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THE ECOLOGY OF FERTILIZATION OF ECHINOID EGGS: THE CONSEQUENCES OF SPERM DILUTION, ADULT AGGREGATION, AND SYNCHRONOUS SPAWNING

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Abstract

Percent fertilization of eggs of the echinoid *Strongylocentrotus droebachiensis* (0. F. Müller) was determined both in laboratory and field experiments. In the laboratory, over 50% of the eggs were fertilized only in relatively dense sperm suspensions ($>10^6$ sperm/l); such suspensions retained their potency for less than 20 minutes. In the field, divers induced individual *S. droebachiensis* to spawn with KCl injections. Along five meter transects running directly downcurrent from spawning males, fixed volumes of seawater presumably containing sperm were drawn into syringes already containing eggs. Within 20 cm of spawning males 60–95% fertilization usually occurred; at distances greater than 20 cm less than 15% of the eggs were fertilized. Higher percentages of eggs were fertilized when current speeds were low (<0.2 m/s); swifter currents quickly diluted sperm so that little fertilization occurred. When several males were induced to spawn synchronously, percent fertilization increased but was generally less than 40% at distances greater than 2 m downstream. These results indicate that production of zygotes could be much less than production of eggs. Life-tables based on estimates of egg production may then be in error, unless adults aggregate and spawn synchronously, countering dilution of sperm by currents.

Introduction

Successful fertilization is a critical step in the life-history of species, particularly for free-spawning marine invertebrates (Mortensen, 1938; Thorsen, 1946; Chia, 1974). Mortensen (1938) noted that although many asteroids produce a large number of eggs, their rates of juvenile recruitment are typically low. He suggested that this discrepancy exists because the probability of eggs encountering sperm in the plankton is low, and that most eggs are never fertilized. In contrast, Thorson (1946) suggested that most eggs of benthic invertebrates are fertilized because adults of both sexes spawn nearly synchronously in aggregations, therefore insuring that sperm and egg encounters occur. In Thorson's (1946) view, low recruitment rates are largely due to predation upon embryos and larvae. Though Thorson's (1946) suggestion has since become more widely accepted, both ideas remain tenable because benthic invertebrates have rarely been observed spawning in the field, and because to date no study has determined field rates of fertilization for any free-spawning invertebrate.

I have thus examined conditions under which spawnings may, or many not, produce fertilized eggs of the sea urchin, *Strongylocentrotus droebachiensis* (O. F. Müller). First, laboratory experiments were conducted to examine the effects of sperm dilution and gamete age on percent fertilization (see Lillie, 1915, 1919; Cohn, 1918; Gray, 1928). Field experiments were then conducted to determine if adult aggregation and epidemic spawning might serve as effective counters to dilution of gametes by tidal

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currents and the effects of gamete aging. The echinoid *S. droebachiensis* was used in this study because: (1) it is a free-spawner, shedding its gametes directly into the sea; (2) adults are motile and can alter their proximity to one another; and (3) the prominent fertilization envelope, which quickly rises from eggs following fertilization (see Tyler and Tyler, 1966), is a convenient indicator of fertilization.

Materials and Methods

Laboratory experiments

The first series of laboratory experiments determined over what range of sperm concentration high percentages of fertilization occur. Gametes were obtained by intracoelomic injections of .55 *M* KCl. Two ml of freshly spawned but settled eggs were pipetted into each of a series of 10 jars containing 3 l of filtered seawater, resulting in a concentration of about 3×10^4 eggs per liter. The water in each jar was thoroughly stirred with a paddle which oscillated at about 20 cycles per minute (Strathmann, 1971). Freshly spawned undiluted semen, or "dry sperm," was quickly run through a series of 10, 10-fold dilutions, thus diluting the dry sperm by an order of magnitude at each step. One ml of each dilution was then added to a single jar of the series; the egg/sperm mixtures were allowed to incubate for 10 minutes before eggs were siphoned off, concentrated and fixed in 2% formalin. Assays for percent fertilization were made by counting the number of elevated fertilization envelopes on the first 100 eggs encountered under 160× magnification. Four replicates of this experiment were conducted. Absolute sperm concentrations were assessed by hemocytometer counts of sperm in water from each of the jars.

A second series of laboratory experiments determined the effects of gamete age after spawning on percent fertilization. In experiments to examine sperm longevity, dry sperm was quickly run through 4, 10-fold dilutions. One ml of the most dilute suspension was then added to each of a series of 7 dishes containing 50 ml of filtered seawater at time 0. One ml of settled eggs was added to the first dish at time 0, and in sequence to the remaining dishes at 10 min intervals thereafter. The eggs added to each dish thus encountered sperm of different ages. Eggs were also added to one jar without sperm to serve as a control against inadvertant contamination of eggs by sperm. The egg/sperm mixtures were allowed to incubate for 10 min before they were fixed, and assays for percent fertilization were performed as above. Three replicate experiments were conducted.

Experiments to examine egg longevity were the converse of those described above for sperm. One ml of settled eggs were added to the dishes at time 0, and fresh sperm dilutions (as above) were added in sequence to each of the 8 dishes at 10 min intervals. The freshly diluted sperm added to each dish thus encountered eggs of different ages. The egg/sperm mixtures were incubated for 10 minutes prior to being fixed and assayed for percent fertilization as above. Three replicate experiments were conducted.

A final laboratory experiment was conducted to determine if high percentages of fertilized eggs would result from induced spawning in an aquarium. A 55 gallon aquarium was filled with filtered seawater and allowed to warm to room temperature (14°C). Sea urchins were injected with KCl solution, and when a male and female began spawning they were strapped, mouth down, to plexiglass plates and rinsed. The plates were then placed 50 cm apart on the bottom of the aquarium. The water within the aquarium was not mixed. Samples of eggs were pipetted from the female's aboral surface at 5 minute intervals during the first 20 minutes after spawning began, and also 20 to 24 hours later. Assays for percent fertilization were performed as described above.

Field experiments

Field experiments examined the effects of adult aggregation, current, and epidemic spawning on percent fertilization. A subtidal valley bordering San Juan Channel, Washington, was located where the tidal current usually flows in the same direction and where *Strongylocentrotus droebachiensis* is common about 10 m deep. The bottom of the valley consisted primarily of fist-sized cobblestones. A five meter transect was set up along the bottom running directly downcurrent. Current direction was determined by releasing dye, and current speed was estimated by timing dye movement along the transect. Several *S. droebachiensis* were collected from the vicinity and stimulated to spawn with KCl injections *in situ*. In initial trials, when a female began spawning she was moved upstream of other spawning animals and samples of eggs were pulled directly from over the gonopores into each of 12, 60 ml syringes. A spawning male was then placed at the head of the transect, and any other spawning sea urchins were moved downstream. Along the transect at a series of distances from the spawning male, 10 ml of water were pulled into each syringe about 10 cm over the substrate. The first syringe was filled upstream from the male as a control to detect any extraneous sperm, the second was filled directly over the male's gonopores to assess maximal fertilizability of the eggs, and one syringe each was filled at 10, 20, 40, 60, and 80 cm, and 1, 2, 3, 4, and 5 m downstream from the spawning male. Except for the control, the syringes presumably contained both eggs and sperm. The syringes were then taken to a skiff where their contents were fixed after incubating for a total of 10 minutes. Eggs within the syringes were later assayed for percent fertilization as described for the laboratory experiments. In later trials, the number of eggs per syringe was controlled by adding .08 ml of settled eggs (about 1600 eggs) to each syringe in the laboratory about an hour prior to performing the field experiments.

Similar experiments were conducted under a variety of current conditions, ranging from slack tide with little current (0.05 m/s) to swift currents (0.8 m/s) to examine effect of current on percent fertilization. Experiments as above were also conducted which simulated limited epidemic spawnings, using three spawning males at the head of the transect.

Questionnaire

Because only one direct observation of echinoid spawning was found in the literature (Randall *et al.*, 1964), over 100 sets of questionnaires were mailed to individuals and marine stations in North America, asking for information regarding diver observations of echinoid spawnings. Intertidal observations of spawning were not solicited because osmotic or thermal stresses associated with exposure to air may induce abnormal spawnings (Fox, 1924; Harvey, 1956), and it is doubtful that gametes or embryos survive at low tide in the intertidal zone.

RESULTS

Laboratory experiments

In laboratory experiments to examine the effects of sperm concentration on percent fertilization, high percentages of fertilization were achieved only with relatively dense sperm suspensions (Fig. 1). Over 80% of the eggs were usually fertilized in suspensions containing more than 10^6 sperm/l, but percent fertilization rapidly declined in more dilute sperm suspensions until essentially no eggs were fertilized in suspensions con-

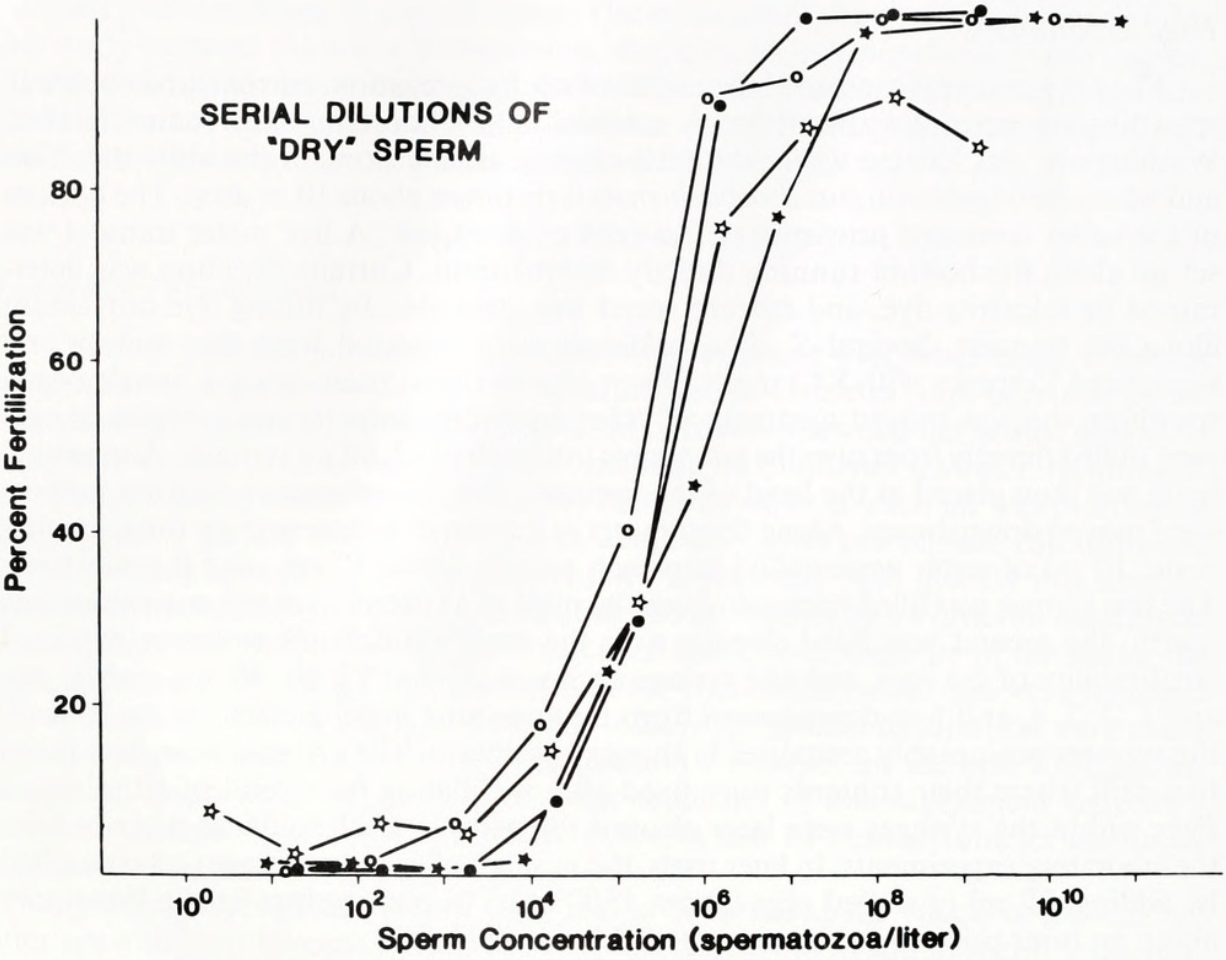

FIGURE 1. Results of four replicate experiments to determine percent fertilization of eggs when constant volumes of a series of 10, 10-fold dilutions of dry sperm (semen) was added to egg suspensions in stirred jars.

taining less than 10^4 sperm/l. Percent fertilization thus rapidly declined when dry sperm was diluted by 6–8 orders of magnitude.

In experiments to examine gamete longevity, diluted sperm suspensions lost potency rapidly so that less than 10% fertilization resulted when eggs were added to 20 min old sperm (Fig. 2A). Eggs, on the other hand, remained fertilizable for at least 90 min after spawning (Fig. 2B).

Results of the experiment to examine fertilization in still water indicated that little fertilization occurred during the first 20 minutes following spawning in extremely still water, but that under these conditions some fertilization continued to occur even 24 hours later (Fig. 3).

Field experiments

Results of the initial series of field experiments show that percent fertilization fell rapidly with increasing distance from spawning males (Fig. 4). Eggs were never fertilized in the control sample taken upstream of the spawning male, whereas over 90% were usually fertilized in the syringe from directly over the spawning male. At distances greater than 10 cm downstream from the male fewer than 25% of the eggs were fertilized, and at distances over 1 m, 10% or fewer of the eggs were fertilized. These low rates of fertilization clearly indicated that the numbers of sperm were limiting, and therefore

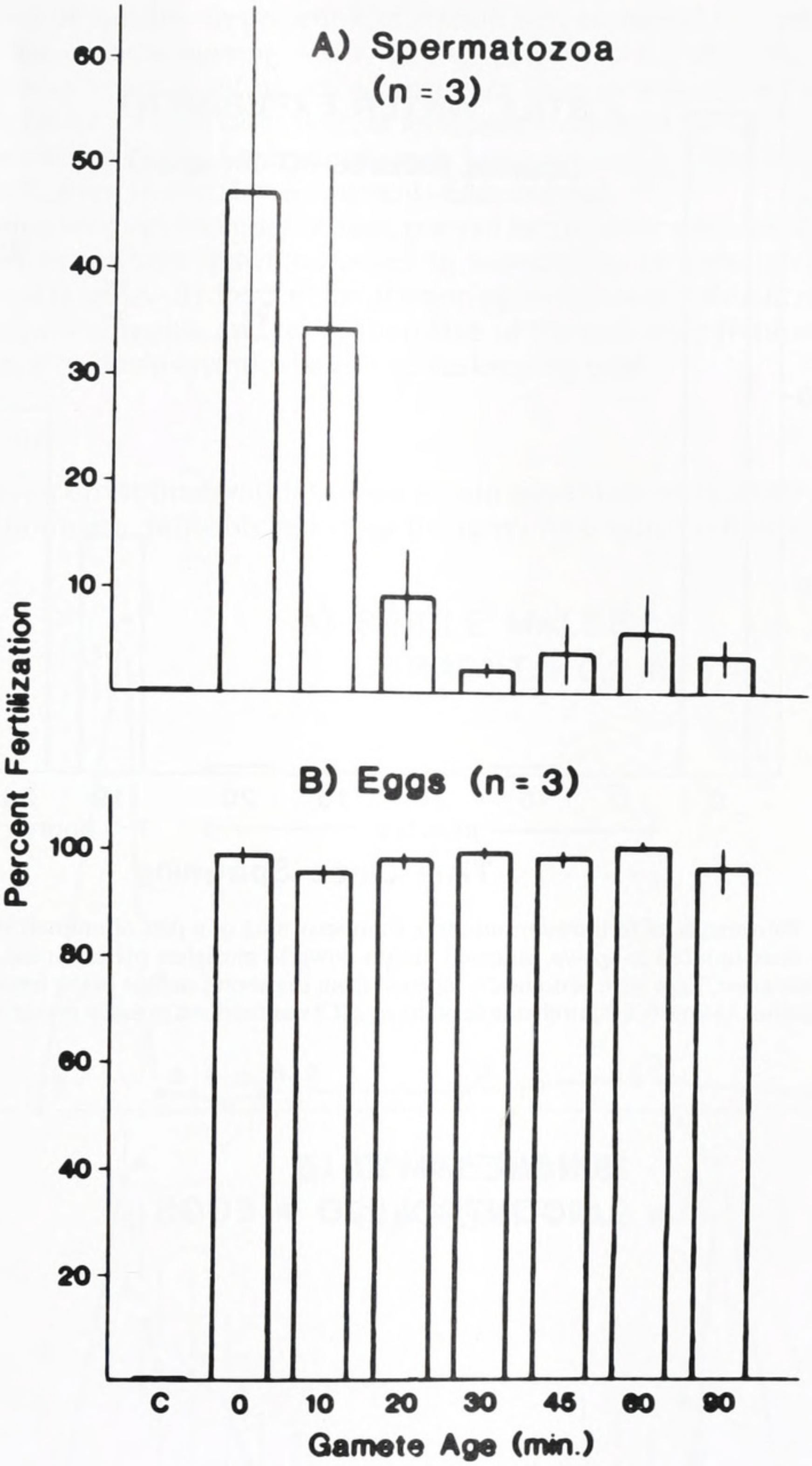

FIGURE 2. Mean percentages of fertilization (±1 standard deviation) resulting from experiments to examine gamete longevity. In A, eggs were added to diluted sperm suspensions of various ages. B is the converse of A, where fresh sperm suspensions were added to eggs of various ages. Three replicates of each experiment were conducted.

that the number of eggs per syringe should be controlled; in subsequent experiments constant numbers of eggs were added to the syringes prior to conducting field experiments. Figure 6A shows that this precaution did not substantially alter the results (for a similar result see Lillie, 1915).

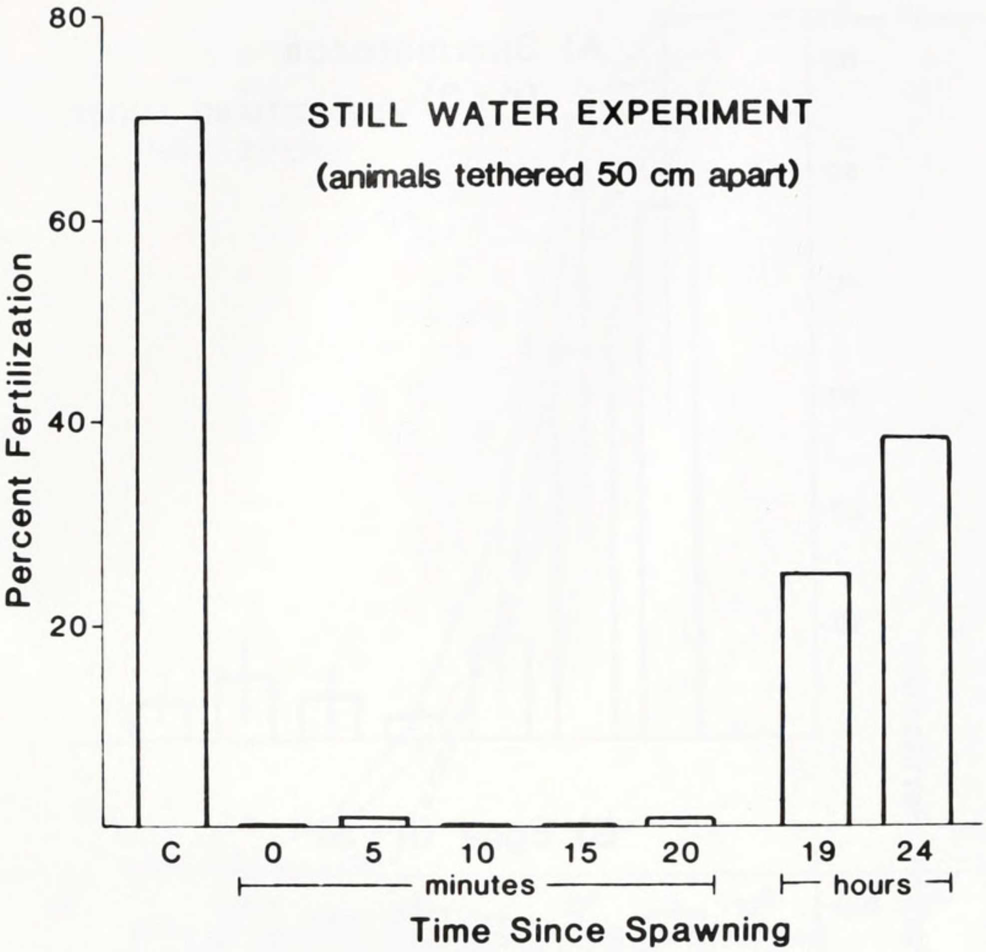

FIGURE 3. Percentages of fertilization resulting from spawning of a pair of animals in still water. A male and female were induced to spawn, strapped mouth down to plexiglass plates, rinsed, and placed in an aquarium 50 cm apart. Eggs were periodically pipetted from the aboral surface of the female and assayed for percent fertilization. At time 0, a control sample of the eggs (C) was fertilized to assess maximal fertilizability.

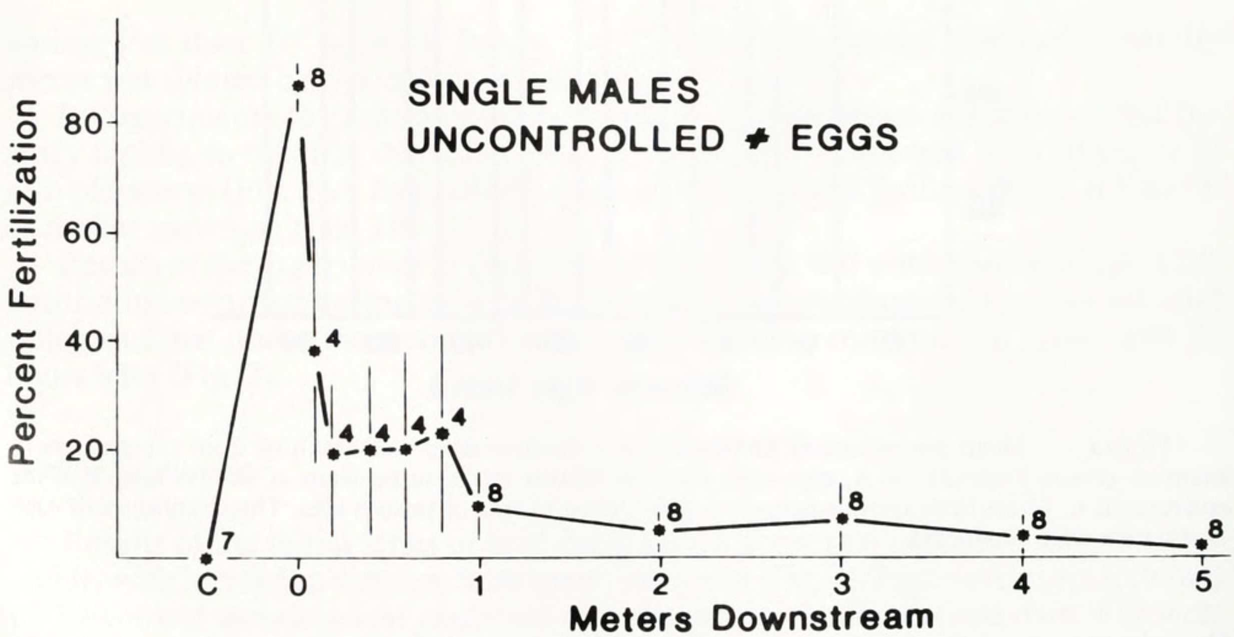

FIGURE 4. Mean percentages of fertilization (±1 standard error) resulting from spawning by single males in the field. Eggs were drawn into the syringes in the field, and 10 ml of water was then pulled into the syringes along a transect running downcurrent from a spawning male. The number above each mean is the number of replicates conducted at that distance from the male.

The effect of current on percent fertilization was examined by conducting experiments in fast or slow-moving water. In fast current (>0.2 m/s; Fig. 5A), percent fertilization was lower at all points downstream than in slower currents (<0.2 m/s; Fig. 5B). However, in both cases percent fertilization declined with increasing distance from the spawning male. At distances over 1 m, percent fertilization was generally less than 20%, even in the slowest currents encountered.

In the simulated epidemic spawnings, percent fertilization was higher at all distances downstream from three spawning males in comparison to experiments where one male was used (Fig. 6A–B). Percent fertilization again decreased with increasing distance from the spawning males, and fewer than 50% of the eggs were fertilized at distances greater than 1 m downstream when three males were used.

Questionnaire

Only seven direct observations of sea urchin spawnings were received in response to the questionnaire, probably reflecting the rarity with which echinoids are observed

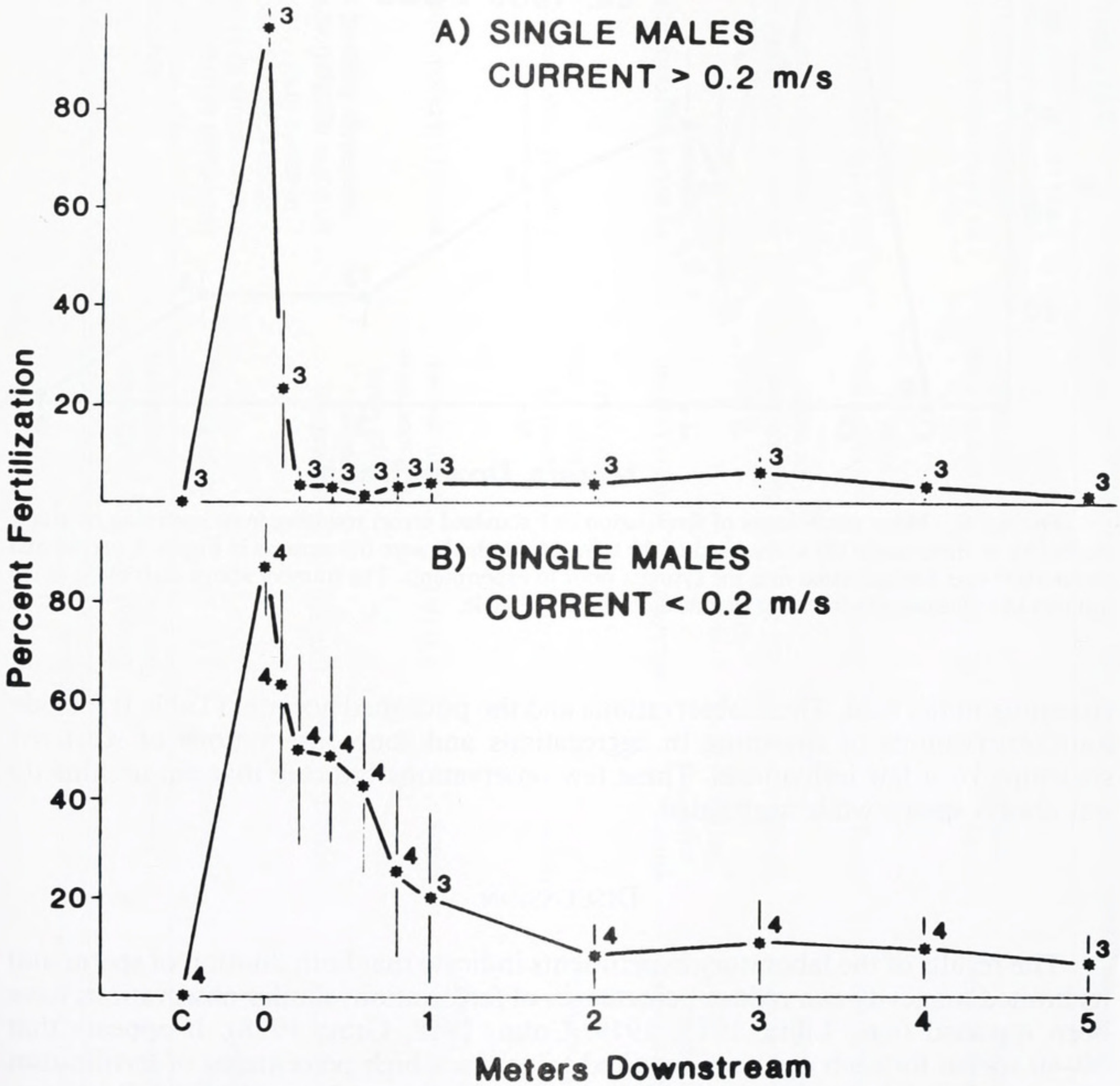

FIGURE 5. Mean percentages of fertilization (±1 standard error) resulting from spawning by single males in currents over 0.2 m/s (A), or under 0.2 m/s (B). Methods were the same as in Figure 4. The number above each mean is the number of replicates conducted at that distance from the male.

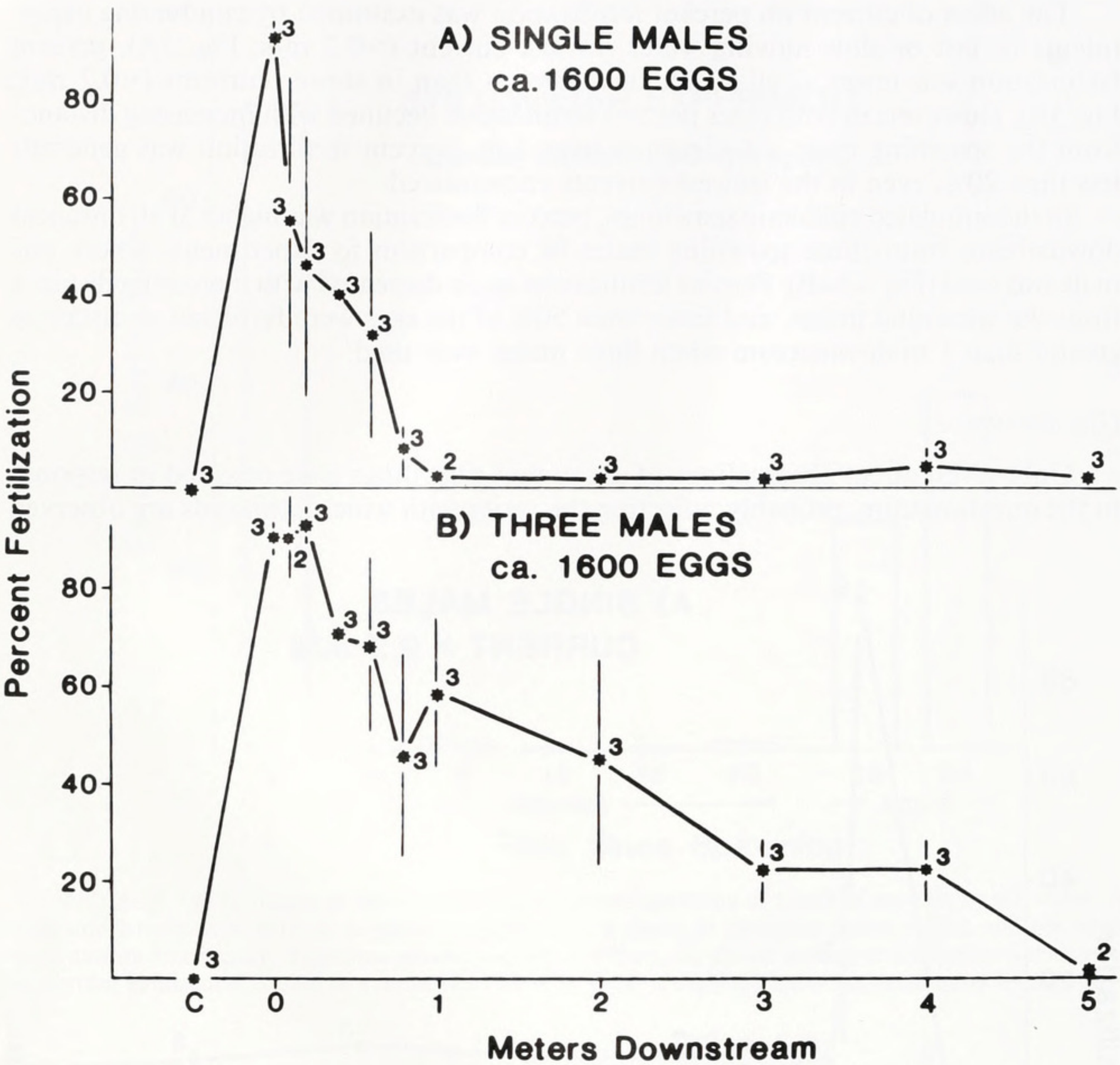

FIGURE 6. Mean percentages of fertilization (±1 standard error) resulting from spawning by single males (A), or three males (B) at the head of the transect. Methods were the same as in Figure 4, except that about 1600 eggs were pipetted into the syringes prior to experiments. The number above each mean is the number of replicates conducted at that distance from the male.

spawning in the field. These observations and the published account (Table I) include four observations of spawning in aggregations and four observations of scattered spawning by a few individuals. These few observations indicate that sea urchins do not always spawn while aggregated.

DISCUSSION

The results of the laboratory experiments indicate that both dilution of sperm and its limited longevity can reduce percentages of fertilization; similar observations have been reported (*e.g.*, Lillie, 1915, 1919; Cohn, 1918; Gray, 1928). It appears that 30–40 sperm for each egg were required to produce high percentages of fertilization (Fig. 1). Several workers have noted similar requirements (Gemmill, 1900; Branham, 1972; Sprung and Bayne, 1984; etc.) which are probably due to the kinetics of random sperm and egg encounters (Rothschild and Swann, 1951). The short potent life of

TABLE I

*Summary of direct observations of subtidal echinoid spawnings**

Species	Season	Density (ind./m^2)	Physical conditions	Spawning behavior	Observer
Diadema antillarum	year-round	1.2–13.4	some current	individuals aggregate year-round; several observations of some individuals within a tight group spawning; upcurrent individuals seemed to spawn first	Randall *et al.*, 1964
Lytechinus pictus	summer	100–500	substantial current; high water temperature	no active aggregation; random individuals spawning; observed several years	R. C. Fay, Pac. Bio-Mar. Labs., Venice, California
Unknown (Apra Harbor, Guam)	spring	75–100	slight current	strongly clumped; mass spawning	G. Pittenger, U.S.C. Mar. Lab., Avalon, California
Heliocidaris erythrogramma	spring	0.1–0.5	no swell or surge; a rare, oily calm	clumped; eggs later seen floating as "rafts"	S. A. Sheperd, Dept. Fisheries, Adelaide, S. Australia
Stronglylocentrotus franciscanus	winter: early spring	unknown	slight current	no active aggregation; scattered individuals spawning	G. Dennis, Comox Diving Services, Comox, British Columbia
Stronglylocentrotus franciscanus	unknown	*ca.* 5	little current or surge	dozens of individuals spawning near other conspecifics crushed by an anchor	J. S. Pearse, UCSC, Santa Cruz, California
Stronglyocentrotus purpuratus	spring	50–60	sea calm; no surge	no active aggregation; scattered groups spawning; crushed *S. franciscanus* nearby	C. T. Mitchell, MBC Applied Env. Sci., Costa Mesa, California
Stronglyocentrotus purpuratus	winter; spring	20–100	moderate surge; low salinity	no active aggregation; random individuals spawning in December and January; mass spawning in April; crushed conspecifics did not induce spawning among intact individuals	R. C. Fay, Pac. Bio-Mar. Labs., Venice, California

* Unpublished *personal communications* were obtained as responses to over 100 sets of questionnaires mailed to individuals and marine stations in North America.

diluted echinoid sperm (Fig. 2) is known as the "respiratory dilution effect" (reviewed by Chia and Bickell, 1983). In essence, sperm in dense suspensions remain quiescent as in the testis, but with dilution the sperm become increasingly active and are rapidly exhausted. Both of these factors restrict the conditions under which field spawnings might produce high percentages of fertilization. Sperm must not only be dense, but must be spawned (or diluted) only minutes prior to encounters with eggs.

In the field, percent fertilization was generally low at distances over 10 cm from spawning males. In laboratory experiments dry sperm diluted by 6–8 orders of magnitude produced similar percentages of fertilization. However, even in the slowest currents encountered, sperm was less than 10 minutes old before it drifted beyond the end of the transect. The limited longevity of diluted sperm thus did not affect results of experiments, and the decreases in percent fertilization observed were probably due to dilution alone. Even if the sperm were long-lived, they would probably be so quickly diluted after spawning that encounters with eggs in the plankton would be rare in any case. The results of all field experiments indicate that if males and females spawn at distances of even a few meters from each other, percentages of fertilization will be very much reduced in comparison to animals that spawn in close proximity.

In all field experiments the syringes contained enough eggs to, in effect, estimate sperm density in the surrounding water. I was thus unable to examine rates of fertilization under conditions where egg suspensions were very dilute. If females spawned some distance upstream from males, possibly inducing downstream males to spawn with a water-born pheromone (see Reese, 1966; Giese and Pearse, 1974), higher sperm-to-egg ratios per unit volume of water downstream from the males might allow higher percentages of fertilization to occur in these areas. However, because echinoid sperm only swim about 2 cm during their potent life (Gemmill, 1900) and do not detect eggs at a distance (reviewed by Rothschild, 1956; Chia and Bickell, 1983), only those eggs that drift directly past spawning males should be fertilized; eggs drifting elsewhere should not encounter sperm.

Percentages of fertilization in slow currents were higher than in swift ones (Fig. 5), presumably due to the increased rate of dilution of sperm in swift-moving water. However, if sea urchins spawn into extremely still water, laboratory results indicate that little fertilization would occur (Fig. 3). Eggs and sperm simply accumulated on the aboral surface of the spawning animals and did not mix, resulting in low rates of fertilization. Because the sperm remained undiluted on the aboral surface of the male, it remained potent and continued to fertilize some eggs for 24 hours after spawning began. These results are almost certainly laboratory artifacts; such extremely still water should rarely, if ever, occur in neritic habitats. In summary, the above results indicate that higher percentages of fertilization in the field will occur if free-spawning animals spawn into quiet, rather than swift-moving water.

When epidemic spawnings were simulated by placing three spawning males at the head of a transect, water downstream presumably contained more sperm per unit volume and thus percentages of fertilization were higher at all distances from the males (Fig. 6). As circumstances that produced high sperm densities also produced the highest percentages of fertilization, percent fertilization would probably be still greater if larger numbers of spawning males had been used.

It therefore appears that adults must first aggregate and then spawn synchronously if high percentages of fertilization are to occur. Mortensen (1938) did not believe that most asteroids exhibit such social behaviors, although at least three asteroid species aggregate or even pair during spawning seasons (Mortensen, 1931; Clemente and Anicete, 1949; Kubo, 1951). In contrast, Thorson (1946) suggested that synchronous spawning of adult aggregations occurs among most species of free-spawning invertebrates. However, Thorson (1946) based his predictions primarily on observations of laboratory spawnings, where a variety of stresses can cause spawning (Fox, 1924; Harvey, 1956) and where aggregation is typically enforced.

Echinoids have often been reported in aggregations during spawning seasons (reviewed by Boolootian, 1966; Reese, 1966), but it has rarely been determined if sea urchins are actually attracted to conspecifics or if they have simply converged upon some common resource such as food, or shelter during daytime (Mortensen, 1943; Moore *et al.,* 1963; Randall *et al.,* 1964; Pearse and Arch, 1969). In the single study where conspecifics have been shown to actively aggregate (Dix, 1969), the behavior was not confined to the spawning season and it was suggested that *Evechinus chloroticus* aggregates not for spawning purposes, but for mutual protection. Tennent (1910) did report that *Lytechinus variegatus* found in groups were often spawned-out, while scattered individuals contained gonads in varying states of maturity. Direct observations of echinoids spawning in the field (Table I) indicate that sea urchins do not always spawn in aggregations. It therefore remains possible that echinoids do not aggregate preparatory to spawning. However, some echinoids do migrate into shallow water during spring and summer, becoming abundant in the shallow subtidal (Elmhirst, 1922; Orton, 1929; Stott, 1931). Echinoids have also been reported to move in feeding "herds" or "fronts" (Foreman, 1977; Mattison *et al.,* 1977; Witman *et al.,* 1982; etc). Regardless of whether these behaviors are truly social interations, higher percentages of fertilization should result if sea urchins spawn during periods of high population density rather than if they spawn while scattered.

Although all experiments simulated synchronous spawnings, the brief potent life of dilute sperm and the absence of fertilization in control syringes filled upstream of spawning males both suggest that no fertilization would occur if spawning was asynchronous. However, the observations in Table I indicate that synchronous local spawning is typical. Presumably, local environmental cues synchronize gametogenic cycles and induce spawning ("proximal causes"; Baker, 1939). In the field proximal cues may be temperature and salinity changes or thresholds, lunar or tidal cycles, changes in quantity or quality of illumination, and increases in phytoplankton or food abundance (reviewed by Giese, 1959; Boolootian, 1966; Giese and Pearse, 1974; Himmelman, 1981; Chia and Bickell, 1983). However, the effects of most proximal cues on spawning remain questionable because portions of a local population commonly become spawned-out weeks before others (MacGinite and MacGinite, 1949), and conversely, because spawnings frequently occur when there has been little or no change in a cue (Giese and Pearse, 1974). Some of the above confusion may arise because it has often been difficult to distinguish between factors that entrain gametogenic cycles and those that initiate spawning. Results of laboratory experiments to examine proximal cues as spawning inducers are difficult to interpret because spawning is often induced by exposing animals to stimuli in quantities that are potentially stressful to adults, gametes, or embryos (Harvey, 1956; see Farmanfarmian and Giese, 1963; Andronikov, 1975; Greenwood and Bennett, 1981).

For echinoderms it is also unclear whether proximal cues might function by inducing entire populations to spawn, or whether they stimulate or stress a few susceptible individuals within a population to spawn. It is widely suggested that once spawning by one or a few animals is initiated, either the gametes or a pheromone released with them induce neighboring conspecifics to spawn (MacGinite and MacGinite, 1949; Hyman, 1955; Rothschild, 1956; Reese, 1966; Giese and Pearse, 1974; Kennedy and Pearse, 1975; Iliffe and Pearse, 1982). Fox (1924) and Lewis (1958) induced sea urchins to spawn by adding sperm suspensions to seawater in the laboratory, and Gemmill (1914, 1920) described similar experiments with starfish. However, many attempts to repeat these experiments have not resulted in spawning (Gemmill, 1900; Palmer, 1937; *pers. obs.*). In the field, Kěckeš *et al.* (1966) induced *Paracentrotus lividus* to spawn by exposure to homogenates of conspecific testes or ovaries, and two of the observations of spawning in Table I were made in the vicinity of sea urchins that had been crushed. In one instance the crushed individuals were conspecific to those that were spawning, but in the second case crushed *Strongylocentrotus franciscanus* may have induced individuals of *S. purpuratus* to spawn. Nevertheless, several of the observations of Table I indicate spawning by only some individuals within a locale.

Though all free-spawning invertebrates encounter the problem of gamete dilution, there is certainly variation in the mechanisms utilized to increase percentages of fertilization. As one example, sperm of some hydromedusae and species of benthic invertebrates chemotactically sense conspecific eggs and swim towards them (Miller, 1979, *in press*). In this case it may be advantageous for sperm to be long-lived or even to remain quiescent until they sense nearby eggs. The evolution of blocks to polyspermy (see Rothschild, 1956) in eggs of free-spawning species clearly indicates that, at least occasionally, eggs encounter sperm in abundance.

In summary, the experiments presented here indicate that if free-spawning adults fail to aggregate prior to spawning, as Mortensen (1938) proposed, percentages of fertilization will often be low. Conversely, if they spawn in aggregations percentages of fertilization will be high, as Thorson (1946) suggested. It remains uncertain as to whether echinoids are gregarious prior to spawning, but it appears that local spawning is often synchronous. If free-spawning invertebrates do not aggregate and then spawn synchronously, life-tables based on estimates of egg production may overestimate fecundity.

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