

D. Ross Robertson

Egg size in relation to fertilization dynamics in free-spawning tropical reef fishes

Received: 4 December 1995 / Accepted: 30 March 1996

Abstract In marine invertebrates that spawn by simply releasing their gametes into the water (free-spawning), fertilization success likely is often limited by low sperm concentrations, due to dispersion of mates and dispersal of gametes by water movements. Production of large, low density eggs might be advantageous when sperm concentrations consistently are low, because large target size might increase egg/sperm encounters, and more low than high density eggs could be produced per clutch. Although average fertilization success in the labrid *Thalassoma bifasciatum* is ~95% in both group spawns (in which multiple males compete for fertilizations by producing large quantities of sperm) and pair (mono-male) spawns, it is slightly lower in pair spawns, due to low level sperm limitation that arises because pair-spawning males release near the minimum number of sperm necessary for maximum fertilization. I examined whether variation in egg size and content in *T. bifasciatum* and other free-spawning fishes is related to variation in spawning mode, to assess whether compensatory production of large, low-density eggs might be contributing to high fertilization success in pair spawns. I found no difference between the volume or density of eggs of (1) pair- and group-spawning females of *T. bifasciatum*, or (2) pair- and group-spawning congeneric species of labrids, scarids, and serranids, or (3) labrids and scarids with vigorous, rapid spawning movements (which could turbulently diffuse gamete clouds) and those with slow movements. Further, egg density does not decline with increasing egg volume among those fishes. Assuming that egg size can affect fertilization success, then sperm limitation seems unlikely to represent a significant problem for pair-spawning *T. bifasciatum*, probably because mates place their vents close together during gamete release. The situation regarding sperm limitation in other fishes, and effects of environmentally generated water

turbulence on it, are less clear. Interspecific variation in the size and content of these fishes' eggs may relate to provisioning of offspring for different larval life-histories.

Key words Fish · Egg size · Fertilization dynamics · Sperm competition, limitation, and economy

Introduction

Egg size, spawning modes, and fertilization success

The explanation of variation in egg size or content is a central part of life-history theory – how much should a female invest in each egg in order to maximize her fitness, given that resource limitation is likely to produce a tradeoff between egg-size and clutch-size (e.g., Vance 1973; Strathman and Vedder 1977; Roff 1992; Stearns 1992). An extensive body of literature examines the life-history implications of variation in the size and content of fish eggs in relation to body and clutch size, the occurrence of parental care, and geographic and seasonal variation in environmental factors likely to affect larval survival (e.g., Bagenal 1971; Ware 1975; Sargent and Gross 1987; Thresher 1988; Duarte and Alcaraz 1989; Elgar 1990; Wootton 1994).

When marine organisms release their gametes freely into the water, fertilization success can be influenced by factors such as water turbulence and the proximity and population density of spawners, which affect sperm concentrations and hence egg-sperm encounter probabilities (Denny and Shibata 1989; Levitan 1991; Levitan et al. 1992; Levitan 1993; Levitan and Petersen 1995). Theory and observations on fertilization kinetics led Levitan (1993) to develop the “sperm limitation” hypothesis to account for variation in egg size among free-spawning benthic marine invertebrates that produce planktotrophic larvae. This hypothesis proposes that (1) the lower limit of egg size is set by effects of target size on the probability of sperm-egg encounters, and (2) large egg size has

D.R. Robertson
Smithsonian Tropical Research Institute (Balboa, Panama),
Unit 0948, APO AA 34002-0948, USA
fax: +507-2280516,
e-mail: stri01.naos.robertsr@ic.si.edu

been selected for in species in which sperm dilution strongly limits fertilization success, because sperm are more likely to encounter large targets. In addition, (3) Levitan (1993) also noted that a reduction in egg density with increasing egg volume might reduce trade-off costs to clutch size that would otherwise be expected to arise from the production of large eggs. Levitan based the sperm limitation hypothesis on a combination of (1) semi-natural experiments with echinoids that indicate sperm limitation of fertilization success is likely to occur in the field, (2) laboratory experiments with and computer simulations of effects of egg size and sperm dilution, longevity and motility on fertilization success, and (3) field observations of factors (e.g., levels of gamete output, population density, habitat distributions) likely to influence the degree of sperm dilution and extent of sperm limitation.

In free-spawning fishes males and females place their vents close together as they simultaneously release gametes. Such spawning behaviors would be expected to produce consistently high fertilization success (Petersen et al. 1992). However, sperm limitation due to sperm rationing might occur in fishes if a declining return for effort meant that the allocation of resources to alternative activities (e.g., growth, defending a territory, or attracting additional mates) enhanced fitness more than did investment in sperm production sufficient to achieve 100% fertilization success (Petersen 1991b). It has recently been shown that sperm limitation does produce small reductions in fertilization success in the labrid fish *Thalassoma bifasciatum* in the field, due to males rationing sperm release to near the minimum sufficient to obtain near maximal fertilization (Shapiro et al. 1994; Warner et al. 1995, and see next section). Such low level sperm limitation occurs in spawnings involving a single male (pair-spawns). In spawnings in which multiple males participate (group-spawns) levels of sperm output are so much higher, due to sperm competition, than in pair spawns, that fertilization success is highly unlikely to be affected by sperm limitation.

Here I assess whether variation in the size and density of planktotrophic eggs of 24 species of free-spawning tropical reef fishes from three families (Labridae, Scaridae, and Serranidae) is related to variation in the potential for sperm limitation. I do so because compensatory increases in egg size might be contributing to high rates of fertilization in situations in which sperm limitation is likely to occur. Such increases in egg size might have co-evolved with decreases in sperm release, particularly in species in which male mating success is high, because in such species it may be cost effective for males to ration sperm and trade-off lowered fertilization rates against obtaining additional mates. I examine three predictions that derive from the sperm limitation hypothesis:

1. Within a species, females that consistently pair-spawn, and thus are consistently exposed to sperm limitation, should produce larger, less dense eggs than females that consistently group-spawn and are highly unlikely to experience sperm limitation.

2. Species that only pair-spawn should have larger, less dense eggs than congeners that typically group-spawn.

3. Species that have rapid, vigorous spawner movements, which could produce sperm limitation by turbulent diffusion of gamete clouds (cf. Levitan and Petersen 1995), should have larger, less dense eggs than species with slow spawning movements and lower potential for such sperm limitation.

Mating modes and the potential for sperm limitation among the study species

Thalassoma bifasciatum

The bluehead wrasse, *Thalassoma bifasciatum* lives on west Atlantic coral reefs. When spawning a female and one or more males rush together up ~0.5 m in the water column, abruptly release gametes while their vents are in very close proximity, then dart separately back to the reef. *T. bifasciatum* has two main modes of spawning, although there is some intergradation. Each day large, distinctively colored terminal phase (TP) males establish temporary spawning territories along reef edges and pair-spawn with single females, which visit those territories only to mate. Concurrently, small initial phase (IP) males that are colored like females form temporary aggregations of dozens to hundreds of individuals at nearby spawning sites. IP males are non-territorial and usually group spawn with females. In a group spawn ~5–10 IP males cluster tightly about a single female during the spawning rush and release of gametes. Both spawning modes are common on large reefs, on which most individuals of this species live (further details in Warner et al. 1975). Except for occasional brief attacks by a TP male on planktivorous fishes feeding on a freshly spawned clutch, there is no parental care.

Differentials between the mating success of females and TP males are high in this species. On large reefs large TP males mate with an average of >40 females/day, while females spawn at most once/day. IP males average 17 spawns/day and effectively fertilize the eggs of one female/day (Warner et al. 1975, 1995). TP males ration sperm release during each pair-spawn in response to the number of females they can expect to spawn with during a day's 1–2 h spawning period, and to female size, which indicates likely clutch size. In doing so they apparently "economize on sperm release by providing the minimum amount of sperm needed to fertilize the egg clutch...." (Shapiro et al. 1994). Further, TP males that mate with large numbers of females release fewer sperm per spawning and fertilize a slightly lower percentage of each clutch than do the least successful TP males (93% vs. 98% fertilization rates, respectively – Warner et al. 1995). In group spawns, individual IP males compete to fertilize eggs by releasing large amounts of sperm. Although IP males ration sperm in relation to the size of the female in each group spawn (Shapiro et al. 1994), sperm production by individual males and by all males

combined is ~6 and ~50–80 times greater, respectively, in a group spawn than in a pair spawn. Initial work indicated that fertilization rates were ~70% in both pair and group spawns (Petersen et al. 1992). However, subsequent more detailed study has shown that, while average fertilization success exceeds 90% in both types of spawns, it is ~2–5% higher in group spawns than in pair spawns (Shapiro et al. 1994; C.W. Petersen and R.R. Warner, personal communications), due to sperm limitation arising from sperm rationing in pair spawns. The results of Petersen et al. (1992) with *T. bifasciatum* also indicated that, under extreme conditions, fertilization success is adversely affected by water turbulence due to wave action. Subsequent work involving more precise measures of water movement has not found evidence of such effects over a range of relatively calm conditions (R.R. Warner and C.W. Petersen, personal communications). One consequence of differences in the levels of sperm competition experienced by TP and IP males is that the testes of IP males are much larger (both absolutely and relative to body size) than those of TP males (Warner et al. 1975).

Other labroid fishes (Scaridae and Labridae)

Labroid fishes generally have spawning behaviors similar to those of *Thalassoma bifasciatum*, although the precise actions of the male and female during spawning do vary among the study species (e.g., Robertson and Hoffman 1977; personal observations). In species of *Sparisoma*, the side-by-side pair make a spiralling upward rush, then abruptly separate and rush back to the bottom. This up-down sequence is notably more rapid in the two smaller species considered here, *S. atomarium* and *S. radians*, than in the two larger species *S. aurofrenatum* and *S. rubripinne* (cf. Thresher 1984). Species of *Halichoeres*, *Novaculichthys*, and *Thalassoma* have a rapid up-down spawning rush similar to that of *Sparisoma*, except that the male is positioned more above the female during a straight (no spiralling) upward rush. Species of *Bodianus* have a relatively slow upward rise by the side-by-side pair, which then slowly separates after releasing gametes. In *Clepticus parrae* the male pushes the torpid female upwards through the water and the pair of fish then simply swim slowly apart after releasing gametes (Robertson and Hoffman 1977; personal observations).

The basic patterns of pair and group spawning outlined above for *T. bifasciatum* are common to many wrasses and parrotfishes, although the relative amounts of those two spawning modes vary among species. Pair-spawning male labroids characteristically have much smaller testes than group-spawning conspecific males (Robertson and Choat 1974; Choat and Robertson 1975; Robertson and Warner 1978; Warner and Robertson 1978). In many labroids, including *T. bifasciatum*, high levels of sperm output often result in large, visible sperm clouds that persist for several seconds after a group

spawn. Such sperm clouds are not seen after pair spawns (personal observations).

In *Halichoeres bivittatus*, sperm limitation due to inadequate sperm release by TP males evidently occurs in pair spawns, because Petersen (1991a) found that rates of fertilization increase by ~7% when IP males participate as satellites in those spawns. His data also indicate that water turbulence due to strong wave action reduces fertilization success, presumably by sperm limitation, in this species.

Serranus species (Serranidae)

Serranus species are simultaneous hermaphrodites, and individuals typically alternate male and female spawning roles during the same spawning period. In *S. tabacarius*, *S. tigrinus*, and *S. tortugarum* all individuals are hermaphroditic, while in *S. baldwini* and *S. psittacinus* a population contains males as well as hermaphrodites. Species of *Serranus* have a spawning rush like that of *Thalassoma*. The number of “males” participating in spawnings also varies, apparently from one to several, and there is interspecific variation in the proportions of pair and “multimale” spawns. Relative allocation to testicular tissue is greater in species with higher levels of “multimale” spawnings, and sperm competition and relatively high sperm output likely occur in multimale spawns (see Petersen 1991b for summary of mating systems and gonad allocation patterns). These serranids lack parental care.

Materials and methods

Study areas

Data on the Caribbean fishes were collected in the San Blas Islands, on the eastern coast of Panama. Data on the eastern Pacific species were collected in the Pearl Islands in Panama Bay. Data on western Pacific labrids were collected at Lizard Island, on the Australian Great Barrier Reef.

Collecting and processing eggs

Except for one set of samples from *T. bifasciatum*, all eggs of labrids and scarids were collected from females that I speared while they were preparing to spawn by rising to an actively courting male. Eggs were stripped from such females within 10 min (usually less) of spearing. Only eggs that flowed freely from a female under light finger pressure on her abdomen and that were perfectly spherical and positively bouyant were used to determine egg volumes.

Because the volume of an egg may be affected by hydration processes occurring both prior to ovulation (Craik and Harvey 1987) and after fertilization (Le Clus 1979) I collected eggs of *T. bifasciatum* both before and after spawning to ensure that any patterns of variation in egg volume and density were not artifacts arising from such hydration processes. In all, four sets of eggs of this species were collected: (1) eggs produced by females that had either group-spawned, or (2) were about to do so, and (3) eggs produced by females that had pair-spawned, or (4) were about to do so. Freshly spawned eggs were collected with a 30 cm mouth-diameter plankton net, within about 10 s of being spawned. Pre-spawning eggs were collected from speared females as described above.

Egg size is known to vary with female size in fishes (e.g., Roff 1992). Hence I recorded the standard length of females of *T. bifasciatum* that I speared to obtain stripped eggs, but did not collect and measure individuals whose eggs I collected after they were spawned. Variation in the mean volume and density of stripped eggs was not significantly related to female length (Pearson $r = -0.22$, $P = 0.255$, for egg volume, and $r = -0.36$, $P = 0.173$ for density; $n = 31$ females ranging from 35 to 82 mm SL). Thus any differences in the sizes of females that produced the eggs I collected seem unlikely to have biased the results. Nevertheless, I made a point of collecting both netted and stripped, and pair- and group-spawned eggs from females of about the same range of sizes.

T. bifasciatum spawns year-round in San Blas (Warner and Robertson 1978), with, on average, 70–75% of the females on a reef spawning on any day (Schulz and Warner 1991; D.R. Robertson and S. Swearer, unpublished work). Females of *T. bifasciatum* show strong fidelity to individual spawning sites, with only 5–15% of their spawns being at sites other than the usual site (Warner 1987). Some females on a reef consistently pair-spawn and others consistently group-spawn, with group-spawning females tending to be smaller than pair-spawning females (Warner 1985, 1987). Group and pair spawning consistently occur at different sites on the same reef. Eggs were collected from group-spawning females at a reef-edge site used regularly and exclusively by an aggregation of several hundred IP males, and from pair-spawning females at sites used regularly by large TP males that had high levels of mating success. Hence it is very likely that a group- or pair-spawning female whose eggs I collected would have consistently spawned in that manner. I made those collections on a large reef that had a population of >5,000 fish.

The five *Serranus* species all spawn in late afternoon (Petersen 1991b, 1995). I obtained samples of eggs from each by capturing fish several hours before their daily spawning period (with a hand-net and a squirt-bottle of dilute quinaldine sulphate anaesthetic), maintaining them in a bag-net floating in the sea, then stripping them shortly after sunset. Because hermaphrodites typically have viable gametes of both sexes, and spawn as both sexes on the same day, those eggs may well have been fertilized. Eggs stripped from female labrids and scarids were unfertilized.

After collection, clutches were stored in jars containing fresh seawater in a cooler and returned to the laboratory. The eggs of all species considered here are spherical and the diameter of the chorion of 15–20 eggs from each clutch was measured with a micrometer under a dissecting microscope at 100× magnification. I use those diameters to estimate the average volume of an egg (calculated for a sphere) in each clutch. After eggs were measured, each clutch was washed ~6 times in distilled water, stored frozen, then freeze-dried. About 50 dried eggs from each clutch were counted and weighed together on a Cahn microbalance to obtain an estimate of the average dry weight of an egg in that clutch. The average density of an egg in a clutch was calculated as average dry weight/average volume of an egg in that clutch.

Mating systems and testicular investment

Data on the mating systems and testicular investment of the Caribbean fishes came primarily from the literature (detailed in Results). Eggs were collected between 1993 and 1995 at the same localities as those studies. In addition I made observations on the spawning of eastern Pacific labrids in the Pearl Islands during a study that involved 3 months of daily monitoring of spawning and recruitment of damselfishes in 1992, and on western Pacific labrids at Lizard Island during one month's fieldwork collecting eggs from such species in the austral summer of 1993–1994.

Data analyses

Statistical tests followed Sokal and Rohlf (1981), and I used the SYSTAT (Wilkinson 1990) computer package to run those tests. Data were log-transformed as needed to equalize variances.

Results

Relationship between volume and content of eggs

Mean egg volumes and densities varied 2.6-fold and 1.6-fold, respectively, among the 20 labrid and scarid fishes considered here in detail (Table 1). There was a strong linear relationship between egg dry weight and volume among those and 16 other labroids ($y = -0.85 + 0.084x$; $r^2 = 0.78$, $P < 0.001$). The intercept of that line did not differ from zero ($P = 0.343$; an intercept >0 would have indicated a decline in density with increasing volume, and an intercept <0 would have indicated an increase in density with volume). Although a power function fitted those data equally well ($r^2 = 0.78$) the exponent of the best-fit function (1.58) indicates that, if anything, large eggs were slightly more dense than small eggs. Thus there was no tendency for egg density to decline with increasing egg size among those labroids.

I examined variation in egg density among the *Serranus* species separately, because their eggs were consistently less dense than those of the labroids (Fig. 1). There also was a strong linear relationship between egg weight and volume in these species ($y = -2.93 + 0.007x$; $r^2 = 0.90$, $P < 0.001$), with the intercept of the regression line not significantly different from zero ($P = 0.258$). A power function with an exponent of 0.38 (indicating a decrease in egg density in larger eggs) fitted those data almost as well ($r^2 = 0.89$) as the linear relationship. Only the lines for the linear regressions for the serranids and labroids are shown in Fig. 1, because the power and linear functions have very similar shapes over the range of egg sizes considered here.

Intraspecific variation in egg size in *Thalassoma bifasciatum*

The unfertilized, stripped eggs of *T. bifasciatum* varied 1.2-fold in volume and 2.4-fold in density. Naturally spawned eggs had a slightly greater (~6%) average volume than stripped eggs (Table 2). The average volume of an egg produced by a pair-spawning female was significantly, but only slightly (~5%), less than the average volume of an egg produced by a group-spawning female. There were no statistically significant differences in the average densities of either stripped or spawned eggs produced by pair- and group-spawning females (Table 2).

Intrageneric variation in egg size and spawning mode

Halichoeres

Levels of multimale spawning by different species varied from close to zero (four species) to approximately half of all matings (three species), to a preponderance of group spawning (two species) (Table 1). Testis sizes were lower

Table 1 Mean (SE) volumes, dry weights and densities of pelagic eggs of 24 species of reef fishes. Spawning mode: pair (P) = one male in each act; group (G) = multiple (~5–10) males in each act; inequality symbol indicates relative amount of each type when both occur. Testis gonad-somatic index (GSI) = testis wet weight as a percentage of body wet weight. For labrids and scarids the two numbers refer to sizes for terminal phase (TP) males (who mainly pair spawn) and initial phase (IP) males (which primarily group spawn), respectively. Single numbers for three labrids that effectively lack group spawning refer to GSIs of pair spawning

males. ? indicates no data. For serranids, the two numbers refer to GSIs of males and hermaphrodites, respectively, in species that have both; a single number refers to the GSI of hermaphrodites in species that lack males. Site: WA = west Atlantic, EP = east Pacific, WP = west Pacific (Australian Great Barrier Reef).

S. psittacinus is a senior synonym of *S. fasciatus* (Allen and Robertson 1994). Sources of data: Robertson and Warner (1978); Warner and Robertson (1978); Hoffman (1980); Warner (1982); Warner and Hoffman (1980); Petersen (1991b, 1995); C.W. Petersen, unpublished data on GSIs of *Serranus* species.

Species	Spawning mode	Testis GSI	Site	Volume (nl)	Dry Weight (µg)	Density (µg/nl)	n
LABRIDAE							
<i>Bodianus axillaris</i>	P	?	WP	199.5 (4.5)	14.4 (0.6)	0.073 (0.002)	2
<i>Bodianus diplotaenia</i>	P	0.09	EP	187.7 (2.9)	12.4 (0.8)	0.066 (0.004)	9
<i>Bodianus rufus</i>	P	0.09	WA	198.8 (2.9)	18.2 (0.8)	0.092 (0.002)	4
<i>Clepticus parrae</i>	P	0.12	WA	157.0 (2.2)	13.3 (0.6)	0.085 (0.004)	6
<i>Halichoeres bivittatus</i>	P = G	0.09/1.02	WA	118.4 (2.0)	8.2 (0.2)	0.068 (0.002)	16
<i>Halichoeres chierchiaie</i>	P < G	?	EP	150.7 (3.4)	9.5 (0.4)	0.064 (0.003)	14
<i>Halichoeres dispilus</i>	P < G	?	EP	102.9 (2.2)	6.0 (0.3)	0.059 (0.003)	13
<i>Halichoeres garnoti</i>	P	0.13	WA	140.6 (2.7)	9.2 (0.5)	0.066 (0.003)	18
<i>Halichoeres maculipinna</i>	P > G	0.21/0.41	WA	98.9 (2.5)	6.1 (0.4)	0.062 (0.004)	6
<i>Halichoeres nicholsi</i>	P	?	EP	137.5 (0.5)	10.4 (0.5)	0.076 (0.004)	2
<i>Halichoeres notospilus</i>	P	?	EP	96.7 (1.5)	5.6 (0.1)	0.059 (0.001)	3
<i>Halichoeres pictus</i>	P	0.02	WA	125.0 (1.2)	8.6 (0.5)	0.073 (0.002)	4
<i>Halichoeres poeyi</i>	P = G	0.44/0.52	WA	130.7 (2.0)	9.6 (0.3)	0.076 (0.003)	15
<i>Novaculichthys taeniourus</i>	P	?	WP	94.0 (2.0)	6.7 (0.4)	0.072 (0.005)	7
<i>Thalassoma bifasciatum</i>	P = G	0.24/2.68	WA	79.6 (0.5)	5.1 (0.3)	0.064 (0.003)	31
<i>Thalassoma lucasanum</i>	P << G	0.97/3.75	EP	75.4 (1.3)	5.7 (0.3)	0.077 (0.004)	15
SCARIDAE							
<i>Sparisoma atomarium</i>	P	0.20/0.27	WA	107.7 (1.7)	6.5 (0.3)	0.061 (0.003)	6
<i>Sparisoma aurofrenatum</i>	P	0.11/0.67	WA	124.4 (3.0)	13.3 (1.7)	0.088 (0.008)	5
<i>Sparisoma radians</i>	P << G	0.76/3.35	WA	96.0 (2.8)	6.7 (0.5)	0.069 (0.005)	6
<i>Sparisoma rubripinne</i>	P < G	0.12/1.57	WA	130.0 (1.3)	11.2 (0.4)	0.089 (0.002)	5
SERRANIDAE							
<i>Serranos baldwini</i>	P > G	0.20/0.04	WA	157.5 (3.7)	6.6 (0.4)	0.042 (0.001)	15
<i>Serranos psittacinus</i> ¹	P > G	0.29/0.20	EP	186.5 (3.1)	9.7 (0.5)	0.052 (0.002)	14
<i>Serranos tabacarius</i>	P	0.19	WA	348.5 (6.7)	20.6 (0.8)	0.059 (0.003)	15
<i>Serranos tigrinus</i>	P >> G	0.08	WA	182.1 (3.7)	10.3 (0.4)	0.057 (0.002)	6
<i>Serranos tortugarum</i>	P < G	0.64	WA	224.9 (3.0)	14.4 (0.7)	0.064 (0.003)	15

in species that exclusively pair spawned than in species with the moderate to high levels of multimale spawning, and lower in TP than in IP males (Table 1).

Average egg volumes varied 1.6-fold among those species, and densities 1.3-fold. For the present analyses I divided the *Halichoeres* species into two groups: "pair-spawners", which have little or no multimale activity (*H. garnoti*, *H. maculipinna*, *H. nicholsi*, *H. notospilus*, and *H. pictus*), and "group-spawners", which have moderate to high levels of multimale spawning (*H. bivittatus*, *H. chierchiaie*, *H. dispilus*, and *H. poeyi* – Table 1). Eggs of Atlantic pair- and group-spawners do not differ in either volume or density (nested ANOVAs: volume – species effect $P < 0.001$, spawning mode effect $P < 0.455$; density – species effect $P = 0.312$, spawning mode effect $P = 0.367$). Among the Pacific species, eggs of pair-spawners had smaller volumes, but do not differ in density from eggs of group-spawners (nested ANOVAs: volume – species effect $P < 0.001$, spawning mode effect $P = 0.049$; density – species effect $P = 0.130$, spawning mode effect $P = 0.219$).

Thalassoma

T. bifasciatum and *T. lucasanum* both pair and group spawn. However, while both spawning modes are common in *T. bifasciatum*, almost all spawning by *T. lucasanum* is group spawning (Warner 1982). The testes of males of *T. lucasanum* are relatively larger than those of males of *T. bifasciatum* (Table 1).

Eggs of *T. bifasciatum* had a slightly (~6%) greater average volume and a moderately (~20%) lower average density than those of *T. lucasanum* (Table 1; *t*-tests, volume $P = 0.001$, density $P = 0.024$).

Sparisoma

Two species of *Sparisoma* almost exclusively pair spawn; their males have small testes. The other two species predominantly group spawn, and their males have large testes (Table 1). Two-way ANOVAs of variation in egg volume and density among pair- and group-spawners

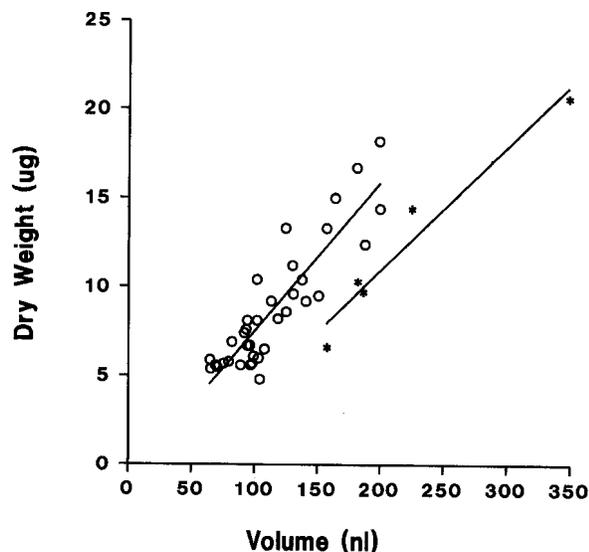


Fig. 1 Relation between mean volume and mean dry weight of spherical eggs of 36 species of labroid (labrid and scarid) fishes (circles), and five species of *Serranus* (stars). Lines are for least squares regressions. Labroids include those in Table 1, plus one scarid – *Cryptotomus roseus* (data point is mean weight and volume for 10 clutches), and 15 other labrids – *Doratonotus megalopsis* ($n = 9$), *Halichoeres chloropterus* ($n = 3$), *H. hortulanus* ($n = 2$), *H. miniatus* ($n = 4$), *H. marginatus* ($n = 3$), *H. margaritaceus* ($n = 5$), *H. melanurus* ($n = 6$), *H. nebulosus* ($n = 2$), *H. trimaculatus* ($n = 7$), *Hemigymnus melapterus* ($n = 6$), *Stethojulis bandanensis* ($n = 2$), *Thalassoma hardwicki* ($n = 7$), *T. janseni* ($n = 6$), and *T. lunare* ($n = 5$). *C. roseus* and *D. megalopsis* are from the West Atlantic, and the remainder from the Australian Great Barrier Reef. *Serranus* species are those listed in Table 2

that have slow and fast spawning movements showed the following patterns:

1. Egg volume. While variation in egg volume was unrelated to spawning mode, eggs of slow-spawners, *S. aurofrenatum* and *S. rubripinne*, were larger than those of fast-spawners, *S. atomarium* and *S. radians* (mode effect $P = 0.113$, speed effect $P < 0.001$). (A significant interaction, $P = 0.001$, occurred because the direction of the difference between egg volumes of pair- and group-spawners was reversed in the two speed classes.)

2. Egg density. While variation in egg density was unrelated to spawning mode, eggs of slow-spawners were more dense than eggs of fast-spawners (mode effect $P = 0.266$, speed effect $P < 0.001$, interaction $P = 0.518$).

Serranus

The five *Serranus* species include two pair-spawners, which have testes at the small end of the size range, two that have low levels of multimale spawning and intermediate sized testes, and one species with large testes in which multimale spawnings are the norm (Table 1). Eggs of the four pair-spawning species varied 2.2-fold in average volume, and 1.4-fold in average density (Table 1). The volume and density of eggs of the single Pacific species (*S. psittacinus*) was well within the range of those characteristics shown by the four Atlantic species (Table

Table 2A, B Average volumes and densities of eggs of pair and group spawning females of *Thalassoma bifasciatum*

A Data

Spawning Mode ^a	Collection method ^b	n	Egg Size Characteristics – Mean (SE)	
			Volume [nl]	Density [$\mu\text{g}/\text{nl}$]
Pair	Strip	16	79.3 (0.6)	0.062 (0.005)
	Net	15	82.2 (1.0)	0.065 (0.006)
Group	Net	15	80.0 (0.9)	0.066 (0.004)
	Strip	15	86.1 (1.4)	0.084 (0.013)

^a Spawning mode: pair spawn – 1 female with 1 territorial TP male; Group spawn – 1 female with ~5–10 nonterritorial IP males

^b Collection method: strip – eggs stripped from ripe females speared immediately before spawning; net – eggs collected with a plankton net immediately after spawning

B Probability values from two-way ANOVAs comparing egg volumes and densities (see data above)

	Volume	Density
Method	<0.001	0.414
Mode	0.026	0.173
Method \times mode	0.119	0.499

1). There was significant interspecific variation in average egg volume and density (Table 1; ANOVAs of volume and of density: both $P < 0.001$). Eggs of the only species that had substantial amounts of multimale spawning, *S. tortugarum*, had a larger average volume than those of three of four pair-spawning species (Tukey *post hoc* comparisons: $P < 0.001$ in each case), and a greater density than eggs of only one of four pair-spawning species (Tukey *post hoc* comparisons: vs. *S. baldwini* $P < 0.001$, vs. *S. psittacinus* $P = 0.090$, vs. *S. tabacarius* $P = 0.967$, vs. *S. tigrinus* $P = 0.626$).

Variation in egg density among labrids

For an analysis of overall patterns of variation within this family I considered only egg density, because there are indications of substantial phylogenetic effects on variation in egg volume – e.g., eggs of the three *Bodianus* species are large (average volume >188 nl in all species), while those of five *Thalassoma* species (two referred to in Table 1, plus three others referred to in Fig. 1) are small (average volume <80 nl in all five species). I divided the 16 species into pair-spawners (species of *Bodianus*, *Clepticus*, and *Novaculichthys*, plus the five *Halichoeres* species listed above), and group-spawners (the remaining four *Halichoeres* species, plus both *Thalassoma* species). Although there was significant interspecific variation there was no consistent pattern of difference in egg density between pair- and group-spawners (nested ANOVA: species effect $P < 0.001$, spawning mode effect $P = 0.344$). Eggs of species with relatively slow spawner movements during and immediately after gamete release (i.e., *Bodianus* species and *Clepticus parrae*) did not differ in density from eggs of species with more rapid

spawner movements (*Halichoeres*, *Sparisoma*, and *Thalassoma*) (nested ANOVA: species effect $P < 0.001$, speed effect $P = 0.093$).

Discussion

At present there is published evidence from only two of the study species (*Thalassoma bifasciatum* and *Halichoeres bivittatus*) that sperm limitation of fertilization success occurs under natural circumstances (Petersen 1991a; Petersen et al. 1992; Shapiro et al. 1994; Warner et al. 1995). Such sperm limitation is low level and occurs only in pair spawns. At least in *T. bifasciatum* sperm limitation is due to sperm rationing rather than sperm depletion. Sperm limitation evidently also occurs in some pair spawns of the labrid *Xyrichthys novacula* (Marconato et al. 1995), and unpublished data referred to in that paper indicate that patterns of sperm rationing and sperm output similar to those found in *T. bifasciatum* occur in pair and group spawns of *Sparisoma radians*. The basic mechanics of the spawning act are similar in all species studied here, and they spawn in modes equivalent to one or both of the main modes found in *T. bifasciatum*. In *T. bifasciatum* sperm competition leads to high levels of sperm output in group spawning, and, coincidentally, probably virtually eliminates the potential for sperm limitation in that spawning mode. Males of *T. bifasciatum* that normally group spawn have large testes, and large testis size and high levels of sperm output also are generally associated with high levels of multimale spawning among the other species studied here. Pair-spawning males of a range of those species spawn repeatedly during a spawning period, have small testes and likely have more limited sperm output than males that typically spawn in groups. Thus it seems reasonable to expect that pair-spawning species generally do have a potential for consistent, albeit low-level, sperm limitation due to sperm rationing that group-spawning species probably do not.

While the sperm limitation hypothesis is concerned primarily with egg size, the relationship between egg density and size has important implications for it. A decline in egg density with increasing egg volume would indicate that some factor in addition to offspring-provisioning is involved in determining egg size. Such a pattern would support the idea that sperm limitation is a major factor affecting egg size characteristics, because a reduction in density would lower the potential trade-off costs (reduced clutch size) to producing large eggs. While a lack of a decline in density with increasing egg size would not disprove the sperm limitation hypothesis, if such a decline does not occur then the sperm limitation hypothesis offers no better explanation for variation in egg size than the hypothesis that size reflects variation in the level of resource investment required for a particular pattern of larval development. I found no evidence that egg density declines with increasing egg volume among several dozen labroid fishes whose eggs vary ~3-fold in average volume. Although the sample of *Serranus* is small ($n = 5$) the simplest explanation for existing data

from that genus is that egg content is directly proportional to volume.

When developing the sperm limitation hypothesis Levitan (1993) relied on data from Strathmann and Vedder (1977), which indicated that egg density declines with increasing volume among four species of his main study genus (*Strongylocentrotus*). However, data obtained by Lawrence et al. (1984) on members of this genus while using a different method do not show such a pattern of variation in egg density. Emlet et al. (1987) concluded that there was no unequivocal evidence of a change in density with egg volume among planktotrophic echinoderm eggs. Using a larger data set, Jaekle (1995) concluded that content is directly proportional to volume in such eggs. Thus egg size/content relationships offer no clear support for the sperm limitation hypothesis in either the fishes considered here or the echinoderms for which the hypothesis was originally developed.

I found little evidence in support of three predictions of the sperm limitation hypothesis that I examined. In *T. bifasciatum*, eggs of group-spawning females are not smaller and denser than those of pair-spawning females. Eggs of species in which group spawning is the usual mode are smaller and more dense than those of pair-spawning congeners in only one of four cases – a minor (~5%) difference in egg volume and a moderate (~20%) difference in egg density between two species of *Thalassoma*. The only other differences detected (between the egg volumes of pair and group spawning females of *T. bifasciatum*, and of Pacific *Halichoeres* species, and of different *Serranus* species) were in the opposite direction to that predicted by the sperm limitation hypothesis. The difference between the egg volumes of the two *Thalassoma* species is so minor as to seem unlikely to affect fertilization kinetics. Although the difference in their egg densities is less minor, there are at least two reasons why it may simply have been a sampling artifact. First, that level of density difference is within the range of difference that occurs among pair-spawning species within each of three other genera (*Sparisoma*, *Serranus*, and *Halichoeres*), and among group spawning species of *Sparisoma* and of *Halichoeres*. Second, differences between the spawning patterns of the two *Thalassoma* species are no greater than those between species using different spawning modes in other genera studied here. Another analysis (Stockley et al., 1996) found no relationship between (stripped) ejaculate size (a crude indicator of the likelihood of sperm limitation due to variation in sperm release?) and egg size among either three pelagic-spawning fishes from two families, or six species of benthic spawners from three families.

Levitan and Petersen (1995) suggested that, in fishes such as those studied here, reduced fertilization success due to sperm limitation might arise from turbulent dispersal of gamete clouds by movements of the spawning fish during and immediately after gamete release. However, the resultant pattern of variation in egg density predicted from this, higher density eggs in slower-spawning species, does not occur in either of the two cases examined.

This study failed to find any convincing, consistent evidence that variation in egg size and density among these fishes is related to variation in the potential occurrence of sperm limitation. Possible reasons for this result include the following:

1. Above some threshold egg size may have no effect on fertilization success in free-spawning marine species in general.
2. Egg size may have such an effect in some taxa but not others, perhaps because a variety of mechanisms may be used to enhance fertilization success by increasing egg-sperm encounter rates (e.g., diffusible egg components that attract sperm – Rossignol and Lennart 1993).
3. Differences in the mechanics of fertilization between invertebrates and fishes may mean that the target-size effect operates only in invertebrates.
4. Sperm limitation may be an important selective factor acting on egg size in invertebrates but not fishes.
5. Egg size may have an effect on fertilization success in fishes but physiological constraints may prevent females from varying the density of their eggs.

Mechanics of fertilization

While sea urchin sperm can penetrate an egg at any point on its surface, in almost all fishes sperm can enter the egg membrane only through the ~5–10 µm diameter micropyle, which represents <<1% of the egg's surface area (e.g., Amanze and Iyengar 1990; Riehl and Kokoscha 1993). Hence an entire egg might not represent a target for fish sperm in the way it does for sea urchin sperm (C.W. Petersen, personal communication). However, because the micropyle represents such a tiny fraction of the surface of an egg, it seems reasonable to expect that fish eggs would be equipped with biochemical and/or structural surface features that facilitate fertilization by guiding sperm to the micropyle, and that a fish sperm that contacts an egg would behave in ways that enhance its chance of encountering the micropyle. This evidently is the case for at least one free-spawning teleost (Amanze and Iyengar 1990). If fish eggs and sperm generally have such characteristics then there is no obvious reason why the entire surface of those eggs should not act as a target (broad sense) and why variation in egg size in fishes should not affect fertilization success in the same way as proposed for sea urchins and other marine invertebrates. Sperm of *T. bifasciatum* are viable for only ~15 s (Petersen et al. 1992). Sperm guides and micropyle-seeking behavior by sperm might be particularly important to fertilization success when sperm are so short lived.

Relative importance of sperm limitation in fishes and invertebrates

Field observations on several echinoderms have shown that there is marked variation in natural fertilization rates consistent with effects of the density and dispersion of

spawners on gamete dilution (see Levitan 1995 for summary). Most existing studies of fertilization rates in this group are based on laboratory and/or field manipulations of “captive” eggs or adults (see Petersen et al. 1992; Levitan 1993, 1995; Levitan and Petersen 1995 for reviews). They show that under calm-water conditions, with males and females in very close proximity, fertilization rates average 80–90%. Those rates drop off rapidly to very low levels with increasing dispersion of mates, decreasing population density, and increasing current velocities.

The best available field data on free-spawning fishes, in two labrids, indicate that average natural fertilization rates exceed 90% (Shapiro et al. 1994; Marconato et al. 1995; Warner et al. 1995). While fertilization rates are higher in multimale spawnings than in pair spawnings of *T. bifasciatum*, those differences are minor: ~2–5% (Shapiro et al. 1994; C.W. Petersen and R.R. Warner, personal communications). The results presented here show that the small size of that difference is not due to compensatory increases in egg size that pair-spawning females make in response to sperm limitation arising from sperm rationing by TP males. These data indicate that, assuming egg size has any effect on fertilization success, then sperm limitation is a relatively trivial problem for this species, even under the most extreme conditions of sperm rationing, and support the idea (Shapiro et al. 1994; Warner et al. 1995) that TP males are finely tuning their sperm output to achieve near-maximal fertilization rates at minimal cost. The suggestion of Petersen (1991a) that sperm limitation might arise because it is more advantageous to allocate energy to obtaining extra mates than to ensure 100% fertilization success of each spawning has been supported by experimental data from *T. bifasciatum* (Warner et al. 1995).

In *T. bifasciatum*, and probably many other species of fishes, the effects of the coordinated actions of the spawners during gamete release overwhelms not only effects of large variation in levels of sperm release due to sperm rationing on the one hand and sperm competition on the other, but also possible effects of turbulent dispersal of gamete clouds by actions of spawners, and may largely eliminate the probable major source of variability in fertilization rates for many invertebrates – degree of separation of mates.

The possibility that seasonally inclement weather might produce significant sperm limitation in tropical reef fishes through its effects on turbulent diffusion of gamete clouds remains an open question. Such environmentally induced sperm limitation might also represent a significant problem for fishes that spawn in environments with consistently strong water currents and high turbulence, e.g., in exposed intertidal situations (cf. Denny and Shibata 1989) and in fast-moving rivers. Studies by Fischer (1981, 1987) and Nakatsura and Kramer (1982) on a serranid and characin, respectively, found that fertilization rates were not infrequently <80% under calm (aquarium) conditions. Any number of factors could have contributed to those diminished fertilization rates – sperm limitation, egg infertility, sperm inviability,

stress and aquarium conditions. Sperm depletion was involved in declines in fertilization success in successive spawnings of the characin, but not in the serranid. Adequate field studies have been performed on so few free-spawning fishes that it would be highly premature to conclude that sperm limitation cannot be a significant problem for them.

Constraints on variation in egg density

There is considerable interspecific, and, more importantly, intraspecific variation in egg density in the fishes considered here. Egg density also is known to vary seasonally within species of fishes (e.g., Kjesbu et al. 1992). This information indicates that the relationship between egg volume and content is not rigidly constrained, by, for example, effects on the fertilization process or early development, and that fishes have some ability to control variation in egg density. Other potential constraints to substantially increasing egg size include the availability of space in the female's body cavity (space limitation). The ability of the sperm limitation hypothesis to explain variation in egg density depends heavily on the assumption that egg production is limited by energy rather than space in the female. Benefits of increased fertilization success arising from production of larger eggs could be offset by the cost of reduced clutch size if the female is space-limited. In *T. bifasciatum* space limitation seems unlikely to be a serious, chronic constraint. In San Blas a female's clutch, on average, is only one-third the size of the largest clutch produced by females of her length ($n = 2579$ females, 90 spawning days; D.R. Robertson and S. Swearer, unpublished data). My calculations based on the data of Warner (1995) from St. Croix indicate that average clutch size of *T. bifasciatum* there is ~43% of the size-specific maximum ($n = 480$ females, 6 spawning days). In the small sample of females collected by Shapiro et al. (1994) (51 fish, unspecified number of days) from Puerto Rico, average clutch size was ~65% of the size-specific maximum. However, the Puerto Rico sample could simply have been taken on 1 or 2 good days. At San Blas average output per female fluctuates considerably over periods of days, and can change by as much as 35% of the size-specific maximum from one day to the next. Hence long time series of data would be needed from other sites to determine whether there is substantial geographic variation in average fecundity, and the potential for space limitation.

If space limitation does represent a serious potential constraint on clutch size its effects could be reduced by the production of smaller clutches at more frequent intervals. Because females of *T. bifasciatum* are capable of spawning on consecutive days, and often do so (e.g., Schultz and Warner 1991), they should have the capacity to reduce effects of space-limitation by spawning every day. While a female of *T. bifasciatum* does not spawn more than once a day (Warner et al. 1975), some *Serranus* species regularly parcel their clutches in multiple spawning acts over a day's spawning period (Petersen

1995, and references cited therein). At least one of those serranids ovulates and/or hydrates eggs throughout the course of a 3-h daily spawning period (Fischer and Hardison 1987). Such a pattern of egg production could allow a fish to release more eggs during one spawning period than it might have space to hold for release in a single spawn.

If, compensatory increases in egg size in response to sperm limitation are not responsible for interspecific variation in egg size among these fishes what factors might be? Levitan (1993) maintained that, because larvae of the congeneric species of sea urchins that he studied develop together at the same time in the same larval habitat, it is unlikely that variation in egg content would be related to interspecific differences in the ecology of young larvae. For fishes such as those considered here there is considerable evidence of variation in larval life histories across a range of taxonomic levels (e.g., Lasker 1981; Victor 1986; Leis 1991). Interspecific variation in egg content among the fishes considered here seems likely to reflect differences in zygote provisioning related to variation in the ecological capabilities of newly hatched larvae, or their developmental trajectories.

Acknowledgements This research was supported by general research funds from the Smithsonian Tropical Research Institute (STRI), and by a grant from the National Geographic Society (No. 4619-91). Work at Lizard Island was supported by a grant to J.H. Choat, of James Cook University (ARC-SIA 9132734). Research in Panama was permitted by the Departamento de Recursos Marinos de la Republica de Panama, and by the Kuna General Congress. Assistance in the field and laboratory was provided by A. Cedeño, E. Peña, S. Swearer, L. Gutierrez, E. Paredes, and C. Guerra. C.W. Petersen generously allowed me to use his unpublished data on the gonadosomatic indices of *Serranus* species. J. Christy, K. Clifton, H. Lessios, D. Levitan, C.W. Petersen and R.R. Warner provided useful criticism of drafts of the paper.

References

- Allen GR, Robertson DR (1994) Fishes of the tropical Eastern Pacific. Crawford House, Bathurst
- Amanze D, Iyengar A (1990) The micropyle: a sperm guidance system in teleost fertilization. *Development* 109: 495-500
- Bagenal TB (1971) The interrelation of size of fish eggs, the date of spawning and the production cycle. *J Fish Biol* 3: 207-219
- Choat JH, Robertson DR (1975) Protogynous hermaphroditism in fishes of the family Scaridae. In: Reinboth R (ed) *Intersexuality in the animal kingdom*. Springer, Heidelberg Berlin New York, pp 263-283
- Craik JCA, Harvey SM (1987) The causes of buoyancy in eggs of marine teleosts. *J Mar Biol Ass UK* 67: 169-182
- Denny MW, Shibata MF (1989) Consequences of surf-zone turbulence for settlement and external fertilization. *Am Nat* 134: 859-889
- Duarte CM, Alcaraz M (1989) To produce many small or few large eggs: a size-independent tactic of fish. *Oecologia* 80: 401-404
- Emler RB, McEdward LR, Strathmann RR (1987) Echinoderm larval ecology viewed from the egg. In: Jangoux M, Lawrence JM (eds) *Echinoderm studies*, Balkema, Rotterdam, vol 2, pp 55-136
- Elgar MA (1990) Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* 59: 283-287
- Fischer EA (1981) Sex allocation in a simultaneously hermaphroditic coral reef fish. *Am Nat* 117: 64-82

- Fischer EA (1987) Mating behavior in the black hamlet – gamete trading or egg trading? *Environ Biol Fish* 18: 143–148
- Fischer EA, Hardison PD (1987) The timing of spawning and egg production as constraints on male mating success in a simultaneously hermaphroditic fish. *Environ Biol Fish* 20: 301–310
- Hoffman SG (1980) Sex-related social, mating, and foraging behavior in some sequentially hermaphroditic reef fishes. PhD dissertation, University of California, Santa Barbara
- Jaeckle WB (1995) Variation in the size, energy content, and biochemical composition of invertebrate eggs: correlates to the mode of larval development. In: McEdward L (ed) *Ecology of marine invertebrate larvae*. CRC, Boca Raton, pp 49–77
- Kjesbu O, Kryvit H, Sundby S, Solemddal P (1992) Buoyancy variations in eggs of Atlantic cod (*Gadus morhua*) in relation to chorion thickness and egg size: theory and observations. *J Fish Biol* 41: 581–599
- Lasker R (1981) Marine fish larvae: morphology, ecology, and relation to fisheries. Washington Sea Grant Program, Seattle
- Lawrence JM, McClintock JB, Guille A (1984) Organic level and caloric content of eggs of brooding asteroids and an echinoid (Echinodermata) from Kerguelen (South Indian Ocean). *Int J Invert Reprod Dev* 7: 249–257
- Le Clus F (1979) Dry mass of yolked oocytes of the south-west African pichard, *Sardinops ocellata*, in relation to maturity stages and spawning cycles, 1972–1974. *S Afr Dept Fish Invest Rept* 119: 1–29
- Leis JM (1991) The pelagic stages of reef fishes: the larval biology of coral reef fishes. In: Sale PF (ed) *The ecology of fishes on coral reefs*. Academic Press, New York, pp 183–230
- Levitan DR (1991) The influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate. *Biol Bull* 181: 261–268
- Levitan DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. *Am Nat* 141: 517–536
- Levitan DR (1995) The ecology of fertilization in free-spawning invertebrates. In: McEdward L (ed) *Ecology of marine invertebrate larvae*. CRC, Boca Raton, pp 123–156
- Levitan DR, Petersen CW (1995) Sperm limitation in the sea. *Trends Ecol Evol* 10: 228–231
- Levitan DR, Sewell MA, Chia FS (1992) How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. *Ecology* 73: 248–254
- Marconato A, Tessari V, Marin G (1995) The mating system of *Xyrichthys novacula*: sperm economy and fertilization success. *J Fish Biol* 47: 292–301
- Nakatsura K, Kramer DL (1982) Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces, Characidae). *Science* 216: 753–755
- Petersen CW (1991a) Variation in fertilization rates in the tropical reef fish, *Halichoeres bivittatus*: correlates and implications. *Biol Bull* 181: 232–237
- Petersen CW (1991b) Sex allocation in hermaphroditic sea basses. *Am Nat* 138: 650–667
- Petersen CH (1995) Reproductive behavior, egg trading, and correlates of male mating success in the simultaneous hermaphrodite, *Serranus tabacarius*. *Environ Biol Fish* 43: 351–361
- Petersen CW, Warner RR, Cohen S, Hess HC, Hewell AT (1992) Variable pelagic fertilization success: implications for mate choice and spatial patterns of mating. *Ecology* 73: 391–401
- Riehl R, Kokoscha M (1993) A unique surface pattern and micropylar apparatus in the eggs of *Lucocephalus* sp. (Perciformes, Luciocephalidae). *J Fish Biol* 43: 617–620
- Robertson DR, Choat JH (1974) Protogynous hermaphroditism and social systems in labrid fishes. In: Cameron AM, Campbell BM, Cribb AB, Endean R, Jell JS, Jones OA, Mather P, Talbot FH (eds) *Proceedings of the Second International Symposium on Coral Reefs, Great Barrier Reef Committee, Brisbane, Vol 1*, pp 217–225
- Robertson DR, Hoffman SG (1977) The roles of female mate choice and predation in the mating systems of some tropical labroid fishes. *Z Tierpsychol* 45: 298–320
- Robertson DR, Warner RR (1978) Sexual patterns in the labroid fishes of the western Caribbean. II. The Parrotfishes (Scaridae). *Smithsonian Contrib Zool* 255: 1–26
- Roff DA (1992) *The evolution of life histories*. Chapman and Hall, New York
- Rossignol DR, Lennarz WJ (1983) The molecular basis of sperm-egg interaction in the sea urchin. *CIBA Found Symp* 98: 268–296
- Sargent RC, Gross MR (1987) Parental care and the evolution of egg size in fishes. *Am Nat* 129: 32–46
- Schultz ET, Warner RR (1991) Phenotypic plasticity in life-history traits of female *Thalassoma bifasciatum* (Pisces: Labridae). 2. Correlation of fecundity and growth rate in comparative studies. *Environ Biol Fish* 30:333–344
- Shapiro DY, Marconato A, Yoshikawa T (1994) Sperm economy in a coral reef fish, *Thalassoma bifasciatum*. *Ecology* 75: 1334–1344
- Sokal RR, Rohlf RJ (1981) *Biometry: the principles and practice of statistics in biological research*, 2nd edn. Freeman, New York
- Stearns SC (1992) *The evolution of life histories*. Oxford University Press, New York
- Stockley P, Gage MJG, Parker GA, Moller AP (1996) Female reproductive biology and the coevolution of ejaculate characteristics in fish. *Proc Roy Soc Lond B* 263: 451–458
- Strathmann RR, Vedder K (1977) Size and organic content of eggs of echinoderms and other invertebrates as related to developmental strategies and egg eating. *Mar Biol* 39: 305–309
- Thresher RE (1984) *Reproduction in reef fishes*. TFH, Neptune City
- Thresher RE (1988) Latitudinal variation in egg sizes of tropical and sub-tropical North Atlantic shore fishes. *Environ Biol Fish* 21: 17–25
- Vance RR (1973) On reproductive strategies in marine benthic invertebrates. *Am Nat* 107: 339–352
- Victor BC (1986) Duration of the planktonic larval stage of one hundred species of Pacific and Atlantic wrasses (family Labridae). *Mar Biol* 90: 317–326
- Ware DM (1975) Relation between egg size, growth and natural mortality of larval fish. *J Fish Res Bd Can* 32: 2503–2512
- Warner RR (1982) Mating systems, sex change and sexual demography in the rainbow wrasse, *Thalassoma lucasanum*. *Copeia* 1982: 653–661
- Warner RR (1985) Alternative mating behaviors in a coral reef fish: life-history analysis. In: Delesalle B, Galzin R, Salvat B (eds) *Proceedings of the fifth International Coral Reef Congress, Antenne Museum – EPHE, Moorea, vol 4*, pp 145–150
- Warner RR (1987) Female choice of sites versus mates in a coral reef fish, *Thalassoma bifasciatum*. *Anim Behav* 35: 1470–1478
- Warner RR (1995) Large mating aggregations and daily long-distance spawning migrations in the bluehead wrasse, *Thalassoma bifasciatum*. *Environ Biol Fish* 44: 337–345
- Warner RR, Hoffman SG (1980) Local population size as a determinant of mating system and sexual composition in two tropical marine fishes (*Thalassoma* spp.). *Evolution* 34: 508–518
- Warner RR, Robertson DR (1978) Sexual patterns in the labroid fishes of the western Caribbean. I. The wrasses (Labridae). *Smithsonian Contrib Zool* 254: 1–27
- Warner RR, Robertson DR, Leigh EG (1975) Sex change and sexual selection. *Science* 190: 633–638
- Warner RR, Shapiro DY, Marconato A, Petersen CW (1995). Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females. *Proc Roy Soc Lond B* 262: 135–139
- Wilkinson L (1990) *SYSTAT: the system for statistics*. Systat, Evanston
- Wootton RJ (1994) Life histories as sampling devices: optimum egg size in pelagic fishes. *J Fish Biol* 45: 1067–1077