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VARIABLE PELAGIC FERTILIZATION SUCCESS: IMPLICATIONS FOR MATE CHOICE AND SPATIAL PATTERNS OF MATING[1]

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Abstract. Fertilization success was measured in the bluehead wrasse, *Thalassoma bifasciatum*, a tropical reef fish with external fertilization of pelagic eggs. This species exhibits intraspecific variation in its spawning behavior; females either spawn with single males (pair spawning) or spawn with a group of at least three and often >20 males (group spawning). Fertilization success averaged ≈75% and did not differ between pair and group spawning, despite an estimated 80-fold increase in sperm release in group spawns. There was also no evidence that pair-spawning males suffered sperm depletion over the course of the spawning period. *Thalassoma bifasciatum* occurs in a variety of habitats and is exposed to varying levels of water turbulence. Fertilization success varied among days, and decreased with rougher water conditions. Within a reef, the calmer spawning sites behind the reef relative to the current had higher fertilization success than those along the side of the reef. These data suggest that while the type of spawning occurring at the site does not affect selection for fertilization success, females may gain fertilizations by selecting particular locations or periods of calmer water conditions to spawn. This is an alternative hypothesis to explain temporal and spatial patterns of mating. We provide detailed methods on how to accurately obtain data on fertilization success. Our technique can be used to study natural spawning in a wide variety of reef fishes and other marine organisms with pelagic eggs, external fertilization, and predictable spawning.

Key words: *Caribbean; external fertilization; Labridae; mate choice; spawning; sperm competition; sperm depletion;* Thalassoma.

INTRODUCTION

Determining the percentage of eggs fertilized in marine organisms, here called fertilization success, is of fundamental importance for understanding both the relative and absolute zygote production of populations, and for understanding patterns of reproductive behavior and mate choice within local populations. For many marine organisms, fertilization occurs externally, after sperm and eggs have been released into the water column, a behavior called free-spawning (Breder and Rosen 1966, Thresher 1984, Giese and Kanatani 1987). Previous investigators have used both theoretical (Denny 1988, Denny and Shibata 1989, Shapiro 1989) and empirical (Pennington 1985, Yund 1990, Levitan 1991, Petersen 1991a, Levitan et al., *in press*, Sewell and Levitan, *in press*; D. Brazeau and H. Lasker, *unpublished manuscript*) approaches to try to understand the factors that determine fertilization success in marine organisms. However, there are few studies that provide accurate estimates of fertilization success from natural spawns of free-spawning species in the field (Petersen 1991*a*; Sewell and Levitan, *in press*). In this paper we describe a simple technique for measuring fertilization success in organisms with pelagic gametes and external fertilization. We then present results on fertilization success obtained from collections of eggs from the bluehead wrasse, *Thalassoma bifasciatum* (Poey), and examine patterns of variation in fertilization success in this species.

At the population level, many estimates of survival in the plankton are based on the assumption that all eggs are being fertilized, and that reductions in recruit-

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ment from population egg production give a rough approximation of planktonic survivorship (reviewed in Young and Chia 1987, Rumrill 1990). If fertilization success is <100%, then planktonic mortality is being overestimated (Denny and Shibata 1989, Rumrill 1990). In addition, if there is systematic temporal or spatial variation in fertilization success, then it must be included in estimates of the contribution of each subpopulation to the larval pool (Denny and Shibata 1989). Within a subpopulation, variance in fertilization success can potentially influence behavioral and life history traits, including spawning-site selection, mate choice, and the evolutionary stability of alternative mating tactics (Shapiro 1989).

Current theory suggests that, because fertilization success is subject to diminishing returns for increasing levels of sperm production, males may not be selected to release enough sperm to achieve 100% fertilization for their spawns (Petersen 1991*b*). There are theoretical studies suggesting that fertilization success should often be <100% (Denny and Shibata 1989, Petersen 1991*b*) and empirical studies suggesting that fertilization success may be <100% under most circumstances (Bauer and Bauer 1981, Nakatsuru and Kramer 1982, Pennington 1985, Levitan 1989, Yund 1990, Petersen 1991*a*, Sewell and Levitan, *in press*; D. Brazeau and H. Lasker, *unpublished manuscript*). However, actual measurements of fertilization success are strikingly absent from discussions of how fertilization success affects the evolution of reproductive behavior, anatomy, and physiology.

The paucity of data on fertilization success of pelagic eggs is not surprising; despite the synchronized spawning of many species, the timing of gamete release for most species is either sporadic or unknown, making the observation and collection of eggs difficult. A second problem involves potential artifacts introduced while collecting eggs. Collection immediately after spawning can interfere with fertilization and developmental processes occurring naturally in the water column (Rollefsen 1932, Markle and Waiwood 1985). However, delays in sampling eggs may result in reduced numbers collected.

Four factors that may influence fertilization success are the number of individuals releasing sperm at one time (Pennington 1985, Denny and Shibata 1989, Shapiro 1989, Levitan 1991), the distance between spawning individuals (Pennington 1985, Denny and Shibata 1989, Yund 1990, Levitan 1991; D. Brazeau and H. Lasker *unpublished manuscript*), sperm depletion (Nakatsuru and Kramer 1982), and the water velocity or degree of turbulent mixing during spawning (Pennington 1985, Denny and Shibata 1989). One species where most of these factors can be examined under natural conditions is the bluehead wrasse *Thalassoma bifasciatum*. The bluehead wrasse exhibits intraspecific variation in spawning behavior. Females may either spawn in aggregations with many males (group spawning), or mate with a single large male in a defended territory (pair spawning) (Warner and Hoffman 1980).

Measurements of testes size, spawning frequency of individual males, and the number of males in group spawns reported in the literature (Warner et al. 1975, Warner and Robertson 1978) indicate that the total amount of sperm released in an average group spawn is ≈80 times that released in a pair spawn. This assumes that an individual's daily sperm release is proportional to his testes size. A qualitative difference in sperm release between group and pair spawns is observable; the sperm cloud is quite visible in group spawns but not in pair spawns. Preliminary data from elsewhere (W. Hunte, *personal communication,* cited in Shapiro 1989) suggests that group spawns have higher fertilization success than pair spawns. If this were true, there should be strong selection for female choice of group-spawning sites.

Another potential source of variation in fertilization success is sperm depletion during the spawning period. Pair-spawning males have small testes and typically spawn 14–25 times per day, but some individuals may spawn as often as 100 times per day (Warner et al. 1975, Warner and Hoffman 1980; R. R. Warner, *personal observation*). This suggests that males may run out of sperm late in the daily spawning period. Sperm depletion has been documented in a freshwater fish in the laboratory (lemon tetra, Nakatsuru and Kramer 1982) but has not been studied in the field for any aquatic organism.

In addition to variation in spawning behavior, different current regimes at spawning sites may affect fertilization success in the bluehead wrasse. It breeds on shallow, relatively exposed reefs that experience high current and water turbulence, two factors believed to reduce fertilization success (Denny and Shibata 1989), but it also spawns at relatively calmer back-reef locations. Thus, by examining fertilization success from reefs experiencing different water conditions we may be able to obtain insights into the importance of both spawning mode and conditions at the spawning site on fertilization success.

Biology of *Thalassoma bifasciatum*

The bluehead wrasse is a common inhabitant of shallow coral reefs throughout the Caribbean. Size and color are sexually dimorphic; females and smaller males have a yellow and black initial-phase (IP) coloration, while larger individuals are brightly colored terminal-phase (TP) males. Some of these TP males are the result of sex change by females (Warner et al. 1975). IP individuals can be reliably sexed by close inspection of the genital papilla.

Spawning occurs in a limited period of ≈110 min in the afternoon the year-round (Warner and Robertson 1978). Females generally migrate from their upcurrent feeding areas to downcurrent segments of the

reef to spawn. Preferred spawning sites consist of a subset of upward projections on the downcurrent periphery of the reef (Warner 1988, 1990*a*).

Spawning follows the typical pattern of tropical reef fishes with pelagic eggs and external fertilization. A pair spawn consists of a female and TP male rushing upward 0.3–1.5 m toward the surface with both releasing gametes at the apex of the spawning rush. Occasionally IP males join the pair just as they are spawning and presumably release sperm in a behavior called streaking (Warner and Robertson 1978). Group spawning consists of a female mating with >1, and typically 5–20, IP males in a spawning rush. When both group and pair spawning occur on the same reef, they occur at different locations (Warner and Hoffman 1980, Warner 1984: Fig. 5). A female spawns on 2 out of 3 d on average, and releases her daily egg production in a single spawn (Schultz and Warner 1991). The transparent eggs are planktonic, ≈ 600 μm in diameter, and positively buoyant once fertilized. Hatching occurs after 20–24 h at 25–28°C. There is no parental care.

Methods

Data were collected during July–August 1989 and during February 1990 at several sites off the northeast coast of St. Croix, Virgin Islands near the West Indies Laboratory. We collected eggs while snorkeling at a water depth of 0–3 m behind the barrier reef in Tague Bay and at several patch reefs east of Tague Bay (see Gladfelter and Gladfelter 1978 for map).

Collection of eggs

To collect eggs from natural spawnings, observers positioned themselves within 2–4 m of a spawning site near the beginning of the daily spawning period. When a spawn was observed, the gamete cloud was marked by a small amount of fluorescein dye released by the observer near the spawn. After waiting a specified period determined experimentally, the observer swept the area around and including the expanded fluorescein cloud for 30 s with a 15 cm brine-shrimp net (maximum mesh size $\approx 100 \times 300$ μm). Preliminary collections indicated that harsher textured nylon nets, such as plankton netting, severely reduced fertilization success. At the end of the sweep, the net was drawn away from the fluorescein cloud, the contents of the net were transferred to a small plastic bag, and the bag was sealed. To verify that eggs collected had come from the observed spawn, we also performed several control collections at the same locations during the spawning period when no spawn had occurred. The procedures were identical.

The next morning the contents of each bag were filtered through 100-μm nylon mesh to collect the eggs, which were then examined under a dissecting microscope and scored as fertilized or unfertilized. By counting eggs ≈ 20 h after spawning, fertilized, developing eggs could be unambiguously distinguished from undeveloped eggs. Developing eggs contain nearly fully developed larvae, since hatching is completed within 24 h. Undeveloped eggs were scored as unfertilized. Only samples with at least 20 eggs were used to estimate fertilization success. After counting, eggs and hatched larvae were released from the West Indies Laboratory dock.

Three possible causes of variation in fertilization success were examined in this study: group spawns vs. pair spawns, early vs. late pair spawning within a day, and water conditions during spawning. Group and pair spawns were compared within days for the subset of reefs with both active group and pair-spawning sites. We examined sequences where at least nine spawns were taken with a single spawning male on a day to determine if sperm depletion by pair-spawning males caused reduced fertilization success late in the spawning period.

We tested for the effects of water mixing on fertilization success in two ways. First, each day was assigned a qualitative water-mixing score from 1 to 5, with 1 representing a calm day with minimal movement and dispersion of the fluorescein cloud and 5 representing a day where the fluorescein cloud moved several metres before collection, and often had dispersed so that its outline was barely visible. We attempted to quantify the dispersion rate of the fluorescein cloud, but found that our variance in estimating the irregular shape was no more precise or accurate than the qualitative scale. Second, we did three pairwise comparisons that appeared to represent general differences in water conditions. On patch reefs, conditions appeared calmer behind the reef relative to the current compared with the side of the reef on any given day. We compared fertilization success for these two spawning habitats on days that we successfully collected multiple spawns in each habitat at a patch reef. In addition to this within-reef comparison, we did two between-reef comparisons. We compared dry season (February) and wet season (July, August) results; water conditions were generally rougher in the dry than in the wet season. Within a season, we compared results from the rougher patch reefs to the calmer back reef. For all of these comparisons, we predicted that calmer conditions with less dispersion of gametes would result in higher fertilization success. However, these last two tests may have been confounded by observer bias. During February we were able to collect eggs on the roughest days, and on rough days in July–August we could only collect eggs at the back-reef location. This would tend to reduce the effects of temporal and spatial differences in water conditions. Due to these biases, we consider the comparisons of fertilization success among days with different levels of water mixing and within days at different sites on a given reef to be stronger tests of the turbulence hypothesis.

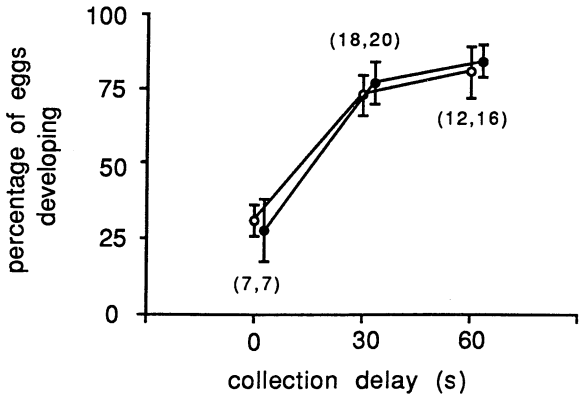

FIG. 1. The relationship between the proportion of eggs developing and delay in the onset of egg collection in spawning *Thalassoma bifasciatum*. ● pair spawns, ○ group spawns. Error bars represent ± 1 SE. Sample sizes for group and pair spawns are in parentheses.

Testing for biases in fertilization success

Our methods of collecting eggs may bias fertilization success measurements. To test for the presence of sampling artifacts we performed several experiments in both the field and the laboratory.

Field controls.—We varied the delay between spawning and initiation of egg collection to determine the minimal collection time that did not reduce our estimate of fertilization success relative to later collections. Three delays prior to the initiation of collection were tested; no delay (0 s), 30 s, or 60 s after spawning. Using days where collections were made at multiple delays for a type of spawn, this minimum time was 30 s for both pair and group spawns (Fig. 1, Table 1). All subsequent methods and results given are for collections made 30 s or more after spawning. The suitability of this delay was later corroborated by laboratory data on gamete viability (see below, *Results: Gamete viability durations*).

In a second field experiment, we determined if the 30-s net sweeps inadvertently enhanced fertilization success. Eggs captured in a net swept through the water pass through a much larger volume of water than they would otherwise. If both types of gametes are still viable during collection, this could cause a higher rate of contact between eggs and sperm and produce artificially high fertilization success. The magnitude of this effect would be proportional to the duration and speed of the sweeps. To test for this potential bias, the fertilization success of eggs collected during 5-s sweeps were compared with those collected in the usual 30-s sweeps. On three days, alternate spawns at the same spawning site were collected using 5- or 30-s sweeps. Both sets of sweeps were made from 30 to 60 s after spawning.

The location of the sample in the gamete cloud may introduce another bias in fertilization success. Eggs located more marginally may be surrounded by both fewer eggs and fewer sperm. If fertilization success depends on sperm concentration, then sweeps that include only the margin of the gamete cloud would have both fewer eggs per sample and lower fertilization success. If this occurred, we would predict a positive relationship between fertilization success and the number of eggs collected for collections within a day. Collections among days cannot be combined to test for this trend; both fertilization success and the number of eggs collected may covary with physical factors, such as current speed, that can vary substantially among days.

Laboratory controls.—Laboratory experiments using artificial fertilizations tested for other potential artifacts introduced by our collecting technique. Individuals were collected at the beginning of the spawning period using a lift net baited with broken sea urchins, were held for a maximum of 4 h, and returned to their home reef after the experiments were completed.

Males and females were held in separate aquaria before use in experiments. To strip females of eggs, individuals were held over a 10 cm diameter glass bowl partially filled with seawater while exerting gentle pressure on their abdomen. Released eggs were rinsed off the fish into the bowl with seawater. Females releasing unhydrated eggs, which are small and opaque, were eliminated from the experiment. To strip males of sperm, individuals were either held over a small bowl

TABLE 1. Two-way ANOVAs of date and delay in collection on proportion of eggs fertilized for pair spawning and group spawning in the wrasse *Thalassoma bifasciatum* (angular transformation).

Pair spawning				Group spawning			
Independent variables	df	MS	*F*	Independent variables	df	MS	*F*
Date	2	0.30	3.36*	Date	4	0.14	1.82
Delay in collection†	2	0.73	9.81**	Delay in collection†	2	0.29	5.59*
Interaction	4	0.07	0.83	Interaction	7	0.05	0.69
Error	34	0.09		Error	23	0.08	
Student-Newman-Keuls multiple-range test:				Student-Newman-Keuls multiple-range test:			
0 s delay vs. 30 s delay			$P < .001$	0 s delay vs. 30 s delay			$P < .05$
30 s delay vs. 60 s delay			NS	30 s delay vs. 60 s delay			NS

* $P < .05$, ** $P < .005$.
† All *P* values for delay in collection are one tailed, with the larger delay predicted to have the higher fertilization success.

in an analogous method to the egg-stripping procedure, or individuals were gently squeezed with their vent underwater. In experiments where eggs and sperm were combined immediately in all treatments, sperm and eggs were simultaneously stripped into the same bowl.

The fertilization success obtained by mixing sperm and eggs immediately after stripping varied considerably and appeared to depend largely on when females were stripped. Females stripped early in a day of experiments sometimes produced unhydrated eggs, while on several days females used late in the day produced hydrated eggs with low fertilization success. To control for the effect of variable female egg quality, when possible we did paired or multiple comparisons, splitting the batch of eggs into 2–3 subsamples. Also, we excluded any replicates where the subsample with the highest fertilization success was $<50\%$.

Artificial fertilizations were used to test the effect of the collecting net, the effect of fluorescein, and the duration of gamete viability.

The sweeping of a net through a gamete cloud can create biases in our estimates of fertilization success by physically damaging eggs, thereby halting development whether or not fertilization took place. This would underestimate the natural fertilization success for a spawn. To test for this effect, eggs and sperm were combined in a glass bowl containing seawater, and immediately split into two aquaria each containing ≈ 3 L of seawater. Thirty seconds after eggs and sperm were combined, a collection net was swept through one of the aquaria for 30 s to mimic egg collections of natural spawns. The water from the net and control treatments was then emptied into separate collection bags, and the eggs were scored the next morning for fertilization success. All replicates included in the analysis ($n = 13$) had at least 50 eggs in each treatment. A significant difference between the control and treatment would indicate that field collections are probably underestimating fertilization success.

The potential effect of fluorescein was tested similarly by artificially fertilizing a batch of eggs, splitting it immediately into two bowls, adding fluorescein to one bowl immediately after splitting, and then, after a 2-min delay, transferring eggs to collection bags containing 2–3 L of seawater. Replicates used to determine fertilization success ($n = 7$) were selected using the same criteria as in the previous experiment.

Gamete viability

If gametes remain viable long after collection, then our estimates of fertilization success could be incorrect. If sperm are inadvertently included in collection bags, maintaining sperm and eggs at relatively high concentrations in plastic bags after collection could result in higher fertilization success. On the other hand, collecting eggs and removing them from sperm before all fertilization has taken place will lead to low fertilization success. These potential biases are irrelevant if either eggs or sperm remained viable for only a short time relative to our 30-s delay before collection.

Three gamete viability experiments were conducted to determine the time range over which fertilization was possible. In the first experiment, both gametes were aged simultaneously, while in the latter two experiments one gamete type was allowed to age in seawater while the other was added immediately after stripping.

To age both gametes simultaneously, eggs and sperm were stripped into separate dishes with ≈ 100 mL of seawater and immediately split into three glass bowls with seawater. Eggs and sperm from one pair of bowls were then immediately combined, and the contents of the other two pairs of bowls were combined at later times. Two minutes after gametes had been combined, contents of the bowls were transferred to a collection bag containing additional seawater. In all gamete viability experiments replicates for determining fertilization success were selected using the same criteria as the other laboratory experiments.

The two experiments that tested for the effects of aging eggs or sperm independently used eggs from one female and sperm from three males. To test for the effects of sperm age alone each of three males was stripped at a different time, and the sperm from each male were put into a separate bowl. While the last male was being stripped, eggs from a single female were simultaneously stripped and split into three containers as in the treatment with both gametes aged. The sperm from each male were then combined with a container of eggs, producing one treatment with fresh sperm and eggs, and two treatments with older sperm combined with fresh eggs from the same female.

To age eggs while keeping sperm fresh, eggs from a single female were split into three containers, and sperm from a male that was stripped simultaneously were added to the first container. At two later times, additional males were stripped, and their fresh sperm immediately added to a separate container of aged eggs.

Statistical analysis

Angular transformations were performed on all data of percent fertilization before performing parametric tests (Sokal and Rohlf 1981). Data were back transformed for estimates of means and standard errors. Back-transformed standard errors were slightly asymmetric but varied $<6\%$; the larger of the two standard errors is given. In cases where data were collected on an ordinal scale (rank of water conditions) or when data were highly non-normal (artificial fertilizations to determine gamete viability), nonparametric statistics were used.

RESULTS

A total of 304 field collections of spawns of *T. bifasciatum* was made on 15 dates during the two field seasons. Of the 227 collections made with a minimum 30-s delay between fertilization and capture, 138 (50%)

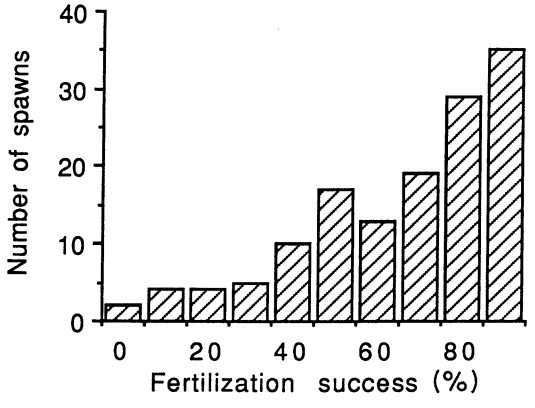

FIG. 2. Frequency distribution of fertilization success for 138 spawns of *Thalassoma bifasciatum* with 20 or more eggs collected. The x-axis scale values are lower bounds for data bars.

had at least 20 eggs and were used in the analysis of fertilization success. The distribution of fertilization success is shown in Fig. 2. The median fertilization success was 76.5%, with a back-transformed mean fertilization success of 73.5%. The seven control sweeps in the water column during the spawning period but not directly after a spawn had very few eggs (median = 1 egg, range 0–7 eggs), so collections appear to be sampling the spawns observed.

Factors affecting fertilization success

There were no differences in fertilization success between pair and group spawns on the same day on a reef (Table 2). This analysis used only data from the seven dates with estimates of fertilization success from at least two group and two pair spawns. As in the analysis of delay in collection (Table 1), there was a significant effect of day of collection on fertilization success. Only 2 of the 77 pair spawns (2.6%) had a streaker join the spawn; these spawns were included with the pair-spawn data.

We found no evidence of sperm depletion in pair-spawning males. Within six sequences, there was no effect of spawn order in the sequence on fertilization success. The 5–11 samples per sequence represented only a portion of the spawns by a male during the day, as we often missed spawns while collecting eggs, and several collected spawns had too few eggs to be included in the analysis. These males represented the individuals with the highest spawning frequency on their reefs. In four of the six cases the correlation between order in the spawning sequence and fertilization success was positive, the opposite direction than would be predicted by sperm depletion, and in no case was the correlation significant (r_s between -0.43 and 0.52, $P > .1$ for all cases).

Water conditions appeared to affect fertilization success. There was a negative relationship between the qualitative score for water mixing on a day and median fertilization success for that day (Fig. 3, $r_s = -0.53$, one-tailed $P = .02$). Fertilization success decreased as water conditions became rougher.

An additional within-reef comparison supported the hypothesis that faster currents and rougher water conditions reduced fertilization success. Spawns at calmer locations directly behind the reef (relative to the water current) had higher fertilization success than did spawns at locations on the side of the reef (Table 3). However, the two between-reef comparisons did not support the hypothesis. Fertilization success was not significantly affected by whether the data were taken during the dry or wet season, or whether the data were taken at patch reefs or the back reef (Table 4).

Tests for potential biases

There was no evidence of biases in fertilization success introduced by the collection technique or the dye.

There was no significant difference in fertilization success between exposing the eggs to a net for 30s or leaving them undisturbed in an aquarium (82.1 vs. 83.8%, paired t test, $t = 0.6$, $P = .56$, $n = 13$ pairs). Similarly, adding fluorescein to eggs did not change fertilization success (fluorescein added = 79.3%, no fluorescein = 79.0%, $t = 0.16$, $P = .88$, $n = 7$ pairs). Finally, varying the net sweep duration when collecting natural spawns had no effect on fertilization success. In a two-way ANOVA with day and duration of sweep, the duration of the sweep did not have a significant effect ($F_{1,22} = 0.13$, $P = .72$), and there was no significant interaction between day and sweep duration $F_{2,22} = 0.89$, $P = .42$).

Within days with >10 samples of at least 20 eggs, there was no relationship between the number of eggs collected per spawn and fertilization success (Spearman rank correlation, r_s between -0.20 and 0.45, $P > .1$ in all cases, $n = 12$–21). Thus, at least for a minimal sample size of 20, there was no evidence for a bias of fertilization success caused by sampling different areas of the gamete cloud on different spawns.

TABLE 2. ANOVA with the effects of date and pair or group spawn on fertilization success in the wrasse *Thalassoma bifasciatum* (angular transformation). $R^2 = 0.33$.

Independent variable	df	MS	*F*
Pair vs. group spawn	1	0.001	0.02
Date	6	0.225	3.80**
Interaction	6	0.098	1.65
Error	68	0.059	

Marginal population means

Category	Percent of eggs fertilized (mean ± 1 SE)
Pair spawning	69.8 ± 4.6
Group spawning	68.9 ± 3.8

** $P < .005$.

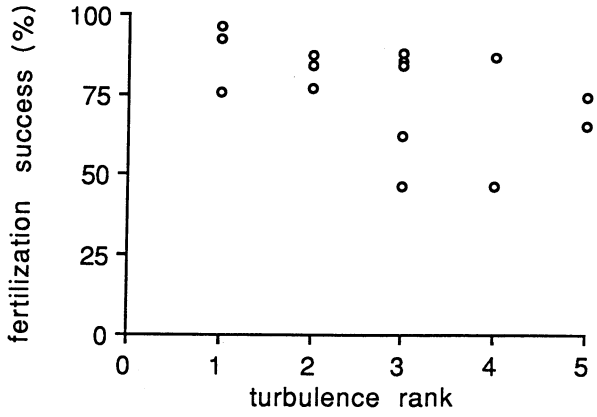

FIG. 3. The relationship between median fertilization success of *Thalassoma bifasciatum* on a given day and the water-turbulence ranking for that day. Spearman's rank correlation $r_s = -0.53$, $P_{[\text{one-tailed}]} = .02$.

The final potential artifact that could have caused a bias in fertilization success was continued gamete viability after collection. Although this effect did not appear to be strong given the negative results from our 60-s delay relative to our 30-s delay (Fig. 1, Table 1), the chance of a Type II statistical error does exist. However, this potential bias was also eliminated with the results from the gamete viability experiments as shown below.

Gamete viability durations

When both gametes were aged, there was a dramatic decrease in fertilization success: 80% (12 of 15) of the trials that had ≥50% fertilization at 0s had a fertilization success of <5% when gametes were aged for 15 s before being combined (Fig. 4). This effect was due to the very short viability of sperm (Fig. 5). Although eggs slowly lost viability (Fig. 5), the average percentage of eggs fertilized at 30s was still 90% of the fertilization success of the 0-s sample. Egg viability only exhibited a statistically significant decline after eggs were fertilized at 60 s (Wilcoxon's signed-ranks test, $Z = 1.96$, $P = .05$). In contrast, the average percentage of eggs fertilized by sperm aged for 30 s had dropped to 7% of the 0-s sample, and no eggs were fertilized in the majority of cases (Fig. 5). These results were not dependent on the technique used for stripping sperm, whether we used new or used glassware, or the order in which we split the gametes for each treatment.

TABLE 3. A two-way ANOVA of the effect of location on patch reef on fertilization success in the wrasse *Thalassoma bifasciatum* (angular transformation). $R^2 = 0.47$.

Independent variable	df	MS	*F*
Behind vs. side of reef†	1	0.13	3.38*
Date	5	0.26	5.52***
Interaction	5	0.04	0.85
Error	55	0.05	

Marginal population means

Category	Percent of eggs fertilized (mean ± 1 SE)
Behind reef	74.4 ± 3.5
Side of reef	64.0 ± 5.2

* $P < .05$, *** $P < .001$.

† *P* value for behind vs. side of reef is one tailed, with behind the reef predicted to have the higher fertilization success.

TABLE 4. A two-way nested ANOVA of the effect of season and reef type on fertilization success in the wrasse *Thalassoma bifasciatum* (angular transformation). $R^2 = 0.30$.

Independent variable	df	MS	*F*
Dry vs. wet season	1	0.11	1.68
Back reef vs. patch reef	1	0.03	0.50
Date nested within season and location	12	0.21	3.38***
Error	123	0.06	

Marginal population means

Category	Percent of eggs fertilized (mean ± 1 SE)
Dry season	78.8 ± 4.2
Wet season	69.5 ± 3.4
Back reef	75.0 ± 4.5
Patch reef	73.6 ± 3.1

*** $P < .001$.

DISCUSSION

This is one of the first studies to measure fertilization success in a free-spawning marine organism under natural conditions. The median fertilization success of 76.5% we obtained for *T. bifasciatum* is not strictly comparable with fertilization success from previous studies that involved artificial manipulations of individuals or gametes. However, our field study agrees with these manipulations in suggesting that the close association of the two sexes during spawning may be the single most important factor determining fertilization success. Higher fertilization success was obtained in these previous studies in cases where male and female gametes originated in closest proximity (fertilization success of at least 80% compared to near 0% for greater distances) (Pennington 1985, Yund 1990, Levitan 1991). The highest fertilization levels in the previous studies involved placement of individuals by the investigator at close distances from one another (*Hydractinia echinata,* Yund 1990), water collected into a syringe of ripe eggs immediately above a male induced to spawn (*Strongylocentrotus droebachiensis,* Pennington 1985), and eggs held in mesh bags a set distance from males induced to spawn or free-spawned eggs fertilized by collected sperm released from a syringe (*Diadema antillarum,* Levitan 1991; *Strongylocentrotus franciscanus,* Levitan et al. 1991). However, under natural conditions we would expect *T. bifasciatum* fertilization success to be at least as high as most

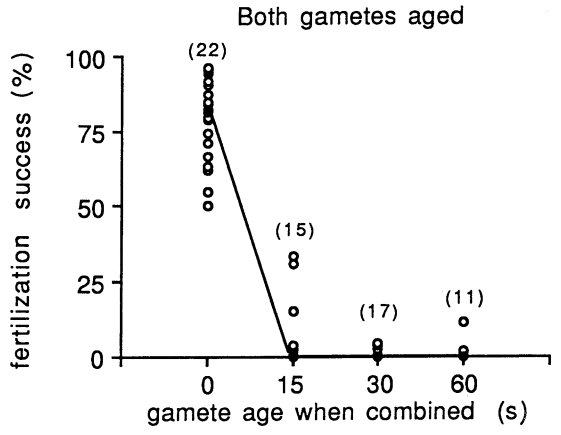

FIG. 4. The fertilization success for sperm and eggs of *Thalassoma bifasciatum* aged in seawater in the laboratory for equal amounts of time before gametes were combined. O represent individual data points, ● represent medians. Sample sizes are in parentheses.

marine invertebrates. Unlike many less mobile marine invertebrates, these fishes normally form close associations during spawning, with their vents millimetres to centimetres apart.

The negative correlation observed between estimated water mixing and fertilization success supports the theoretical analysis of Denny and Shibata (1989) and the empirical results of Pennington (1985), which showed that increasing current velocity and water turbulence decreased fertilization success. The strong effect of date of collection on fertilization success (Tables 1–4) appears to be due to differences in water conditions among days (Fig. 3). This result was also supported by the within-reef comparison, with the calmer spawning sites behind the reef relative to the current direction having a higher fertilization success than sites at the side of the reef.

The effect of water conditions on fertilization success suggests that the contribution of different habitats to the zygote pool will be variable. Specifically, habitats that experience rougher conditions may contribute less to the zygote pool compared to what would be predicted by individual fecundities alone. Denny and Shibata (1989) hypothesized that the increased turbulence in the intertidal zone might reduce the input of intertidal subpopulations of marine organisms to the zygote pool. To the extent that the fertilization success is reduced in a habitat relative to the population average, the effects of selection for specific traits in that habitat, and selection for choosing that habitat over others, will be diluted by their decreased contribution to the gene pool. All of the subpopulations in our study are capable of contributing to the zygote pool; even under our roughest collecting conditions fertilization success averaged above 40%. Although there is variance in fertilization success among habitats, we found no habitats with the castastrophically low fertilization success ($\leq 1\%$) that Denny and Shibata (1989) suggested may be occurring in the surf zone.

If large differences in fertilization success did exist between the pair- and group-spawning modes, strong selection for female choice of the more productive mode would be expected. However, there was no evidence that the type of spawn (pair vs. group) affected fertilization success. Previous results by Warner (1987) demonstrated female choice of specific spawning sites but not of specific males at those sites. On most reefs, some females migrate to group-spawning sites, while others mate at pair-spawning sites (Warner 1985, 1987). There are no apparent differences among these females in growth rates or size-specific fecundity, but larger females do tend to mate at pair-spawning sites (Warner 1985). The results from group vs. pair spawns conflict with results cited by Shapiro (W. Hunte, *personal communication,* cited in Shapiro 1989), but the details of

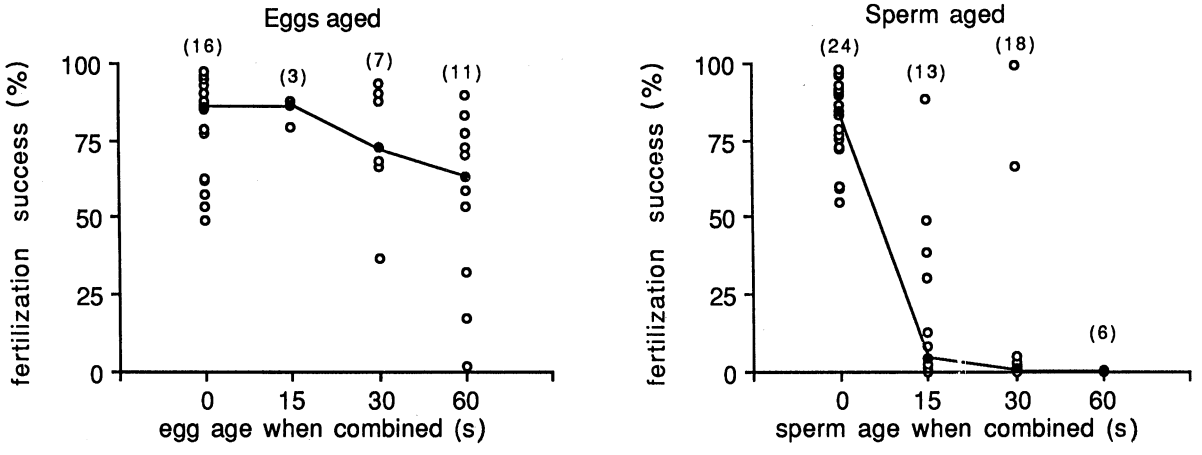

FIG. 5. The fertilization success of *Thalassoma bifasciatum* eggs in the laboratory for different ages of sperm and eggs with the other gamete added with no aging. See Fig. 4 for format.

Hunte's study were not reported, prohibiting any speculation into the causes of the discrepancy between the data of Hunte and our own.

The equivalence of fertilization success between the group and pair spawns is intriguing, given the estimated 80-fold difference in sperm release between the two types of spawns. We can offer two potential reasons to account for this nonintuitive result. First, group spawns may produce turbulence reducing the concentration of gametes faster than in pair spawns. Second, the juxtaposition of male and female vents during gamete release may be much closer in pair spawns than in group spawns. This could lead to a higher spatial overlap in gamete clouds, and reduce the effect of lower absolute sperm production in pair spawns.

There was no evidence of sperm depletion by pair-spawning males during a spawning period. This result is consistent with behavioral data that showed no evidence of mate choice influenced by limited sperm supply in *T. bifasciatum* (van den Berge and Warner 1989).

For fertilization success to affect the evolution of female mate choice, differences among males, spawning sites, or spawning times that affect fertilization success must be assessable by females. Differences in fertilization success must also exist over a short enough temporal and spatial scale to allow individual females to spawn at differing locations or times that would result in a higher fertilization success. Our results show that females cannot increase their fertilization success by discriminating between group and pair spawns or by spawning early rather than later in the spawning period. However, within a day females may achieve higher fertilization success by spawning behind the reef relative to current direction vs. at the side of the reef. In *T. bifasciatum,* popular mating sites are at the mean downcurrent point (Warner and Hoffman 1980, Warner 1988, 1990*b*). Day-to-day variation in current direction can induce females to spawn at sites offering the best fertilization success. Warner (1986) found that if females do shift their mating from the site to which they are normally faithful, they do so when current shifts result in their normal site being upcurrent. Downcurrent spawning sites have usually been interpreted as adaptations to avoid reef-based egg predation (Randall and Randall 1963, Warner et al. 1975, Johannes 1978, Shapiro et al. 1988). Our results suggest an alternative hypothesis to account for mating-site location. In general, variation in fertilization success may strongly affect the spatial and temporal patterns of mating.

Among days, there also appears to be selection for females to spawn on calmer days that have higher fertilization success. This pattern of higher fertilization success associated with calmer water condition was also found in a second wrasse, *Halichoeres bivattatus* (Petersen 1991*a*). The proportion of female *T. bifasciatum* spawning each day varies (Schultz and Warner 1991), but the causes of this temporal variation are not known. A negative correlation between the proportion of females spawning on a day and rougher water conditions would support the hypothesis that females tend to avoid days with lower fertilization success, but this could also be explained by other variables, such as food levels or the risk of predation on adults or gametes.

Within a day, over half of the variance in fertilization success remained unexplained after the effects of reef location were taken into account (Table 3). There are at least three potential causes for this variation, although the relative contribution of each is not known. First, because our collections of eggs are subsamples of spawns, some of the variation in fertilization success may represent sampling error. In addition, if fertilization success is heterogeneous through the gamete cloud, then subsampling a spawn could result in a higher variance than would be expected from random sampling error alone. Second, within-day variance in pair and group spawns may be caused by variation in the proximity of the male or males and female during gamete release, the speed of the spawning rush, the height or angle of the spawning rush, or local short-term variation in water mixing at the spawning site. Finally, variance in fertilization success within group spawns might also be caused by variation in the number of males participating in the group spawn.

By assuming fertilization success to be constant, researchers have ignored its possible effects on female choice and male-male competition for mating sites. Many studies of mating systems have implicitly assumed that fertilization success is constant within a species, and that changing the number of spawning males increases competition for a fixed number of zygotes (e.g., Warner and Hoffman 1980, Charnov 1982, Warner 1987, Petersen 1987, 1990). Similar fertilization success for group and pair spawns supports this assumption for *T. bifasciatum,* but this may not be true in other species. In a recent study, Petersen (1991*a*) found that in another wrasse, *Halichoeres bivattatus,* pair spawns with streakers had higher fertilization success than pair spawns without streakers.

For planktivorous reef fishes, preferred feeding areas are at upcurrent sites, the opposite end of the reef to the preferred downcurrent spawning locations. In *T. bifasciatum* females migrate between feeding and spawning sites. However, sessile filter-feeding marine invertebrates may face conflicting selective pressures for preferred settlement sites, and higher current velocities may increase feeding rates but decrease fertilization success. How these factors affect settlement and reproductive patterns in sessile marine organisms is currently not known.

Variation in fertilization success has the potential to affect temporal patterns of spawning, the degree of spawning aggregation, spawning behavior, mate choice, and investment patterns of marine organisms. By providing both a technique to measure fertilization success in organisms with predictable spawning and short-lived

sperm and data showing that predictable variance exists in fertilization success, we hope to stimulate work in this little-studied area of marine biology.

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