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Influence of Sperm and Phytoplankton on Spawning in the Echinoid *Lytechinus variegatus*

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Abstract. The cues triggering large-scale broadcast-spawning events in marine invertebrates are not fully understood. Using the sea urchin *Lytechinus variegatus*, we tested the effectiveness of a variety of potential spawning cues in eliciting a spawning response. In the laboratory, during two consecutive spawning seasons, about 400 isolated sea urchins were exposed to phytoplankton, sperm, or eggs, singly or in combination. The likelihood of spawning, time to spawning, and spawning behavior were recorded for both sexes. Sperm was most successful at inducing spawning. No response to eggs was noted. Phytoplankton alone did not trigger spawning, but when a phytoplankton cue was followed by the addition of sperm, spawning behavior was induced, the time between addition of sperm and spawning was reduced, and the variance among individuals in the time of spawning initiation was reduced. Males spawned sooner in response to cues than females and rarely spawned spontaneously in phytoplankton or control treatments. A semilunar pattern in the sensitivity to spawning cues was noted. During time periods when sea urchins were less ripe, the ratio of spawning males to spawning females increased. Our results indicate that seasonal and lunar cycles, together with the presence of phytoplankton, increase the sensitivity of these sea urchins to spawning cues and the precision of their responses to conspecific sperm.

Introduction

Many marine invertebrates reproduce by releasing their gametes directly into the water column (Giese and Kanatani, 1987), where they can obtain high fertilization rates by synchronizing release of sperm and eggs (Oliver and Babcock, 1992; Lasker *et al.,* 1996; Levitan *et al.,* 2004). They are thought to achieve this synchronization by sensing external cues. Despite numerous studies, external cues for spawning remain, in some cases, poorly understood (reviewed by Giese and Kanatani, 1987; Babcock *et al.,* 1992; Mercier and Hamel, 2009).

Studies on sea urchin spawning cues suggest that the lunar cycle, temperature change, phytoplankton, and sperm may all play roles in initiating and synchronizing spawning events (comprehensively reviewed by Mercier and Hamel, 2009). Although these studies differ in their conclusions about which cue is most important, they generally agree that successfully synchronized spawning in sea urchins is probably due to a compound effect of several factors (Mercier and Hamel, 2009).

Field observations of spawning events in the sea urchins *Diadema antillarum* Philippi (Levitan, 1988), *Evechinus chloroticus* Valenciennes (Lamare and Stewart, 1998), and *Strongylocentrotus droebachiensis* O. F. Müller (Gaudette *et al.,* 2006) provide evidence of lunar periodicity in some species (reviewed by Pearse, 1975). Patterns of gametogenesis have also been linked to a lunar cycle in some (Lessios, 1991; Muthiga, 2005), but not all (Lessios, 1991; Williamson and Steinberg, 2002) sea urchin species.

Sea urchin spawning events have also been noted to occur close, in space and time, to phytoplankton blooms (Himmelman, 1975; Gaudette *et al.,* 2006). Experiments with *S. droebachiensis* found spawning in response to a variety of phytoplankton taxa and also revealed that the response time of females to phytoplankton was decreased by the addition of sperm (Starr *et al.,* 1990, 1992).

Spawned materials are also thought to be an important cue in echinoderms (Beach *et al.,* 1975; Miller, 1989; Unger and Lott, 1994). Sperm has been experimentally shown to induce spawning in *S. droebachiensis* (Starr *et al.,* 1990), although later studies suggested that the response to sperm depends on the presence of phytoplankton (Starr *et al.,*

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1992). In contrast, the sea urchin *Lytechinus variegatus* Lamarck has been reported to show no spawning response to conspecific sperm (McCarthy and Young, 2004).

The evidence that temperature changes result in natural spawning events is also equivocal. Several spawning events have been observed to follow temperature increases (see, *e.g.,* Himmelman *et al.,* 2008) or decreases (see, *e.g.,* Tsuji *et al.,* 1989), but such changes are often correlated with other environmental conditions, such as phytoplankton blooms, that might be the actual spawning trigger (reviewed by Mercier and Hamel, 2009). Laboratory observations often note spawning in response to temperature shifts, but these shifts are often so rapid that they constitute thermal shocks rather than simulating natural cues (Himmelman, 1999).

Even if the variety of cues that might induce spawning events could be untangled, scant evidence is available to indicate how these cues differentially influence male and female spawning. A large number of marine invertebrates (Thorson, 1950; Levitan, 1998) and marine algae (Clifton and Clifton, 1999) have shown sexual dimorphism in spawning—males initiate spawning before females—but very few studies have determined sex-specific responses to spawning cues. In *S. droebachiensis*, males tended to spawn before females in response to phytoplankton (Starr *et al.,* 1992), but when sperm were mixed with the phytoplankton, the response time of females was reduced to equal that of males. Males in that study did not respond to sperm (Starr *et al.,* 1992), so although the observation of males initiating spawning before females was well supported, the proximate explanation remains unknown.

The uncertainty is due, in part, to the rarity of recorded, natural observations of spawning events (Babcock *et al.,* 1992) and, in part, to the paucity of laboratory experiments that have tried to tease apart the many factors that are correlated during these observations. Cues that might be proven effective in temperate systems (see, *e.g.,* Starr *et al.,* 1992) might not be effective in other environments that are less seasonal, tidal, or wave exposed. The study we report here was intended to clarify the extent to which phytoplankton, gametes, and the lunar period function as spawning cues, both independently and combined, in the more tropical *Lytechinus variegatus*. *Lytechinus variegatus* is a common and ecologically important herbivore in the Gulf of Mexico and Caribbean (*e.g.,* Rose *et al.,* 1999; Valentine *et al.,* 2000). We considered how these cues influence the likelihood of spawning, time to spawning, and spawning behavior in both sexes.

Materials and Methods

Specimens of *Lytechinus variegatus* were obtained from shallow sea-grass islands in St. Joseph's Bay on the Gulf Coast of Florida. Previous research indicated that the spawning season extended from late spring to early fall (Beddingfield and McClintock, 2000; McCarthy and Young, 2002), and that guided our seasonal sampling scheme. Individuals were collected on one of 12 dates in the 2008 and 2009 spawning seasons from March to November, with most sampling concentrated in the summer, when preliminary data suggested it was a more predictable season for collecting ripe animals (DRL, pers. obs.). A total of 92 and 299 sea urchins were tested in 2008 and 2009, respectively. Individuals ranged from 2.9 to 7.5 cm in diameter; this size range was consistent over all experimental trials. An online supplement at http://www.biolbull.org/supplemental/ provides the dates of collection and testing for all individuals, the treatment applied to each individual, and their sex (if known), test diameter, and response.

In the fall of 2008, sea urchins were kept in covered laboratory tanks at temperatures ranging from 22.6 to 25 °C (in recirculated artificial seawater), and water salinity mirrored natural levels. In 2009, sea urchins were kept outdoors in uncovered ambient wet tables. They were given at least 24 h to acclimate to laboratory and wet-table conditions and were tested within 7 days of collection. Each urchin was used for one trial only. Although the circadian rhythm of sea urchins was not explicitly accounted for in this study, 318 of the 393 tested individuals, or 80% of the urchins, were tested between the hours of noon and 1700.

Treatments consisted of the appropriate gametes or phytoplankton (depending on trial) in 600 ml of artificial seawater (in 2008) or filtered seawater (in 2009) in 1000-ml beakers. Temperature was held at room temperature in 2008 (22.6 to 25 °C) and at ambient water temperature in 2009. In 2008, five different treatments were administered: (1) control, (2) sperm, (3) phytoplankton, (4) phytoplankton and sperm, and (5) eggs. In 2009, five treatments were used: (1) control, (2) sperm, (3) phytoplankton, (4) phytoplankton and sperm, and (5) phytoplankton followed by sperm. In general, the treatments were established in each beaker (described below) and then a randomly selected sea urchin was placed into the beaker. The exception was the "phytoplankton followed by sperm" treatment, in which the sea urchin was placed into the beaker with phytoplankton and sperm was added after 75 min. This last treatment was intended to most closely mimic natural conditions in which a few males might release sperm into a phytoplankton bloom, perhaps inducing additional individuals to spawn.

The "control" treatment consisted of 600 ml of seawater (artificial seawater in 2008 and filtered seawater in 2009). The "sperm" treatment was the addition of 1 ml of dry sperm to seawater. Dry sperm was collected from individual urchins induced to spawn by injection of 0.5–1 ml of 0.55 mol l^{-1} KCl (as in Levitan, 1993). Donor sperm from two to seven individuals was mixed together and stored on ice for a maximum of 15 min before use. The number of males used was determined by the volume of sperm needed to

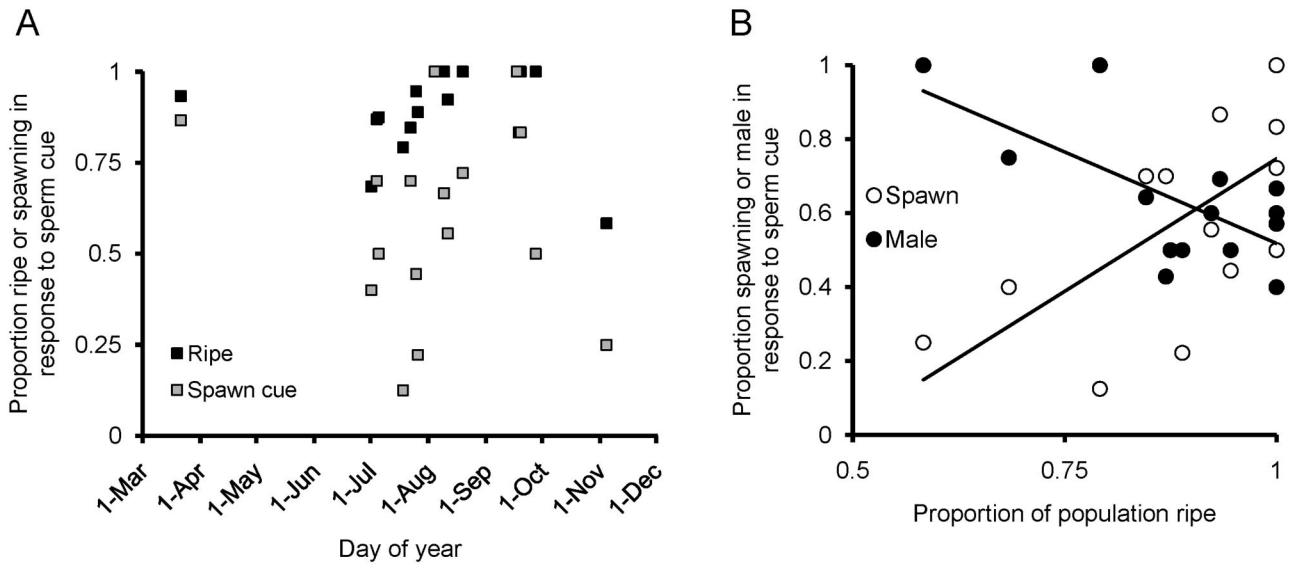

Figure 1. Patterns of reproductive ripeness, defined as response to KCl injection, and response to spawning cues. (A) The proportion of ripe individuals (black symbols) and proportion of ripe individuals that spawned in response to the presence of sperm (grey symbols) as a function of season. (B) The proportion of individuals spawning in response to the presence of sperm (open symbol) and the proportion that spawned in response to the presence of sperm that were male (black symbol) as a function of the proportion of the population that was ripe.

conduct the experiments. We did not distinguish between an effect of sperm and that of any other materials besides spermatozoan cells that might be released during spawning. The "phytoplankton" treatment was the addition of 2 ml of each of three phytoplankton species (*Rhodomonas salina* (Wislouch) D. R. A. Hill & R. Wetherbee; *Rhodomonas lens* Pascher et Ruttner; and *Prorocentrum micans* Ehrenberg—each at stock concentrations of approximately 5×10^8 per liter). Phytoplankton was cultured in natural daylight with an f/2 enrichment medium (Strathmann, 1987) in 0.45-μm-filtered, sterilized, seawater. The "phytoplankton and sperm" treatment was the combination of sperm and phytoplankton as described above. The "egg" treatment was the addition of 1 ml of concentrated eggs (from 2 to 5 female donors). The "phytoplankton followed by sperm" treatment was set up as a "phytoplankton" treatment, and then 1 ml of dry sperm was added 75 min after the sea urchin was placed into the beaker.

Each trial lasted 185 min, during which the spawning status of urchins was visually verified at 5-min intervals. Spawning times, number of times gametes were released, spawning behavior, diameter, and sex were recorded. Sex was determined by observation of released gametes. During the 2009 trials, if no spawning was observed during the experimental period, we injected sea urchins with KCl to determine ripeness and sex of each individual. Sex was scored as "undetermined" if KCl injection did not trigger spawning. In total, 83 sea urchins were tested under control conditions, 93 were tested using a sperm treatment, 99 were treated only with phytoplankton, 63 were tested with sperm and phytoplankton, and 44 were exposed first to phytoplankton and then to sperm.

Results

Seasonal patterns of ripeness

We define a "ripe" individual as one that spawned during the experiment or in response to KCl injection immediately after the experiment. Most sea urchins were ripe from March through September. Some animals, typically males, released sperm through the end of the experimental period in early November (Fig. 1a). From mid-July through September, over 90% and often 100% of individuals were ripe. Fewer were ripe in the early summer and late fall. A complete list of individuals, test date, treatment, and response is provided in the supplemental material online at http://www.biolbull.org/supplemental/. These patterns match published data for this species (Beddingfield and McClintock, 2000; McCarthy and Young, 2002). Sea urchin diameters ranged from 2.9 to 7.5 cm (average 4 cm). Neither males and females nor ripe and unripe (and therefore unsexed) urchins differed significantly in size ($P > 0.05$, ANOVA).

On the seven dates on which all animals were ripe, the sex ratio was exactly 1:1 (59 males and 59 females), so significant deviations from this sex ratio in our experiments are functions of either sex-dependent ripeness or sex-dependent spawning responses. The response of sea urchins to any

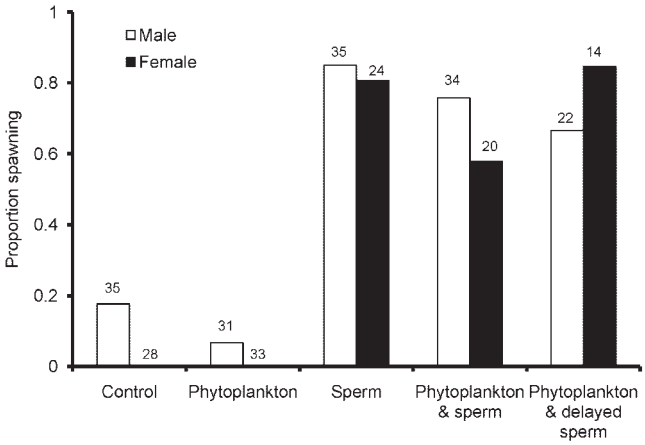

Figure 2. Proportions of males (open bars) and females (black bars) that spawned in response to cues. Number above each bar is sample size. Analysis using 2009 data, as these individuals were sexed *via* KCl injection after experiments. Animals in treatments with sperm spawned more often than those in treatments without sperm; the presence of phytoplankton did not influence spawning. Males and females did not differ significantly in response to these treatments, although only males were observed to spawn at low levels in control and phytoplankton (alone) treatments.

treatment with sperm depended on the proportion of the population that was ripe: increased ripeness resulted in increased response of both sexes to the sperm cue ($R^2 = 0.44$, $P < 0.01$, Fig. 1b). The spawning sex ratio also depended on the sperm spawning cue: with increased population ripeness the sex ratio approached 1:1 ($R^2 = 0.47$, $P < 0.01$, Fig. 1b). When ripeness decreased in early summer and late fall, most individuals that responded to the presence of sperm were male. In November, at the end of the spawning season, only males responded to sperm.

Spawning responses

Egg treatments never induced spawning in any replicate in the 2008 trials and were discontinued for the 2009 trials. In 2009, because we determined the fraction of ripe animals after each trial, we could calculate the proportion of ripe animals (of each sex) that spawned in response to each spawning cue. Control and phytoplankton treatments were ineffective at inducing high levels of spawning (<20%), and these treatments were not significantly different from each other (Fig. 2). The addition of sperm, with or without phytoplankton, induced between 50% and 90% of sea urchins to spawn, significantly higher than treatments without sperm (ANOVA, Table 1). Sex had no significant influence on the likelihood to spawn (Table 1), but a small proportion of males spawned in control and phytoplankton (without sperm) treatments. Females were never observed to spawn in control or phytoplankton (without sperm) treatments (Fig. 2).

Table 1

Analysis of variance testing the proportion of ripe individuals that spawned each experimental day as a function of the presence of sperm or phytoplankton, by sex

Source	df	Type III SS	MS	*F*	*P*
Sperm	1	8.43	8.43	79.19	<0.0001
Phytoplankton	1	0.03	0.03	0.28	0.60
Sex	1	0.01	0.01	0.07	0.80
Error	99	10.56	0.11		
Total	102	19.12			

No interactions were significant, so these terms were removed from the analysis. The addition of sperm induced a higher proportion of sea urchins to spawn. The addition of phytoplankton did not significantly increase the likelihood of spawning over that of controls. Males and females did not differ significantly in the likelihood of spawning across these treatments, but only males spawned in control treatments and when exposed to phytoplankton (albeit at a low frequency; Fig. 2).

Lunar periodicity: In the summer of 2009, when the test dates were most concentrated, a lunar pattern was noted, with most individuals responding to any sperm treatment when associated with the full or new moon (Fig. 3). Although most individuals (89%, S.E. 3%) spawned in response to KCl injection over the summer 2009 sample dates (indicating that most individuals contained ripe gametes), individuals were significantly more likely to spawn to the presence of sperm in experimental trials during the week leading up to and including new and full moons than during the intervening week of waxing or waning moons (chi-square = 8.28, $P < 0.01$, df = 1 for combined sexes over two time periods; all trials conducted on the week leading up to and including the full or new moons *versus* trials

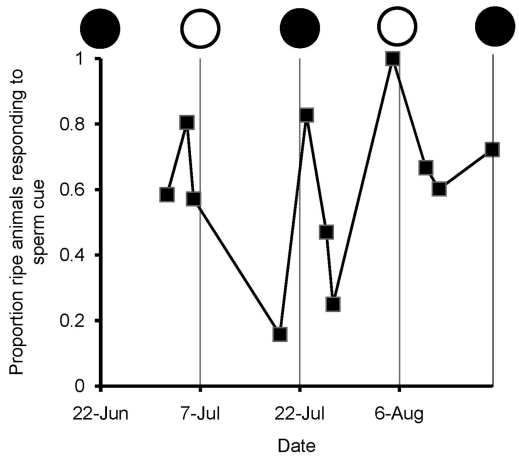

Figure 3. Proportion of ripe individuals spawning over lunar period. Solid circles indicate new moon; open symbols indicate full moon. Data collected in the summer of 2009. Individuals were significantly more likely to respond to the presence of sperm when tested near the new and full moons (chi-square test, see text).

Table 2

Analysis of covariance testing the main effects of treatment, sex, and the covariate of seasonal date on the time from treatment (addition of sperm, phytoplankton, sperm-and-phytoplankton, or phytoplankton-and-delayed-sperm) to initiation of spawning

Source	df	Type III SS	MS	*F*	*P*
Treatment	2	17,604.62	8,802.31	18.52	<0.0001
Sex	1	6,137.06	6,137.06	12.91	0.0005
Date	1	34,082.01	34,082.01	71.72	<0.0001
Error	118	56,076.20	475.22		
Total	122	115,458.50			

No interaction terms were significant, so these terms were removed from the model.

conducted on the week leading up to the waxing or waning moons).

Time of spawning response to experimental cues: The time interval between the introduction of the cue and the sea urchin spawning response was examined with an ANCOVA with main effects of treatment (sperm, phytoplankton and sperm, phytoplankton followed by sperm), sex, and a covariate of date. Both the main effects and the covariate were significant, but no interaction terms were (Table 2). Sperm and phytoplankton-and-sperm treatments did not differ significantly, but both significantly induced spawning before the phytoplankton-and-delayed-sperm treatment (Fig. 4a). However, when the time interval was defined as from the introduction of the sperm (rather than from any cue), the phytoplankton-and-delayed-sperm treatment significantly reduced the time interval to spawning, as compared to sperm and phytoplankton-and-sperm treatments (Fig. 4b): 54 rather than 93 min (least square means reported). In the phytoplankton-and-delayed-sperm treatment, males initiated spawning an average of 26 min earlier (39 min, S.E. 6) than females (65 min, S.E. 8). Sex did not interact significantly with response to these cues (Table 2). Phytoplankton therefore did not induce spawning, beyond what was noted in controls, but did reduce the response time to sperm for both males and females.

Season affected the time to response to sperm (Fig. 5). For the sperm, phytoplankton-and-sperm, and phytoplankton-and-delayed-sperm treatments, the response times increased progressively over the course of the spawning season ($P < 0.0001$, Table 2).

The average standard deviations, across dates, in time to spawning response to the sperm (18.5 min) and phytoplankton-and-sperm (19.3 min) treatments was nearly twice as great as that in response to the phytoplankton-and-delayed-sperm treatment (10.7 min).

Spawning behavior: During the 2008 trials, sea urchins that eventually spawned first became more active and began climbing the sides of the beakers, becoming partially exposed to air. During the 2009 trials, we recorded the time spent engaged in this "spawning behavior" during each trial (Fig. 6). All sea urchins that spawned engaged in it, but not all sea urchins that engaged in it were observed to spawn

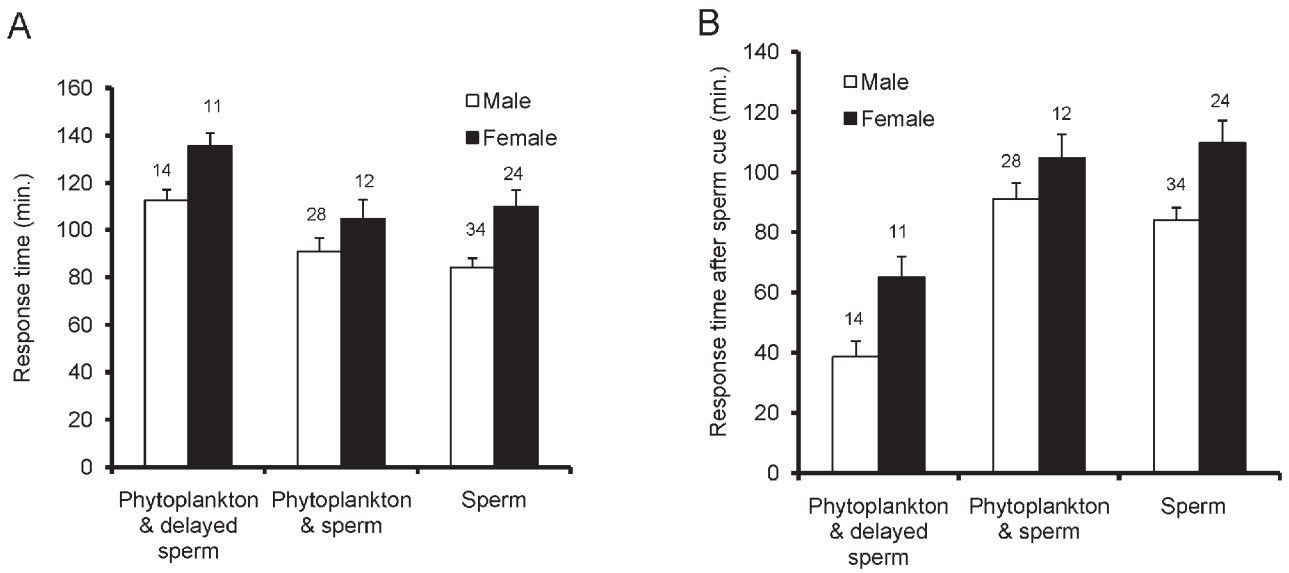

Figure 4. Response time of males (open symbols) and females (filled symbols) to presence of sperm (standard error bars). (A) Response time as defined by addition of any cue. (B) Response time from addition of sperm. Number above each bar is sample size. Note that, when sperm was added after phytoplankton, the response time was cut by about 50%. Males initiated spawning before females in all cases.

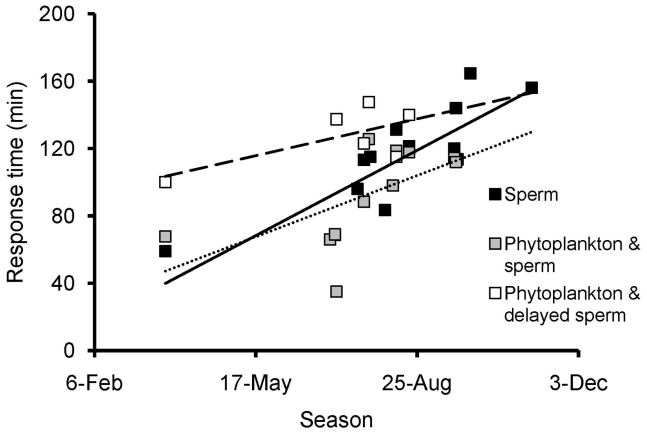

Figure 5. Response time after addition of sperm as a function of season. Spawning in response to addition of sperm was progressively delayed over the course of the spawning season.

during the experimental period. An ANOVA indicated significant effects of treatment and sex (males, females, and unripe individuals that did not respond to KCl injection), but not their interaction, on the length of time engaged in spawning behavior (Table 3). All individuals in phytoplankton and sperm treatments exhibited significantly more spawning behavior than did those in control treatments. Those in treatments with phytoplankton and/or sperm did not differ significantly in this behavior. Males exhibited significantly more spawning behavior (84 min) than did females (67 min) or unripe individuals (37 min). Females exhibited marginally (nonsignificantly) more spawning behavior than unripe individuals ($P = 0.087$).

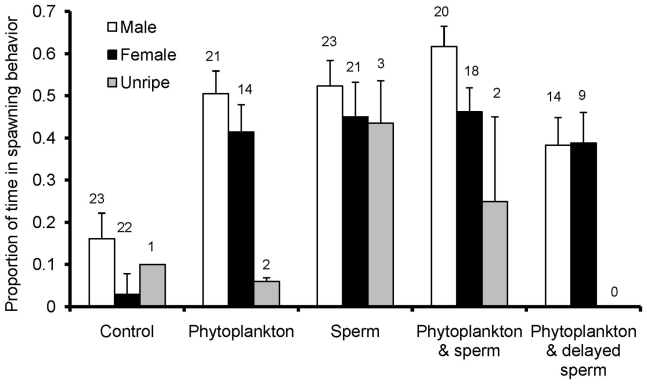

Figure 6. Time spent engaged in spawning behavior, defined as climbing up the sides of the beaker, for each treatment, by male (open bars), female (black bars), and unripe (grey bars) individuals (standard error bars). Number above each bar is sample size. Behavioral data collected only from 2009 trials. All treatments (phytoplankton and/or sperm treatments) elicited similar and greater spawning responses than did the control treatment. Males, followed by females, and unripe individuals exhibited the longest time engaged in spawning behavior.

Table 3

Analysis of variance testing treatment and sex on time spent in spawning behavior

Source	df	Type III SS	MS	*F*	*P*
Treatment	4	75,472.02	18,868.00	8.34	<0.0001
Sex	2	24,099.65	12,049.78	5.32	0.0056
Error	186	421,003.96	2,263.46		
Total	192	518,527.31			

Sex included males, females, and unripe (unsexed) individuals. The Interaction term was not significant and was removed from the model.

Discussion

This study aimed to test the effectiveness of gametes and phytoplankton, both independently and combined, as spawning cues in the sea urchin *Lytechinus variegatus*. We found that (1) spawning rates were highest in the days up to and including the new and full moons, (2) males rarely spawned in control and phytoplankton treatments (females never did), (3) both males and females spawned in response to conspecific sperm and/or associated spawned materials, (4) males spawned before females in response to cues, and (5) phytoplankton primed males and then females to spawn earlier and more precisely to a sperm cue. Our results suggest that spawning in *L. variegatus* occurs as a result of a cascade of events: (1) seasonal effects on gametogenesis, (2) semilunar effects on sensitivity to cues, and (3) increases in spawning behavior and the response time to conspecific sperm cues in response to the presence of phytoplankton, coupled with (4) release of sperm by a few males, resulting in (5) a synchronous spawning by the population.

Our assessment is congruent with previous suggestions that a combination of temporal, environmental, and conspecific cues work in concert to initiate and synchronize spawning (reviewed by Mercier and Hamel, 2009). Starr *et al.* (1992) also found that a combination of sperm and phytoplankton induced more rapid spawning in *Strongylocentrotus droebachiensis* females (but not males), but they noted that sperm alone could not induce spawning, whereas we found that phytoplankton alone did not increase the likelihood of spawning above control levels. Starr *et al.* (1992) also found that the combination of sperm and phytoplankton synchronized the response time to the cues, whereas we found that, whenever spawning took place, males initiated spawning first.

The evidence that temperature induces spawning events is mixed (reviewed by Mercier and Hamel, 2009). We conducted preliminary experiments with raised temperatures (results not reported) and found spawning in response to unnaturally high temperatures; but unlike that in response to the treatment cues reported here, the spawning was not preceded by spawning behavior, so we assume that it was

simply a stress response to temperature shock. Although we have no evidence of spawning in response to more moderate temperature shifts, we cannot rule out the possibility that temperature shifts play a role in spawning synchrony in *L. variegatus*.

Our results show that both males and females of *L. variegatus* are induced to spawn by conspecific sperm, contrary to the results of a previous study (McCarthy and Young, 2004). A likely explanation for the difference is that McCarthy and Young (2004) used a 30-min window of time for spawning response. In our study, the spawning response to sperm averaged around 100 min without prior exposure to phytoplankton. Phytoplankton greatly reduced the delay between the sperm cue and spawning, and lunar period greatly increased the likelihood of a response to sperm; both of these factors were considered by McCarthy and Young (2004) as possible explanations for the lack of response that they noted.

Phytoplankton did not induce spawning in males or females above control levels in our trials. We cannot rule out that different concentrations of phytoplankton or different species of phytoplankton might have induced spawning, but our results clearly indicate that the sea urchins did detect phytoplankton in our experiments. The addition of phytoplankton resulted in spawning behavior and decreased the response time to the presence of sperm, but these effects appear to take some time. Sperm treatments and sperm-and-phytoplankton treatments did not differ significantly in response time; only when phytoplankton was added before sperm did its effects become evident. Phytoplankton concentration is likely to be less ephemeral than sperm concentration in the water surrounding sea urchins. Higher concentrations of phytoplankton or longer exposure might induce sea urchins to spawn even more rapidly in response to sperm, especially once one or several males leak sperm, thereby initiating a synchronized spawning event.

The presence of a phytoplankton primer decreased the time between the introduction of the sperm cue and the spawning response. This primer also decreased the variance among individuals in when gametes were released. Natural observations of spawning individuals of *L. variegatus* in St. Joseph Bay suggest that spawning events can last for at least an hour (Simon and Levitan, unpubl. data). Spawning events that last more than an hour have been noted in other species of sea urchins (Levitan, 2002) and echinoderms (Hamel and Mercier, 1996), during which several males initiate spawning, then additional males join, and then after about an hour females initiate spawning. This pattern is a fairly good match with the time delay of less than an hour between the introduction of sperm and the large-scale initiation of male spawning, followed by an approximately 30-min delay before females initiated spawning. The standard deviation of 10 min among sea urchins in response to cues is reasonably short compared to spawning events that last on the scale of an hour or more. Our experiments were conducted with isolated individuals, and the degree of synchrony might increase further if sea urchins were in chemical or physical contact with each other, so that cues could feed back and be magnified among individuals.

Sperm in *Lytechinus variegatus* start to age after they become diluted in seawater: compared to freshly diluted sperm, sperm 1 h after dilution require an order of magnitude higher concentration to fertilize 50% of eggs (Levitan, 2000). Studies of sperm and egg dispersal in St. Joseph Bay indicate that gametes disperse away from the spawning aggregation within minutes of release (Simon and Levitan, unpubl. data). This suggests that gamete dispersal may be a more important limitation on fertilization than gamete aging (eggs tend to be more resistant to aging than sperm in echinoids—Pennington, 1985). Thus the ability to fertilize is dependent on the degree of synchrony in relation to the length of time individuals release gametes (see Lotterhos and Levitan, 2010, for a theoretical investigation of this relationship).

The observation that male sea urchins responded earlier than females is consistent with what is known about most broadcast-spawning marine invertebrates (reviewed by Thorson, 1950; Levitan, 1998) and algae (Clifton and Clifton, 1999). Field experiments on the consequences of sex biases in the timing of spawning revealed that spawning late was much more costly for males when they competed for fertilizations. In addition, males that spawned 30 min before other males were able to win in sperm competition for eggs released by females at greater distances (Levitan, 2005). These experiments suggest that spawning early may be advantageous for males, permitting them to win in sperm competition; and that spawning later might be advantageous for females, after sperm has had time to accumulate in the water column (Levitan, 2005).

Lytechinus variegatus was observed spawning on 13 June 2009 in St. Joseph Bay, Florida (Simon and Levitan, unpubl. data). The spawning was noted in an aggregation 2 m in diameter of several hundred sea urchins piled one or two high. Sea urchins were noted moving toward the spawning aggregation from several meters away. The spawning behavior noted in our experiments was climbing. Although climbing has been noted in the field in a variety of marine invertebrates during spawning observations (reviewed by Levitan, 1988), climbing in the laboratory might also reflect attempts by individuals to move toward the source of the spawning cue. Note that the natural spawning event (Simon and Levitan, unpubl. data) was 6 days after the full moon, suggesting that although spawning might peak near the new and full moons, events can also be triggered at other times. *Diadema antillarum* also shows a propensity to spawn near the new moon, but sea urchins were observed to spawn for 3 out of the 4 weeks of the lunar cycle (Levitan, 1988). When local circumstances are right, such that a critical mass

of individuals are in close proximity and ripe, and a few males leak sperm, spontaneous spawning events might happen at less predictable times. Observations of spawning in *Strongylocentrotus* sea urchins have indicated that physical discontinuities, such as crevices in a rocky subtidal habitat, can delineate spawning and nonspawning populations (Levitan, 2002). These observations of patchy and unpredictable spawning that characterize many records of spawning (Babcock *et al.,* 1992) suggest that the right oceanographic conditions must coincide with localized cues (*e.g.,* sperm) to synchronize spawning events.

Synchrony in spawning is necessary for successful fertilization. Individuals that spawn even 15–30 min too early or too late may release gametes that never find mates (Levitan *et al.,* 2004). The use of a variety of cues at different time scales can increase precision in spawning, even when the process of gametogenesis can take weeks to months (Soong *et al.,* 2006). Other species may use different cues, appropriate for their environments, such as temperature, wave action, or tidal changes, in systems where those cues are more variable (reviewed by Mercier and Hamel, 2009). In addition, sedentary organisms may rely on cues such as sunrise (marine algae, Clifton, 1997) or sunset (corals, van Veghel, 1994) to trigger spawning when individuals might not be able to aggregate closely enough to detect conspecific signals. Given the strong negative selective pressure on individuals spawning at the wrong time (see, *e.g.,* Levitan *et al.,* 2004), we should not be surprised that external fertilizers use the cues that are available and reliable in their own environments.

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