



Spawning and Maturation of Aquaculture Species



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LABORATORY SPAWNING OF CORAL REEF FISHES: EFFECTS OF TEMPERATURE AND PHOTOPERIOD

G. Joan Holt*

The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive,
Port Aransas, Texas 78373-5015 USA

*Tel: (361) 749-6749; Fax: (361) 749-6749; e-mail: joan@utmsi.utexas.edu

and

Cecilia M. Riley

Gulf Coast Bird Observatory, 103 West Highway 332

Lake Jackson, Texas 77566 USA

ABSTRACT

The culture of marine tropical fish would help safeguard coral reefs and develop a new source of organisms for the aquarium trade. Although many of the freshwater tropical species sold to the public are now cultured, more than 90% of all ornamental marine organisms are still collected from the wild. Increasing pressures on natural populations of coral reef animals due to their expanding popularity in the aquarium trade has stimulated interest in the culture of marine tropical fish. Over the past few years, we have conducted experimental studies with several species of coral reef fishes including pygmy angelfish *Centropyge argi*, spotfin hogfish *Bodianus puchellus*, bluehead wrasse *Thalassoma bifasciatum*, and clown wrasse *Halichoeres maculipinna*. All are protogynous hermaphrodites, have sexual size dimorphism, and some show sexual dichromatism. Spawning was induced by manipulating temperature and photoperiod. Environmental conditions of at least 22 C, 11 h light (13 h dark) and 15 min dawn and dusk induced regular spawning in paired adult *C. argi*, *T. bifasciatum*, and *B. puchellus*. *H. maculipinna* spawned primarily during simulated summer conditions. All species produced small, spherical, pelagic eggs containing a single oil globule that hatched within 18-26 h. Fecundity was strongly affected by day-length and temperature in all species, and by length of time in captivity. Newly-hatched larvae were small with rudimentary development. Though offered a wide variety of food beginning on d 3 after hatching, no larvae survived to metamorphosis.

INTRODUCTION

The culture of marine tropical fish conserves natural reef resources by offering alternatives to wild capture and develops a new source of organisms for the aquarium trade. The aquarium hobby draws 10-20 million enthusiasts who keep more than 90 million tropical fish (Andrews 1990). Two-thirds of the aquarium hobbyists worldwide live in the United States. With this popularity, it is not surprising that there is concern for the impact of the aquarium hobby on natural populations of marine organisms and their habitats. Although many of the freshwater tropical species sold to the public are now cultured, more than 90% of all marine ornamental organisms continue to be collected from the wild.

Increasing pressures on natural populations of coral reef animals due to their expanding popularity in the aquarium trade has

stimulated interest in developing culture techniques for marine tropicals. Controlled spawning through temperature and photoperiod manipulations to simulate seasonal changes and bring about gonadal maturation has been successful with several temperate species (Arnold et al. 1976; Arnold 1978). Slight changes in day-length in the tropics may act as seasonal cues for tropical organisms (Wolda 1989). Coral reefs experience seasonal changes in day-length of 0.5-2.0 h and temperature differences of 10-12 C at their northern and southern limits (Rezak et al. 1985). Lobe1 (1978) reported that *Centropyge potteri* reproduction was influenced by both seasonal and lunar changes. Likewise, many Caribbean reef fishes in Jamaica show a high level of spawning activity between January and May (Munro et al. 1973) and seasonal spawning peaks have been reported for other tropical marine teleosts (Johannes 1978; Foster 1987). Thus, egg

production in tropical reef fishes may be tied to seasonal changes.

This study was initiated to determine if coral reef fishes could be induced to spawn in captivity using photoperiod and temperature cues. Over the past few years we have conducted experimental studies with several species of coral reef fishes including pygmy angelfish *Centropyge argi*, spotfin hogfish *Bodianus puchellus*, bluehead wrasse *Thalassoma bifasciatum*, and clown wrasse *Halichoeres maculipinna*. All are protogynous hermaphrodites, have sexual size dimorphism, and the clown wrasse and bluehead wrasse show sexual dichromatism. There are scattered reports of these popular ornamental fish species spawning in captivity but not in any controlled or predictable manner.

Pygmy angelfish are pair spawners with relatively small territories (Moyer et al. 1983). Several species of *Centropygi* have spawned in captivity (Bauer and Bauer 1981; Hioki and Suzuki 1987; Karanikas 1989; Hioki et al. 1990). The spotfin hogfish spawns in harems or in pairs and both sexes are similar (Thresher 1984). The dichromatic bluehead wrasse and clown wrasse spawn in harems or large multi-male groups or occasionally in pairs (Warner and Hoffman 1980; Thresher 1984). There are no reports of aquarium spawning in the spotfin hogfish or clown wrasse but two *Thalassoma* spp. have spawned in captivity (Thresher 1984).

The overall goal of this research was to develop a protocol for predictable spawning of coral reef fishes. This paper reports spawning and fecundity results and data on egg size and hatch rates for the four species of Caribbean reef fishes.

MATERIALS AND METHODS

Tropical reef fish were hand-collected from the Florida Keys or from reefs near Veracruz, Mexico. Paired adults were maintained in separate 300- or 900-L, fiberglass tanks and fed twice daily with commercial flake food and raw shrimp or fish. Rocks covered with algae and invertebrates (live rock) were stacked in the tanks to simulate the reef habitat (Fig. 1A). Effluent water from spawning tanks was airlifted into an external filter box fitted with four vertical plates covered with

polyester filter material as substrate for nitrifying bacteria (Fig. 1B). Crushed oyster shell was added to buffer pH. Water returned from the filter box at a rate of 2.1 L/min resulting in a total tank water exchange every 2.4 h. Using light timers and heat pumps, photoperiod and water temperature were manipulated to simulate seasonal changes. Fish were subjected to environmental manipulations that ranged from 10 h light and 17-20 C for the simulated winter season to 13 h light and 25-30 C for summer. Spring and fall conditions were 11 h light and 21-24 C. Spawning tanks were equipped with both overhead and underwater lights (Fig. 1A) on separate timers to produce a 0.25 h dawn and a 0.25 h dusk. Salinity was maintained at 32 to 36 ppt, pH=8.2, NO₂ and NH₃ <0.03 ppm

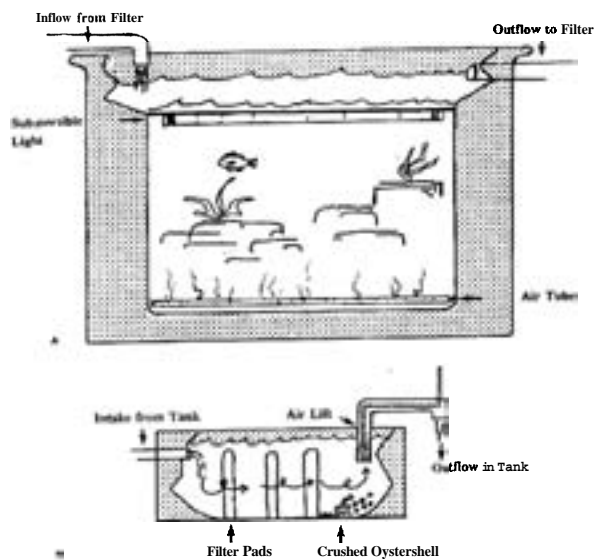


Figure 1. A) 300-L (60 x 90 x 60 h cm) fiberglass spawning tank with live rock, internal underwater light, and surface level water outflow for filtration. B) External biological filter box of fiberglass resin coated plywood fitted with vertical filter plates.

Eggs were collected in 150- μ m mesh bags (10 x 20 cm) attached to the intake pipe inside the filter box. A subsample of eggs was measured and the remaining embryos were placed in 5 μ m filtered sea water in small (1-6 L) glass aquaria, at 25 C, pH = 8.2, salinity = 32-36 ppt (to match the spawning salinity). Only floating eggs were considered fertilized and percent hatching was calculated as the number of viable larvae on the morning after hatching divided by the number of fertilized eggs.

During the study three pairs of a pygmy angelfish and bluehead wrasse and two pairs each of spotfin hogfish and clown wrasse were exposed to laboratory controlled annual cycles. The mean sizes of the captive fish are given in Table 1. Fecundity was examined for individual females exposed to one or more annual cycles. Fecundity was measured as the total number of eggs produced per fish, regardless of fertility.

RESULTS

During their first 6 mo in captivity, adult fish were subjected to environmental changes that mimic typical seasonal changes in northern tropical latitudes (Fig.2). After an abbreviated summer, fall, and winter, spawning began when conditions were changed to spring. Spring conditions of 22 C, 11 h light, 13 h dark, and 0.25

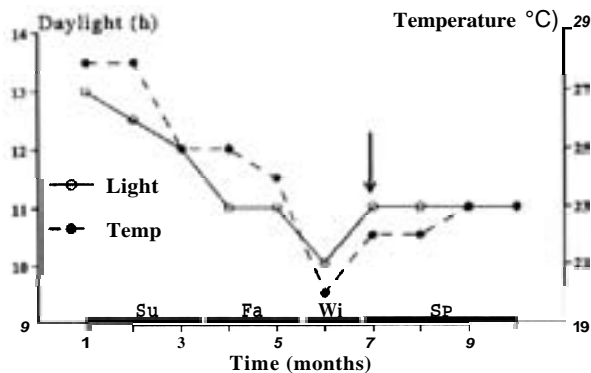


Figure 2. Photoperiod and temperature regime used to induce laboratory spawning of adult *Centropyge argi*, *Thalassoma bifasciatum*, *Halichoeres maculipinna* and *Bodianus puchellus*. Adults collected in summer were subjected to an abbreviated annual seasonal cycle in the laboratory. Lengths of each simulated season are represented by bars at the bottom of the graph, where Su=summer, Fa=fall, etc. Arrow indicates date when spawning began.

h dawn and dusk, induced continuous daily spawning in pygmy angelfish, bluehead wrasses, and spotfin hogfish. Clown wrasses began to spawn during simulated summer conditions (25 C; 13 h light). All species produced small spherical, pelagic eggs containing a single oil globule that hatched within 18-26 h.

Pygmy angelfish spawned 15-35 min before total darkness after an elaborate courtship lasting 30-45 min. Duration of the actual spawning event was less than 1 min. The large rocks and rubble in the tank were used during courtship. During the final spawning burst, the male pushed the female toward the surface into the open water and spawning occurred approximately 50 cm above the tank floor. Pygmy angelfish spawned continuously over a broad range of day-length and temperature conditions (10-13 h light and 21-27 C). Daily fecundity was strongly influenced by photoperiod and temperature with highest fecundity occurring at 11-13 h light and 24-25 C and lowest fecundity at 10 h light and 21 C. Spawning ceased when water temperature was increased to 28 C or decreased below 20 C. The number of eggs produced averaged 100 eggs/female (N=478) (Table 2) with a mean percent fertilization of 87% (Table 3). The small spherical eggs (0.73 mm in diameter) contained a single oil globule and eggs hatched after approximately 18 h at 24 C (Table 3).

Bluehead wrasses spawned at midday following a courtship during which the male displayed by swimming up and down in the water column (looping). The final spawning event involved the pair rushing up toward the surface and releasing a gamete cloud. Daily egg production by individual female bluehead wrasse varied from 3-7221 and was influenced by

Table 1. Sizes (mm SL) of males and females of four species of marine tropical fish spawned in captivity at UTMSI under temperature photoperiod regulation.

	Males		Females	
	Mean	Range	Mean	Range
Pygmy angelfish (<i>Centropyge argi</i>)	5.43	4.8-6.0	4.06	3.6-4.6
Bluehead wrasse (<i>Thalassoma bifasciatum</i>)	12.04	10.5-14.0	6.87	5.4-9.2
Clown wrasse (<i>Halichoeres maculipinna</i>)	11.10	7.8-14.4	7.25	7.4-8.0
Spotfin hogfish (<i>Bodianus puchellus</i>)	13.00	10.0-16.0	8.42	6.8-10.0

Table 2. Summary of egg production data for four species of marine tropical fish spawned in captivity at UTMSI under temperature photoperiod regulation. N= the number of spawns, Mean = mean daily number of eggs produced by an individual female when spawning occurred, Max= maximum number of eggs produced in any one day, Temp/light= temperature (°C) and day length of maximum egg production, Range= range of temperatures (°C) for good egg production.

	N	Mean eggs	Max eggs	Temp/light optimum	Range
Pygmy angelfish (<i>Centropyge argi</i>)	478	100	648	24.5/11h	23-25.5
Bluehead wrasse (<i>Thalassoma bifasciatum</i>)	529	425	7221	27.0/13h	27-30
Clown wrasse (<i>Halichoeres maculipinna</i>)	114	144	662	27.5/13h	24-29
Spotfin hogfish (<i>Bodianus puchellus</i>)	494	366	1980	25.5/11h	23.5-27.5

Table 3. Egg size (mm), percent fertilization, percent hatching, and longest larval survival for four species of marine tropical fish spawned in captivity at UTMSI under temperature photoperiod regulation.

	Egg Size	Fertilization	Hatch	Larval Survival
Pygmy angelfish (<i>Centropyge argi</i>)	0.73	87%	97%	7 days
Bluehead wrasse (<i>Thalassoma bifasciatum</i>)	0.56	61%	88%	7 days
Clown wrasse (<i>Halichoeres maculipinna</i>)	0.59	78%	78%	5 days
Spotfin hogfish (<i>Bodianus puchellus</i>)	0.85	56%	98%	21 days

photoperiod, temperature and fish size. A small female (5.6 cm) averaged 257 eggspawn during the first spawning cycle but during a second spawning period when the female had grown to 6.5 cm SL, she produced 504 eggspawn. Spawning occurred between 10-13 h light and 21-30 C, but fecundity increased with rising temperature and was highest at 25-29 C, and lowest at 10 h light. Spawning ceased when temperatures reached 20 C or lower. The 0.56 mm diameter eggs hatched after incubating 26 h.

Clown wrasses spawned under summer conditions in the early afternoon after a simple courtship display by the brightly colored male. The male and the female rushed upwards toward the surface in the final spawning ascent. Highest fecundity (Table 2) occurred at 13 h light and 27.5 C. There was no spawning below 22 C or at 11 h light. Percent fertilization of the 0.59 mm eggs was 78% (Table 3).

The spotfin hogfish spawned in the late afternoon (1630- 1730). Courtship consisted of the larger of the pair (presumably the male) displaying, with dorsal and anal fins erect, in front of the smaller fish by bobbing up and down horizontally. The final spawning rush by the pair

was very short in duration. Maximum fecundity occurred at 25.5 C and 11 h light. In one pair, an individual female spawned every day for 1 mo averaging 703.1 eggspawn with a range of 25 to 1691 eggspawn. This was followed by a week of little or no spawning and then another month of spawning at a reduced rate (average of 423.6 eggspawn). During the first year of spawning, when conditions were favorable, this female spawned continuously missing only a week or so at any one time. The eggs were relatively large (Table 3) and hatched in 18-24 h.

DISCUSSION

Courtship of pygmy angelfishes in this study was identical to that described by Bauer and Bauer (1981) for six species of *Centropyge* based on reef and tank observations and by Moyer et al. (1983) for pygmy angelfish in the Netherlands Antilles. Bauer and Bauer (1981) reported spawning every month of the year averaging 119 eggs/female with a percent fertility of 80%, similar to our data of 100 eggspawn with 87% fertilized, but we found spawning to be limited by temperature extremes.

There are no previous reports of the captive spawning behavior of bluehead wrasses but spawning observed in this study is similar to that reported for natural pair spawning (Feddern 1965; Reinboth 1973; Thresher 1984). An increase in fecundity but not in frequency of spawning with increased size of the female (Feddern 1965; Schultz and Warner 1991) was observed in our captive fish. Our mean fecundity data was lower than that reported by Schultz and Warner (1991) for natural populations of bluehead wrasses but the maximum fecundity we recorded (1350 eggs for 45-50 mm and 7221 eggs for > 60 mm SL female) was greater. We were unable to find any published reports of captive spawning for spotfin hogfish, or clown wrasses, but in our laboratory each of these species spawn during specific daylight hours as suggested in Thresher (1984).

Spawning of these tropical fish species conform to the "continuous spawning strategy" proposed by Bauer and Bauer (1981) in that fish generally spawn regularly every day when temperature and day-length are constant at appropriate levels. Our laboratory data suggest a variant of this strategy may occur in subtropical latitudes where temperature and day-length are reduced during winter, causing cessation or reduction of spawning during short-day and low-temperature periods. Extremely high temperatures (29 and 30 C) resulted in reduced fecundity in the pygmy angelfish.

Temperature and photoperiod have not typically been described as important determinants of fecundity in tropical fish. Moyer and Nakazono (1978) found that in the more temperate waters of Japan, *C. interruptus* spawned from May to October when temperatures increased to 21 or 22 C, while Munro et al. (1973) established that in Jamaica many shallow reef species spawn during the season when water temperatures are minimal (below 28 C). As we found in this study, water temperatures between 22 and 28 C appear to be optimal for spawning in many coral reef fishes.

Conditions for controlled spawning of several marine tropical fishes have been identified. These species, as well as most coral reef fishes, produce tiny eggs and larvae that require special rearing conditions. The small planktonic larvae

develop quickly and need food at 36-48 h after hatching. The development of an adequate rearing protocol is at present a major problem in raising these and many other reef fishes in captivity. To help resolve this problem the diets of wild planktonic larvae captured in light traps over coral reefs were examined (Riley and Holt 1993). Micro-zooplankton, found to be important larval prey, are under culture in the lab and tank design and water quality parameters are being evaluated to identify optimal growing conditions.

The long-term goal of this research is to develop aquaculture techniques for raising reef fish in captivity to increase our understanding of their ecological requirements, to preserve rare and endangered species, and to reduce harvesting pressure on natural populations.

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LITERATURE CITED

- Andrews, C. 1990. The ornamental fish trade and fish conservation. *J. Fish Biol.* 37: 53-59.
- Arnold, C.R. 1978. Maturation and spawning of marine finfish, pp. 25-27. *In:* Carl J. Sindermann (ed.), Proceedings of the seventh U.S.-Japan meeting on aquaculture, marine finfish culture, Tokyo, Japan, Oct. 3-4, 1978. NOAA Tech. Rep. NMFS 10.
- Arnold, C.R., J.L. Lasswell, W.H. Bailey, T.D. Williams and W.A. Fable, Jr. 1976. Methods and techniques for spawning and rearing spotted seatrout in the laboratory. 30th Annual Conference of the Southeastern Association of Game and Fish Commissioners 13: 167-178.
- Bauer, J.A. and S.E. Bauer. 1981. Reproductive biology of pygmy angelfishes of the genus *Centropyge* (Pomacanthidae). *Bull. Mar. Sci.* 31: 495-513.

- Feddern, H.A. 1965. The spawning, growth, and general behavior of the bluehead wrasse, *Thalassoma bifasciatum* (Pisces: Labridae). *Bull. Mar. Sci.* 15: 896-941.
- Foster, J.A. 1987. Diel and lunar patterns of reproduction in the Caribbean and Pacific sergeant major damselfishes *Abudefduf saxatilis* and *A. troschellii*. *Mar. Biol.* 95: 333-343.
- Hioki, S. and K. Suzuki. 1987. Reproduction and early development of the angelfish, *Centropyge interruptus*, in an aquarium. *J. Fac. Mar. Sci. Technol.* 24: 133-140.
- Hioki, S., K. Suzuki and Y. Tanaka. 1990. Development of eggs and larvae in the angelfish, *Centropyge ferrugatus*. *Jap. J. Ichthol.* 37: 34-38.
- Johannes, R.E. 1978. Reproductive strategies of coastal marine fishes in the tropics. *Environ. Biol. Fish.* 3: 65-84.
- Karanikas, J. 1989. The spawning of the flame angel *Centropyge loriculus*. *Sea Scope, Spring*, 7: 1-2.
- Lobel, P.S. 1978. Diel, lunar, and seasonal periodicity in the reproductive behavior of the pomacanthid fish, *Centropyge potteri*, and some other reef fishes in Hawaii. *Pacific Science.* 32: 193-207.
- Moyer, J.T. and A. Nakazono. 1978. Population structure, reproductive behavior and protogynous hermaphroditism in the angelfish *Centropyge interruptus* at Miyake-jima, Japan. *Jap. J. Ichthyol.* 25: 25-39.
- Moyer, J.T., R.E. Thresher and P.L. Colin. 1983. Courtship, spawning and inferred social organization of American angelfishes in the Genera *Pomacanthus*, *Halocanthus* and *Centropyge*; (Pomacanthidae). *Environ. Biol. Fish.* 9: 25-39.
- Munro, J.L., V.C. Gaut, R. Thompson and P.H. Reeson. 1973. The spawning seasons of Caribbean reef fishes. *J. Fish. Biol.* 5: 69-84.
- Reinboth, R. 1973. Dualistic reproductive behavior in the protogynous wrasse *Thalassoma bifasciatum* and some observations on its day-night changeover. *Helgoländerw. Meeresunters.* 24: 174-191.
- Rezak, R., T.J. Bright and D.W. McGrail. 1985. Reefs and banks of the Northwestern Gulf of Mexico: Their geological, biological, and physical dynamics. A Wiley-Interscience Publication, John Wiley & Sons, NY.
- Riley, C.M. and G.J. Holt. 1993. Gut contents of larval fishes from light trap and plankton net collections at Enmedio Reef near Veracruz, Mexico. *Revista de Biología Tropical.* 41(1):53-57.
- Schultz, E.T. and R.R. Warner. 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.
- Thresher, R.E. 1984. *Reproduction in Reef Fishes*. T.F.H. Publications, Neptune City, NJ. 399 p.
- Warner, R.R. and S.G. Hoffman. 1980. Local population size as a determinant of mating system and sexual composition in two tropical marine fishes (*Thalassoma* spp.) *Evolution.* 34: 508-518.
- Wolda, H. 1989. Seasonal cues in tropical organisms. Rainfall? Not necessarily! *Oecologia.* 80: 437-442.