

Sea urchin spawning in benthic boundary layers: Are eggs fertilized before advecting away from females?

Philip O. Yund¹ and Susanne K. Meidel

School of Marine Sciences, Darling Marine Center, University of Maine, Walpole, Maine 04573

Abstract

Past work on fertilization in echinoids and other egg-broadcasting, free-spawning invertebrates suggests that these organisms might be extremely sperm limited in the field unless individuals spawn in close proximity and under nearly ideal flow conditions. However, virtually all previous experiments have used one or more techniques (surrogates for males and females, and short sampling duration) that bypassed two potentially important aspects of echinoid reproductive biology: the release of gametes in viscous fluids that cling to tests and spines, and extended longevity of eggs and undiluted sperm. We hypothesized that these attributes might interact with some flow regimes to facilitate time-integrated fertilization. Consequently, we explored fertilization processes in sea urchins induced to spawn in a benthic boundary layer in a flow-through flume, with a male 0.5 m upstream of a female. Our observations and data suggest that at free-stream flow velocities of 2.5 and 8.5 cm s⁻¹, gametes were slowly and continually advected from the aboral surfaces of spawning animals. Eggs on the surface of the aboral mass were often fertilized before they ablated from the surface; many advected eggs were fertilized after being trapped in the vortex downstream of the female. Gamete advection and fertilization continued for several hours, with the actual time course depending on flow velocity. Fertilization levels declined only slightly with increasing flow velocity. These results suggest that fertilization in echinoderms and other free-spawners with viscous, long-lived gametes could be much less sperm-limited than currently envisioned and have additional implications for population dynamics and selection on gamete characteristics.

Sexual reproduction via the release of sperm into the water column (free-spawning) is widespread among sessile and sedentary invertebrates and other marine organisms (e.g., algae, fish). The potential for gametes to be rapidly diluted after release and for sperm availability to limit successful fertilization has led to considerable recent interest in assessing fertilization levels under field conditions (reviewed in Levitan and Petersen 1995; Levitan and Sewell 1998; Yund 2000). Many free-spawners that brood eggs appear to possess sophisticated mechanisms for filtering dilute sperm out of the water and, hence, exhibit high fertilization levels under a broad range of ecological conditions (reviewed in Yund 2000). However, fertilization in egg broadcasters is generally thought to be extremely sensitive to short-term ambient sperm concentrations; hence, fertilization in these systems is expected to be sperm limited (Levitan and Sewell 1998). If sperm availability limits reproductive success at the population level, sperm limitation could affect population dynamics by reducing population growth at low density (a form of the Allee effect; Levitan 1991; Pfister and Bradbury 1996). Even intermittent sperm limitation of some individuals could have implications for the evolution of gamete characteristics (Levitan and Irvine 2001; *but see* Podolsky 2001; Randerson and Hurst 2001) and other aspects of life history strategies (Yund 2000).

Fertilization in broadcast-spawning echinoids is widely

expected to be limited by sperm availability. Because of the ease with which gametes can be manipulated and fertilization assayed, echinoids are an important model system for fertilization studies and have been the subject of numerous short-term field experiments (Pennington 1985; Levitan 1991; Levitan et al. 1992; Levitan and Young 1995; Wahle and Peckham 1999). As a group, these studies suggest that sperm limitation will be quite severe unless individuals spawn simultaneously, in close proximity, and under nearly ideal flow conditions. Consequently, Allee effects from sperm limitation at low population density have been incorporated into models of sea urchin population dynamics and harvest rates (Quinn et al. 1993; Pfister and Bradbury 1996). However, to date, there have been no measurements of fertilization levels in natural spawns of echinoids. All estimates of field fertilization levels are derived from the experimental studies cited above and models that are largely consistent with the conditions in these experimental approaches (Vogel et al. 1982; Denny 1988; Denny and Shibata 1989; Young 1994; *but see* Denny et al. 1992 for consideration of positive effects in more complex flows). However, if past experiments do not adequately mimic natural spawning conditions, current expectations about the severity of sperm limitation in this group might be unfounded (Yund 2000; Jumars et al. 2001).

All of the experimental field fertilization studies on echinoids cited above share an important feature: they were conducted with very short gamete exposure times (ca. ≤10–20 min) on the assumption that the rapid dilution of gametes and a limited period of gamete viability would render longer term assays irrelevant. With one exception (a portion of the work reported in Levitan 1991), these studies also employed experimental proxies for males (hypodermic syringes filled with concentrated sperm), females (eggs held in either

¹ Corresponding author (philyund@maine.edu).

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sperm-permeable baskets or syringes), or both. But these experimental protocols deviate greatly from expected conditions in natural spawns. Many sea urchins release gametes in a viscous fluid that resists dilution; after initial release, concentrated sperm can cling to the test and spines and diffuse away for hours (Thomas 1994a; Young 1994). Although dilute sperm are viable for only minutes (Chia and Bickell 1983), viable sperm continue to diffuse away from concentrated clumps for at least several hours (Meidel and Yund 2001). By contrast, when the plunger of a syringe (mimicking a male) is depressed, the single pulse of sperm released is immediately advected downstream (pers. obs.). Eggs are also released in a viscous fluid and cling to females after release, suggesting that they might be fertilized before dispersing into the water (Thomas 1994a; Young 1994). Eggs of some species are viable for at least several days (Meidel and Yund 2001), if not longer (Epel et al. 1998). Because eggs are negatively buoyant, even eggs that have been advected away from a female might not enter the free stream flow but could instead remain in a benthic boundary layer and be entrained in flow structures. If so, they could be fertilized throughout their period of viability if sperm subsequently become available from an upstream male. Hence, the interaction of gamete viscosity and longevity with non-turbulent field flow regimes has the potential to produce time-integrated fertilization levels that are much higher than those documented in short-term studies (Meidel and Yund 2001). Have past fertilization experiments with echinoids circumvented some of the very reproductive strategies that might have evolved to counteract gamete dilution (Yund 2000)?

We explored some of these issues for green sea urchins (*Strongylocentrotus droebachiensis*) that were induced to spawn in a benthic boundary layer in a flow-through flume. This flow regime is relevant for sea urchins living on relatively flat surfaces (e.g., bedrock) and spawning while exposed to nonoscillatory (e.g., tidal) currents. We asked whether gamete release and fertilization under these conditions is a short-term or time-integrated process and observed the behavior of spawned eggs in boundary layer flows. We used contemporaneous samples at different egg locations to evaluate whether fertilization occurred before or after eggs diffused from the aboral surface of the female. All experiments were repeated at two different free-stream flow velocities, permitting a preliminary assessment of the sensitivity of fertilization to flow variation.

Materials and methods

Flume—All experiments were conducted in a flow-through flume (modified from the design of Muschenheim et al. 1986) in the flowing seawater laboratory at the University of Maine's Darling Marine Center. The flume was constructed of 1.2-cm-thick acrylic with internal dimensions of 238.4 (length) \times 50.7 (width) \times 15.2 cm (depth) and filled with water to a depth of 10 cm. A width-to-depth ratio of 5 was selected to minimize sidewall effects on flow (Nowell and Jumars 1987). The 10-cm water depth permitted us to work with up to 3.5-cm test height *S. droebachiensis*

while keeping subject height to less than 35% of the water depth to avoid impeding flow within the flume (per Nowell and Jumars 1987; spines are assumed to have a negligible effect on cross-sectional area).

To keep water input constant, seawater (pumped from the Damariscotta River estuary) entered the flume via gravity feed from a cylindrical 200-liter tank with a constant head pressure. Water from the head tank entered the flume via a T-shaped plastic pipe (external diameter 6.5 cm), which was mounted with the top bar of the T oriented perpendicular to the long axis of the flume, 4.5 cm from the upstream end. Flow was adjusted with a valve located between the head tank and T-bar. Water exited the T-bar through a series of holes in the upstream edge and was directed toward the upstream wall. Water pressure was equalized across the channel via a porous filter made of crumpled fishing net, held in place with a sheet of commercial plastic lighting grate. Laminar flow was induced via a collimator placed immediately downstream of the grate. The collimator consisted of plastic drinking straws, 14.3 cm in length with 0.5 cm internal diameter, glued parallel to one another and completely filling the channel of the flume. Before each experimental trial, the collimator was rinsed with fresh water and replaced. Care was taken to eliminate all bubbles trapped within the collimator. After traveling the length of the channel, water exited the flume through five lower and four upper polyvinyl chloride (PVC) pipes (inside diameters 4.6 and 5.7 cm, respectively). Each pipe had a horizontal section 41.5 cm long and was fitted with a ball valve followed by a 90° elbow and a vertical section of 34–36 cm length.

Male and female mounting stations were established in the flume to hold spawning animals in place. Each station consisted of a pair of rubber bands oriented perpendicular to one another and encircling thin (0.1 cm thick) plates glued to the bottom of the flume. The center of each station, which coincided with the gonopore of the spawning sea urchin, was 25 cm from the side walls of the flume (*y*-axis). The center of the male station was 70 cm downstream (*x*-axis) of the collimator, and the center of the female station was an additional 50 cm downstream of the male. The bottom of the flume was then covered with a thin (~0.4 cm) layer of coarse sediment (grain size distribution by weight: 14.1%, $\leq 63 \mu\text{m}$; 21.0%, 64–249 μm ; 8.2%, 250–499 μm ; 56.7%, $\geq 500 \mu\text{m}$). The sediment served both to increase bottom roughness (resulting in a well-established benthic boundary layer over a larger portion of the flume) and to bury the plates used to restrain animals.

Velocity profiles—Fertilization experiments were conducted at two free-stream flow velocities. At each flow speed, we assayed velocity profiles at two locations in the flume by timing the horizontal advection of drops of fluorescent dye at 1-cm depth increments. Dye advection was timed by tracking location relative to transparent grids attached to both walls of the flume. These grids were marked at 1-cm intervals (1–9 cm) along the vertical (*z*) axis, and at 10, 20, and 40 cm along the longitudinal (*x*) axis. We used a stopwatch to time dye drops released from a pipette attached to a metal stand. Each assay was replicated six times, and the average was used to estimate flow velocity at that

depth. Velocity profiles were assayed immediately following the end of each experimental trial at the center of both the male and female station (i.e., at $y = 0$ cm). The last two trials in the high-velocity experiment (trials 5 and 6), were conducted in a single day and so shared the same velocity profile.

Fertilization trials—Fertilization trials at the low velocity (free stream flow = 2.5 cm s^{-1} , $n = 7$) were conducted 14 February–5 March 2001 and at the higher velocity (free stream flow = 8.5 cm s^{-1} , $n = 6$) 4–10 April 2001. At the beginning of each trial, one male and one female of similar size were selected from a pool of field-collected animals that had previously been biopsied to determine gender and held in flow-through tanks at ambient seawater temperature. Experimental animals had a test diameter of 5.50 ± 0.05 cm (mean \pm SE) and a corresponding test height of 2.93 ± 0.04 cm, but because spines and tube feet elevate the sea urchin slightly, gonopores on the aboral surface were located about 3.4 cm above the substratum. Both animals were injected with 2.5–4.0 ml of 0.5 mol L^{-1} KCl and placed in a plastic container. Once the female started to spawn, eggs were pipetted from the aboral surface for controls (*see below*); the animal was then very carefully placed in the flume and secured at the female station with the two rubber bands. As soon as the male started to spawn, he was similarly located at the male station. Females and males were generally placed in the flume within 1–6 min of one another. Positioning sea urchins in the flume resulted in little apparent disturbance of the egg or sperm pool that had started to accumulate on the aboral surface and that continued to form after deployment.

Once a discrete egg pool had accumulated on the aboral surface of the female, the trial was initiated and egg samples were collected after 15 min and 1 h, and then at additional 1-h (low velocity) or 30-min (high velocity) intervals. Sampling ended when either eggs or sperm were no longer visible on the spawning animals. At each interval, samples were collected at the following locations: (1) top of the egg mass on the aboral surface, (2) substratum immediately downstream of the female (except trials 3 and 7 at low velocity and trial 4 at high velocity), (3) plankton (except at 15 min in the low-velocity trials). Samples were collected by pipetting eggs from the surfaces of the egg pool and substratum and by catching eggs in a plankton net positioned over the outflow pipes of the flume (5-min sample duration at low velocity and 2.5 min at high velocity). During low-velocity trials, we also used a pipette to sample a fourth location: the bottom of the egg mass on the female's aboral surface. Eggs sampled from the female or the substratum were added to 10 ml aged (24–48 h) seawater plus 2 ml 37% formaldehyde in 20-ml glass vials. Eggs sampled from the plankton were rinsed from the plankton net with aged seawater and fixed with formaldehyde within 3 min of collection. Fertilization levels were calculated as the percentage of a random subsample of ~ 200 eggs (in the case of plankton samples, 50–100, or if <50 collected, all present) with a fertilization envelope. A two-way analysis of variance (JMP 4.0, SAS Institute) was used to test for time and position effects on the percentage of eggs fertilized (data were arcsine transformed prior to analysis). The total number of eggs exiting the flume

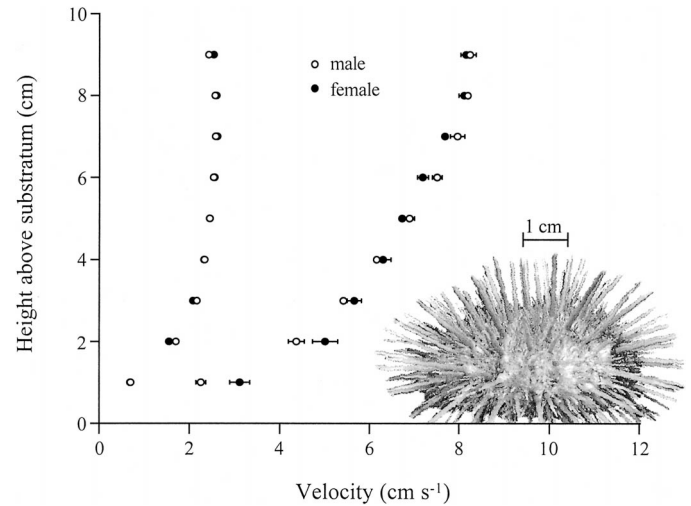


Fig. 1. Velocity profiles at the male and female stations at the conclusion of each experimental trial. Points represent the mean of seven (low velocity) and five (high velocity) measurements, and error bars (often obscured by the symbols) represent one standard error. The image of the sea urchin is to scale; gametes are released and accumulate on the aboral surface.

per unit time was estimated by counting the total number of eggs in an aliquot of the plankton sample.

Two control samples of eggs (held at 3°C) were assayed for (1) ability to fertilize at the start of each trial and (2) the presence of false fertilization envelopes (or sperm contamination). For both controls, dry eggs were pipetted from the surface of the female as soon as spawning started and added to 10 ml aged seawater in 20-ml glass scintillation vials. For fertilization controls, we added a small amount of sperm pipetted from the sperm cloud above the spawning male in the flume. Vials were gently agitated three times during a 15-min period, following which the fertilization process was stopped with the addition of 2 ml formaldehyde. Controls for false envelopes or sperm contamination were casually inspected at the beginning of a trial and then fixed and quantified (per the flume samples) at the end of each trial.

Results

Velocity profiles—Dye visualizations indicated that at both free-stream flow velocities, a well-developed benthic boundary layer was established throughout most of the flume. Velocity data collected immediately after each fertilization trial revealed consistent vertical flow profiles at the male and female stations during experiments (Fig. 1). At the lower velocity, the boundary layer (defined as the region where velocity $<99\%$ of free stream flow; Denny 1988) ended about 4 cm off the substratum, whereas at higher velocity, it extended up to about 8 cm (Fig. 1). As a consequence, gamete release in the low-velocity experiment occurred near the upper edge of the boundary layer, whereas in the high-velocity trials, gametes were released more than midway down the boundary layer (Fig. 1). Near-bottom flows (1 and 2 cm above the substratum) at the female station were 15–38% higher than at the male station during the high-velocity

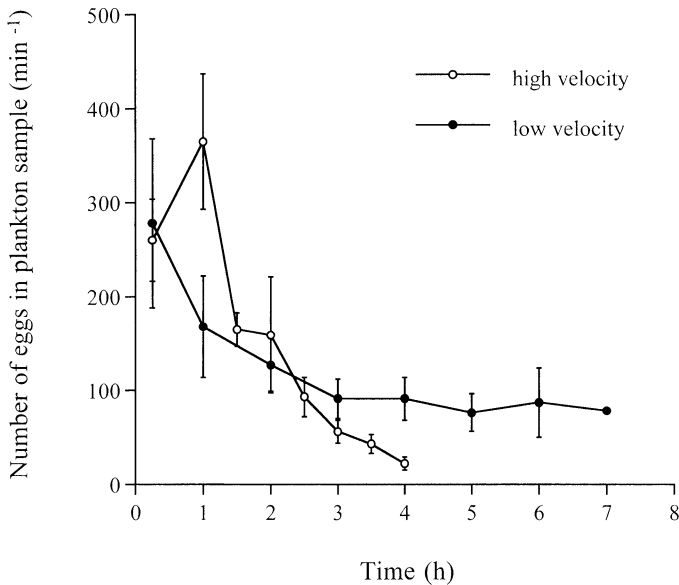


Fig. 2. Temporal pattern of eggs exiting the flume (as sampled with the plankton net) at the two different free-stream flow velocities. Each point represents the mean of seven (low velocity) and six (high velocity) measurements, and error bars (often obscured by the symbols) represent one standard error.

experiment (reflecting a location 0.5 m further downstream of the male), but velocities at the height of gamete release were comparable (Fig. 1). Note that the actual height of gamete release (Fig. 1; 3.4 cm) is slightly higher in the water column than the test thickness of the experimental animals because tube feet and spines hold the underside of the test slightly above the substratum. No edge effects were detectable within 10 cm of the center of the flume (i.e., along the y-axis; unpubl. velocity profile data). Flow in the downstream portion of the flume did not appear to be affected by backflow upstream of the exit gates (pers. obs. from dye visualizations).

Fertilization trials—Qualitatively, gametes behaved much as predicted. Masses of sperm and eggs initially accumulated on the aboral surfaces of the males and females, then these piles slowly shrank as gametes were ablated from the surface. Eggs that were advected from the aboral surface were often entrained in a vortex that formed downstream of the female and either continued to recirculate within the vortex or fell to the substratum. Over time, eggs in the vortex or on the substratum were advected downstream and exited the flume. We did not attempt to determine what fraction of eggs were transported through the free stream flow versus the benthic boundary layer. At low velocity, the total number of eggs exiting the flume per unit time (as collected in the plankton net) declined steadily from a maximum of 278 min⁻¹ at 1 h to a minimum of 78 min⁻¹ at 8 h (Fig. 2). In the high-velocity experiment, the number of eggs exiting initially declined from 260 min⁻¹ at 30 min to a maximum of 365 min⁻¹ at 1 h, then declined steadily to a minimum of 22 min⁻¹ at 4 h (Fig. 2).

Both time and sample location affected fertilization levels

Table 1. Effect of time and sample location on fertilization. Analysis of variance on arcsine-transformed fertilization levels with time and sample location as main effects.

Source	df	SS	F ratio	p > F
Low-velocity experiment				
Time	6	1.22	6.71	<0.0001
Sample location	3	28.74	315.15	<0.0001
Error	148	4.50		
High-velocity experiment				
Time	7	1.03	3.03	0.0006
Sample location	2	4.97	51.00	<0.0001
Error	102	4.97		

in the low-velocity experiment (Table 1). Post hoc tests indicated that fertilization levels were significantly lower at the first sampling interval (Tukey honestly significant difference [HSD], $p < 0.05$), whereas minor differences among later intervals were not significant (all Tukey HSD, $p > 0.05$; Fig. 3A). Fertilization levels differed significantly among all sampling locations, with the exception of the substratum and plankton, which were indistinguishable (Tukey HSD). Fertilization was lowest at the bottom of the egg mass on the aboral surface, intermediate on the surface of the egg mass, and highest in the plankton and substratum samples (Fig. 3A). Although significantly lower, fertilization levels on the surface of the egg mass nevertheless averaged ~50% of the level in contemporaneous substratum and plankton samples (after the first sample, 43.4% vs. 93.1 and 89.4%, respectively; Fig. 3A). Fertilization levels of eggs sampled from the bottom of the mass on the aboral surface were low at all sampling intervals, fluctuating between 1 and 12% (Fig. 3A). Overall, we estimate that approximately 87% of the eggs were fertilized in this experiment (calculated as the average of the fertilization levels of plankton samples at each time point weighted by the proportion of the eggs exiting the flume at that time).

A similar pattern emerged in the high-velocity experiment (Fig. 3B), although fertilization levels were somewhat lower at all stations sampled. Both time and sampling location significantly affected fertilization levels (Table 1). Fertilization levels in the first sample were significantly lower than at 1, 2, 3, and 3.5 h (Tukey HSD; Fig. 3B). Fertilization levels in samples from all three stations differed significantly (Tukey HSD), with values for plankton samples slightly lower than those for substratum samples (Fig. 3B). Fertilization levels of eggs from the surface of the egg mass again averaged ~50% of the level in comparable substratum and plankton samples (34.5% vs. 79.7 and 68.4%, respectively, after the first interval). Because fertilization levels in plankton samples were intermediate to those for the substratum and aboral surface samples, eggs exiting the flume must have been derived from a combination of different locations. The bottom of the egg mass on the aboral surface was not sampled in the higher velocity experiment. Overall, ~58% of the eggs were fertilized in this experiment, indicating a 33% decrease in total fertilization over a 3.4-fold increase in free-stream velocity.

Controls to ensure that eggs could be fertilized at the be-

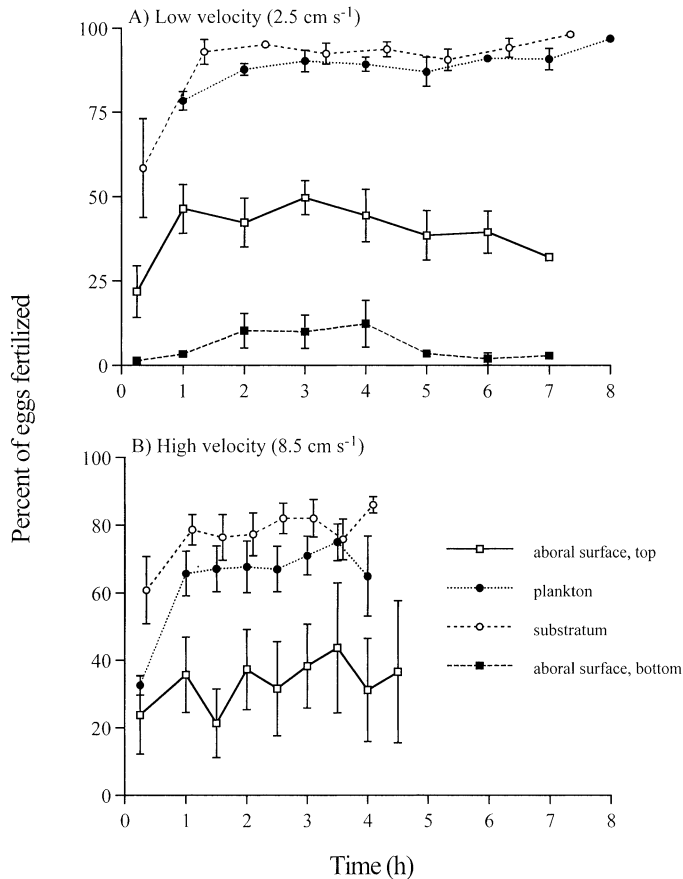


Fig. 3. Temporal pattern of fertilization levels at main stream flow velocities of (A) 2.5 and (B) 8.5 cm s⁻¹. Symbols represent values for contemporaneous samples collected at four different locations: surface of the mass of eggs on the aboral surface of the female, bottom of the mass of eggs on the aboral surface of the female, substratum immediately downstream of female, and exiting the flume (collected with a plankton net). Sample sizes range from $n = 5$ to 7 (see text for details), and error bars represent one standard error.

gining of the trials yielded very high fertilization levels (low velocity, $97.8 \pm 0.8\%$, $n = 7$; high velocity, $99.1 \pm 0.3\%$, $n = 6$). Controls for false fertilization envelopes indicated a very low incidence of false envelopes in lab samples (low velocity, $0.0 \pm 0.0\%$, $n = 7$; high velocity, $0.3 \pm 0.2\%$, $n = 6$).

Discussion

In the well-established benthic boundary layer of our flume (Fig. 1), eggs and sperm clung to sea urchins, and fertilization continued for extended periods of time. Eggs were continually ablated from the aboral surface (pers. obs.) and exited the flume (Fig. 2). Fertilization levels on the aboral surface were generally about half those in contemporaneous substrata or plankton net samples (Fig. 3). This comparison suggests that about half of the fertilization events took place before eggs were advected away from the aboral surface. Many of the advected eggs did not immediately en-

ter the free stream flow but were instead trapped in the vortex downstream of the female, either recirculating in that eddy or falling to the substratum. In the higher velocity experiment, fertilization levels in substratum samples were slightly higher than in samples of eggs exiting the flume (Fig. 3). Hence, fertilization apparently continued after eggs were advected from the aboral surface of the female, and many fertilizations might have occurred in the eddy downstream of the female. Although gamete advection and fertilization continued for hours at both flow velocities, the supply of gametes was exhausted more quickly at higher velocity (Fig. 2). At both velocities, fertilization levels increased significantly between 15-min and 1-h samples, suggesting that short-term assays might underestimate fertilization. Overall, these results suggest that the current paradigm of sea urchin fertilization as a short-term water column process might not be valid. The interaction of innate gamete properties with flow in benthic boundary layers could enhance fertilization levels beyond that predicted by experiments that bypass life history adaptations and inject gametes into free stream flow (Jumars et al. 2001).

Unfortunately, we lacked the instrumentation to quantify turbulence intensity in our experimental flows. However, turbulence is likely to play a major role in dispersing and diluting gametes and will thus affect both fertilization levels and the site of fertilization (water column vs. egg mass). Turbulence intensity in our flume experiment is likely to have been relatively low because we used a collimator to straighten the entrance flow and employed a low roughness substratum (coarse sand). Thus, our conclusions are limited to low-turbulence flows, and future work should address the effects of turbulence on fertilization in boundary layer flows.

Although the flow regimes that we explored were relatively benign, they nevertheless appear to be quite relevant to natural populations. Portions of the *S. droebachiensis* population inhabit shallow, high-energy, wave-swept environments, but in the northwest Atlantic, this species is abundant to depths of 12–20 m and occurs on wave-protected as well as wave-exposed surfaces (McNaught 1999; Wahle and Peckam 1999; Witman and Dayton 2001). Free-stream flow velocities in the deeper or more protected portions of sea urchin habitat are routinely <10 cm s⁻¹ on calm days (Nova Scotia, Balch et al. 1999; Gulf of Maine, J. D. Witman and C. Siddon unpubl. data). Turbulence intensity is expected to be low in nature in well-established boundary layer flows over relatively flat, low-roughness surfaces, such as the flat ledges of granite bedrock that are common on this coast.

Although our data indicate that a substantial portion (about 50%) of the fertilizations occurred on the surface of the egg mass on the aboral surface of the female, we believe that our sampling technique actually resulted in an underestimate of this effect. The continual export of eggs from the flume (Fig. 2), combined with a time series of relatively high instantaneous fertilization levels on the egg mass (Fig. 3), suggests that a new layer of eggs was continually fertilized as each older layer was ablated away. However, our simple pipette technique could not sample only the single most exposed surface layer of eggs. Each sample would actually have contained a mix of eggs from the surface and deeper within the mass. Because fertilization levels were

substantially lower at the bottom of the mass (Fig. 3A), inclusion of eggs from deeper levels is likely to have reduced the fertilization estimates nominally attributed to the surface layer. More careful sampling of the surface layer of eggs might have revealed that a greater percentage of the fertilizations occurred before eggs were advected from the mass.

In addition to this sampling problem, two other potential experimental artifacts could also have led us to underestimate the proportion of fertilizations occurring on the egg mass by artificially inflating fertilization estimates for our plankton samples. First, physical damage can produce a false fertilization envelope, and we did not test for successful development of apparently fertilized eggs. Because only eggs in the plankton samples were exposed to substantial disturbance (in the course of traversing the exit tubes of the flume), false fertilization envelopes from egg damage could have inflated fertilization estimates in the plankton versus the other two samples. Second, eggs collected in the plankton net were likely exposed to a dilute sperm solution during the interval necessary to obtain an adequate sample. Again, we have no independent test of this possible bias, but if it occurred, it could only have resulted in an underestimate of the proportion of fertilizations occurring in egg masses on the aboral surface of the female.

Comparison of time-integrated fertilization levels of eggs exiting the flume at the two different free-stream flow velocities (Fig. 3; $\sim 87\%$ at 2.5 cm s^{-1} and 58% at 8.5 cm s^{-1}) indicates overall higher fertilization levels and less reduction in fertilization with velocity than demonstrated in short-term experiments (Pennington 1985) or predicted by models that assume complete and instant gamete solubility in seawater (Denny 1988). Because gonopores were within the benthic boundary layer, the flow velocities at the height of the gonopores were lower than the free-stream velocities (Fig. 1; ~ 2 and 6 cm s^{-1} , respectively). This is not an experimental artifact, but a reasonable approximation of how benthic boundary layers will interact with spawning animals in nature. Reduced velocities within well-developed boundary layer flows might mitigate some of the effects of variation in free-stream velocity. The relatively high fertilization levels that we report were also influenced by the position of the females directly downstream of males; fertilization levels away from the main axis of flow would probably have been reduced.

Evidence to date suggests that the strong selective pressure exerted by sperm limitation has resulted in life history adaptations that maximize successful fertilization in many free-spawning marine organisms (Yund 2000). In perhaps the best studied system to date, fucoid algae, which typically occur in sea urchin habitat (and like sea urchins, broadcast both male and female gametes) spawn in response to drops in dissolved inorganic carbon levels (Pearson et al. 1998) that are associated with the cessation or minimization of water movement (depending on the species; Pearson and Brawley 1996; Serrão et al. 1996). As a result, nearly 100% of eggs are fertilized (Brawley 1992; Pearson and Brawley 1996; Serrão et al. 1996), and a phenomenon associated with high sperm concentrations (polyspermy) might limit reproductive success under some environmental conditions (Brawley 1992; Serrão et al. 1999). Yet water motion in

typical fucoid habitat (exposed rocky shores) is substantial much of the time, and so fucoids could arguably be expected to be just as subject to sperm limitation as sea urchins. If initial field fertilization experiments with fucoids had induced spawning under the turbulent, oscillatory, wave-driven flows that are prevalent in this habitat, instead of striving to understand natural spawning patterns and processes, we might have a different perspective of fertilization in these organisms. Has a past focus on dramatic hydrodynamic regimes (vs. those in which spawning might occur) and experimental approaches that bypassed reproductive strategies that minimize gamete dilution resulted in an overly pessimistic assessment of the likelihood of fertilization in echinoids?

The interactive effects of gamete viscosity, longevity, and boundary layer flows that we have explored here are probably relevant to fertilization in a variety of other free-spawning taxa. Numerous taxa release gametes in viscous fluids (Thomas 1994b) and have extended periods of gamete viability (Bolton and Havenhand 1996; Williams and Bentley 2002). Boundary layer flows can be particularly relevant for infaunal invertebrates. Our interest in echinoderms arose because of the convenience of this model system and the central role that experiments on echinoderms have played in formulating the sperm limitation paradigm, not because we thought that adaptations to minimize the potential effects of sperm limitation were especially unique in this group. However, we readily acknowledge that different mechanisms could be important in other groups. Although we feel our results are likely to be directly applicable to a number of other taxa, we mainly advocate the general principle of incorporating relevant life history characteristics when designing fertilization experiments with diverse organisms.

Our results have implications for at least two other issues in the fertilization ecology of free-spawning marine organisms. First, the ongoing debate about whether selection by sperm limitation acts on egg, jelly coat size, or both (Levitan and Irvine 2001; Podolsky 2001; Randerson and Hurst 2001) implicitly assumes that fertilization occurs when sperm encounter individual eggs suspended in the water column. But the fertilization consequences of variation in egg and jelly coat size can be very different if sperm routinely encounter large masses of closely packed eggs. Second, models of population dynamics of free-spawners have started to incorporate Allee effects from sperm limitation at low population densities (Quinn et al. 1993; Pfister and Bradbury 1996). Although our work does not preclude the existence of such an effect (indeed, below some population density it is inevitable), it suggests that the threshold density for fertilization failure in echinoderms could be far lower than currently envisioned. Sperm availability in the field (as measured by time-integrated, rather than short-term, assays) can be high even several meters from possible sperm sources (Meidel and Yund 2001).

Although our current results raise doubts about the prevailing paradigm for fertilization in echinoids and other broadcasting free-spawners, they generate more questions than they answer. We have explored the fertilization consequences of only one easy-to-generate flow regime, over a very limited range of free-stream flow velocities and with

males and females separated by a single fixed distance (oriented parallel to flow). Fully evaluating the issues we raise here requires additional empirical work on velocity, turbulence, distance, direction, and population density effects on fertilization in boundary layer flows and in the more complex oscillatory flow regime of breaking waves. In addition, better information on the hydrodynamic conditions under which echinoderms actually spawn in nature is sorely needed to assess what flow regimes are relevant to spawning in natural populations.

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