

# Gamete Dilution and Fertilization Success Among Broadcast Spawning Octocorals

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**Abstract.** Applications of life history theory to benthic invertebrates have traditionally focused on factors affecting survival and recruitment of larvae. Among broadcast spawning species reproductive patterns also affect rates at which eggs are fertilized. These rates are implicitly assumed to be high and uniform, but neither of these assumptions may be accurate among broadcast spawning species. We conducted a series of dilution experiments with gametes of the Caribbean gorgonian *Plexaura A* in which male and female branches were maintained in isolation during the 5 day period of spawning following the full moon of August 1991. Each evening, freshly released eggs were mixed with water from 18 liter tanks which contained branches from male colonies. Eggs were mixed with water from male containing tanks at densities of 0.8, 0.4, 0.08, 0.008 or 0.0008 of the original sperm concentration. Fertilization rates were variable but decreased dramatically at 0.01 of the original sperm concentration and approached 0.0 at 0.001 of the original concentration. In situ dye release experiments, conducted to determine the dilution rates in natural settings, indicate that sperm densities drop to levels at which fertilization is unlikely within meters of a colony. Fertilization rates among broadcast spawners may be low and must be considered in analyses of reproductive strategies.

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## Introduction

Most analyses of benthic invertebrate reproductive strategies have focused on fecundity and the subsequent survival of larvae as the rate limiting steps controlling successful recruitment (for instance, Vance 1973a,b; Strathmann 1978 1985; Strath-

mann and Strathmann 1982; Roughgarden 1989). This approach dates back to Thorson's (1946 1950) conclusion that mortality of larvae is usually high and limits settlement rates among many marine benthic species. Thus, most analyses have considered reproductive strategies in which the number of settling larvae varies as a function of the size, feeding biology and longevity of the larvae. Assumptions which are implicit in these analyses are that most eggs are fertilized and that the proportion of eggs fertilized is independent of reproductive strategy. This assumption may be incorrect for species which broadcast sperm or both sperm and eggs. Studies on sea urchins (Pennington 1985; Levitan 1991; Levitan et al. 1991 1992), hydroids (Yund 1990), soft corals (Brazeau and Lasker 1992) and fish (Peterson 1992) have shown that proportions of eggs fertilized are variable and can be affected by local conditions such as population density and current regime. Thus fertilization may be a limiting step in the life histories of some marine species. In this paper we present data on the effects of sperm density on fertilization rates of a gorgonian coral and, using dye studies, identify the spatial scale over which male colonies can effectively fertilize eggs.

Most of the work on spawning and fertilization rates has been conducted on sea urchins. Pennington (1985) conducted a series of field experiments using *Strongylocentrotus droebachiensis* eggs and found fertilization rates of less than 15% among eggs placed greater than 20 cm from a male. Levitan and coauthors have characterized 1, the kinetics of fertilization of *Strongylocentrotus franciscanus* eggs (Levitan et al. 1991); 2, the effects of *S. franciscanus* density on fertilization rates (Levitan et al. 1992) and 3, the effects of individual size and population density on fertilization rates of *Diadema antillarum*

eggs (Levitan 1991). As in Pennington's work, Levitan's studies indicate that fertilization only occurs within short distances of spawning sites. Working with a hydroid, Yund (1990) found low (<20%) fertilization of *Hydractinia echinata* eggs at distances greater than 3m from males and no fertilization beyond 7 m.

Estimates of naturally occurring fertilization rates have been restricted to single reports for a coral reef fish and a soft coral. Petersen (1991) reports rates of fertilization of *Halichoeres bivittatus* which ranged from approximately 20 to 100% with a mean of 88%. The lowest fertilization rates occurred on days with the greatest water motion. Brazeau and Lasker (1992) used the ratio between the number of eggs in polyps and the number of embryos brooded on the surface of *Briareum asbestinum* colonies as an index of fertilization. *In situ* experiments suggested that fertilization dropped markedly as distance increased from 10 to 100 cm, and numbers of naturally occurring zygotes on colonies at two different sites suggested fertilization rates of 25% or less and rates that varied markedly between both sites and years. Zygote production correlated with differences in water movement, with colonies from the site with quieter waters producing more larvae.

The conclusion that one cannot assume high rates of fertilization of either spawned or brooded eggs is again suggested by the modelling efforts of Denny (1988) and Denny and Shibata (1989). They modeled probabilities of fertilization under conditions of turbulent flow. They modelled flow under a range of current regimes and found that eggs only 10 cm from the sperm source would have fertilization rates of 1% and lower (under the conditions they considered most probable for surf-zone environments).

## Methods

Fertilization rates of eggs of the Caribbean gorgonian *Plexaura A* were studied at the San Blas Field Station of the Smithsonian Tropical Research Institute (STRI) in Panama. Like many gorgonians *Plexaura A* is a gonochoric broadcast spawner (Brazeau and Lasker 1989). Females release eggs over a 4–6 day period starting 3 d after the late May full moon and then during each full moon period through early September. Egg release is synchronous starting each evening approximately 30 min after sunset and continuing for up to 90 min. The eggs are large (600–750  $\mu\text{m}$ ) and in still water will float to the surface but are readily advected throughout the water column on all but the stillest nights.

Fertilized eggs can be observed cleaving several hours after collection. Sperm release is not readily visible in the field but observations of branches kept in tanks suggest that sperm are released either prior to or simultaneous with egg release.

The effects of sperm density on fertilization rates were determined in a series of experiments conducted following the full moon of July 1991. Two days prior to the full moon colonies containing large eggs or spermaries were collected from reefs in the San Blas Point region of the San Blas Islands and were transported to 18 liter aquaria at the STRI field station. Water in the tanks was vigorously exchanged every 2–4 h throughout the day, then once before dusk (approximately 6 P.M.), once at midnight and then again at 7 A.M. The water in the aquaria was unfiltered and the water intake at the field station is over 1 km from the nearest male *Plexaura A* colony. Males and females were maintained in separate tanks. Colonies maintained in this fashion released gametes over a 5 day period starting 4 days after the full moon, the same days as colonies in the field. On each of 4 nights eggs were collected from two tanks. Those eggs were then mixed with water from a tank containing branches from four different male colonies. The combined size of the branches was 920 linear cm of tissue (approximately  $69 \times 10^3$  polyps). Equipment limitations (the lack of a compound microscope) prevented us from directly determining sperm density in the sea water from male tanks. The same male and female colonies were used on each of the four nights.

Groups of 50 eggs each were incubated in 125 ml of sea water in polystyrene containers which contained either 100, 50, 1, 0.1 or 0.01 ml of water from the male tank. These amounts correspond to densities of 0.8, 0.4, 0.08, 0.008 and 0.0008 of the original sperm density in the male tanks. The containers with sperm and eggs were placed in mesh bags and suspended over the side of a dock where they were subject to agitation by gentle wave action. The experiments were terminated after approximately 12 h when 2 ml of formalin was added to each container. Each of the eggs was examined on a later date for signs of fertilization and development. Cleaving cells are readily observed in developing embryos 4 h after fertilization and after 12 h embryos have undergone gastrulation and their surface has taken on a pockmarked raisin-like appearance. Virtually all embryos which survive to this stage develop into planulae.

Previously, Brazeau and Lasker (1989) hypothesized that *Plexaura A* may develop parthenogenetically. That conclusion was based on the appar-

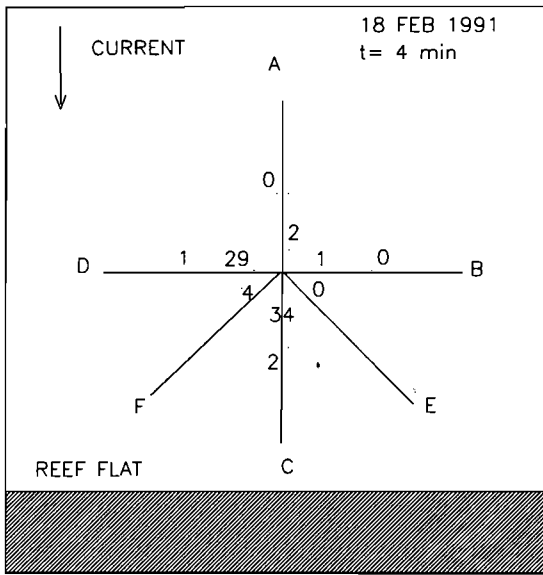


Fig. 1. Sampling pattern for dye release experiments and dye concentrations 4 minutes after dye release at Korbiski Reef on February 18, 1991. Sample points at 2, 5 and 10 m were located at the intersection of the dashed circles and the axis. Dye concentrations (ppb of the 10% Rhodamine B solution) are listed at the points they were collected from. The dominant onshore current depicted in this example was unusual among the replicate experiments. In the other four replicates the dominant current flow ran parallel to the reef flat.

ent absence of males on reefs in the San Blas Point area. Since that time we have identified a small number of male colonies. Additionally we have now completed aquarium experiments in which female branches were maintained in aquaria either with male branches or alone. Those experiments demonstrate fertilization in the presence of males. There have also been rare instances of egg development in the absence of males. However, we have not been able to exclude contamination as the source of those developing eggs and we now believe that the vast majority (over 99.99%) of the eggs which develop in the field are fertilized.

We used in situ dye studies to assess the dilution of sperm around colonies. Experiments were conducted at Korbiski Reef, a site with abundant *Plexaura* A. Five replicate experiments were conducted on days characterized by low wave and current action. Thus the observed dilution rates should provide data on the minimal levels of dilution and maximal residence time on the reef. At the start of each experiment 10 ml of a 10% Rhodamine B solution (Forestry Suppliers, Jackson, MS) were released 0.5 m above the bottom. The dye release point was on a sand and rubble plain which sloped

from a reef flat to a slope break at approximately 5 m depth. The release point was at 2.5 m depth and was 15 m from the reef flat and 20 m from the slope break. Water movement at the site was characterized by an oscillatory onshore component which was driven by wave action and by a current parallel to the reef slope which most commonly ran to the NE. Prior to the dye release, a grid was set up and sampling sites were marked along the major axis of the grid at distances of 2, 5 and 10 m from the release point (Fig. 1). The grid was always labelled such that the A-axis was upstream of the dye release. The 10 ml of dye was placed into a balloon and release was effected by breaking the balloon. Water samples were collected at the 0 and 2 m sampling sites at the time of release ( $t=0$ ) and at 2, 4, 8, 16 and in some cases 32 min. after release. Divers collecting the samples did not swim through the forward boundary of the dye cloud and movements were always kept to a minimum. Samples at the 5 and 10 m stations were not sampled at times 0, 2 and 4. Water samples were collected in glass scintillation vials which contained air and were filled by removing the vial cap.

Approximately 2 min were required for two divers to complete the samples from the 0 and 2 m points and 4 minutes to collect the samples from the 5 and 10 m points. Samples collected at the release point at time=0 were actually collected approximately 1 min after the dye was released. The diver collecting the samples at the 0 and 2 m marks generally remained on the bottom and collected samples with as little swimming as possible. The diver collecting samples from the 5 and 10 m sampling sites swam on the surface and/or around the periphery of the dye cloud whenever possible.

An additional dye release experiment was conducted in which the dye concentration was determined at a drogue which was released simultaneously with the dye. The drogue was a 2 m length of nylon rope which was weighted slightly at one end and had a small piece of styrofoam at the other end. The size of the styrofoam float was adjusted such that the top of the drogue floated several centimeters below the water surface.

Vials containing the water samples were capped, sealed with parafilm, wrapped in paper and returned to the laboratory for fluorometric measurement of dye concentration. A Turner Model 111 fluorometer, equipped with a 546m $\mu$  primary filter, a 590m $\mu$  secondary filter and a high sensitivity sample holder was used for measurements of fluorescence. The fluorometer was allowed to warm up for >2 h prior to measurements. Eleven standards made from serial dilution of the original 10% so-

lution (but following the procedures of Wilson 1968) were used to quantify the unknowns. All standards and unknowns were at the same temperature.

Current measurements at the point of dye release were made with a General Oceanics mechanical current meter with a low speed rotor (minimum current sensitivity 2 cm/sec). The current meter was moored 1.5 m above the point of dye release and was free to rotate as current direction varied. Additional, lower resolution estimates of current speed were made from observations of the position of the leading edge of the dye cloud at the sample times. Position of the dye cloud was recorded to the nearest meter at those times.

**Results**

Dilution of water from the male tanks markedly reduced fertilization rates of *Plexaura* A eggs (Fig. 2). The experiments can be broken into two groups, those which were conducted 30–31 July 1991 and those conducted on 1–2 August 1991. The July experiments were characterized by low fertilization rates regardless of dilution whereas fertilization rates in the August experiments were high (>50%) at the 1.25 and 2.5-fold dilutions. In all cases fertilization rates were virtually nill at the 1250-fold dilution.

*Dye experiments.* Dye concentrations dropped markedly over both time and distance (Fig. 3). Current speeds during the different experiments are presented in Table 1. The sensitivity of the current meter is 2 cm/s and values lower than that can not be interpreted as accurate estimators of current speed. In most cases current meter derived flow rates were less than 2 cm/s. A second estimate based

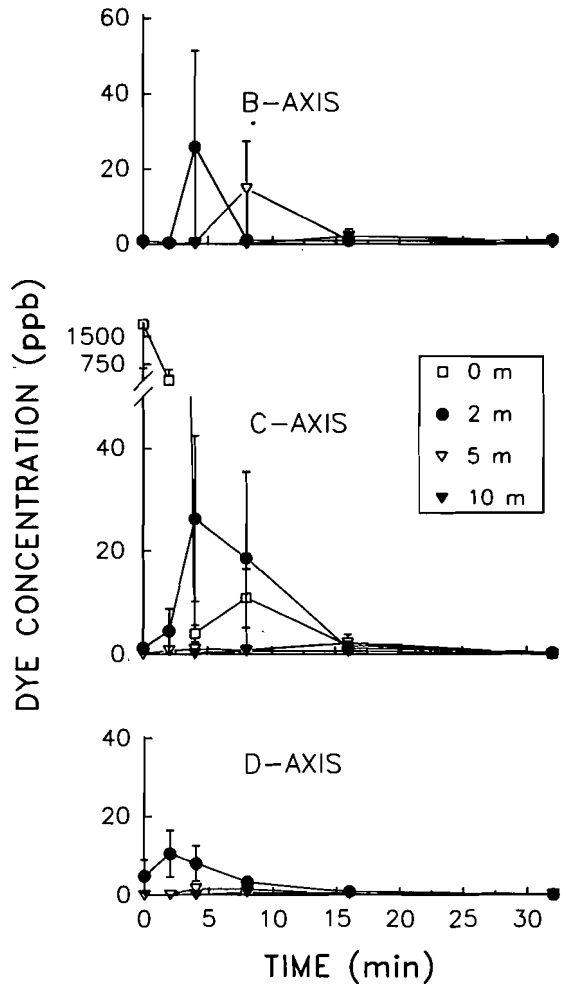


Fig. 3. Average dye concentrations at points 2, 5 and 10 m from the point of dye release along the B, C and D axis. Error bars are standard errors of the mean. Concentrations at the point of release (0 m) are presented with C-axis data.

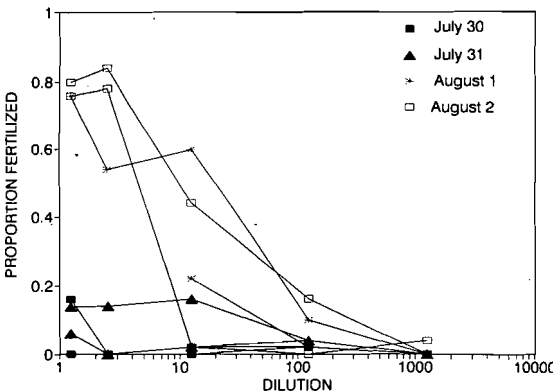


Fig. 2. Proportion of *Plexaura* A eggs which developed after 12 h incubation in water from a tank containing four male colonies as a function of dilution of the water from the male tanks.

Table 1. Current velocities measured at Korbiski Reef, San Blas Islands during dye release experiments.

Date	Velocity (cm/s)	
	Current Meter	Movement of dye cloud
6 July 1990	1.1	1
26 September 1990	0.4	1
30 November 1990	1.8	2
5 January 1991	—	4
18 February 1991	2.9	4
2 May 1991	1.6	1
1 June 1991	0.6	2

on the movement of the visible portion of the dye cloud indicates the mass transport of the dye was usually greater than the current meter reading.

The dye was mixed with 10 ml of sea water, but the mixture was less dense than sea water and upon release the slightly buoyant dye tended to rise. The dye reached the surface within the first minute after release. Dye at the surface (the upper 10–20 cm) often travelled more quickly than the dye 0.5 m above the bottom (the sampling depth), and the surface layer often followed a slightly different track than the deeper water. On those occasions in which a distinct surface layer formed the surface water was apt to be swept onto the reef flat. The dye cloud both on the surface and at depth was visible through the first 16 min of the experiment. Dye was seldom visible to the naked eye at 32 min both as a function of the visible advection of the dye cloud out of the sampling grid and dilution.

Observation of the movement of the dye cloud indicates the dye was not rapidly transported away from the sampling area, but concentrations of the dye dropped rapidly with both distance from the release point and with time (Fig. 3). The average pattern (Fig. 3) was for the dye to spread downstream with the longshore current and on to the reef flat as a result of wave action. At any one point the dye concentration rose rapidly as the dye cloud reached the site and then slowly dissipated. The highest concentrations were observed along axis B and C. The pattern was extremely variable between days. Some dye was detected just upstream of the release on some days, corresponding to the days with the lowest current and the greatest effect of

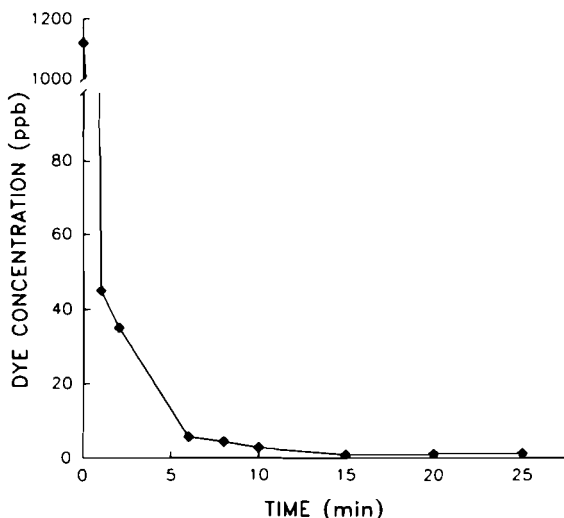


Fig. 4. Dye concentrations of water samples collected alongside a drogue released simultaneously with the dye.

wave-driven oscillatory water movements. At the points 2 m from the point of dye release, the maximum observed dye concentrations were 103 ppb which is equivalent to a dilution factor of  $10^7$ . At points other than those directly downstream or at distances of 5 or 10 m dilution factors were most often  $10^9$  or greater.

Dye concentrations in the drogue release experiments are depicted in Fig. 4. Currents during the experiment were virtually null and during the course of the experiment the drogue had drifted approximately 10 m to the southwest of the release point after 15 minutes and then reversed direction and was approximately 5 m south of the release point after 25 minutes. As in the other experiments the released dye became vertically mixed within minutes of release but then travelled in visually stratified layers. The drogue did not exactly track the centroid of the visible dye cloud and the drogue's movements were somewhat more responsive to the surface layer than other layers in the water column. After 2 min dye at the drogue was diluted by a factor of  $10^7$ . The concentration at the release point was only slightly less than half of that at the drogue. Concentrations at both the drogue and source points fell to less than 10 ppb after 10 min.

## Discussion

The sperm dilution experiments demonstrate that sperm density has a large effect on fertilization rates of *Plexaura A* eggs and the dye release studies indicate that sperm released by a colony are very rapidly diluted as water movement carries them away from their source. However, neither of these experiments exactly mimics the processes by which sperm are released and dispersed into the water column and then fertilize eggs. Thus the interpretation of our results is dependent on understanding the process of sperm release and fertilization and comparing those with our experiments.

Absolute sperm densities in our experiments were not measured, and it is possible that the differences in fertilization rates observed between the 30–31 July and 1–2 August experiments were related to variation in sperm density. Egg release varies on a daily basis and such a finding would not be surprising in sperm release as well. However, the presence of uniform but low fertilization rates at low dilutions (i.e. the higher sperm concentrations) on 30 and 31 July suggests that sperm concentrations were saturating and that the reduced fertilization rate was related to some other factor such as egg maturation or viability.

Although absolute densities were not measured we can estimate an upper bound for the densities in the dilution experiments. These estimates are based on the numbers and size of spermaries from a large male colony collected from Korbiski Reef in 1990 just prior to spawning. Spermary size and the number of spermaries per polyp were determined from dissection and measurements of 10 polyps. Density of sperm/testes was determined from histologic sections. Sections ( $7\mu\text{m}$  thick) were stained with azocarmine B (Yevich and Barszcz 1982) and counts of numbers of sperm found in "transects" across spermaries were made at  $1260\times$ . Polyps contained 10.8 spermaries (S.D.=2.0,  $n=10$ ) and those spermaries were  $256.3\mu\text{m}$  diameter. The polyp contained on average  $8 \times 10^6$  sperm/polyp. *Plexaura A* colonies contain approximately 75 polyps per linear cm of branch (Lasker 1990), that branches in the tanks were approximately 920 cm in total linear length and the aquaria had volumes of 18 liters. If all of the sperm were released at once density of sperm would be  $6 \times 10^6$  sperm/ml. This estimate is an upper bound since all of the sperm are not released on a single night and some of the spermaries in many of the polyps are immature. If the estimated sperm densities of approximately  $10^6$ /ml are correct, then the sperm concentration-fertilization relationship of *Plexaura A* is very similar to the sea urchins data reported by both Pennington (1985) and Levitan et al. (1991). As in our experiments with *Plexaura A*, Pennington and Levitan both report marked decreases in fertilization rate at concentrations between  $10^4$ /ml and  $10^3$ /ml.

The relationship of sperm density in the experiments with those on the reef is less clear. As calculated above a single polyp produces  $8 \times 10^6$  sperm. We have not observed sperm release on the reef, but observations of colonies in aquaria (M.A. Coffroth pers. comm.) suggest that it occurs over an approximately 10–30 min period and is characterized by the release of a slightly viscous, clear fluid similar in appearance to mucus. Using spermary content as an estimate of sperm production, then complete sperm release from a single polyp over a 10 min spawning period would yield a release rate of  $1 \times 10^4$  sperm/s. The initial density of released sperm can be calculated if one assumes, that the volume of fluid containing the sperm is negligible at the moment of release and that the sperm are released into a quasi-laminar flow. In that case, after 1 s the sperm would be distributed in a volume of water equal to a cone whose base has a radius determined by the swimming speed of the sperm and a height (distance away from the polyp) based

on the current speed. In a 2 cm/s current and with sperm swimming at  $100\mu\text{m/s}$  the volume of this cone is  $2 \times 10^{-4}$  ml and the density of sperm is  $6 \times 10^7$  sperm/ml. These estimates, which must be viewed as rough approximations, suggest that the initial density of sperm used in the dilution experiments is comparable to that which occurs in the field within centimeters of the polyp.

The dilution experiments suggest that a 100 to 1000-fold dilution of sperm can dramatically reduce fertilization rates. In the dye release experiments such dilutions occurred within the first 2 min of the experiment. If average concentration is calculated as a function of diameter of the dye cloud then dilutions of  $10^{-3}$  will occur once the dye cloud has spread into a sphere with a 10 cm radius. Observations during the experiments show that occurs within 1 minute. Indeed, the very highest observed concentration during the experiments was 7700 ppb (a  $10^6$  dilution of the dye).

These estimates of dilution suggest that fertilization in the natural setting is virtually impossible. However, *Plexaura A* eggs are fertilized in nature (Lasker, unpublished data), suggesting that sperm density in the water column is locally greater than that expected from the very simple model of sperm dispersal and fertilization presented here. Extrapolation of our laboratory results to the field also requires evaluation of some factors which increase fertilization rates relative to those observed in aquaria as well as factors which should reduce fertilization relative to that observed in experiments. Firstly, the water containing gametes can be thought of as a conveyer belt moving past colonies some of which contribute sperm and some eggs. Thus density of sperm in the water surrounding any given egg will accumulate as sperm are added by many colonies. Secondly, each time an egg passes in close proximity to a male colony there is opportunity for it to be exposed to the relatively greater concentrations of sperm as they are released by the colony. The transport of sperm (or eggs) across the reef and past opposite sex colonies also has the negative effect of limiting the exposure of the sperm (or eggs) to only that fraction of eggs (or sperm) that were released in the short time the sperm (or egg) is in the vicinity of the opposite sex colony. Finally, our discussion of dilution has assumed that dye (and thus sperm) are uniformly distributed. During the first several minutes of dye dilution we observed a distinct concentration gradient between the center and edges of the dye cloud. Additionally, sperm appear to be released in a mucous-like substance. If sperm do not fully mix, local concentrations (on a scale of cm and mm) may be

substantially greater than that suggested by a simple diffusion model. This latter phenomena is suggested for gorgonians by the extremely patch distribution of zygotes on *Briareum asbestinum* colonies (Brazeau and Lasker, 1992).

It also is important to note that in our experiments, eggs were sealed in 125 ml containers with the sperm. The extended exposure of eggs and sperm in a calm environment could enhance fertilization rates. For instance, Levitan et al (1991) identified a significant positive relationship between fertilization rate and the length of the laboratory experiments. However, the scale over which time was a factor was that of tens of seconds, and the length of the experiment had 40 fold smaller effect on fertilization rate than did sperm concentration. Petersen (1991) did not detect any difference in fertilization rates of fish eggs collected from spawning events after either 30 or 60 seconds. The data of these authors suggests that fertilization occurs early in an experiment and that extending the experiment has little effect on that rate. Thus sealing eggs and sperm into a container may have slightly enhanced fertilization rates, but other effects probably had a much greater influence on fertilization than the extended exposure used in our experimental protocol.

Although the opportunities for fertilization may not be quite as low as suggested by the dilution and dye release experiments, it is clear from these data that uniformly high fertilization rates cannot be assumed for *Plexaura A* gametes. Thus selection for enhanced fertilization rates should be included in models of "reproductive strategy" among broadcast spawning species. Furthermore, fertilization rate may actually limit population growth for some species and in some settings. Lessios (1988) and Levitan (1988) have both speculated that the slow recovery of *Diadema antillarum* following the 1983 mass mortality, may be related to density related reductions in fertilization rate. Whether or not such effects are important components of the population dynamics of broadcast spawners should be explored.

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