within the first few seconds after mixing of sperm and eggs (Levitan et al., 1991), and subtidal environments–apart from wave-swept shores –have eddy structures dominated by periods of calm water with intermittent and slow-moving eddies. We assume that fertilization will be similar to that under laboratory conditions in which gametes are placed in a discrete water parcel (scintillation vial) for periods of time and concentrations equivalent to field situations.

Under these circumstances, we use a turbulent-diffusion model (Denny, 1988; Denny & Shibata, 1989) to estimate the transport, diffusion, and concentration of released gametes. We then apply a gamete kinetics model (Vogel et al., 1982; Levitan et al., 1991; Levitan, 1993) to translate these gamete concentrations and the time period during which eggs and sperm are in the same water mass into estimates of the proportion of eggs that are fertilized. We measure the appropriate parameters for the former in the field and the latter in the laboratory. This combination is a simplistic description of the interaction of flow and fertilization, but by comparing the results of the model to empirical estimates of diffusion and fertilization, it is possible to determine the accuracy of the model under a limited set of conditions. One problem we cannot address directly is how increasing the spatial scale of the model beyond the scale we can compare empirically affects our predictions. Our indirect solution to this problem is to vary some parameter values over three orders of magnitude to determine the robustness of our conclusions.

Predicting diffusion of sperm. Babcock et al. (1994) modified equations presented by Denny (1988) to predict the sperm concentration (c in sperm/m^3) at any downstream position from a point of release (x = the direction of advection, y = direction perpendicular to x, and z = height off the sea floor, in meters) will be

$$S = \frac{W_s}{2\pi u \sigma_y \sigma_z} \exp\frac{-y^2}{2\sigma_y^2}\left(\exp\frac{(-z+h)}{2\alpha_z^2} + \exp\frac{-(z-h)}{2\sigma_z^2} + \exp\frac{-2(D-Z-h)^2}{2\sigma_z^2}\right) \quad (1)$$

where the standard dispersion coefficients are

$$\sigma_y = \alpha_y(u^*/u)x^{\beta_d} \quad (2)$$

$$\sigma_z = \alpha_z(u^*/u)x^{\beta_d} \quad (3)$$

and where the dispersion coefficients α_y and α_z are equal to 2.2 and 1.25, respectively (Denny, 1988), $\beta_d = 1$ (Denny, 1988). The sperm release rate is Q (sperm/s), D is the water depth (m), H is the height of sperm release (m), and u is the mean current velocity (m/s). The friction velocity (u_*) is calculated from

$$u_* = \kappa((u_2 - u_1)/(\ln z_2 - \ln z_1)) \quad (4)$$

where κ is von Karman's constant (0.4) and u_1 and u_2 are the velocity at two heights (z_1 and z_2 m/s) off the substratum. This equation provides approximate values for u^* in the log section of a well-defined boundary layer (Wimbush & Munk, 1970; Wimbush, 1976; Young et al., 1992).

The modification of Babcock et al. (1994) was to allow for sperm to reflect both off the sea floor and off the water surface back into the water column. These modifications become increasingly important when spawning takes place close to the sea floor and in shallow water.

Predicting fertilization. From these estimates of sperm concentration, fertilization rates can be calculated with the model of Vogel et al. (1982; see also Levitan et al., 1991; Levitan, 1993). This model assumes that sperm attach to the first egg they contact, regardless of whether fertilization occurs (the Don Ottavio model, Vogel et al., 1982). This model incorporates concentration of virgin sperm (S_0 sperm/μl) and eggs (E_0 eggs/μl), sperm half-life (τ s), and two rate constants, β_0 and β. The first, β_0 (mm^3/s), is the rate constant of sperm and egg collision, based on the sperm swimming velocity (mm/s) and the cross sectional area of the egg (mm^2). The second, β (mm^3/s), is the rate constant of fertilization. The proportion of eggs fertilized is predicted by

$$\phi_\infty = 1 - \exp(-(\beta_0 S_0/\beta E_0)(1 - e^{-\beta_0 E_0 \tau})). \quad (5)$$

The time of sperm-egg contact (the time an egg spends in a sperm suspension) "t" can be substituted for "τ" when "t" is less than "τ" (Vogel et al., 1982; Levitan et al., 1991). Because sperm longevity depends on sperm concentration, we used the regression

$$log\ \tau = (\log \text{sperm}/\mu\text{l} \times 0.313) + 3.05 \quad (6)$$

to estimate "τ" (pooled data from three echinoid species, Levitan, 1993). Under all the conditions we model, sperm longevity is not an issue, because sperm are quickly diluted to concentrations too low to achieve fertilization long before they lose their potency. Thus, for our purposes, the sperm-egg contact time is the relevant time period.

Using the kinetics model to predict the proportion of eggs fertilized in the field requires estimates of gamete concentration and sperm-egg contact time. Gamete concentration can be calculated by Eq. 1. Sperm-egg contact time can be calculated from the time period required for a sperm cloud to drift past a batch of stationary eggs. If the eggs are suspended in the water and drift, the percent of eggs fertilized can also be calculated. If all the urchins spawn at some constant rate (Q), then the concentration of sperm at any one location will be constant. The path along which a batch of eggs drifts, through a series of overlapping sperm plumes, can be divided into a finite number of small cells. The velocity of the

drifting eggs provides the time period during which the eggs reside in each cell of constant sperm concentration. The cumulative fertilization of eggs can be calculated from the percent of virgin eggs fertilized in each cell.

2.2. Laboratory estimates of fertilization dynamics

To estimate the rate constants (β_0 and β) for the fertilization-kinetics model, eggs were exposed to serial dilutions of sperm in the laboratory. Spawning was induced with a 3-ml injection of 0.55 M KCl. Fifty unfertilized eggs, from the same female, were placed in each of 18 scintillation vials with 9 ml of 0.45-μm-filtered seawater. One ml of dry (undiluted) sperm was added to 9 ml of filtered seawater to create a stock suspension. A 1-ml aliquot of the stock suspension was then transferred to the first vial and mixed. A 1-ml aliquot of this suspension was transferred to the next and the process was repeated until we had made six 10-fold dilutions. Each concentration was replicated three times. The vials containing eggs and sperm were swirled periodically and incubated at 20°C for 1 h. Eggs were then examined under a compound microscope for the presence of a raised fertilization membrane. The stock sperm suspension was fixed in formalin and sperm concentration determined with a hemacytometer (10 replicate counts of 4×10^{-6} ml samples).

2.3. Field test of fertilization model

Predictions of Denny's model of sperm concentration (Eq. 1) were tested by comparing the model's predictions to in situ measures of fluorescein dye diffusion. Also tested was the combination of Denny's model of sperm concentration (Eq. 1) with Vogel et al.'s model of fertilization kinetics (Eq. 5) by means of in situ measures of fertilization of eggs held in sperm-permeable containers. These comparisons were made at four distances from a sperm/dye source (0.25, 0.50, 1.00, 2.00 m) in seven replicate experiments over a range of flow conditions.

This study was conducted on the northwest side of Burroughs Cay, Bahamas, in ≈2 m of water, within a dense population of *Clypeaster rosaceus*. The substratum consisted of a flat, hard rock pavement with shell rubble, clumps of algae, and occasional small coral heads.

The experimental protocol, for each replicate, was to (1) collect individuals, obtain eggs and sperm, and place eggs in Nitex containers; (2) place a line, marked at points 0, 0.25, 0.50, 1.00, and 2.00 m from the release point, on the sea floor parallel to the water current; (3) measure flow velocity, (4) release dye at the "0" point and to sample the dye cloud at the four down-current points; and (5) deploy Nitex containers at the four down-current points and release sperm from a single male at the "0" point.

Gametes, collected by injecting urchins with ≈3 ml of 0.55 M KCl, were used within 1 h of collection. Sperm were stored dry, and eggs were diluted to

maximize viability. All the sperm from each male (range 2.5 to 9.5 ml), minus 0.5 ml, were used as a replicate. The 0.5-ml subsample was fixed in formalin and retained for later sperm counts. Eggs (1 ml of dilute eggs) were placed in plastic millipore Petri dishes with the flat surfaces replaced by 153-μm-mesh Nitex.

A nylon meter tape was positioned parallel with the current. Current direction was checked by release of a small amount of fluorescein dye. Flow velocity (m/s) was estimated immediately before the experiment, by recording of the time required for 5 ml of concentrated dye to travel 2 m at two heights above the substratum (0.1 and 1.0 m). Next, to estimate diffusion, we released 10 ml of stock fluorescein dye, at a rate of 1 ml/s, 15 cm above the substratum with a syringe (pointed upward to imitate spawning). At 0.25, 0.50, 1.00, and 2.00 m downstream, 50-ml water/fluorescein samples were collected with a syringe. Divers made collections 15 cm above the substratum by floating along with the dye cloud and sampling in the middle of the dye cloud when it reached each sampling point. The absorbance of these samples and the fluorescein stock solution at 480 nm were later measured with a spectrophotometer.

The egg-filled containers were then deployed at the same points (0.25, 0.50, 1.00, and 2.00 m) downstream along the transect line. The containers were held 15 cm above the substratum between a weight and a plastic fishing float. The mesh surfaces of the containers were oriented perpendicular to the direction of flow. All the sperm from a single male (minus the 0.5-ml subsample) were released (upwards from a syringe) at the upstream end of the transect, 15 cm off the bottom, at a rate of 1 ml/s. After 20 min, the egg containers were collected and placed in individual glass jars. Several hours later, the eggs were scored, under a microscope, for percent fertilization. The experiment was repeated seven times.

We also measured the distribution and abundance of *Clypeaster* at this study site to provide base-line data for the population parameters in our simulations. Population density was estimated with 40 haphazardly thrown 3.14-m^2 circular quadrats. Dispersion of adults was estimated by comparison of the distance from a random point to the nearest *Clypeaster* with the distance between that individual and its nearest neighbor (76 random points).

3. Results

3.1. Comparison of diffusion equation to diffusion of dye

The variables for Eq. 1 were measured to predict sperm concentration at four distances (0.25, 0.50, 1.00, 2.00 m) from a sperm source, over a range of flow conditions (0.06 to 0.14 m/s). We compared the estimates of sperm concentration to empirical measures of dye concentration. Using Eq. 4, we estimated u_* from the measure of water velocity at 0.1 and 1.0 m. We calculated the water velocity (u) at a height of 0.15 m (the height of sperm release and the height of experimentally held eggs) from a log-log plot of velocity vs. height. The sperm-

release rate (Q, number/s) was calculated from the concentration of sperm (number/ml, from hemacytometer counts of the subsample) at a release rate of 1 ml/s. The water depth (D) was 2 m.

The predicted sperm concentration could also be calculated from the empirical estimates of dye diffusion. Because the initial sperm and dye concentrations were known for each replicate, and the release rate (1 ml/s) was identical for sperm and dye, a prediction of sperm concentration could be calculated from the estimate of dye concentration measured with the spectrophotometer (Fig. 1). A plot of each paired measurement (for each distance and replicate) of estimated sperm concentration (Fig. 2) indicates that, under these flow conditions, Eq. 1 accurately predicts the mean rate of dye diffusion (Model II regression slope = 1.007, 95% CI = 0.232, not significantly different from 1; intercept = 0.012, 95% CI = 0.955, not significantly different from 0, correlation coefficient = 0.837). The diffusion of fluorescein dye and sperm may be similar at the moderate flow speed we encountered. The sperm we released from the syringes spread out in a plume. Thomas (1994) noted that echinoid sperm dispersed in a plume at current velocities over ca 0.1 m/s, and Benzie et al. (1994) measured sperm dispersal in the asteroid *Acanthaster planci* and reported that sperm dispersed as predicted by

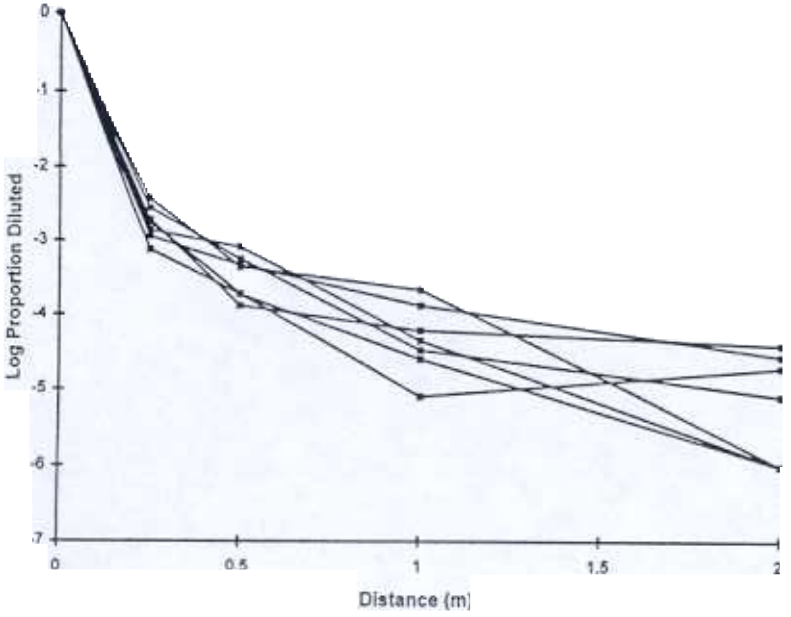

Fig. 1. Concentration of fluorescein dye as a function of distance downstream from release point. Concentration plotted as the log proportion of the released stock solution. Each trial is plotted separately. Lines indicate ranges.

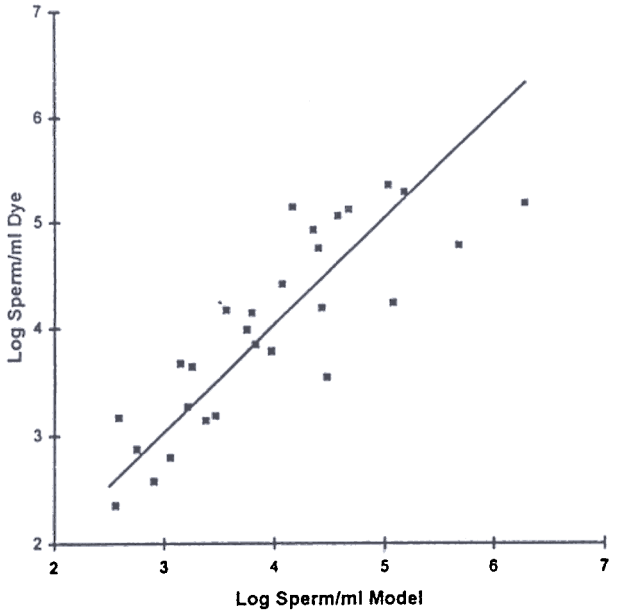

Fig. 2. Comparison of sperm concentration measured by diffusion of fluorescein dye and predicted by Eq. 1. Each datum is the paired measurement for each replicate and distance (correlation coefficient = 0.837).

plumes of neutrally buoyant particles. These studies suggest that Eq. 1 also approximates the rate of sperm diffusion at moderate to high levels of flow.

3.2. Comparison of predicted fertilization to in situ fertilization

Predicting fertilization success in the field using Vogel et al.'s (1982) model requires estimates of β_0, β, sperm-egg contact time, and the concentrations of sperm and eggs. We used the Marquardt method of nonlinear regression to estimate values of β_0 and β by iteration from the laboratory experiments (SAS Institute, 1988). The results of this analysis were highly significant ($p < 0.001$, $r^2 = 0.996$, $n = 18$) and provide estimates of β_0 (0.0106) and β (0.00177) (Fig. 3). In the laboratory, fertilization decreased from over 90% to less than 5% within two orders of magnitude of sperm concentration, indicating the sensitivity of fertilization to changes in sperm concentration that can occur within centimeters or seconds in the field (compare to Fig. 1).

Sperm-egg contact time (t) was estimated from the time required to extrude the volume of sperm released at 1 ml/s for each replicate (mean = 5.4 s, SE = 1.0 s). On the assumption that rates of advection overwhelm the rate of diffusion and

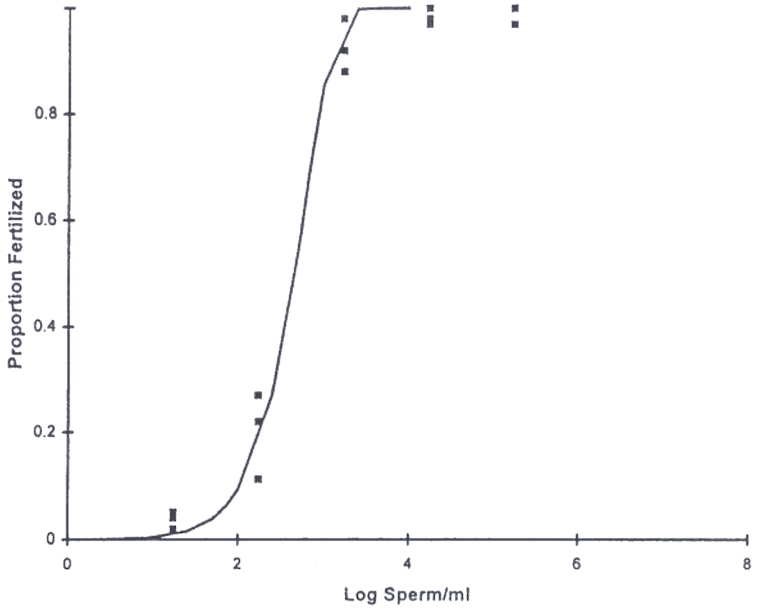

Fig. 3. Laboratory assay of the proportion of eggs fertilized as a function of sperm concentration. A series of eight 10-fold dilutions of collected sperm. Fertilization determined for the last six dilutions. The experiment was replicated three times. Regression line is the best fit of the data using Eq. 4 and the Marquardt method of iteration (SAS Institute, 1988, $r^2 = 0.997$, $p < 0.001$, $n = 18$).

shear forces in the x plane (Denny, 1988, pers. comm.), the time required to extrude the volume of sperm would equal the time for the sperm cloud to pass by the Nitex containers.

Sperm concentration was estimated from Eq. 1 for each replicate and distance (from above). Egg concentration was calculated from the number of eggs (mean = 74.8, SE = 14.9, $n = 23$) in each replicate and the volume of the Nitex containers (19 ml). Conditions for each trial are listed in Table 1.

The predicted fertilization success of eggs held in Nitex containers was compared to the actual fertilization success of eggs in these containers (Fig. 4). Replicates with fewer than nine eggs at the end of the experiment were not included in the analysis (5 out of 28 samples). Loss of eggs was the anticipated cost of using the largest possible mesh size to decrease any boundary-layer effect of the container. To determine whether our predictions of fertilization were significantly different from our measures of fertilization, we used a paired t-test comparing the predicted estimate of fertilization with each empirical measure. The results indicate no significant difference of predicted and empirical values ($t = 1.119$, $p = 0.275$). The correlation between the predicted and empirical values was weak (correlation coefficient = 0.194), but the slope (0.82, 95% CI = 0.371) and intercept (0.164, 95% CI = 0.185) were not significantly different from 1 and 0

Table 1
Amount of sperm released and flow conditions for each trial

1	9.5	4.67×10^7	0.08	0.13	0.087	0.009
2	8.0	7.82×10^7	0.06	0.17	0.072	0.019
3	6.0	2.25×10^7	0.12	0.18	0.129	0.011
4	2.0	1.40×10^8	0.13	0.19	0.139	0.011
5	5.5	7.50×10^7	0.08	0.17	0.091	0.016
6	4.0	1.17×10^8	0.11	0.17	0.119	0.011
7	2.5	1.32×10^8	0.06	0.07	0.062	0.002

[a] Equal to the release rate (Q, sperm/s) because the sperm suspension was released at 1 ml/s.
[b] Flow velocity at 0.1 and 1.0 m off the substratum and the calculated velocity at 0.15 m (u at the height of sperm release).
[c] From Eq. 2.

respectively, indicating that the mean predictive ability was accurate but not precise. A weak correlation is not surprising, given that the flow measurements and in situ experiments were paired but not conducted simultaneously and given the unpredictable nature of turbulence, and given possible artifacts associated

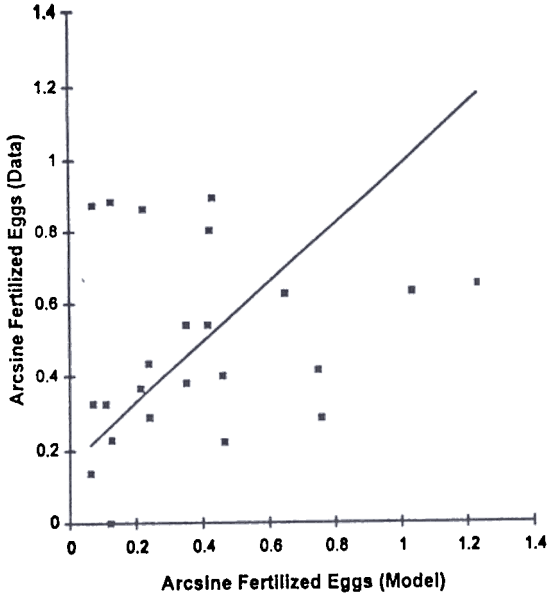

Fig. 4. Comparison of percent of eggs fertilized measured empirically from in situ experiments and from predictions based on Eqs. 1 and 5 (correlation coefficient = 0.194). The predictions are based on each trial's data for current velocity, sperm release rate, sperm-egg interaction time, and egg concentration (Table 1).

with the Nitex containers. In spite of these sources of variance, the model predicted the mean levels of fertilization measured empirically.

3.3. Distribution and abundance of natural populations

The population of *Clypeaster rosaceus* at Burroughs Cay was confined to an area roughly 50–100 m in diameter. Outside of this patch, we found no individuals. In a casual inspection, we could not detect any habitat differences associated with the boundary of this population. The population density of *Clypeaster rosaceus* within this aggregation was $3.88/m^2$ (SD = 1.43). The distribution of *Clypeaster* within the quadrats was not significantly different from a Poisson distribution (chi-square = 37.7, $p > 0.5$, $n = 40$ quadrats), indicating a random distribution. At the spatial scale of the sampling quadrats, a random distribution was also indicated by a Morisita's index (Vandermeer, 1981) of 1.053, where a value of 1 indicates a random distribution (higher numbers indicate a clumped distribution). The mean distance between a random point and the nearest individual sea biscuit was 0.226 m (SD = 0.143). The mean nearest neighbor distance was 0.214 m (SD = 0.157). These two distances are not significantly different (Student's "t" = 0.48, $p > 0.5$), indicating that pairs of individuals are also randomly spaced.

3.4. Simulation of natural populations

In our simulation, we placed hypothetical females in the centers of 2-dimensional arrays that varied in size from 2 × 2 m to 128 × 128 m. Each array was subdivided into 0.1-m cells, and hypothetical males were randomly assigned to these cells at different densities (0.0625 to 8 males/m^2). In each simulation, all males released sperm at one of three rates: 1×10^6, 1×10^7 (the mean empirical measure–Tyler et al., 1956; our unpublished observations of several echinoid species), and 1×10^8 sperm/s per individual. Current velocity (u) was also varied over three rates: 0.01 m/s, 0.10 m/s (mean empirical measure), and 1.00 m/s. The frictional velocity was 10% of the current velocity (based on empirical measures). We calculated the cumulative sperm concentration from all males at every 0.1-m cell (Eq. 1) from the center of the array (the release point of eggs) to 10 m beyond the edge of the array, along the x axis (the direction of advection). Beyond this distance sperm concentration becomes negligible (Fig. 5).

From the center of each array the female released a clutch of eggs, which traveled along the x axis at a velocity equal to the current velocity (0.01, 0.10, or 1.00 m/s). The cumulative fertilization of these eggs as they traveled through each 0.1-x-0.1-m cell was calculated from Equation 5. We used a sperm-egg contact time equivalent to the time the eggs would reside in each 0.1-m cell of water (10.0, 1.0, 0.1 s for the three current velocities. Because fertilization is insensitive to egg concentration, the diffusion of eggs was not considered, nor were the eggs of co-occurring females. Egg concentration was arbitrarily held constant at 0.01/μl. Because fertilization was sensitive to the actual placement of males, we conducted

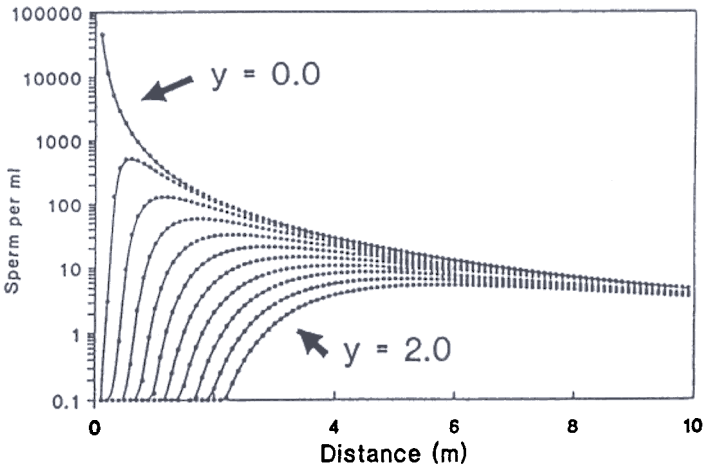

Fig. 5. Sperm concentration, as estimated by Eq. 1, along the x axis (distance downstream) when sperm are released at 0.2-m intervals along the y axis (perpendicular to x). The sperm-release rate (Q) is 1×10^7 sperm/s, and the water velocity (u) is 0.1 m/s.

100 replicates of the simulation for each array size, population density, current velocity, and sperm release rate. Each replicate used a different random placement of males within the array.

The simulation indicates that both the population density and the spatial extent of the population are important to fertilization success (Fig. 6). At the mean current velocity (0.10 m/s) and sperm release rate (1×10^7 sperm/s) noted empirically, fertilization success remained below 90% for all areas smaller than 1,024 m^2 and at densities below 16 individuals/m^2 (8 males/m^2) or 16,384 spawning individuals. Fertilization success approaching 100% occurred only in the areas (16,384 m^2) at the highest densities (16 individuals/m^2), 262,144 spawning individuals. The density of *Clypeaster* at Burroughs Cay was $4/m^2$. At this density, a population size of over 65000 individuals is needed to ensure greater than 90% fertilization success.

When spawning rate or current velocity shifted one order of magnitude in each direction, the absolute levels of fertilization shifted as well. Increases in current velocity resulted in increased diffusion rate and decreased sperm-egg contact time, leading to decreased levels of fertilization. Increases in sperm release rate resulted in increased sperm concentration and levels of fertilization. The only condition where most eggs were fertilized under almost all population conditions was that in which when sperm release rate was one order of magnitude higher and simultaneously current velocity was one order of magnitude lower than measured empirically. The only condition where very low levels of fertilization were noted under all population conditions was that in which when sperm release rate was one order of magnitude higher and simultaneously sperm release rate was one order of magnitude lower than empirical estimates. Within these two extreme

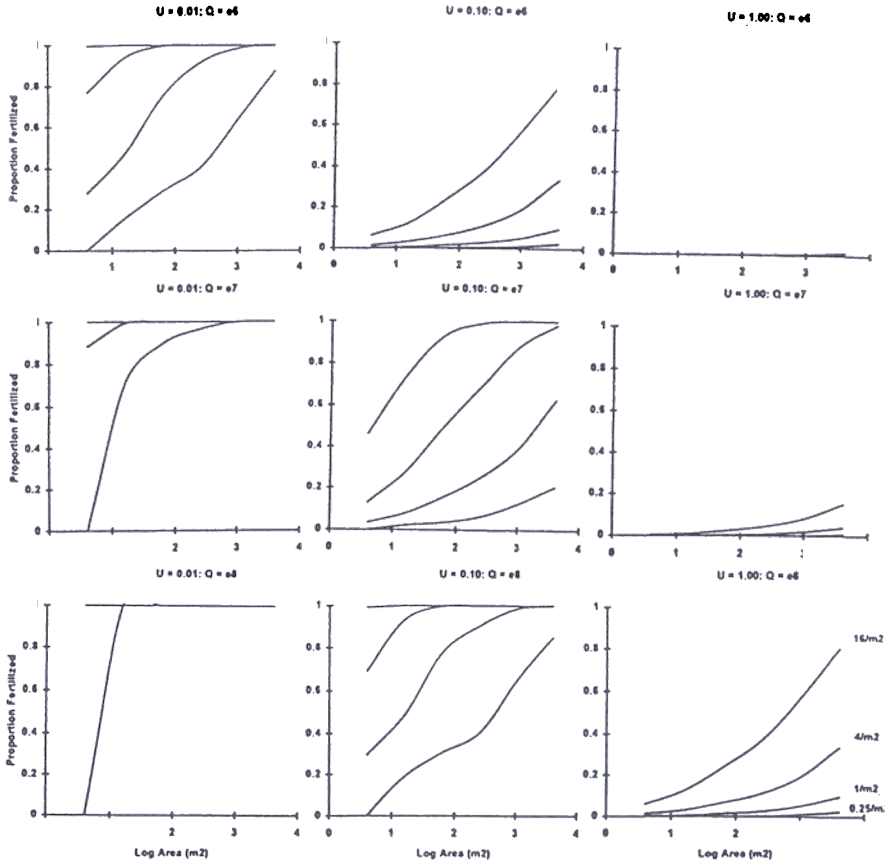

Fig. 6. Simulation results indicating the proportion of eggs fertilized as a function of the size of the array (area of population) and population density. The naturally occurring population density at Burroughs Cay, Bahamas, is $4/m^2$. Current velocity (U) is varied from 0.01 to 1.00, sperm release rate (Q) is varied from 1×10^6 to 1×10^8 sperm/s. The empirical measure of current velocity (0.10 m/s) and sperm release rate (1×10^7 sperm/s) is depicted in the center graph. In all graphs, the top line is $16/m^2$, followed by $4/m^2$, $1/m^2$, and $0.25/m^2$ on the bottom (see lower right graph for labels).

boundaries, fertilization was sensitive to both changes in the spatial extent and density of the population.

This model assumes 100% synchrony during spawning; any deviation from full synchrony is equivalent to a decrease in the density of spawning animals. If, for example, only 6.25% of the individuals spawn synchronously (a reasonable value, Levitan, 1988), then for a *Clypeaster* population at ambient flow conditions ((0.10 m/s), sperm release rates (1×10^7 sperm/s), and densities ($4/m^2$) in an area of 16,384 m^2, the percent of eggs fertilized is only 20%.

There is also an important interaction among population size, dispersion, and

density. In our simulations, for example, 64 individuals can spawn at two levels of clumping–at a density of $4/m^2$ in an area 4×4 m or at a higher density of $16/m^2$ in a smaller area, 2×2 m (Fig. 6). In this comparison, the 64 clumped individuals at a higher density have a higher fertilization success (0.014%) than does the dispersed population (0.007%). The results of comparing the percent increase in fertilization with clumping show a general trend of increased benefits when population size is small and fertilization success is low (Fig. 7). When fertilization is high, and the scope for improvement is small, then clumping obviously cannot increase fertilization success to a large degree. However, because the relationship between sperm concentration and fertilization success is not linear (e.g. Fig. 3), the relationship between population size and the benefits of clumping is also not linear. In fact, in many comparisons, clumping is most beneficial at intermediate population sizes.

4. Discussion

The proportion of released eggs that become zygotes depends on the probability that sperm will locate and then fertilize eggs. The distribution of sperm once they are released is a function of flow conditions and male variables including spawning synchrony, reproductive output, distribution, and abundance, but the importance of the distribution and abundance of males to fertilization success has been questioned. Experimental studies, although they demonstrate the importance of population size and density (Pennington, 1985; Levitan, 1991; Levitan et al., 1992) were conducted at small spatial scales, and previous theoretical treatments (Denny & Shibata, 1989) have suggested that the number of spawning males in not important to fertilization success because only the closest male would influence levels of fertilization.

To address the question of scale and fertilization, we tested a fertilization model at a logistically tractable spatial scale and then used the model to simulate large-scale spawning events. In our tests, over a typical range of flow conditions and male sperm production, we could not distinguish between predicted and empirical estimates of either dye diffusion or fertilization of eggs in situ. Two independent tests failed to reject the predictions of the fertilization model. Because fertilization is very sensitive to small changes in sperm concentration (Fig. 3) and sperm concentration can decrease 4–5 orders of magnitude within 2 m of a sperm source (Fig. 1), any major discrepancy between model predictions and empirical data should have been evident. The simplifying assumptions we have made do not influence the usefulness of the model under these moderate flow conditions.

The results of our simulations over a range of population sizes (2 to >250000 individuals) suggest that sperm can be limiting even in large synchronously spawning populations. Under the flow velocities and population parameters we modelled, fertilization success was limited by the availability of sperm unless population size was in the hundreds of thousands. The only exception to this case

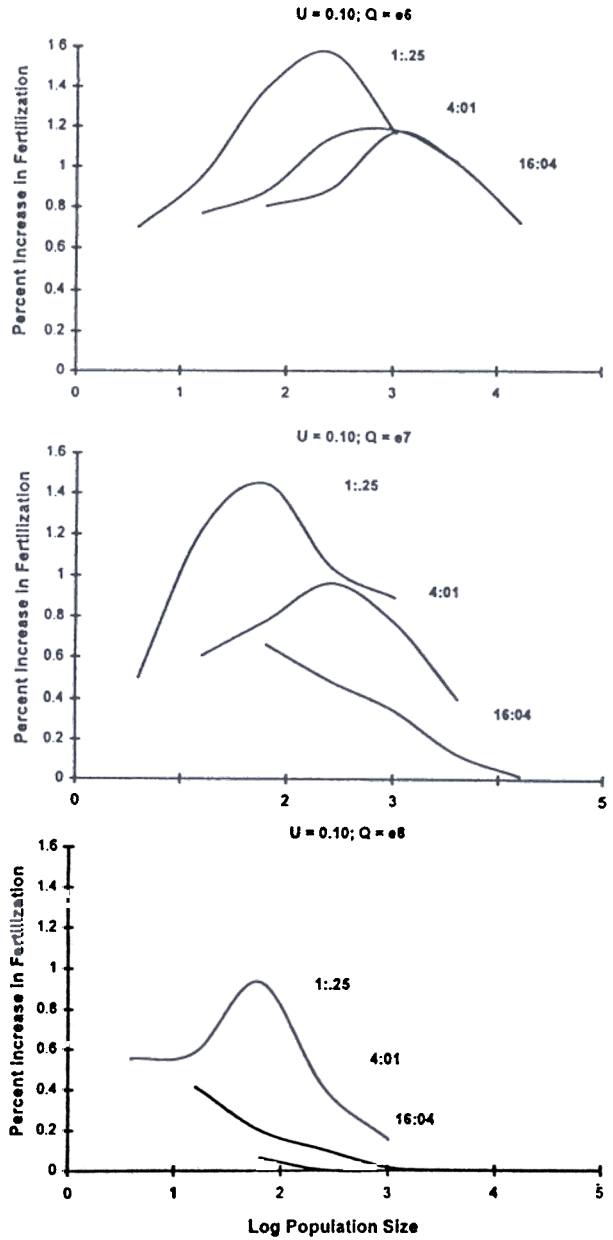

Fig. 7. Simulation results indicating the percent change in the proportion of eggs fertilized when population density increases fourfold while population size is held constant (changing the degree of clumping in the total population). Top line is a change from $0.25/m^2$ to $1/m^2$, followed by $1/m^2$ to $4/m^2$, and $4/m^2$ to $16/m^2$.

occurred when two conditions were met simultaneously: individuals released an order of magnitude more sperm than measured empirically, and flow velocity was minimal (0.01 m/s).

When Denny & Shibata (1989) suggested that only the nearest male would substantially influence fertilization, they had considered only sperm from up-current males drifting over eggs, but when eggs can drift, increasing population size increases the chance that an egg will pass over one or more spawning males, regardless of the flow conditions.

If sperm are limiting, and fertilization success is influenced by the number of spawning individuals over a large range in natural population sizes, then the benefits of being in a large and crowded population (the Allee effect, Allee, 1931) may be more important than generally recognized (Levitan et al., 1992). The influences of the Allee effect and intraspecific competition on population dynamics are often considered not to overlap; the former is thought to act only at very small population sizes, when mates are limiting, and the latter in much larger populations, when resources are limiting (e.g. Begon et al., 1986; Levitan, 1991; Levitan et al., 1992). However, if fertilization success varies over the full range of natural abundances, then the Allee effect can alleviate some of the effects of competition (e.g. food limitation) and vice versa (Levitan, 1991). Therefore, sperm limitation not only reduces the number of zygotes potentially produced (Pennington, 1985) but can also subtly –because fertilization success is difficult to quantify –alter the dynamics of natural (Levitan, 1991; Knowlton, 1992) and harvested (Orensanz, 1986; Quinn et al., 1995) populations.

Our simulations also indicate an interaction between population size and clumping (Fig. 7). Clumping can greatly increase fertilization success when population size is small or intermediate (as has been documented in previous experimental studies, Levitan et al., 1992) but is progressively less important as fertilization approaches 100%. The population of *Clypeaster* at Burroughs Cay is estimated to comprise between 10000 and 40000 animals at a density of $4/m^2$. In this population, local clumping should have very little influence on fertilization success if spawning synchrony is high; indeed we did not detect any clumping in this species during the breeding season. The predicted nonlinearity of the benefits to aggregation at low and intermediate population sizes suggests that the intensity of selection to aggregate will vary in an unpredictable fashion. Early considerations of fertilization success (e.g. Belding, 1910; Sparck, 1927; Allee, 1931; Mortensen, 1938; Gross & Smyth, 1946; Thorson, 1946), as well as recent results from small-scale experiments (Pennington, 1985; Levitan, 1991; Levitan et al., 1992; Babcock & Mundy, 1992), imply that aggregation increases fertilization success. Nevertheless, mobile, free-spawning invertebrates do not universally aggregate when spawning (Pennington, 1985; Levitan, 1988; Pearse et al., 1988; Babcock et al., 1992). Aggregative behavior will evolve when the benefits (e.g. prey defense, physiological tolerance, mating success) outweigh the costs (e.g. resource limitation, spread of pathogens) (Allee, 1931; Jackson, 1985). As population size increases past some intermediate threshold, the benefits of aggregation to fertilization success will decline (Fig. 7), as the costs (e.g. resource

limitation) increase. All else being equal, the degree to which free-spawning animals aggregate should reflect this balance. Therefore 100% fertilization might not be expected in populations (aggregated or not) that experience resource limitation or some other cost of aggregation. In fact, large-scale spawning events have often been observed in which fertilization was generally high (50–90%), but not 100%, and aggregation was not seen (Babcock & Mundy, 1992; Oliver & Babcock, 1992; Sewell & Levitan, 1992). Observations of small-scale spawning events typically involve tighter clumping (Randall et al., 1964; Pennington, 1985; Run et al., 1988; Young et al., 1992) and high levels of fertilization (Petersen, 1991; Petersen et al., 1992) or sporadic spawning of isolated individuals at low density (Pennington, 1985; Levitan, 1988; Pearse et al., 1988) with lower levels of fertilization (Babcock et al., 1992). This last scenario may simply reflect the best attempt at reproduction for rare individuals.

These experiments and simulations examine reproductive success from a female perspective, but the conclusion that reproductive success (number of eggs fertilized) will increase with population size and density should also hold from a male perspective if all males are equally successful at competing for eggs and the sex ratio is maintained at 1:1. It will be interesting to examine the effect of male competition (e.g. Yund & McCartney, 1995) to determine whether the trade-offs between aggregation and fertilization are gender-specific.

These simulations predict that the Allee effect operates throughout a large range in population sizes as fertilization success asymptotically approaches 100%. This form of the Allee effect is very different from the threshold view, where at some critical but relatively small population size, populations grow rather that diminish (see, e.g. Begon et al., 1986). In free-spawning organisms (both plants and animals) the Allee effect and intraspecific competition can interact, leading to nonintuitive dynamics and social (e.g. dispersion) behaviors. The relationship between individual reproductive success and population size will ultimately depend on the sensitivity of gamete production, fertilization success, and offspring survival to changes in population size (Levitan, 1991; Levitan et al., 1992).

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