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Spawning events in small and large populations of the green sea urchin *Strongylocentrotus droebachiensis* as recorded using fertilization assays

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Abstract

During the winter and spring of 2002 and 2003, we used time-integrated fertilization assays to monitor sperm availability in three populations of the green sea urchin *Strongylocentrotus droebachiensis* in Maine: a naturally occurring population of >40,000 urchins and two smaller groups (<1,000) of transplanted urchins isolated from other aggregations. Episodes of sperm release coincided in two populations 10 km apart, suggesting that urchins were responding to a widespread environmental signal. We observed significant lunar periodicity in sperm release events for both of these populations. However, extensive spawning as shown by fertilization rates near 100% and a dramatic drop in gonad mass only occurred in the large natural population around the onset of thermal stratification and the spring phytoplankton bloom. By contrast, in the two small populations we observed low fertilization rates and little or no change in gonad mass. We speculate that a subset of males in these populations responded to a common external spawning signal, but that mass spawning is more likely to occur in large, dense populations where sperm concentrations reach high enough levels to trigger spawning in less responsive urchins.

For benthic free-spawning organisms that release gametes in the water, timing of spawning is critical to reproductive success, especially with respect to two processes: fertilization and larval development. First, the degree of spawning synchrony between males and females will strongly influence fertilization rates because of the limited longevity and rapid dilution of gametes (Pennington 1985; Levitan 1995). Second, spawning should occur at a time of year when pelagic larvae have a high probability of surviving and developing (Himmelman 1999). One way to synchronize gamete release and ensure early survival is to link the spawning process to environmental cues that predict favorable conditions (Giese and Kanatani 1987; Pearse 1990; Himmelman 1999). Despite the vast literature on reproduction in broadcast spawners, our understanding of environmental cues remains speculative largely because of the difficulty of recording spawning events with high temporal resolution.

Although the literature on reproduction in marine organisms reflects a long-standing interest in environmental spawning cues (e.g., Giese and Kanatani 1987; Himmelman 1999), our understanding of the dynamics of fertilization during spawning has mainly developed over the past two decades. The influence of the physical environment, particularly advection and diffusion, on fertilization success has been studied intensively (Pennington 1985; Denny and Shibata 1989; Yund and Meidel 2003). Because of the rapid dilution of gametes, marine free-spawners at low population density or in smaller aggregations may face severe fertilization failure (i.e., Allee effect; Petersen and Levitan 2001). Consequently, low per-capita reproductive performance may prevent depleted populations from recovering and even lead to local extinction (Quinn et al. 1993; Pfister and Bradbury 1996).

It is feared that a reproductive failure scenario applies to the green sea urchin, *Strongylocentrotus droebachiensis* (O.F. Müller) in the Gulf of Maine. This free-spawner has been intensively harvested in New England and in the Canadian Maritimes since the late 1980s. Now many populations have been depleted, repeating the familiar fate of exploited sea urchin populations elsewhere (Keesing and Hall 1998; Andrew et al. 2002). In addition, urchins over extensive areas along the Atlantic coast of Nova Scotia are subject to occasional mass mortalities caused by a protozoan parasite (Scheibling and Hennigar 1997).

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Although per capita gamete production can rise with the increase in algal food availability that occurs as urchin densities decline, it is not likely to offset the consequent drop in fertilization success (Wahle and Peckham 1999).

The question of whether depleted marine free-spawners are subject to severe reproductive failure because of sperm limitation remains unresolved (Levitan and Petersen 1995; Yund 2000). Reports of direct measures of fertilization success during spawning have been scarce and fortuitous (Yund 2000). Although echinoids have been extensively used in fertilization and spawning studies (e.g., Levitan et al. 1992; Starr et al. 1993; Yund and Meidel 2003), few workers have quantified fertilization success during natural echinoid spawnings (Levitan 2002). In other taxa, in which fertilization has been measured during natural spawning, fertilization success is reported to be highly variable (reviewed by Levitan and Petersen 1995; Yund 2000). Interpreting the degree of sperm limitation from measures of fertilization success at specific points in time remain difficult because of limited knowledge of the environmental factors triggering spawning and a failure to adequately document the time course and spatial extent of spawning in populations.

No field studies have convincingly demonstrated the relation between environmental cues and spawning for sea urchins. Several studies have suggested that spawning in *S. droebachiensis* is triggered when urchins detect an increase in phytoplankton during the spring bloom (Himmelman 1975; Starr et al. 1993; Vadas and Beal 1999). Studies of other echinoids have documented an association with the lunar cycle (e.g., Kennedy and Pearse 1975) or temperature change (e.g., Lamare and Stewart 1998; Vadas and Beal 1999). Reports on other marine organisms that co-exist with the green sea urchin (e.g., *Fucus* spp.; Pearson et al. 1998; Berndt et al. 2002) infer that specific hydrodynamic conditions trigger gamete release.

Recently, two studies of *S. droebachiensis* have demonstrated the utility of time-integrated fertilization assays in evaluating sperm availability and characterizing spawning events (Meidel and Yund 2001; Wahle and Gilbert 2002). Fertilization assays measure the fertilization rate of mature ova placed in mesh containers in the field and can provide a detailed record of natural spawning events over the spawning season. These assays have the potential to provide greater temporal resolution of the spawning process than gonad indices, which are difficult to sample more frequently than once or twice per week (e.g., Starr et al. 1993; Vadas and Beal 1999).

In the present study, we used time-integrated fertilization assays at various distances from aggregations of sea urchins in the field to better understand the factors inducing spawning and to gain insight into the severity of sperm limitation during natural spawning events. Our specific objectives were (1) to monitor sperm availability over the spawning season in and near sea urchin aggregations of different sizes, and (2) to assess the association of spawning events with changes in temperature, phytoplankton, sea state, and lunar periodicity.

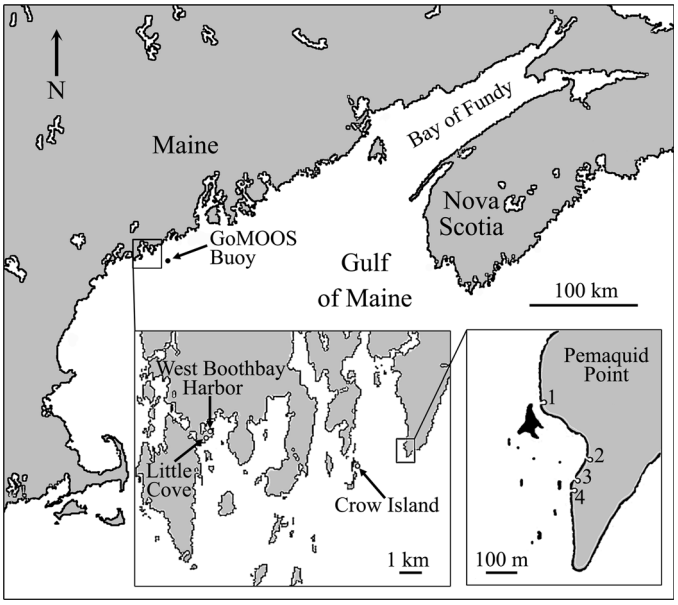

Fig. 1. Location of the three sites in the Gulf of Maine. At Pemaquid Point, black areas show the positions of the natural aggregations and the numbers 1–4 indicate the location of the fertilization assay stations. Little Cove was the site of a transplanted urchin aggregation; a caged aggregation was maintained at West Boothbay Harbor. Crow Island was the source of urchins transplanted to Little Cove and West Boothbay Harbor.

Methods

Study sites and populations—Our study was conducted during the spawning season of the green sea urchin in the Gulf of Maine (March to May; Stephens 1972; Meidel and Scheibling 1998; Vadas and Beal 1999) in 2002 and 2003. We studied spawning at three sites along the coast of Maine (Fig. 1), each with an urchin aggregation of a different size.

The largest was a natural population on the exposed rocky shore at Pemaquid Point (43°50′N, 69°31′W). This site is particularly exposed to south and southwest wind where fetches range from 6.6 (SW) to >100 km (S). Pemaquid Point was one of six state-managed sea urchin conservation areas, and harvesting was not permitted. It supported one of the largest remaining, accessible urchin aggregations within a 10-km radius. Diving surveys conducted at the end of the 2003 spawning season revealed that urchins occurred in several distinct patches ranging in area from 4 to 3,700 m^2 at depths between 2 and 15 m below mean low water (Fig. 1). The density of urchins (>2 cm in diameter) in the patches varied from 10 to 42 individuals m^{-2}, and we estimate the total population within the conservation area to be between 40,000 and 80,000 individuals. A NORTEK Vector current meter placed at 0.5 m above the barrens at Pemaquid Point (10 m deep) over a complete spring-neap tidal cycle (10–25 May 2003) showed that mean flow velocity was 0.07 m s^{-1} (SD = 0.05).

The second site, which we will refer to as Little Cove, was located just outside West Boothbay Harbor and approximately 10 km west of Pemaquid Point (Fig. 1). It

is a well-protected cove with a narrow degree of exposure to southeast wind (maximum fetch is <9 km). We chose this location because of its isolation from other urchin populations and its accessibility for servicing fertilization assays. During an initial dive survey of this site in February 2002 we found about 100 adult sea urchins aggregated on a small ledge (<10 m^2) at 2 m below mean low water. At that time, we added 900 urchins, which we collected from a natural population at Crow Island (and this made the initial density ~ 100 individuals m^{-2}). Subsequent dives during the experiment indicated that most of the urchins remained on the ledge, but by May the aggregation had declined to about 400 individuals. By February of the next year (2003) the urchins had completely disappeared from Little Cove, as had occurred in a number of other locations that year (possibly a result of predation by the Jonah crab, *Cancer borealis*, during the previous summer). We transplanted 1,000 more urchins from Crow Island to Little Cove for the 2003 assays and the population declined in a similar manner as during the previous year. The mean current velocity recorded when the Vector current meter was deployed near the urchin aggregation at 1.5 m depth from 24 April to 08 May 2003 was 0.02 m s^{-1} (SD = 0.02).

The third site was inside West Boothbay Harbor where initial studies with time-integrated fertilization assays had been conducted by Wahle and Gilbert (2002). This site is a protected embayment with a mainly muddy bottom and no naturally occurring urchins. Maximum fetch at this site is >1.4 km. The flow regime at 0.1 m above the bottom, 2 m below the mean low water (as assessed by Wahle and Peckham 1999), was nondirectional with velocities <0.02 m s^{-1}. In February 2002, we transferred 500 urchins from Crow Island to this site and divided them equally between two wire mesh cages (110 × 65 × 50 cm). One cage was placed 2 m below mean low water next to a pier and was used for the fertilization assays. The second cage was placed at the same depth next to a second pier, located 160 m away, and these urchins were used for periodic determinations of gonad indices. The use of the second cage meant that the fertilization rates near the first cage would not be affected by decreasing numbers of urchins. Urchins in both cages were fed kelp every other week.

Fertilization assays—We obtained gametes for the fertilization assays from adult urchins that were maintained at ambient seawater temperatures in the flowing seawater facilities at the Maine Department of Marine Resources and the University of Maine's Darling Marine Center. For each assay period we used eggs from two to four females. The eggs were obtained by injecting 2 mL of 0.5 mol L^{-1} KCl solution into the peristomial cavity of the urchins. Several urchins were injected at once with different needles to avoid sperm contamination. The females were spawned into a dry glass beaker kept on crushed ice. To select the best eggs, we examined a subsample of eggs for each spawned female under a compound microscope. Only high-quality eggs (>95% mature, >90% fertilizable, and >90% without a raised vitelline envelope) were used in assays except on four occasions late in the season when it was difficult to obtain good eggs (~15% of the eggs had false envelopes). A raised envelope at this stage was not likely to have resulted from sperm contamination, as eggs did not develop further when they were incubated for 2 d. Yund (pers. comm.) similarly observed seasonal egg deterioration, leading to a higher occurrence of false envelope. To control for changes in egg quality, we inspected 300 eggs at the beginning and end of each assay for (1) the incidence of false envelopes and (2) the egg viability after being fertilized with dilute fresh sperm. Finally, as a control we set aside a 0.2-mL aliquot of dry eggs so we could determine egg viability at the end of the assay. The eggs were kept in 10 mL of aged seawater at 4°C during the field assay.

We used time-integrated fertilization assays to monitor ambient sperm availability at our study sites. We deployed fresh unfertilized eggs (0.8 mL) in 35 μm Nytex mesh containers (4 cm diameter, 10 cm long), which were similar to those used by Wahle and Gilbert (2002). The mesh size was fine enough to retain the eggs but was permeable to sperm. Each egg-filled container was placed inside an open-ended section of PVC pipe (12-cm diameter × 20-cm long) to be protected from direct wave action, and the pipe was secured to a concrete or lead block. At Little Cove and West Boothbay Harbor where wave action was not a threat, we perforated the PVC pipes with four 5-cm holes to promote flow (see Discussion for potential biases that these holes may have created). At each station, we deployed a pair of baskets separated by 1–3 m to provide a measure of variability in sperm availability. We ran the assays during the entire spawning season (early March to early May) in both 2002 and 2003, except that sampling was only started in early April at Pemaquid Point in 2002.

To assess spatial variation in sperm availability during natural spawning, we placed fertilization assays at fixed distances, which we will refer to as "stations," from the urchin aggregations of interest. We considered the particularities of each site in situating our assay baskets. At Pemaquid Point, we placed containers in surge channels and tide pools that were connected to the open water, even at low tide (Fig. 1). No urchin was present in any of these pools or channels. Stations 1 and 2 were established in surge channels located 15 and 100 m, respectively, from the largest aggregation (which we estimated to consist of 37,000–70,000 urchins) located at 2–12 m depth at mean low water. Stations 3 and 4 were in pools located further along the shore, approximately 15 m from a smaller aggregation (about 5,000 urchins) at 5 m depth. We conducted assays at stations 3 and 4 in 2002 and at all four stations in 2003.

At Little Cove, we determined fertilization rates at three distances, 0.30, 15, and 30 m from the 1,000-urchin aggregation on the ledge (each station was at a pier in the cove). Finally, at West Boothbay Harbor, fertilization assays were conducted at two stations along the pier, 0.2 and 10 m from one of the cages containing 250 urchins.

Although we intended to conduct assays at 2-d intervals, in some cases the interval was 1, 3, or 4 d due to weather and tide conditions. At the end of each assay, we transferred the eggs with a pipette to a vial and fixed them with 4% saline formalin. In the laboratory, we examined a sample of ~300 eggs under a compound microscope to

determine the percentage of eggs fertilized. Baskets with damaged or lost eggs (when <30 eggs could be counted) were eliminated from the analysis. We considered eggs fertilized if they had undergone at least one cell division. Fertilization success was taken as the ratio of embryos with two or more cells to the total number of viable eggs and embryos counted.

For each study site, we statistically evaluated the overall spatial variability in fertilization rates over the spawning season using one-factor analyses of variance (ANOVAs) in which location was a factor with two to four levels depending on the number of stations. Given that spatial effects would only occur when sperm was in the water column, we excluded from the analysis days with levels below 3% fertilization at all stations. This served to homogenize the variances among locations. Still, variances were not always homogenous at Little Cove, but nonparametric tests did not alter our conclusions. We presented the ANOVA results as suggested by Conover (1999). This and subsequent ANOVAs were conducted using SPSS software.

Gonad indices—Gonad indices (wet mass of the gonads as a percentage of total body mass) provided an index of how much spawning had occurred. During the 2002 and 2003 seasons, we made periodic determinations of the gonad index on urchins (≥14 ind.) collected at the three study sites, as well as Crow Island, where the transplanted urchins were collected. Sampling was less frequent at Pemaquid Point because of the difficulty of getting to this shore with scuba gear during the winter, and at Little Cove because we did not want to deplete this already small population. In both years at Pemaquid Point, we sampled urchins in the grazing front adjacent to the shallow kelp bed and in the barrens itself where most of the gametes in the aggregation are produced (Wahle and Peckham 1999). Statistical comparisons of gonad indices were made using a one-factor ANOVA, followed by Fisher's Least Significant Difference (LSD) tests to identify when significant drops in gonad size occurred at each site. When the assumptions of normality and homogeneity of variance were not satisfied, we used the nonparametric Mann–Whitney test.

Environmental monitoring and correlations—We examined time series of environmental factors that are suspected to influence spawning in sea urchins and other taxa. Data on chlorophyll *a* (Chl *a*) concentration and temperature (measured at 1 and 20 m) were obtained from the Gulf of Maine Ocean Observing System (GoMOOS) buoy located 16 km southeast of Pemaquid Point (buoy E0109 Central Maine Shelf; 43°42′47″N, 69°21′20″W; http://gomoos.org). Satellite imagery provided complementary Chl *a* data that indicated the timing and spatial extent of phytoplankton bloom in the region (Sea-viewing Wide Field-of-view Sensor; http://www.seasurface.umaine.edu/frames_sw.html). We also recorded temperatures at 2-h intervals using a temperature logger secured to the bottom at Little Cove (2002–2003) and a logger suspended 1 m below a surface buoy at Pemaquid Point (2002). Sea conditions at Pemaquid Point were quantified by evaluating the daily average of the southern wind component. The nearest available wind data were obtained from the National Oceanic and Atmospheric Administration (NOAA) buoy (44007: 43°31′53″N, 70°08′39″W; http://www.ndbc.noaa.gov/station_page.php?station=44007) located 55 km southwest of Pemaquid Point. Tidal amplitude data were obtained from the NOAA water level station in Portland, Maine (station 8418150; http://www.tidesonline.nos.noaa.gov). Time series of these environmental data (temperature, Chl *a*, southern wind component) were inspected visually for changes coinciding with fertilization events.

We used a periodic regression to test for lunar cycles in sperm availability since this method is preferable to categorical ANOVA for detecting a complex lunar pattern (deBruyn and Meeuwig 2001). The fertilization data for Little Cove and Pemaquid Point were treated separately. We pooled the measurements for the 2 yr (only stations 3 and 4 used at Pemaquid Point, as only these were studied both years, and the far station was excluded at Little Cove because sperm was commonly absent at this station). Each assay was assigned to the lunar day on which the assay ended. The lunar month was divided into 360° to give each lunar day an angular equivalent. Fertilization rates were averaged for each lunar day and then log transformed so that the data conformed to assumptions of normality and homogeneity of variances. These assumptions were better satisfied than with the conventional arc sine transformation used for proportional data. The regression analysis was done using the following model:

$$\log \textsc{Fert} = b_0 + b_1 \sin\theta + b_2 \cos\theta + b_3 \sin 2\theta + b_4 \cos 2\theta$$

where Fert is the fertilization rate on a particular lunar day, b_0 the mean fertilization rate for the lunar month, and b_1 to b_4 are model coefficients that define phase shift and amplitude. The sin 2θ and cos 2θ terms allow the detection of a semilunar cycle (two peaks per lunar month), whereas peaks with different amplitude necessitate the incorporation of sin θ or cos θ terms in the model. We use SAS/STAT software to perform the regression analysis.

Results

Spawning activity as indicated by fertilization assays and gonad indices—In the control tests of egg viability, 96% ± 0.4% (mean ± SE, $n = 61$) of the eggs were fertilizable at the beginning of the assays. This rate dropped to 67% ± 32% ($n = 3$) after 1 d, 60% ± 6% ($n = 54$) after 2 d, 37% ± 14% ($n = 10$) after 3 d, and 6.6% ± 3% ($n = 2$) after 4 d.

Pemaquid Point: Sperm availability in the large natural population, as measured by fertilization assays, varied over the season. We found at this site no difference among stations in sperm availability over the entire spawning season of 2002 (ANOVA, df = 43, $F = 0.284$, $p = 0.60$) and 2003 (one-factor ANOVA, df = 74, $F = 0.103$, $p = 0.96$). Throughout the season, sperm release occurred intermit-

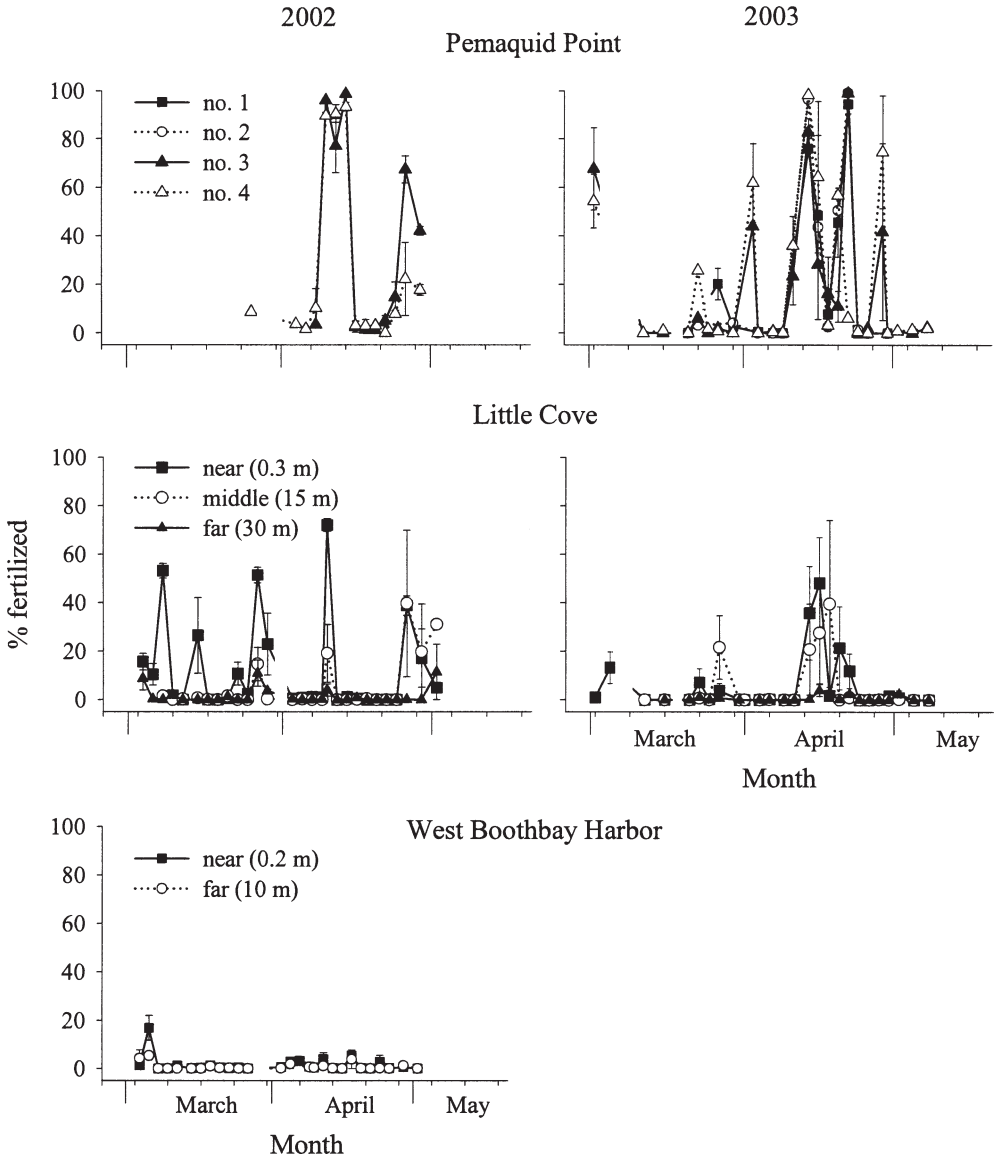

Fig. 2. Mean (±1 SE) fertilization rate (percentage of embryos at or beyond the two-cell stage) for *Strongylocentrotus droebachiensis* at fertilization assay stations during the spawning season in 2002 and 2003. Data points indicate the date assays ended. Breaks in the lines indicate gaps in the time series.

tently. In March of both years, we observed minor sperm release events during some assays (Fig. 2). In March 2003, we were not consistently able to obtain mature eggs for the assays, possibly because of delayed development resulting from the colder winter. As a result, there were gaps in the March time series. Nevertheless, the fertilization assays at Pemaquid Point typically showed some evidence of male spawning in March (e.g., 03 March 2003). The most significant evidence of spawning, however, occurred in April of both years. The first of these events (08 to 14 April 2002) was intense and sustained, with fertilization rates approaching 100% over three consecutive assays (6 d) at the two stations sampled. The second event of that year (22 to 29 April 2002) was weaker, and a specific ANOVA applied to this event revealed that the fertilization rates differed significantly between the two stations (ANOVA, df = 11, $F = 5.396$, $p = 0.043$), indicating that sperm supply varied locally, ranging from 67% at station 3 to 22% at station 4 (Fig. 2). In 2003, the highest fertilization rates occurred from 11 to 14 April and the average at the four stations varied from 78% to 98%. However, this event was less accentuated than the mid-April events in 2002. The next fertilization event, which occurred between 20 and 22 April 2003, was also more gradual, but still approached 100% at stations 1, 2, and 3, but was only 6% at station 4. We observed a final fertilization event from 26 to 29 April 2003, with high variability among stations.

The gonad index of urchins at the grazing front at Pemaquid Point dropped markedly just after the peaks of sperm availability in 2002 and 2003. Change in gonad size

on the barrens at Crow Island (our source for the transplanted urchins) paralleled the decline observed at the grazing front at Pemaquid Point in 2002 (23.1% ± 2.0% in February to 6.1% ± 0.8% in May; data not shown). On the barrens, gonads were smaller than at the grazing front and gonad size dropped less dramatically but still significantly over the period of the fertilization assays in 2002 (LSD, $p = 0.003$) and 2003 (Mann–Whitney test, $Z = -2.261$, $p = 0.023$).

Little Cove: In the transplanted population, fertilization rates varied over time and among the three sampling stations at different distances from the sperm source. Fertilization was consistently greatest at the station nearest the aggregation and decreased toward the more distant stations, and more so in 2002 (ANOVA, df = 66, $F = 12.405$, $p < 0.001$; LSD, near vs. middle, $p < 0.004$; near vs. far, $p < 0.0001$) than in 2003 (ANOVA, df = 55, $F = 3.195$, $p = 0.049$; LSD, near vs. middle, $p = 0.691$; near vs. far, $p = 0.021$; mid vs. far, $p = 0.058$). This confirmed that our transplanted aggregation was the source of the sperm at Little Cove. Despite the proximity of the nearest station to the aggregation, fertilization rates rarely exceeded 50% and only once reached 72%. Spawning activity was also less sustained than at Pemaquid Point as we observed only once three consecutive assays where fertilization rates exceeded 35% at both the near and the middle stations.

In further contrast to Pemaquid Point, we observed in both years no reduction in gonad size at Little Cove over March and April of both years (Fig. 3), although there were numerous sperm-release events. Although Pemaquid Point and Little Cove were about 10 km apart, on many dates sperm-release occurred at both locations (Fig. 2). The timing of spawning events at the two sites was significantly correlated when we analyzed the data on weekly means for both years ($r^2 = 0.56$, $p = 0.021$, $n = 15$).

West Boothbay Harbor: At this site we consistently observed little evidence of spawning as fertilization rates averaged below 5% during most of the season and only reached 20% once at one of the two stations (Fig. 2). Thus, no distance effect was detected. As at Little Cove, the gonad index at West Boothbay Harbor did not change during March and April for the urchins maintained in the separate cage (at the adjacent pier) for gonad index determinations. Near the end of the experiment in late April, mean gonad mass of urchins in the cages serving the fertilization experiments was similar to that of the urchins used to track gonad indices, confirming the two groups followed similar trajectories ($t = -0.804$, df = 28, $p = 0.43$). Significant decreases in gonad size did finally occur in both cages in 14 May 2002, several days after we had discontinued the fertilization assays (Welch's statistic applied to the pooled data for the two cages, $t' = 4.320$, df = 32.26, $p = 0.0001$; Zar 1999).

Relation of spawning to environmental factors—At Pemaquid Point, male spawning showed a lunar cycle; males spawned significantly more around new and full moon phases (spring tides), and levels of spawning were similar during the two phases ($\log \text{FERT} = 0.96 + 0.50 \cos 2\theta$, $R^2 = 0.31$, df = 25, $p = 0.0028$; Fig. 4). At Little Cove, our 2-yr time series also revealed significant semilunar periodicity in sperm release ($\log \text{FERT} = 0.56 - 0.26 \sin \theta - 0.33 \cos \theta + 0.27 \cos 2\theta$, $R^2 = 0.36$, df = 28, $p = 0.0095$; Fig. 4) with predominant spawning activity around the full moon. Whereas the lunar cycle explains up to 36% of the variation in sperm availability at these sites, the balance of the variation is probably explained by other factors.

Although we did not obtain a temperature record for Pemaquid Point in 2003, due to a malfunctioning logger, temperatures in the region were markedly colder in 2003 than 2002. At Little Cove, temperature averaged 5.9°C in 2002 (March to mid-May) versus 3.9°C in 2003 (Gaudette unpubl. data). Similarly, at the offshore GoMOOS buoy, water temperature for the same period was also 2°C warmer in 2002 than in 2003 (5.3°C vs. 3.3°C, respectively). Still, in spite of the colder temperature, the major spawning events at Pemaquid Point occurred in mid-April both years. Surface temperatures at the GoMOOS buoy during the mid-April spawning in 2002 were 4.6°C versus 2.8°C during the mid-April spawning in 2003 (Fig. 5). Also, the cumulative degree-days at the buoy from February to the onset of spawning was 320°C in 2002 versus 251°C in 2003. Despite these differences, the onset of the major spawning events occurred in mid-April in both years, when thermal stratification was beginning at GoMOOS buoy (Fig. 5). During these events the temperature difference between 1 and 20 m was as great as 1.0–1.4°C. Thus, absolute temperatures or degree-days do not appear to be as strongly related to the time of spawning as the events surrounding the onset of stratification.

The major spawning events at Little Cove and Pemaquid Point also coincided with a major increase in Chl *a* concentration in April (Fig. 5). The spring phytoplankton bloom is well known to be associated with the onset of thermal stratification as surface temperatures warm (Sverdrup 1953). In 2002, the Chl *a* fluorescence measured at the offshore GoMOOS buoy was low during March and early April, and then increased suddenly on 12 April. Chlorophyll measurements were not available for the GoMOOS buoy after 17 April, due to equipment failure, but SeaWiFS satellite images indicated a large-scale bloom that lasted several weeks (Fig. 6). Similarly, in 2003 GoMOOS buoy measurements, which became available after 27 March, revealed the development of a phytoplankton bloom the second week of April. SeaWiFS satellite images indicate that this spring bloom was region-wide in adjacent coastal waters.

At Little Cove, we observed the highest fertilization rates on 10 April 2002, 2–3 d prior to the chlorophyll increase recorded at the GoMOOS buoy. The fertilization assays indicated more frequent spawnings from mid-April 2003 onward—thus after the onset of the spring phytoplankton bloom. It was not possible to relate other specific spawning events to chlorophyll fluctuations since chlorophyll data were not available at our specific sites.

Our data provided no evidence of spawning being directly related to wave and swell conditions. We observed sperm-release events at Pemaquid Point during both rough

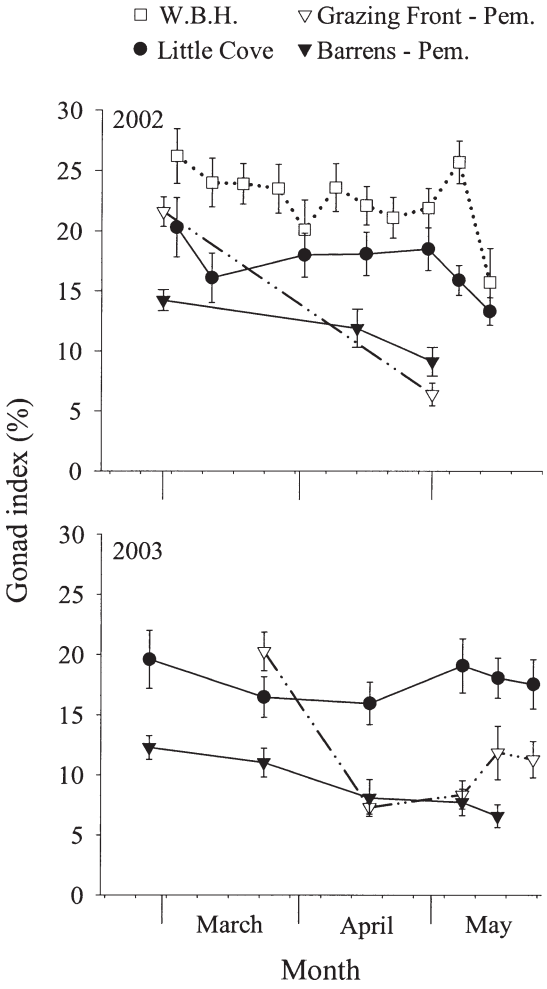

Fig. 3. Mean (±1 SE) gonad index of the sea urchin, *Strongylocentrotus droebachiensis*, at West Boothbay Harbor (W.B.H.), Little Cove, and Pemaquid Point (Pem.) during the spawning season in 2002 and 2003.

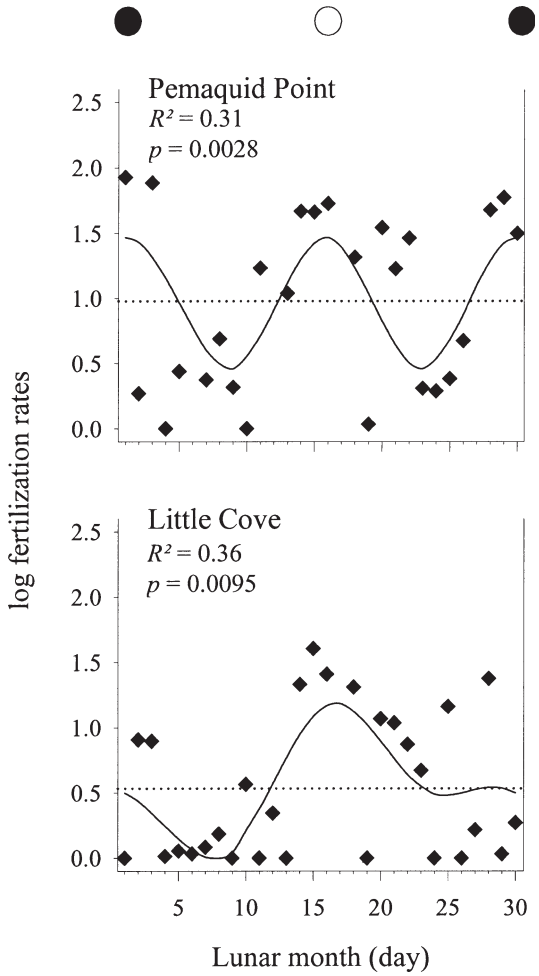

Fig. 4. Relation of sperm availability to lunar phases at Pemaquid Point (stations 3 and 4 only) and Little Cove (near and mid stations only). Points are the averages of fertilization rates recorded at the end of assays over the 2-yr study pooled by lunar day. The solid line is from the periodic model (Little Cove, log FERT = 0.62 − 0.26 sin θ − 0.34 cos θ + 0.27 cos 2θ; Pemaquid Point, log FERT = 0.98 + 0.50 cos 2θ). The horizontal dashed lines indicate the mean fertilization rate (log transformed). Full moon (open circle) corresponded to lunar day 16.

and calm conditions (i.e., 08–14 April 2002 and 20 April 2003, respectively; Fig. 5). Additionally, the coincident spawning at Pemaquid Point and Little Cove could not be explained by sea state since the former was an exposed shore and the latter a protected inlet with little wave action.

Discussion

Spawning pattern described by fertilization assays—Experiments with time-integrated fertilization assays have begun to fill the void in our understanding of the spatial and temporal variation in the availability of sperm in small spawning populations of the green sea urchin, *S. droebachiensis* (Meidel and Yund 2001; Wahle and Gilbert 2002). Our fertilization assays, using eggs for the better part of their viable life (>72 h), enabled us to monitor male spawning activity over the entire spawning season. Taken together, our data on fertilization rates and gonad indices showed that male spawning at Pemaquid Point and Little Cove occurred intermittently and with variable intensity. At Little Cove and West Boothbay Harbor, spawning events were small and led to little or no reduction of gonad mass. Two previous studies using time-integrated fertilization assays (Meidel and Yund 2001; Wahle and Gilbert 2002) also described a similar weak spawning intensity for small aggregations (<350 individuals). Such small-scale sperm releases seem to occur independent of aggregation size, however. At Pemaquid Point, we also observed small sperm releases, even before most of the females had mature gametes (e.g., 28 February–02 March 2002). Therefore, we believe that small-scale releases of sperm may be a characteristic of the reproductive behavior of this species, and henceforth refer to this phenomenon as "trickle spawning."

In marked contrast to trickle spawning events, we recorded at least three large spawning events at Pemaquid Point (08–14 April 2002, 09 April 2003, and 13 April 2003) in which we measured fertilization rates approaching 100%. During each of these events, spawning was most likely

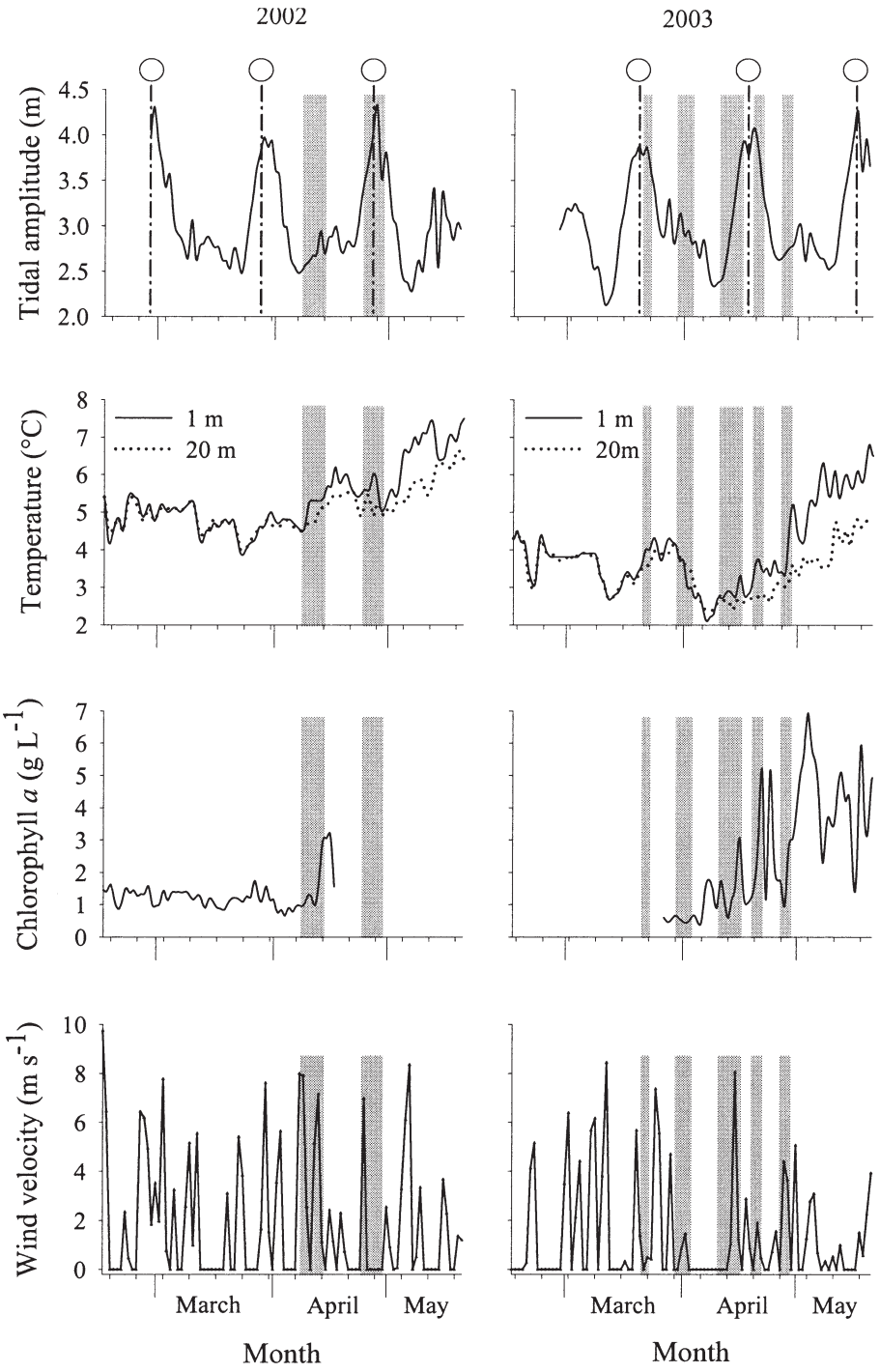

Fig. 5. Changes in tidal amplitude, temperature at the surface (1 m) and 20 m deep, Chl *a*, and south wind vector velocity during the spawning season of *Strongylocentrotus droebachiensis* in 2002 and 2003. Tide amplitude data are from the NOAA water level station 8418150; temperature and chlorophyll data are from the GoMOOS buoy E0109, and wind data are from the NOAA buoy 44007. Open circles with dashed line correspond to full moon date. Shade areas indicate periods when the fertilization rate at Pemaquid Point was 40% or greater at one or more stations.

occurring throughout the area as sperm concentrations were near saturation levels at all stations (except for station 4 on 13 April 2003). It is noteworthy that we observed coincident high fertilization rates among widely spaced stations (>50 m) that were located no less than 15 m from the nearest urchin aggregation. In contrast, at Little Cove, where the nearest baskets were within centimeters of the sperm source and the PVC pipes had openings to facilitate

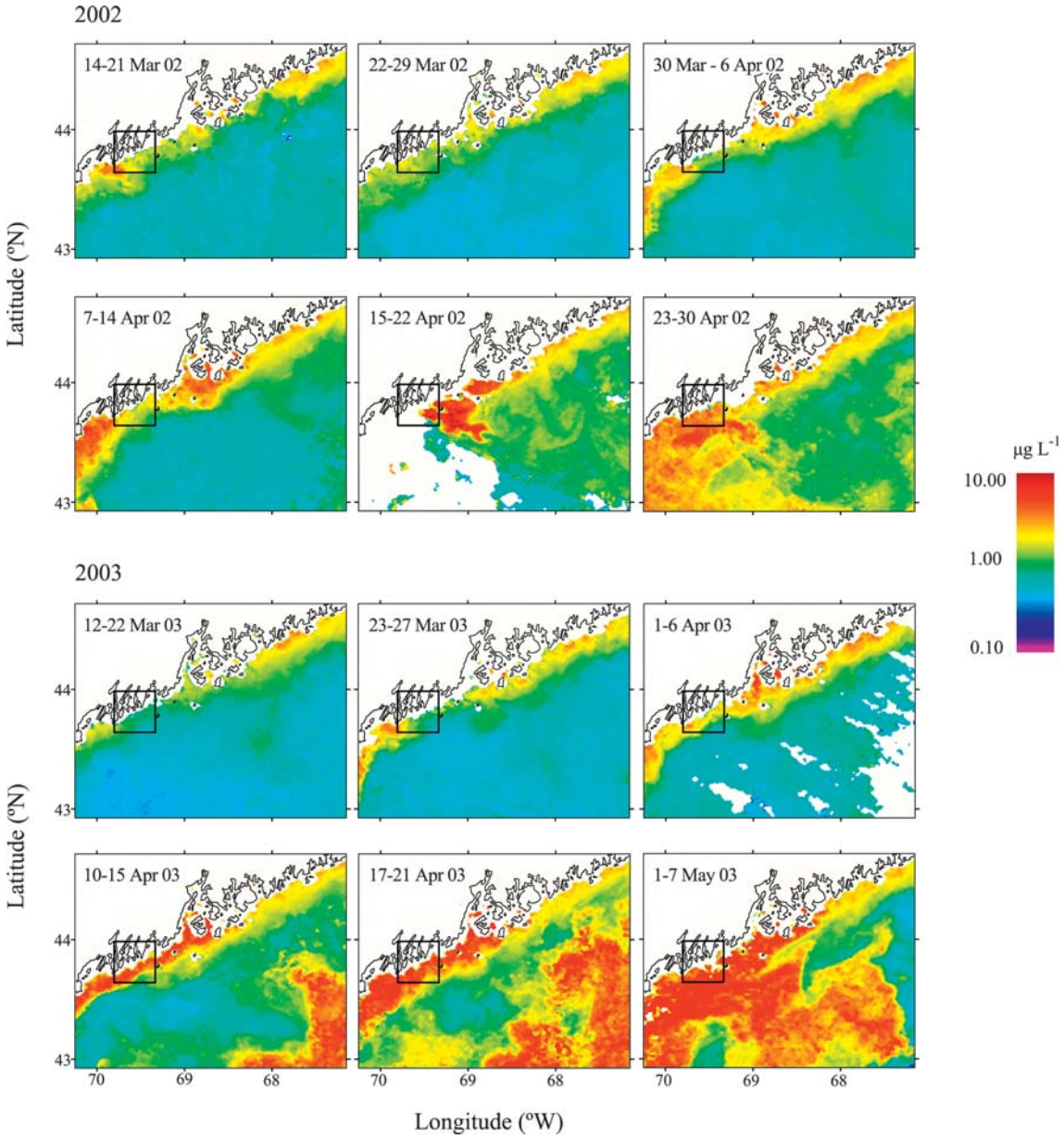

Fig. 6. Mean Chl *a* concentration for periods of 4–10 days in the Gulf of Maine as estimated by the SeaWiFS satellite. The squares indicate our study area. The major spawnings observed at Pemaquid began between 08 and 10 April 2002 and between 11 and 14 April 2003.

flow, we never observed fertilization rates greater than 72% and rates diminished significantly with distance from the urchin aggregation. These results are consistent with Levitan and Young's (1995) spawning model that spacing between individuals is less important to fertilization rates in larger, more widespread populations.

We recognize that fertilization rates are likely to be affected by factors such as the number of spawning individuals and the rate of spawning, as well as currents and turbulence, none of which were quantified during the assays. Potential artifacts of egg baskets on fertilization rates under varying conditions of flow and turbulence are not well understood. Therefore site comparisons of fertilization rates are to be done with caution. Nonetheless, the apparent low sperm availability we observed at Little Cove and West Boothbay Harbor is consistent with the absence of a drop in gonad indices at these sites, suggesting spawning did not occur to the extent it did at Pemaquid Point. At Pemaquid Point, it is unlikely that the higher wave-induced flow conditions explain the higher fertilization rates since we observed high fertilization rates under both rough and calm conditions (e.g., 08–14 April 2002 and 20 April 2003; Fig. 5). Therefore, we consider it likely that the difference in sperm availability among sites was real, and we conclude that the fertilization assays together with the gonad indices indicate that massive, synchronous

spawning events occurred at Pemaquid Point around mid-April of both years, but not at the other two experimental sites.

The two other main limitations of using fertilization assays to study spawning are that (1) it holds the eggs stationary for an unnatural period of time, and (2) fertilization assays only recorded male spawning activity. Unfortunately, we do not know if naturally spawned eggs would have been fertilized at a similar rate, because we could not determine when or if females released their eggs relative to male spawning events and flow conditions, or how long eggs stay in the benthic boundary layer after being spawned (Yund and Meidel 2003). Nonetheless, gonad indices provided the necessary corroboration of the degree to which spawning by both sexes had occurred in our study populations, albeit at a more coarse temporal scale. Although it would have been desirable to gather larger samples more frequently for gonad analysis to track the progress of male as well as female spawning, the numbers of urchins needed to make that assessment would have run into the hundreds, and we did not wish to deplete the populations under study. A method to evaluate female spawning on the same short time frame as the fertilization assays would be extremely beneficial to our understanding of fertilization dynamics during natural spawning.

Relation of spawning to environmental factors and a conceptual model—Synchronous spawning of males and females is critical to reproductive success in species that release their gametes into the water, but the mechanism by which it is achieved remains unclear for *S. droebachiensis*. Although many marine taxa likely use external environmental cues to synchronize spawning (Giese and Kanatani 1987), few studies have convincingly identified the specific cues involved (Himmelman 1999). To date our understanding of the role of environmental cues in *S. droebachiensis* has been limited to direct observation in the laboratory (Starr et al. 1990, 1992) or evaluating the timing of spawning by loss of gonad mass in the field (Starr et al. 1993; Vadas and Beal 1999).

Our time series of fertilization assays, gonad indices, and environmental monitoring suggest that a combination of cues influence spawning in *S. droebachiensis*. First, the synchrony between Pemaquid Point and Little Cove in fertilization events suggests that male spawning is caused by a large-scale environmental cue. These spawning events were correlated with the lunar cycle with peak intensity at the new and full moons at Pemaquid Point. However, at Little Cove, our model revealed a semilunar cycle with a significantly stronger peak around the full moon. Meidel and Yund (pers. comm.) also observed more spawning activity around the full moon in a small and protected population of *S. droebachiensis*. More research is required to better evaluate the generality and extent to which lunar or tidal cycles influence spawning patterns in the sea urchin.

Considering that spawning in high flow and turbulence would rapidly dilute gametes (Denny and Shibata 1989), one might predict that spawning would preferentially occur during calm periods. For instance, it is well documented that *Fucus* species release their gametes only during calm periods to favor fertilization (e.g., Pearson et al. 1998; Berndt et al. 2002). However, we found no evidence that spawning in the green sea urchin is correlated with sea state. Recent modeling by Denny et al. (2002) suggests previous models have overstated the rate of dilution under turbulent conditions and that contact between eggs and sperm may be facilitated at higher rates of flow and turbulence than initially expected. Moreover, examples of taxa that preferentially spawn in rough conditions suggest adaptive mechanisms to compensate for dilution effects (e.g., Shanks 1998). In the green sea urchin, aggregating is one mechanism that could alleviate gamete dilution in rough conditions.

Our findings suggest green sea urchin spawning is more tightly linked to the spring phytoplankton bloom. We observed that the major spawnings in both years at Pemaquid Point and Little Cove occurred around the onset of thermal stratification and the spring phytoplankton bloom in nearshore waters. The synchrony between spawning in *S. droebachiensis* and the spring bloom has been reported in previous studies (Himmelman 1975; Starr et al. 1993; Vadas and Beal 1999), although not with the temporal or spatial resolution of this study. Further, laboratory experiments by Starr et al. (1990, 1992) showed that cells of various phytoplankton species and their extracts induced spawning in the green sea urchin. The latter reports speculate that spawning at the onset of the phytoplankton bloom could confer a number of advantages, a major one being abundant food for larvae.

In marked contrast to the substantial spawning events at Pemaquid Point was the absence of major spawning at Little Cove and West Boothbay Harbor, although gonad mass did eventually fall in May, several days after we discontinued the fertilization assays. We considered the possibility that handling during transplanting the urchins to Little Cove or their captivity at West Boothbay Harbor may have caused a delay in the natural spawning. Given the similar lunar periodicity of the Little Cove and Pemaquid Point populations, and prior evidence of delayed spawning in undisturbed small aggregations of green sea urchins (Meidel and Scheibling 1998; Meidel and Yund 2001), we believe that possibility to be unlikely. One interpretation consistent with prior evidence is that the delay is related to low sperm concentrations, as sperm (or associated pheromones) is well known to induce or enhance spawning in conspecifics (male and female) of *S. droebachiensis* (Starr et al. 1990, 1992) and other echinoderms (e.g., Hendler 1991). Many studies of spawning echinoderms report that males spawn before females (e.g., holothurins, McEuen 1988; ophiuroids, Hendler 1991; *Strongylocentrotus* spp., Levitan 2002). Delayed spawning by females until sufficient sperm concentrations are detected may be a strategy to avoid gamete wastage. On the other hand, spawning order among males may influence the quantity and quality of offspring (Marshall et al. 2004*b*).

We therefore envision a chain of environmental cues and positive density-dependent feedback that under the right conditions lead to a mass synchronous spawning. Whereas environmental cues are likely to play a role in the timing

and synchrony of gamete release, feedback internal to the population may reinforce the spawning response. In our study, the coincident spawning events at Pemaquid Point and Little Cove point to a common external spawning cue. On the whole, the numerous spawning events were correlated with the lunar cycle, whereas the massive spawning observed at Pemaquid Point occurred at the onset of thermal stratification and the spring phytoplankton bloom. We speculate that if sperm or pheromone concentrations are high enough during these events, they would stimulate spawning by less responsive males as well as by female urchins, thus increasing the synchrony and intensity of spawning. In small aggregations or sparse populations, internal feedback would likely be weaker because sperm (or pheromone) would be rapidly dispersed and diluted. Similarly, it has been suggested that spawning synchrony by the rockpool anemone, *Oulactis mucosa*, may be difficult to achieve at low density because of the dilution of spawning pheromones (Marshall et al. 2004*a*).

Much research in recent years has focused on the density-dependence of fertilization success among free-spawners (reviewed by Levitan 1995). Most of the fertilization models derived from these studies assume that the proportion of population spawning is independent of population size or density (e.g., Levitan and Young 1995; Claereboudt 1999). However, this assumption would be invalid if the mass spawning events depended on a positive feedback mechanism.

In conclusion, our monitoring of gonad indices, sperm availability, and environmental factors in sea urchin aggregations of different size over the course of the spawning season has provided valuable insights into the environmental correlates of spawning and the influence of aggregation size, not only on fertilization success, but on the spawning process itself. Further studies of natural populations are needed to improve our understanding of density-dependent effects on spawning and fertilization success and their link to future recruitment.

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