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In situ Rates of Fertilization Among Broadcast Spawning Gorgonian Corals

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Abstract. Fertilization rates among marine benthic taxa have implicitly been assumed to be uniformly high in most analyses of life history evolution, but *in situ* fertilization rates during natural spawning events are rarely measured. Fertilization rates of the Caribbean gorgonians *Plexaura kuna* and *Pseudoplexaura porosa* were measured at a site in the San Blas Islands, Panama, by collecting eggs downstream of colonies during synchronous spawning events during the summer months in the years 1988–1994. Eggs collected by divers were incubated, and the proportion of eggs that developed was determined. Proportions of eggs developing suggest fertilization rates that vary from 0% to 100%. Monthly means ranged from 0% to 60.4%. Failure of gametes to develop can be attributed to sperm limitation, as eggs collected during spawning had higher fertilization rates if incubated with an excess of sperm. *Plexaura kuna* fertilization rates were highest during the July spawning events. Fertilization of *Plexaura kuna* eggs was usually lower during the first two nights of the 4–6 night spawning event. The proportion of eggs being fertilized when collected from a given place and time was highly variable, with one peak in the frequency distribution at or below 20% fertilization, and a second group of samples with greater fertilization rates. High variance in fertilization rates is evident at all levels of analysis: between replicate samples, times within nights, and among nights and months. This variance can be attributed to a combination of the effects of heterogeneity in the water column as gametes are diluted, spawning behavior of the gorgonians, and the current regime. Fertilization rates are often low and may represent a limiting step in recruitment during some years. Low fertilization rates may also be an important component of the life history evolution of these species.

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Introduction

One of the primary goals of benthic ecology has been the determination of the factors limiting populations. For most of this century, such efforts have been concentrated on post-recruitment events in the life history of the organism (*i.e.*, Connell, 1961, and hundreds of subsequent studies). Missing from such analyses are the processes that directly or indirectly affect the number of larvae that settle. The importance of such processes was recognized by many authors (*e.g.*, Thorson, 1946), but the difficulties of measuring phenomena such as survival of larvae in the water column led to research that virtually ignored larval ecology in favor of the more knowable post-settlement dynamics of populations. More recently, larval ecology has been rediscovered (Young, 1990; Grosberg and Levitan, 1992), and a wide variety of work has documented the importance of larval dynamics and the circumstances under which recruitment may limit populations (Connell, 1985; Gaines and Roughgarden, 1985; Gaines *et al.*, 1985; Roughgarden *et al.*, 1988).

As in the earlier treatments, most studies of larval ecology have also glossed over a potentially limiting but seemingly unknowable component of the life cycle of broadcast-spawning taxa: the proportion of gametes that are fertilized and that complete early development. This bias reflects the general difficulties of determining the fate of gametes and embryos after they are released into the water column.

Thus, most analyses of benthic invertebrate reproductive strategies have focused on fecundity and the subsequent survival of the larvae as the rate-limiting steps controlling successful recruitment (for instance, Vance, 1973; Strathmann, 1978, 1985; Strathmann and Strathmann, 1982; Roughgarden, 1989). Assumptions that are implicit in these analyses are that eggs are readily fertilized, that fertilization rates are relatively uniform over time and space, and that the proportion of eggs fertilized is independent of reproductive strategy.

The assumption that fertilization is uniformly great may be incorrect for many species, and a growing body of literature suggests that fertilization has the potential to be a limiting step in the life histories of some benthic species. Pennington (1985), Yund (1990), Levitan (1991), Levitan *et al.* (1991, 1992), Babcock *et al.* (1992, 1994), Brazeau and Lasker (1992), Oliver and Babcock (1992), Benzie *et al.* (1994), Benzie and Dixon (1994), Yund and McCartney (1994), and Levitan (1995) have experimentally demonstrated the potential for sperm limitation in the fertilization of a variety of benthic species. These measurements, in concert with the modeling efforts of Denny (1988) and Denny and Shibata (1989), suggest that eggs are often sperm limited.

Measurements of fertilization rates from naturally spawning events have yielded rates that are substantially below 100% (Table I). The most extreme of those rates have been those of *Briareum asbestinum,* which has been observed over multiple years and sites. Observed rates were as low as those suggested by experimental data (Brazeau and Lasker, 1992). However, in many cases fertilization rates during natural spawning events have been surprisingly great, as among *Acanthaster planci* (Babcock and Mundy, 1992). Although the rates presented in Table I are generally below 100%, most are also well above 0%. Apparent fertilization rates that are lower than 100% could be a function of egg viability or biases introduced by sampling techniques. The fertilization rates that have been reported, although consistent with the hypothesis of sperm limitation, do not demonstrate sperm limitation. Furthermore they suggest that sperm limitation may be taxon-, site-, and time-dependent. If these restrictions held, sperm limitation could contribute to the differential success of populations and species at differing sites.

Most field measurements of fertilization rates have also been restricted to either single spawning events or sites. Thus both the extent and source of variation in fertilization rates are unknown for most taxa. Characterization of this variation, when it has been conducted, has proven to be extremely interesting. Brazeau and Lasker (1992) examined fertilization rates of *Briareum asbestinum* at two sites in Panama over two years. They reported 0.0% fertilization rates from one of those sites (*i.e.,* complete reproductive failure), suggesting that some sites with seemingly healthy populations may have little impact on the survival of a species. Petersen and colleagues (Petersen, 1991; Petersen *et al.,* 1992) reported on fertilization rates of wrasses, both across reefs and across days, and they identified environmental conditions that may enhance fertilization success and explain variance in fertilization success. These results suggest that fertilization success, by controlling the relative contribution of individuals, may be an important variable in the evolution of the life history of a species.

Table I

Fertilization rates determined from in situ *collections of spawned eggs or from incubations of eggs with sea water samples*

Species	Fertilization rate	Reference
Briareum asbestinum (gorgonian)	<0.01–6.5%	Brazeau and Lasker, 1992
Montipora digitata (coral)	0.2–49.0%	Oliver and Babcock, 1992
Acanthaster planci (asteroid)	23–83%	Babcock and Mundy, 1992
Actinopyga lecanora (holothurian)	67–78%	Babcock *et al.,* 1992
Bohadshia argus (holothurian)	0–96%	Babcock *et al.,* 1992
Cucumaria miniata (holothurian)	92 (1–100%)	Sewell and Levitan, 1992
Holuthuria coluber (holothurian)	9–83%	Babcock *et al.,* 1992
Halichoeres bivittatus (wrasse)	20–100%	Petersen, 1991
Thalassoma bifaciatum (wrasse)	76% (0–100%)	Petersen *et al.,* 1992

In this paper we report fertilization rates for the broadcast spawning Caribbean gorgonians *Plexaura kuna* and *Pseudoplexaura porosa* at a site in the San Blas Islands, Panama. We document variation in fertilization rates over spawning events that occurred over 15 months during the period 1988–1994, and we show that the low fertilization rates can be attributed directly to sperm limitation at this site.

Materials and Methods

Fertilization success of the gorgonians *Plexaura kuna* (previously discussed as *Plexaura* A, Lasker *et al.,* 1996) and *Pseudoplexaura porosa* was measured by determining the proportion of spawned eggs which initiated development. *P. kuna* is a gonochoric, broadcast spawner that releases its eggs during 4–6 day spawning events that occur four days after the full moon during the months of June–September or May–August depending on the timing of

the full moon (Brazeau and Lasker, 1989). In Panama spawning is restricted to a 60-min period starting 0–40 min after sunset, and most of the spawning occurs during the middle 30 min of the release period. *Pseudoplexaura porosa* is also a gonochoric broadcast spawner, and it spawns over a 3-day period. Its spawning event usually overlaps with *P. kuna* on 2–3 nights. A second, distinct *Pseudoplexaura* sp. was observed on two nights and collections of its eggs were also made when colonies were observed spawning. Collections of all species were made at the northeast corner of Korbiski Reef, a small reef located near the San Blas field station of the Smithsonian Tropical Research Institute (STRI). Gorgonians, and *P. kuna* in particular, dominate the benthos in this area (see descriptions in Lasker and Coffroth, 1985; Lasker, 1990). Collections were made at arbitrarily selected locations on the reef at depths of 2–5 m.

Gametes used in experiments were collected from colonies maintained in 18-l aquaria at the STRI field station. Colonies were collected several days prior to spawning and transferred to aquaria in which the seawater was replaced 5–7 times daily. Colonies maintained in this fashion spawned on the same nights as *in situ* colonies, but spawning in aquaria usually lagged behind *in situ* spawning by 30–90 min.

We assayed released eggs for fertilization during spawning events between 1988 and 1994. Collections during 1988–1991 were made at arbitrarily selected positions immediately downstream of female colonies that were releasing eggs. Divers were usually 1–3 m from the colony and collected the large (>600 μm diameter) eggs in 60-ml syringes. Starting in 1992, eggs were collected by SCUBA divers positioned along transects several meters downstream of large clusters of colonies releasing eggs. In all years sites for collection were chosen to minimize collection of eggs from other colonies and other species. Divers surveyed the collection areas for eggs from other colonies both before and after the collections. Collections believed to be contaminated with eggs of unknown origin were not considered in the analyses. Upon collection, eggs were either transferred to a 1-liter-capacity plastic bag (1988–1991) or were left in the syringes (1992–1994). After 0.5-2 h, eggs were counted, placed in seawater that had been collected prior to the start of spawning (henceforth referred to as sperm-free seawater) and then incubated in 120-, 500-, or 1000-ml containers. Concentrations of eggs were kept below 500 in 1000-ml containers, 200 in 500-ml containers, and 75 in 120-ml containers. All containers were soaked in seawater for at least 2 weeks prior to use, and then rinsed and dried prior to use. The containers were suspended in mesh bags off of a dock at the STRI field station. The containers, which were slightly buoyant, bounced with the mild sea swell at the laboratory site, and the eggs, which were buoyant, were distributed throughout the water in the container. Seawater in the containers was changed at 12 h and when applicable at 36 h. The number of developing embryos observed either 12 h (1993, 1994) or 36 h after spawning (1988–1992) was used as our estimator of the number of eggs that had been fertilized. Number of embryos at 12 h was determined using a dissecting scope at 20×; number of embryos and/or planulae at 36 h was determined without magnification.

In 1993 and 1994 we also collected water samples simultaneous with our egg collections in order to measure the "fertilization potential" of the water. These samples were transported back to the STRI field station where they were used to incubate eggs that had been collected out of aquaria that contained female colonies alone. Incubations were started 0.5–2 h following collection of the water samples. *Plexaura kuna* sperm remain viable for 2–4 h (Lasker, unpub.). As in the field samples, eggs were assayed the following morning to determine the proportion fertilized.

In order to determine whether *in situ* fertilization was sperm limited, a series of paired samples were collected in August 1992. Eggs were collected on the reef in syringes as described above. Eggs from two syringes were pooled and counted and one half of the eggs were then incubated in sperm-free water, while a second half of the eggs were incubated using water from an aquarium containing a male colony. After 12 h the number of developing embryos was determined in each sample. Sperm density was not determined, but in more recent experiments we have found that sperm densities in such tanks are typically $>10^5$ sperm/ml.

Our collecting techniques incorporated several potential biases, and a series of experiments were conducted to assess biases in our estimates of fertilization. Eggs that are collected in syringes are maintained in water that presumably contains sperm. Fertilization is a function of sperm density for *Plexaura kuna* (Lasker and Stewart, 1993). Sperm density in the water column would presumably steadily decline as a function of turbulent mixing. Thus eggs would normally be exposed to ever decreasing concentrations of sperm as they were transported off the reef. Eggs collected and then maintained in syringes for 0.5–2.0 h could have elevated fertilization rates. As a test of such an effect, paired samples of eggs were collected by divers stationed next to each other. These samples were immediately carried to an overhead boat. In an initial experiment, in July 1993, one of the two samples was immediately diluted 20 times and then both samples allowed to develop overnight. In August 1993 we further tested for a "syringe effect" by taking one of the paired samples and immediately washing it with sperm-free water. The eggs were rinsed with 200 ml of seawater in a 150-μm-mesh Nitex filter device that kept the eggs submerged and off the screening at all times. The

eggs were then transferred to a 120-ml container and thereafter treated like all other field-collected eggs.

An additional set of washing experiments was also conducted using virgin eggs collected from aquaria that contained only female colonies. Virgin eggs were (1) incubated with sperm overnight without any washing [control]; (2) incubated for 1 min in seawater containing sperm, then washed and incubated with sperm-free seawater [washed]; (3) incubated for 1 min in sperm-free seawater, then washed and incubated overnight in water containing sperm [control for effects of washing on eggs]; and (4) incubated with sperm for 1 min, then washed and incubated overnight in seawater containing sperm [control for effects of washing on fertilized eggs]. These treatments assessed whether the rinsing procedure damaged eggs and reduced apparent fertilization rates.

To assure the identity of collected eggs, samples were gathered immediately downstream of spawning colonies. Thus eggs were collected within 1–5 m of their source colony and, depending on current speed, were probably being collected within 0.5–3 min of release. We have not observed any delay in maturation of eggs released by *Plexaura kuna* colonies (unpub. data). However, in other species, fertilization rates are affected by the amount of time exposed to sperm (Levitan *et al.*, 1992). Therefore our observed fertilization rates could be artificially low. To test for such an effect we conducted two surveys. On August 10, 1993, two divers collected eggs being released by a single *Pseudoplexaura* sp. colony. This colony was the only female colony releasing eggs at the time of the collection (1955–2058) and identity of the eggs is virtually certain. One diver collected eggs from approximately 1 m away and the second from 6–8 m downstream. Eggs were collected and incubated as previously described. Numbers of developing embryos were determined the following morning.

A second assay for potential downstream fertilization was made by collecting seawater downstream of the reef and then using that water to incubate virgin eggs. Downstream water was obtained by releasing a drogue buoy during the period of peak egg release and then collecting water from around the buoy 5–20 min later. The water was transported back to the field station and replicate samples of 50 virgin eggs incubated in the water in a 120-ml container. The drogue could only be deployed on nights when the prevailing currents would not wash the buoy onto the reef. On all nights a water sample was collected from the reef and used to incubate virgin eggs.

The proportion of eggs that had developed was assayed at 12 h (1993–1994) and 36 h (1988–1992) after spawning. This estimator of fertilization could underestimate fertilization success by incorporating post-fertilization mortality. In 1991, 20 groups of 50 eggs were incubated in water containing sperm and the number observed developing was determined at both 12 and 36 h after the start of the incubation.

Results

Fertilized eggs began cleaving several hours after exposure to sperm. After 4 h embryos were at the 16–64 cell stage of development. After 12 h individual cells were no longer visible and the stereoblastula exhibited a characteristic raisin-like wrinkled appearance. Between 12 and 36 h, embryos elongated and took on the appearance of a planula, but only a few of the planulae were actively swimming at 36 h. Twenty groups of 50 eggs that were incubated with sperm had mean survivorship of 45.6% (S.D. = 2.2) at 12 h and 37.0% (S.D. = 5.9) after 36 h. The decrease in survivorship was significant (F = 46.5, df = 1, 19, $P << 0.001$). The fertilization rates observed at 12 h ranged only from 44 to 50%, and over this range there was not a significant relationship between the initial fertilization rate and the decline over the subsequent 24 h (fertilization at 12 h *vs* 36 h, r^2 = 0.11). However, the result does indicate that mortality of developing eggs during the 1988–1992 observations led to an underestimate of fertilization of 9%.

Simple 20-fold dilution of the water in which the eggs were collected had no effect on fertilization (Fig. 1), indicating either that the dilution was insufficient to elicit a response or that there were only minimal numbers of post-collection fertilizations. Diluted and undiluted incubations were split evenly between Diver 1 and Diver 2 collections. Variance between samples, including simultaneous and adjacently collected paired samples, was extremely high. Between-sample variance made up 66% of the total variance among those samples, with between-pair (*i.e.*, time) variance contributing 34% of the total variance. The washing experiments indicated that holding eggs in syringes with seawater from the reef had a small but significant effect on fertilization rate (Fig. 2). Measuring the effect of washing was made difficult by the high level of sample to sample variance (std. dev. of fertilization rates among control samples, 20%; percent of variance explained by error term, 34%; and percent of variance explained by between-paired samples, 65%). On average, washing reduced fertilization rate by 4%, but as is evident from Figure 2, the variance in the effect was large [std. dev. of (control fertilization rate − washed fertilization rate) = 17%]. When analyzed as a two-way ANOVA (arctan transformed values) there was no significant difference between washed and control fertilization rates, and the slope determined by RMA regression analysis (Niklas, 1994) did not differ from 1.0 (Fig. 2). (The experiments for filtration effects indicate that eggs could still be fertilized following filtration. Eggs in the control had a fertilization rate of 67%; eggs that were added to sperm-con-

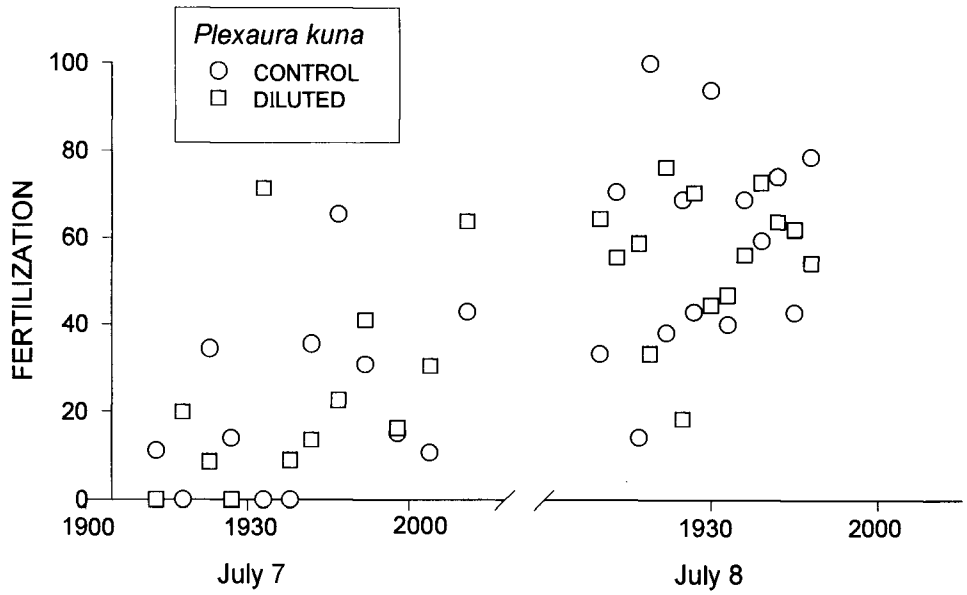

Figure 1. Proportions of *Plexaura kuna* eggs fertilized in individual samples collected on two nights at Korbiski Reef in 1993. Diluted and undiluted samples were collected from within 1.5 m of each other at identical times. Half of the samples were diluted 20-fold after collection, but dilution had no effect on fertilization rates (see text).

taining seawater but immediately washed had a fertilization rate of 15%, indicating that washing could prevent post-collection fertilizations. Eggs that were washed and then incubated with sperm-containing water had a fertilization of 60% and those that were exposed to sperm, washed and then again incubated with sperm had a fertilization rate of 71%. These latter controls indicate that mechanical effects of washing on both fertilized and unfertilized eggs were negligible.)

Fertilization rates in the water column as eggs drifted away from the reef had minimal effect on our field estimates of fertilization. *Pseudoplexaura* sp. eggs that were allowed to float in the water column for an additional 5–7 m after release did not have greater fertilization rates than eggs collected 1 m downstream of the colony (38% at 1 m *vs.* 30% at 6–8 m; *t*-test $P > 0.20$, $n = .013$ paired samples). Water collected immediately downstream of the reef during *Plexaura kuna* spawning events was often capable of fertilizing eggs, but fertilization rates in those experiments never exceeded 4%. Those values are probably maximal estimates of downstream fertilizations, because the water samples were taken only on days with low current flow (approx. 2.5 cm/s), when mixing of the sperm would be lowest, and because incubations with those water samples (*i.e.*, exposure to sperm) lasted 12 h instead of the minutes of exposure that would occur naturally.

The number of samples obtained during each of the days/months/years was highly variable (Table II). Differences between years and months reflect differing collecting effort and differences between days within months reflect the availability of eggs at the Korbiski site. The number of days sampled (3–4) in each month usually reflects our inability to collect unambiguously mono-specific *Plexaura kuna* collections on the last two days of the spawning event when *Pseudoplexaura porosa* also spawned.

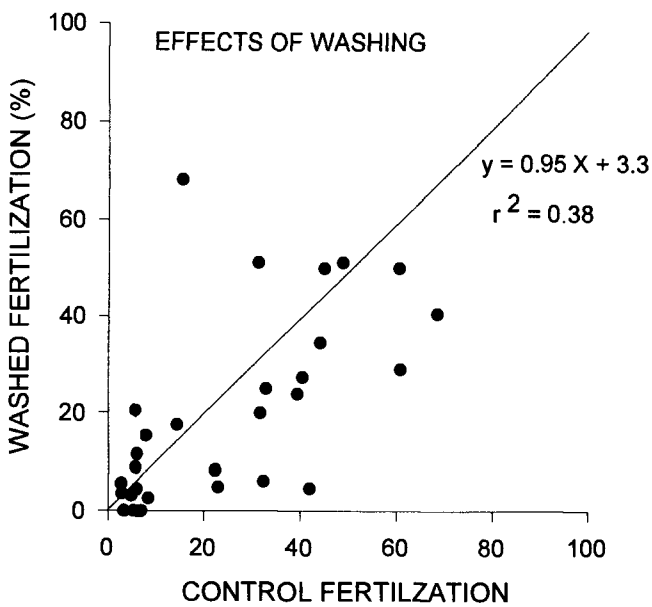

Figure 2. Effects on *Plexaura kuna* fertilization rates of washing eggs immediately following collection.

Table II

Collection sites and numbers of samples collected at Korbiski Reef, Panama, during spawning events

Date	Site	Number of days	Number of samples	Number of eggs
June 1988	A	2	2	108
July 1988	A	2	8	1,084
August 1988	A	1	2	48
June 1989	A	3	10	678
July 1989	A	6	26	3,526
June 1990	A	2	4	318
July 1990	A	3	13	2,881
August 1990	A	5	13	3,192
May/June 1991	A	3	16	247
June/July 1991	A	5	29	3,132
August 1991	A	1	2	209
July 1992	A	4	145	3,251
August 1992	A	3	36	1,983
July 1993	T	4	109	2,802
August 1993	T	4	84	3,049
June 1994	T	4	60	2,119
July 1994	T	5	61	3,401
August 1994	T	3	62	2,230

A, arbitrarily selected colonies; T, transect located downstream of colonies.

Fertilization rates of *Plexaura kuna* eggs from individual samples were most commonly below 50% and were extraordinarily variable. Figure 1 presents fertilization rates of eggs collected on two days. Fertilization rates on those days were among the highest observed in the study. Fertilization rates tended to rise at the start of spawning, but as is evident in Figure 1 this pattern is itself variable. The increase suggests that either sperm concentration or egg receptivity changed over the course of the spawning event. The data from the 1- and 6-m *Pseudoplexaura* collections suggest no short-term change in egg receptivity, and in laboratory experiments we have been able to fertilize *P. kuna* eggs shortly after they were released (unpub. data). These data suggest that the rise in fertilization rates observed early in the spawning event was probably driven by the release and accumulation of sperm in the water column.

As is evident in Figure 1, regardless of whether mean fertilization rates were great or small, samples taken by individuals spaced only meters apart could vary by 100% or more. The samples illustrate the extraordinary variability in fertilization rates between samples that were taken within 0.5–2 m of each other. Similarly, collections taken by the same individual at the same location varied between collections as well. (An average collection required 2–5 min to gather.) When averaged across days, much of the variability disappeared and there was a general tendency for the first and last day(s) of spawning in each month to have lower fertilization rates than the middle 2–3 days (Fig. 3).

Monthly average fertilization rates of *Plexaura kuna* eggs were well below 100% and were also highly variable. In some months there was virtual reproductive failure of the Korbiski Reef *P. kuna* population (Fig. 4). Fertilization rates were highly variable over both months and between years. The highest fertilization rates occurred during July 1993 spawning events. The values in 1993 and 1994 tend to be among the highest, which may be attributed to the shorter incubation and greater embryo survivorship at 12 h compared to 36 h. However, low fertilization rates were observed among those two years as well. The lowest fertilization rates occurred in August 1988, and June, July, and August 1990 (0, 4.1, 3.1, and 2.9%, respectively).

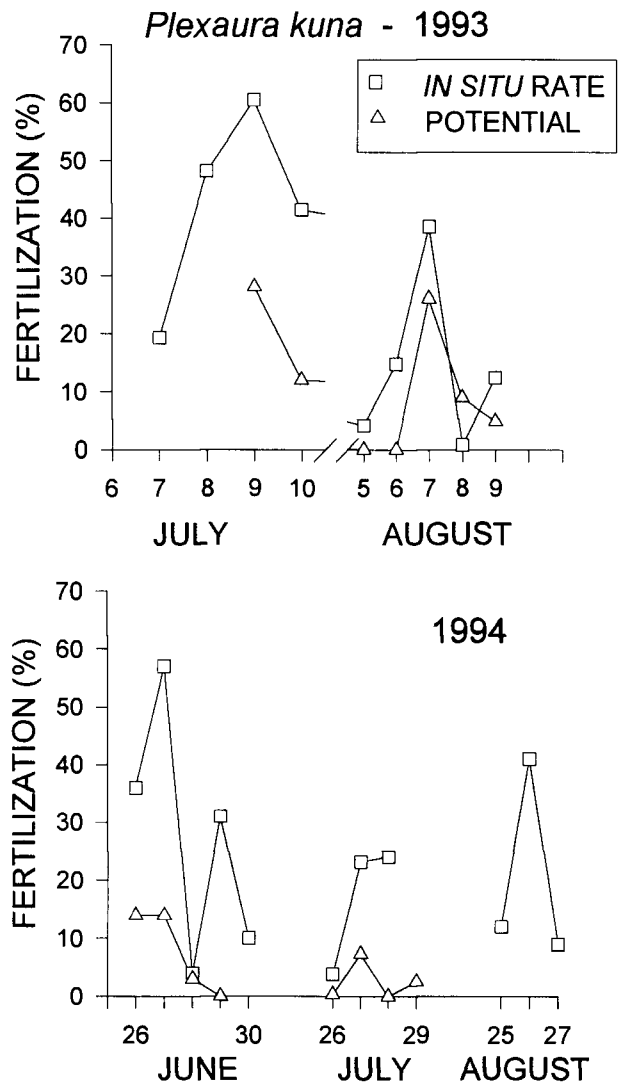

Figure 3. Daily averages of proportion of eggs fertilized in samples of eggs that were collected on Korbiski Reef (*in situ*) or were incubated with water from Korbiski that was collected at the same time as the *in situ* egg samples (potential).

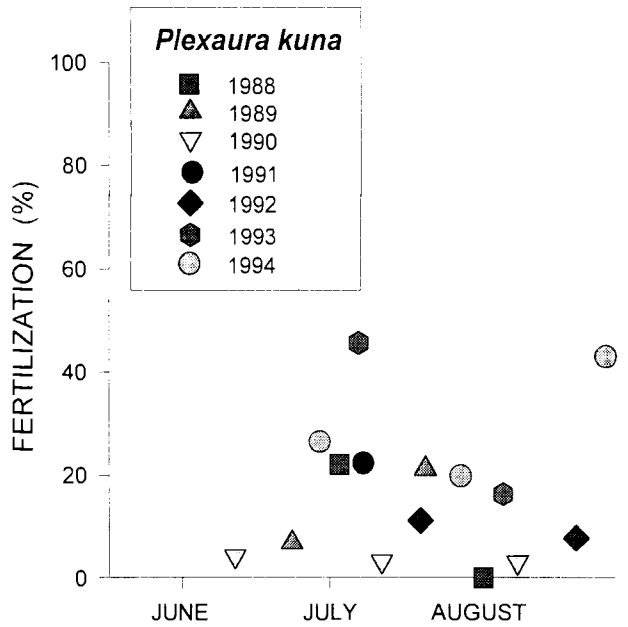

Figure 4. Proportions of *Plexaura kuna* eggs of collected eggs that were fertilized during spawning events at Korbiski Reef between 1988 and 1994. Values are weighted averages of data from 1–5 days of collections during each month.

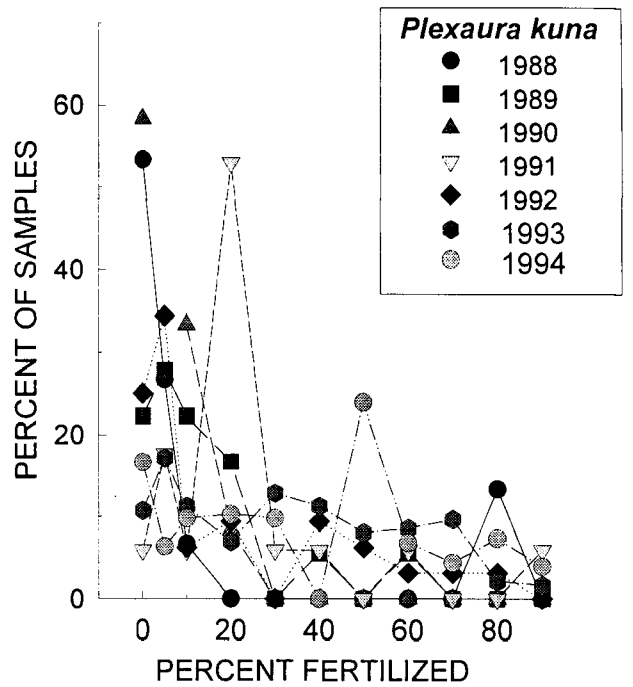

Figure 5. Frequency distribution of fertilization rates of *Plexaura kuna* eggs collected in samples from Korbiski Reef in the San Blas Is., Panama.

Variance in fertilization rates was driven by the tendency for eggs from individual samples (*i.e.*, single syringes) to have somewhat bimodally distributed fertilization rates (Fig. 5). The frequency distribution of proportion of samples having a given level of fertilization is usually characterized by a large peak at or below 20% fertilization and either a very broad tail at higher fertilization rates or in a number of cases a second, smaller peak at a greater fertilization rate. This pattern was much less pronounced in 1993 when relatively high rates of fertilization were observed. However the pattern returned when samples were partitioned between months or days.

Measures of fertilization potential were significantly correlated with the daily average *in situ* rates (Fig. 3, $r = 0.64$). Fertilization potentials were almost always lower than the field rates, which may reflect a decrease in sperm activity associated with the 0.5–1 h delay between collection and the introduction of eggs to the water sample. There was no significant correlation when the fertilization potential of individual samples was compared to the fertilization rate of simultaneously collected eggs (Fig. 6).

Eggs collected *in situ* and then incubated with additional sperm always had greater fertilization rates than controls that were not supplemented with additional sperm (Fig. 7). The increase was dramatic as among controls the greatest fertilization rate was 38% and the lowest fertilization rate among the treated samples was 68%. The average difference between paired control and treatment was 72%.

Pseudoplexaura porosa fertilization rates were determined on 19 days between 1990 and 1994. Mean fertilization rates over those days were 32% (1990), 85% (1992), 39% (1993), and 50% (1994). Fertilization rates were bimodally distributed as among *Plexaura kuna* samples but, for *Pseudoplexaura porosa* samples, the second peak occurred at a greater fertilization rate and included a greater

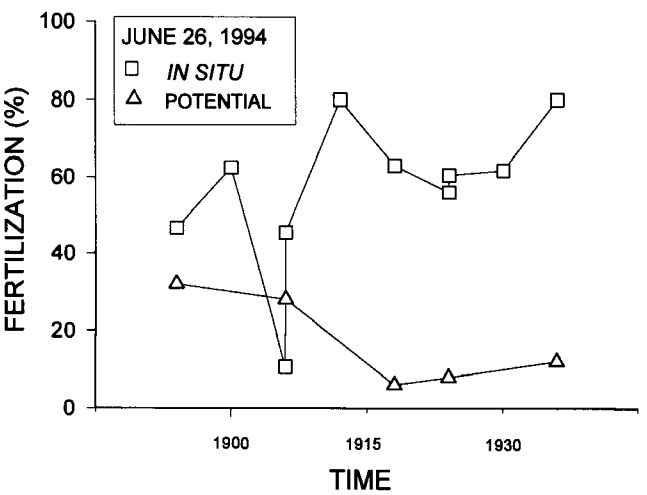

Figure 6. Proportions of eggs fertilized in individual samples of eggs that were collected on Korbiski Reef (*in situ*) or were incubated with water from Korbiski that was collected at the same time as the *in situ* egg samples (potential).

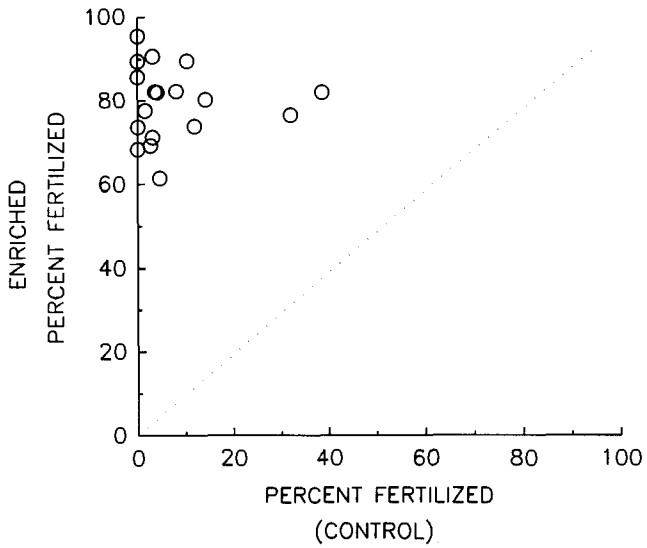

Figure 7. Fertilization rates among *in situ* samples of eggs incubated with and without an excess of sperm. Dotted line represents expected relationship if enrichment with sperm had no effect on fertilization rate.

percentage of the samples. Fertilization rates between days exhibited the same degree of variance as shown among the *P. kuna* samples (Fig. 8).

Discussion

Regardless of whether one looks at the data from individual samples or from daily or monthly averages, both *Plexaura kuna* and *Pseudoplexaura porosa* eggs have fertilization rates that were almost always well below 100% and often approached 0%. Indeed, the majority of *P. kuna* samples collected at Korbiski had rates <20%. On virtually every night, there were samples with near-zero fertilization rates, and on some occasions rates were uniformly low, as in 1990. During 1988–1991, samples were collected immediately downstream of arbitrarily selected colonies, and some of the low rates may simply be a function of the colonies chosen. There is no *a priori* reason to suspect that the colonies sampled in 1990 were unlikely to produce zygotes, but given the relatively small number of colonies involved (2–4 per night), the low values could be a random sampling effect. Even if the low values recorded in 1990 are specific to only some colonies, the commonness of low values indicates that on any given night some (and perhaps many) of the colonies on a reef can have almost complete reproductive failure. That failure is driven not by their production of gametes, but by fertilization rates.

Fertilization rates during 1993 and 1994 were among the greatest observed. Some of this effect may be attributable to the shorter incubation times employed during those years, but our comparisons of survival at 12 and 36 h suggest that any such effect would be of smaller magnitude (9%) than the range of variance observed. Furthermore, the difference in incubation regimen cannot explain the presence of near 0% fertilization on two of the nights during August 1994.

Our results also indicate that measures of fertilization based on the ability of water samples to fertilize eggs are generally accurate but probably obscure much of the fine-scale variance in fertilization rates. In our experiments the "fertilization potential" of field-water samples explained only 41% of the variance in the field samples. As Figures 3 and 6 indicate, fertilization potential characterized much of the overall pattern of fertilization rates, but differed from field data in absolute magnitude and exhibited less variance.

Our August 1992 sperm supplementation experiments indicate that the low fertilization rates can unambiguously be attributed to sperm limitation. The consequences of sperm limitation are twofold. First, *Plexaura kuna* has extraordinarily low rates of recruitment in the San Blas (Lasker, 1990, and unpub. data). Although there are probably multiple causes for the recruitment failure, fertilization success places yet another barrier in the cascade of events necessary to generate years with high recruitment. Years with high fertilization rate may not generate high recruitment rates, but high fertilization success is probably a necessary prelude to years with successful recruitment.

The other striking feature of the data is the variability of fertilization rates that can be found at every level of analysis. The source of that variance is ultimately linked to the processes that control the densities of eggs and sperm in the water column, as it is these densities that control *P. kuna* fertilization success. Both the production of gametes and the current regime that dilutes those gametes contribute to the variance in gamete densities and the variance in fertilization success.

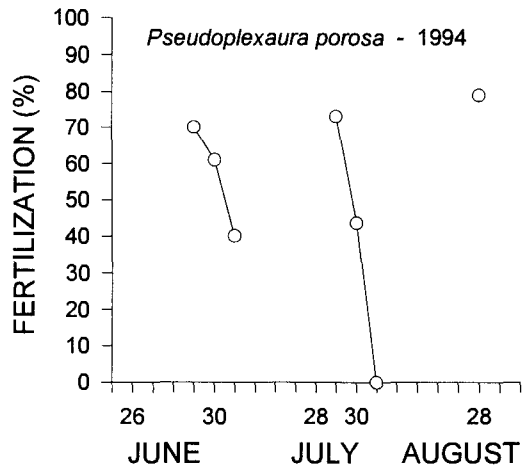

Figure 8. Daily average of proportions of *Pseudoplexaura porosa* eggs fertilized in samples collected from Korbiski Reef on different days.

The pattern of variance in *P. kuna* fertilization rates that we have observed at Korbiski has been driven by variation occurring at a number of different scales. At the level of months and days, there has been the trend throughout our observations for July fertilization success to be greater than June and August. Similarly the first and last day(s) of spawning usually have the lowest rates of fertilization of each month. Much of the day-to-day and month-to-month variation is probably driven by numbers of gametes being released. That and changes in the direction of currents that carry sperm either toward or away from female colonies may explain the daily and monthly pattern. The current direction effect is especially important for *Plexaura kuna* at Korbiski because there are only three male clones present on the reef and current direction can direct their gametes away from our collection sites. This is well illustrated in the 1994 fertilization rates where the decrease in fertilization rate on June 28, 1994 (Fig. 3) can be explained by the current having a different orientation than on either the preceding or following nights.

On a finer scale the variance in fertilization rates that occurs between samples collected within minutes or meters of each other is probably related to fine-scale variance in the release or mixing of sperm as they are released from male colonies. Observations of dye clouds released shortly before spawning events indicate that dye, and presumably sperm, do not diffuse uniformly as they are advected downstream (Lasker and Stewart, 1993). Instead, a highly heterogeneous series of structures are formed that remain surprisingly coherent over a scale of centimeters and meters (*i.e.,* over seconds and minutes depending on current speed). These same types of heterogeneities have also been observed by Moore *et al.* (1992, 1994), in studies of the turbulent diffusion of odor plumes. They have observed heterogeneities on the scale of centimeters and seconds.

The results of the analyses of spawning on Korbiski Reef in the San Blas Islands indicate that the potential for sperm limitation identified in many of the previous studies of broadcast spawning is realized in at least some species. *Plexaura kuna* and *Pseudoplexaura porosa* are common and more importantly represent two extremes in distribution pattern. *P. kuna* is clonal (Lasker, 1984, 1990; Coffroth *et al.,* 1992) and at some sites spreads extensively via fragmentation. Although *P. kuna* at Korbiski is locally very dense, the reef is dominated by two female clones and there are only three male clones on the reef. Furthermore, the male clones are among the smallest clones on the reef. Consequently, there are few males at Korbiski relative to the number of female colonies. In this context *P. kuna* populations can be thought of as groupings of relatively rare, albeit very large, individuals that are often distantly spaced. Under these circumstances the finding of sperm limitation and low fertilization success is not surprising. In contrast, at Korbiski and at many Caribbean localities, *Pseudoplexaura porosa* is common (over 40 large colonies in the 200 m^2 area from which we collected) and has a 1:1 sex ratio. The finding of low fertilization success among the *P. porosa* egg samples suggests that sperm limitation is likely to occur in a wide variety of species and settings.

Our results as well as previous studies (Table I) all demonstrate that fertilization rates are often low and that rates will vary depending on environmental effects such as turbulence and biotic factors such as the numbers of sperm released and spawning behaviors. The effects of these factors operate at two levels, population dynamics and life history evolution. At the population level they may limit population dynamics. Many marine species recruit sporadically or at extremely low levels. One factor that may contribute to these recruitment rates is fertilization. Both Lessios (1988) and Levitan (1988) have speculated that very low population densities following the *Diadema antillarum* pandemic (Lessios *et al.,* 1984) may have reduced fertilization rates and have retarded recovery of *D. antillarum* populations. Similarly, the low fertilization rates observed for *P. kuna* are consistent with the low recruitment rates that we have observed (Lasker, 1990).

Regardless of whether the total number of eggs fertilized limits population growth, variation in fertilization rates has important implications for our understanding of broadcast-spawning species. If the proportion of an individual's gametes which become zygotes predictably varies with respect to that individual's morphology, behavior, or choice of habitats, there will be differential (and perhaps heritable) differences in each genotype's contribution to the next generation. This selection will occur independently of whether the population is actually limited by the supply of zygotes. Thus there should be strong selection pressure for enhanced fertilization success and, to the extent that behavioral, morphologic, or growth strategies can predictably affect fertilization rates, this selection has helped to mold reproductive strategies of broadcast-spawning species (Levitan, 1995; Levitan and Petersen, 1995).

The variance in fertilization success between months and years suggests that predictable reproductive success for many broadcast-spawning species may only occur on a scale of years and decades. This may not be a serious constraint for gorgonians, which are long lived. However, broadcast spawning cannot be expected to be successful unless an individual reproduces over many years, or produces large numbers of gametes and releases them in a fashion that minimizes sperm limitation. This places a premium on both longevity and the production of large numbers of gametes, in other words, long-lived individuals that produce many gametes. This seeming contradiction in strategies can be reconciled if one considers that many

of the broadcast-spawning taxa are modular. Modular or clonal growth allows genetic individuals to become extremely long lived and to grow to large spatial size. As a consequence, an individual that lives long and produces prodigious numbers of gametes is created.

It is also clear from the *P. kuna* data that ecologic traits affecting size or spacing also affect fertilization. Clonal propagation, by spreading *P. kuna* clonemates across substantial areas (Coffroth *et al.*, 1992), allows *P. kuna* clones to sample across the spatial variance in fertilization success as well as the temporal variance. The same potential for spatial and temporal variance in fertilization should also exist for wind-pollinated plants. Among many of these species, modular/clonal growth produces large, long-lived structures (*e.g.*, trees or large areal expanses of a grass clone) (Silander, 1985).

Iteroparity across many spawning events increases the odds of spawning at a time favorable to fertilization success, but an alternative strategy is to reduce the likelihood of sperm limitation by increasing the number of sperm released and exhibiting behaviors and gamete traits that enhance the probability of fertilization. Echinoderms provide good examples of these strategies. Work by Babcock and Mundy (1992), Babcock *et al.* (1994), and Benzie *et al.* (1994) has demonstrated that *Acanthaster planci* realizes high fertilization rates by releasing large numbers of sperm during spawning events. Other species such as *Strongylocentrotus franciscanus* and *Diadema antillarum* (prior to the 1983 mass mortality) may rely on high population densities to assure fertilization (Lessios, 1988; Levitan, 1988, 1993; Levitan *et al.*, 1992). Yet other species aggregate and thereby enhance fertilization (Young *et al.*, 1992).

If fertilization rates have played an important role in the life history evolution of broadcast-spawning species, then we should be able to observe tradeoffs between fertilization-enhancing features and the rest of the phenotype that vary with the degree of sperm limitation. For instance, Levitan (1993) has argued that echinoid egg size affects fertilization and that the tradeoff between number and size of eggs is also affected by population density and fertilization success. *P. kuna* and *P. porosa* appear to differ in fertilization rates, and it will be important to determine whether tradeoffs related to sperm limitation can be observed among these and other gorgonians in traits such as sperm longevity, egg size and viability, colony fecundity, distribution, and clonal spread.

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