

Variation in Fertilization Rate in the Tropical Reef Fish, *Halichoeres bivittatus*: Correlates and Implications

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Abstract. Fertilization rates were estimated for natural spawns in the tropical wrasse, *Halichoeres bivittatus*. Fertilization rate averaged 88%, but varied with both sea conditions and with the addition of males (streakers) to pair spawns. As sea conditions became rougher, the mean fertilization rate for a day decreased. This effect was due to the two days with the roughest sea conditions and the lowest mean fertilization rates; there was no obvious trend when these two days were excluded from the analysis. On a given day, pair spawns, with a single male and female, had fertilization rates approximately 7% lower than spawns where 1–2 streakers joined the pair spawn. These results suggest that variation in fertilization rate must be considered as a potential selective force in shaping reproductive behavior in fishes with external fertilization of pelagic eggs.

Introduction

The social and mating systems of tropical reef fishes, especially the more colorful and abundant species like the wrasses (Pisces: Labridae), have been the focus of a large body of research (Robertson, 1972, 1981; Robertson and Choat, 1974; Robertson and Hoffman, 1977; Warner and Robertson, 1978; Thresher, 1979; Warner and Hoffman, 1980a, b; Moyer and Yogo, 1982; Tribble, 1982; Kuwamura, 1984; Warner, 1984a, b, 1987, 1988; Nemtsov, 1985; Hoffman, 1985; Ross, 1986; Lejeune, 1987; Victor, 1987; Baird, 1988). Much of this work has involved documenting spawning behavior, quantifying the reproductive success of various mating tactics, and speculating on

the adaptive significance of morphological and behavioral differences between and within the sexes.

The wrasses have reproductive behaviors typical of free-spawning tropical reef fishes, with spawning occurring at downcurrent sites of reefs. Spawning consists of one to over ten males and a female rushing upward 0.2 to several meters toward the surface and releasing gametes into the water column at the apex of the spawning rush. Although past workers have proposed many alternative mechanisms for the evolution of reproductive behaviors in tropical reef fishes, one fundamental factor has been almost completely ignored: the effect of fertilization rate on individual reproductive success and spawning behavior (for exceptions see Shapiro, 1989; Petersen, 1991). In this paper, I provide measurements for fertilization rate in the tropical wrasse, *Halichoeres bivittatus*, examine patterns and the potential causes of variation in fertilization rate, and discuss the role that fertilization rate may play in the evolution of reproductive behavior in this species.

Selection on males has been generally thought to act such that individuals produce enough sperm to fertilize all of the eggs of a female, implying that investigations of variation in fertilization rate would be uninteresting. However, as the amount of sperm released in a spawn increases, the number of additional fertilizations for a given number of sperm released should decrease, reducing the benefit for producing more sperm (Petersen, 1991). There is no reason to expect that the benefits to males for producing additional sperm will outweigh the costs of sperm production until all eggs are fertilized. Under these circumstances, the selected level of sperm production may not be enough to fertilize all available eggs, and the fertilization rate should be less than 100% (Petersen, 1991).

The extent to which fertilization rate is important in discussions of mating-system evolution depends on how

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much variance exists in fertilization rate and how much of that variance is predictable. Given that fertilization rate may not always be 100%, it is still not clear whether fertilization rate plays a significant role in determining male and female mating strategies in organisms that have external fertilization of pelagic eggs.

In many tropical reef fishes, there is more than one male mating behavior within a population (e.g., Warner *et al.*, 1975; Robertson and Warner, 1978; Warner and Robertson, 1978; Thresher, 1984; Petersen, 1990). Spawning sites may be occupied by either a large territorial male who mates singly with the female (pair spawning) or by a group of smaller males who mate together with the female (group spawning). In addition, small non-territorial males are known to rush in and join pair spawns at the moment of gamete release, a mating behavior called streaking (Warner *et al.*, 1975; Thresher, 1984). In many species, there may be several active spawning locations within the range of an individual female, providing females with a choice of conditions for spawning. The extent that males choose or compete for spawning locations and females choose either spawning locations or the identity of the male-role spawners based on fertilization rate is unknown.

Reproductive Biology of *Halichoeres bivittatus*

The slippery dick, *Halichoeres bivittatus*, is the most common Caribbean wrasse found over shallow reefs and seagrass communities. Smaller males and females have an initial-phase (IP) drab coloration; large terminal-phase (TP) individuals are more brightly colored and are invariably male (Warner and Robertson, 1978). TP males may be the result of sex change, or merely a coloration change from IP males (Warner and Robertson, 1978). Sex ratio is only slightly biased towards females (1:1.2), and IP males outnumber TP males approximately 2.3:1 (Warner and Robertson, 1978).

Spawning occurs daily in the mid-afternoon over the lee edges of reefs and over turtle grass (Warner and Robertson, 1978). In pair spawning, gravid females enter the territories of TP males, are approached by the TP male, and after one to several rushes by the male toward the female both rise quickly 0.3 to over 1 m in the water column in a fast spawning rush. Gametes are released at the apex of this spawning rush, after which both fish dart back to their normal positions closer to the bottom. The eggs are fertilized externally as they begin to float away from the location of the spawn. The TP male continues to court additional females, and may spawn over 20 times in a day (pers. obs.). Streaking occurs when an IP male joins the spawning pair at the apex of their spawning rush and presumably releases sperm. Only TP males were observed pair spawning in this study; IP males were observed

streaking pair spawns and, on one occasion, approximately 10 IP males took part in a group spawn. Both pair and group spawning appear to be common in this species (Warner and Robertson, 1978). All data reported here are from pair spawns with or without streakers.

Materials and Methods

Data were collected during February 1990 along the northeast coast of St. Croix, Virgin Islands, and during March 1990 in the San Blas Islands of Panama. At St. Croix, collections of eggs were made in shallow water (1–4 m depth) while snorkeling behind the barrier reef approximately 1 km west of Tague Bay. In the San Blas, collections were made while snorkeling at 1–3 m depth at Smithsonianupoo reef adjacent to the Smithsonian Tropical Research Institute San Blas Field Station.

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 for at least 30 s, the observer swept the area around and including the expanded fluorescein cloud for 30 s with a 6-inch brine-shrimp net (maximum mesh size approximately $100 \times 300 \mu\text{m}$). Collections of eggs from another wrasse (*Thalassoma bifasciatum*, Petersen *et al.*, 1992) indicated that harsher-textured nylon nets, such as plankton netting, severely reduced the fertilization rates while the brine-shrimp net had no effect. 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. For each spawn, the location of the spawn was recorded, and the number of streakers that joined the spawn (0–2) was noted.

The next morning the contents of each bag were filtered through $100 \mu\text{m}$ nylon mesh to collect the eggs, which were then examined under a dissecting microscope and scored as fertilized or unfertilized. By counting eggs 16–20 h post-spawning, fertilized, developing eggs could be unambiguously distinguished from undeveloped eggs. Developing eggs contain nearly fully developed larvae, because hatching is completed within 24 h. Undeveloped eggs were scored as unfertilized. Estimates of fertilization rate for spawns relied on collections with at least 20 eggs.

To test whether the 30-s delay before collection was adequate to allow fertilization to occur, on three days collections were alternated between 30-s delays and 60-s delays. A significant increase in fertilization rate in the 60-s sample would suggest that the 30-s samples underestimate fertilization rate. In addition, collections were made at spawning locations during the spawning period but not immediately after a spawn to verify that eggs collected

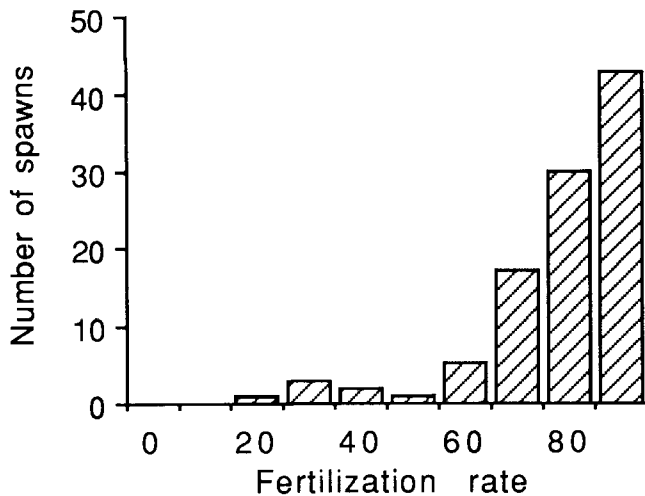


Figure 1. Frequency distribution of fertilization rates for all spawns with ≥ 20 eggs. The lower bound for the 10% categories is given, the last category included two spawns with 100% fertilization of collected eggs.

after spawns were not in the water column from spawns elsewhere.

On each date, a qualitative score of sea conditions was made during the spawning period. This score varied from 1 for the calmest conditions to 5 for the roughest conditions experienced during the study. These data were used to test for decreased fertilization rate on days with higher water velocities and increased water mixing. Based on theoretical studies of water turbulence and fertilization rate, increased water mixing was predicted to reduce sperm concentrations and reduce fertilization rates (Denny and Shibata, 1989).

The possibility that sperm depletion in males during the daily spawning period causes lower fertilization rates was examined by testing for a negative correlation between fertilization rate and the order of collection of spawns for a specific male during a spawning period. Only sequences with at least seven samples were used for this purpose.

For statistical analysis, fertilization rate data were angularly transformed before applying parametric statistical techniques. In cases where the sea-condition ranking or the spawning-order ranking was used, non-parametric statistics were employed.

Results

A total of 180 collections of eggs were taken over 19 days. Of the 180 collections, 102 (57%) had the minimum of 20 eggs and were used in the analysis of fertilization rate. The mean fertilization rate of these collections was 88.1% (median = 87.3%) (Fig. 1). The number of eggs collected from a spawn varied from 0 to 536 (median = 26.5; for collections with at least 20 eggs, median = 65.5). The three control collections had 0–4 eggs, im-

plying that eggs in collections do come largely from the spawn that was observed immediately prior to sampling.

There was no evidence that collecting eggs after 30 s detrimentally affected the estimate of fertilization rate. Eggs collected from spawns after 60 s had virtually identical fertilization rates to spawns collected after 30 s ($F_{1,15} = 0.001$, $P = 0.98$).

Spawning date had a significant effect on fertilization rate. Over the 18 dates with at least two collections with 20 eggs, fertilization rate varied significantly among dates (ANOVA, $F_{17,84} = 8.31$, $P < 0.001$).

One of the causes of variation in fertilization rate among days appeared to be sea conditions. There was a negative correlation between the qualitative score of sea condition and the mean fertilization rate for a day, with rougher days having lower fertilization rates (Fig. 2, $r_s = -0.44$, $P_{[1-tailed]} < 0.05$, $n = 18$ days). This analysis used only spawns without streaking; an identical trend existed for the spawns with streakers ($r_s = -0.48$, $P_{[1-tailed]} < 0.05$, $n = 16$). Much of the variation in fertilization rates among dates was due to the two dates that had the roughest sea conditions and also had the lowest mean fertilization rates. Excluding these two days, there was no significant effect of sea conditions on fertilization rate (both types of spawns, $P_{[1-tailed]} > 0.25$).

Spawns with streakers had significantly higher fertilization rates than pair spawns without streakers collected on the same day. To test for changes in fertilization rate with the presence of streakers, date of collection and the presence of streakers were examined simultaneously in a two-way ANOVA for the subset of days with collections from pair spawns both with and without streakers ($n = 16$ dates). Using this database, both the presence of a streaker joining the pair spawn and the day a spawn was collected

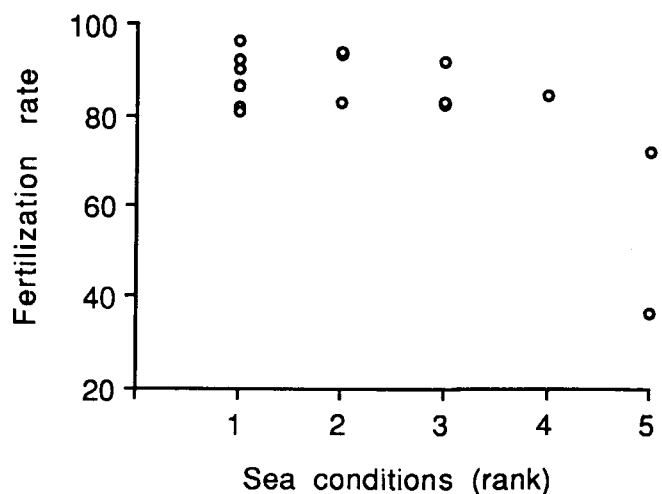


Figure 2. The relationship between sea conditions (rank score) and mean fertilization rate on a day for all spawns without streakers with ≥ 20 eggs.

had a significant effect on fertilization rate (Table I). Spawns with streakers had an average of 6.6% higher fertilization rate on a day (range = -4 to 18%) compared with pair spawns without streakers.

The identity of the male did not appear to affect fertilization rate. On two days collections were made simultaneously from three males on the same back reef area within 30 meters of one another. There was a significant effect of date ($F_{1,19} = 6.41$, $P = 0.02$), but no significant effect of male identity ($F_{2,19} = 0.56$, $P = 0.58$).

There was also no evidence that fertilization rate for spawns by a male changed in any systematic way during the spawning period. Over the course of the study, there were three sequences of 7–10 spawns successfully collected from a male. These sequences occurred over a period of 33–48 min. The order in the spawning sequence was not significantly correlated with fertilization rate ($r_s = -0.25$ to 0.64, $P_{[1-tailed]} > 0.1$ in all cases). Thus, the fertilization rate data does not provide evidence for sperm depletion by TP male *H. bivattatus*.

Discussion

Fertilization rate has been a previously ignored aspect of the reproductive biology of tropical reef fishes. The result that lower fertilization rates occur on days of rougher water conditions and higher current velocity is consistent with a theoretical treatment of the effect of turbulence on fertilization rate (Denny and Shibata, 1989) and with empirical data for marine invertebrates (Pennington, 1985; Levitan, 1989) and another marine fish (Petersen *et al.*, 1992).

A difference in fertilization rate was also observed between pair spawns and pair spawns joined by streaking males. The most likely cause of the increase in fertilization rate in spawns with streakers is a higher total amount of sperm released with the addition of another male. However, this result could also be caused by some other factor associated with streaking. For example, a slower spawning ascent or a lower spawning rush height might be associated both with increased fertilization rates and with a higher probability of additional males joining the spawn. Although these alternatives are possible, I will assume that the most straightforward interpretation is correct, that streakers directly increase fertilization rate in *Halichoeres bivattatus*. If so, the result that streaking increases fertilization rate indicates that some of the assumptions that have been made about reproduction in reef fishes have been violated and that fertilization rate must be considered as a potential selective force in shaping spawning behavior.

If females attempt to maximize fertilization rates in their spawns, irrespective of the identity of the male that fertilizes the eggs, then these data provide evidence for a conflict of interests between the TP territorial male and

Table I

Two-way mixed-model ANOVA of the effect of presence of streakers and collection day on fertilization rate

Dependent variable—Fertilization rate (angular transformation)				
Independent variable	df	MS	F	P
Day of collection	15	0.10	6.62	<0.001
Presence of streakers	1	0.12	6.77	<0.05
Interaction	15	0.02	1.1	>0.25
Error	65	0.016		

$R^2 = 0.74$.

the spawning female. Females gain fertilizations by having a streaking male join the spawn, while the TP male will almost certainly lose fertilizations due to sperm competition with the streaking IP male. Territorial males chase IP individuals away from the spawning site during the spawning period, and many, if not all, of these are presumably males and potential streakers. In addition, immediately before spawning, males sometimes exhibit a behavior called “looping” (see Thresher, 1984, for a description and drawing). Looping consists of the TP male going through a series of quick rushes upward near the spawning site. Looping has traditionally been thought of as a courtship behavior, but appears to have a second function of exposing potential streakers in *H. bivattatus*. In several instances, IP individuals streaked while the male was looping, and were immediately chased by the male. Thus, looping appears to be a tactic by territorial males to expose potential streakers before spawns.

Although there is convincing evidence that males attempt to exclude streakers, there is no convincing evidence that females alter their behavior to increase their fertilization rate by increasing the probability of successful streaking. In the Mediterranean wrasse, *Symphodus tinca*, females avoid nesting males with large numbers of peripheral males (van den Berge *et al.*, 1989). Fertilization rates of the demersal eggs were near 100% and did not differ for nests with and without frequent spawning by peripheral males, so spawning site selection did not appear to be based on differences in fertilization rate (van den Berge *et al.*, 1989).

In the seabass, *Serranus fasciatus*, Petersen (1987) interpreted hesitation by female-role spawners before beginning the spawning rush as a behavioral tactic to reduce streaking. When the spawning partner hesitated, males often broke off spawning and patrolled the spawning area, chasing any individuals that were close enough to the spawning location potentially to streak. However, the increased fertilization rate for spawns with streakers in *H. bivattatus* suggests a different interpretation for this be-

havior. Hesitations by females before beginning the spawning rush in tropical reef fishes could be a female mating tactic to increase streaking rate, and not a female response to imperfect positioning by the TP male prior to spawning or a tactic to reduce streaking rate. This hypothesis needs to be tested with comparative data, including species with and without streaking males. Females in *H. bivattatus* often hesitate several times before ultimately spawning with a TP male (pers. obs.).

Despite the possible advantages of female spawning behavior that increases fertilization rate, these modifications may be highly constrained by predation pressure during spawning. Behaviors that may increase the chance of a nearby male seeing and joining the spawn, such as slowing down the spawning rush, may also increase the vulnerability of the female to predators. In two instances during this study, a lizardfish (*Synodus* sp.) struck at the fish in a pair spawn at the apex of the spawning rush. Although neither attempt was successful, predators may limit the advantages of female behaviors that could increase their visibility to streakers but at the same time make them more vulnerable to predators. Thresher (1984) has noted that many of the observations of reef-fish being preyed upon have occurred during spawning.

In another Caribbean wrasse, *Thalassoma bifasciatum*, fertilization rates do not differ between pair spawns and group spawns (Petersen *et al.*, 1992), despite an estimated 80-fold increase in sperm released in group spawns relative to pair spawns. Group spawns typically involve at least five males, and may produce higher levels of turbulence and water mixing, reducing the concentration of gametes faster than in pair spawns. Group spawns may also lack the close juxtaposition of a male and female during gamete release that exists between the pair-spawning male and female with or without streaking (Petersen *et al.*, 1992). Thus, fertilization rate comparisons between different types of spawns may depend both on the number of males in the spawn and the type of spawn. This difference for spawns with streaking and group spawns compared with pair spawns should be tested for other species to determine its generality.

In transforming male mating success into reproductive success, researchers have had to rely on approximations of how fertilizations are divided among males in cases of sperm competition. The approach has varied from assigning all of the reproductive success to the male using the alternative reproductive pathway due to his higher sperm production and proximity to the female (Gross and Charnov, 1980) to dividing the fertilization evenly among all males in the spawn (Warner and Hoffman 1980a, b; Petersen 1987, 1990). In all of these studies, fertilization rate was assumed constant. This study shows that additional males alter the fertilization rate, and, although it is not clear how fertilizations are divided among the two

types of males, it is clear that our assumptions of how mating success is translated into reproductive success in these fishes needs to be reappraised.

These results suggest two avenues for future research in the reproductive biology of tropical reef fishes. First, variation in female mate choice, spawning site selection, and temporal patterns of spawning may be at least in part a response to variation in fertilization rate. Second, the spawning behavior of both males and females needs to be reconsidered as a potential tactic to increase individual fertilization rates.

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Literature Cited

- Baird, T. A. 1988. Abdominal windows in straight-tailed razorfish, *Xyrichtys martinicensis*: an unusual female sex character in a polygynous fish. *Copeia* 1988: 496-499.
- van den Berge, E. P., F. Wernerus, and R. R. Warner. 1989. Female choice and the mating costs of peripheral males. *Anim. Behav.* 38: 875-884.
- Denny, M. W., and M. F. Shibata. 1989. Consequences of surf-zone turbulence for settlement and external fertilization. *Am. Nat.* 134: 859-889.
- Gross, M. R., and E. L. Charnov. 1980. Alternative male life histories in bluegill sunfishes. *Proc. Nat. Acad. Sci. U.S.A.* 76: 6937-6940.
- Hoffman, S. G. 1985. Effects of size and sex on the social organization of reef-associated hogfishes, *Bodianus* sp. *Environ. Biol. Fish.* 14: 185-197.
- Kuwamura, T. 1984. Social structure of the protoyous fish *Labroides dimidiatus*. *Publ. Seto Mar. Biol. Lab.* 29: 117-177.
- Lejeune, P. 1987. The effect of local stock density on social behavior and sex change in the Mediterranean labrid *Coris julis*. *Environ. Biol. Fish.* 18: 135-141.
- Leviton, D. R. 1989. Life history and population consequences of body size regulation in the sea urchin *Diadema antillarum* Phillipi. *Ph.D. Dissertation*. University of Delaware, Newark.
- Moyer, J. T., and Y. Yogo. 1982. The lek-like mating system of *Halichoeres melanochir* (Pisces: Labridae) at Miyake-jima, Japan. *Z. Tierpsychol.* 60: 209-226.
- Nemtzov, S. C. 1985. Social control of sex change in the Red Sea razorfish *Xyrichtys pentadactylus* (Teleostei, Labridae). *Environ. Biol. Fish.* 14: 199-211.
- Pennington, J. T. 1985. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. *Biol. Bull.* 169: 417-430.
- Petersen, C. W. 1987. Reproductive behaviour and gender allocation in *Serranus fasciatus*, a hermaphroditic reef fish. *Anim. Behav.* 35: 1601-1614.
- Petersen, C. W. 1990. The relationships among population density, individual size, mating tactics, and reproductive success in a hermaphroditic fish, *Serranus fasciatus*. *Behaviour* 113: 57-80.

- Petersen, C. W. 1991. Sex allocation in hermaphroditic seabasses. *Am. Nat.* (in press).
- Petersen, C. W., R. R. Warner, S. Cohen, H. C. Hess, and A. T. Sewell. 1992. Variation in pelagic fertilization success: implications for production estimates, mate choice, and the spatial and temporal distribution of mating. *Ecology* (in press).
- Robertson, D. R. 1972. Social control of sex reversal in coral-reef fish. *Science* 177: 1007-1009.
- Robertson, D. R. 1981. The social and mating systems of two labrid fishes, *Halichoeres maculipinna* and *H. garnoti*, off the Caribbean coast of Panama. *Mar. Biol.* 64: 327-340.
- Robertson, D. R., and J. H. Choat. 1974. Protogynous hermaphroditism and social systems in labrid fishes. *Proceedings Second International Symposium Coral Reefs* 1: 217-225.
- Robertson, D. R., and S. G. Hoffman. 1977. The roles of female mate choice and predation in the mating systems of some tropical labroid fishes. *Z. Tierpsychol.* 45: 298-320.
- Robertson, D. R., and R. R. Warner. 1978. Sexual patterns in the labroid fishes of the western Caribbean. II: The parrotfishes (Scaridae). *Smith. Contrib. Zool.* 255: 1-26.
- Ross, R. M. 1986. Social organization and mating system of the Hawaiian reef fish *Thalassoma duperrey* (Labridae). Pp. 794-802 in *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes*, T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura, eds. Ichthyological Society of Japan, Tokyo.
- Shapiro, D. Y. 1989. Sex change as an alternative life-history style. Pp. 177-195 in *Alternative Life-history Styles in Animals*, M. N. Burton, ed. Kluwer Publishers, Dordrecht, The Netherlands.
- Thresher, R. E. 1979. Social behavior and ecology of two sympatric wrasses (Labridae: *Halichoeres* spp.) off the coast of Florida. *Mar. Biol.* 53: 161-172.
- Thresher, R. E. 1984. *Reproduction in Reef Fishes*. T.F.H. Publications, Neptune City, NJ.
- Tribble, G. W. 1982. Social organization, patterns of sexuality, and behavior of the wrasse *Coris dorsomaculata* at Miyake-jima, Japan. *Environ. Biol. Fish.* 7: 29-38.
- Victor, B. C. 1987. The mating system of the Caribbean rosy razorfish, *Xyrichtys martinicensis*. *Bull. Mar. Sci.* 40: 152-160.
- Warner, R. R. 1984a. Mating behavior and hermaphroditism in coral reef fishes. *Am. Sci.* 72: 128-136.
- Warner, R. R. 1984b. Deferred reproduction as a response to sexual selection in a coral reef fish: a test of the life historical consequences. *Evolution* 38: 148-162.
- Warner, R. R. 1987. Female choice of sites versus mates in a coral reef fish, *Thalassoma bifasciatum*. *Anim. Behav.* 35: 1470-1478.
- Warner, R. R. 1988. Traditionality of mating-site preferences in a coral-reef fish. *Nature* 335: 719-721.
- Warner, R. R., and S. G. Hoffman. 1980a. Local population size as a determinant of mating system and sexual composition in two tropical reef fishes (*Thalassoma* spp.). *Evolution* 34: 508-518.
- Warner, R. R., and S. G. Hoffman. 1980b. Population density and the economics of territorial defense in a coral reef fish. *Ecology* 61: 772-780.
- Warner, R. R., and D. R. Robertson. 1978. Sexual patterns in the labroid fishes of the western Caribbean, I: The wrasses (Labridae). *Smith. Contrib. Zool.* 254: 1-27.
- Warner, R. R., D. R. Robertson, and E. G. Leigh, Jr. 1975. Sex change and sexual selection. *Science* 190: 633-638.