Maturation and Spawning of Marine Finfish[1]

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INTRODUCTION

Laboratory maintenance and spawning of important marine fish species has been necessary to gather life history and reproductive information. Species whose native stocks have been depleted by natural and/or man-made changes in the environment or those species that have a potential for mariculture (Johnson et al. 1977) have been among those maintained in a laboratory situation to obtain this information.

The bulk of early information on reproduction has been attained from gravid fish collected and brought to laboratories during the spawning season. For example, salmon and striped bass have been kept in tanks and manually stripped of eggs and milt (Stevens 1966; Bardach et al. 1972). More recently, gonadal and/or pituitary hormone injections have been used in conjunction with stripping. However, with this technique many of the eggs were voided as immature oocytes in various stages of development. Hormonal injections are valuable in inducing spawning for those species which release all their eggs in a single spawn (one time spawners). However, if the fish is a continuous spawner (spawns over a long time period with many separate spawns) the use of hormone injections usually causes all the eggs to be released, only a small percentage of which are at a stage which can be fertilized. Often the brood fish die or gonadal tissue is permanently damaged from these hormone and stripping procedures. Variations in this basic technique in which the stripping process is eliminated have also been reported; and gravid fish injected with hormones have been induced to spawn naturally in a laboratory situation (Kuo et al. 1974; Hoff et al. 1977; Garza et al. 1978).

Research on the life history of fish depends on a continuous and predictable supply of eggs and milt. Methods involving hormone injections do not always ensure an adequate supply of gametes. But, this earlier research with salmon, striped bass, and other species provided a number of techniques to collect fish, treat diseases, control water quality, and feed fish in open and closed systems (Lasker and Vlymen 1969; Spotte 1970; Bardach et al. 1972).

Pickford and Atz (1957) and Hoar (1959) recognized that temperature and photoperiod were two of the most important spawning stimuli in temperate and subtemperate fish species. Temperature changes are most important for maturation in some temperate species while in other species photoperiod plays the major role (Kuo et al. 1974). Generally, in spring spawners, a rise in temperature and an increase in photoperiod induces maturation and the inverse is true for the fall spawners. These increases or decreases in temperature and photoperiod must be determined with each species before laboratory spawning can occur.

Another critical limit in spring or fall spawners is an obligatory refractory (winter) period that must be met and passed through before laboratory spawning can occur. This period is usually of a few months in duration in temperate and subtemperate fish but may be very long in subtropical species. This period also varies for one time spawners and continuous spawners. Once the refractory period and the necessary lengths and rates of change from each of the four annual seasons to another are determined, temperature and photoperiod control should induce spawning and provide a large volume of usable eggs and milt.

Temperature and photoperiod control, with knowledge of the refractory period and seasonal limits, may permit a decrease in the annual cycling time between spawns making more than one spawn per year possible.

Once the temperature and photoperiod for a species have been determined, spawning may be delayed or prolonged by changing the water temperature a few degrees up or down from the optimum. This technique prolongs the spawning season and produces eggs on demand. Care must be exercised with the rate and timing of water temperature changes to prevent resorption of eggs.

Egg and milt production may be maximized with continuous spawners by "holding" the temperature and photoperiod constant when a species has started spawning. A species may be spawned more than 12 consecutive months when spawning temperature and photoperiod are set in this manner (Arnold et al. 1976).

METHODS AND MATERIALS

Fish utilized for spawning experiments were taken near Port Aransas, Tex., from estuarine and gulf populations of each species. Red drum, *Sciaenops ocellata*, were taken by hook-and-line and beach seine. Spotted seatrout, *Cynoscion nebulosus*, and red snapper, *Lutjanus campechanus*, were collected by hook-and-line. Southern flounder, *Paralichthys lethostigma*, were taken by hook-and-line and trammel net. Red drum and southern flounder were taken during their fall spawning period and spotted seatrout in the early spring season. Immature red snappers taken in early fall were placed in the spawning tank. The snapper tank was covered with nylon netting to prevent fish from jumping out of the tank.

Red drum (750 mm TL), spotted trout (356 mm TL), and southern flounder (males 254 mm TL, females 305 mm TL) were kept as brood fish. Fish were transferred to the laboratory in fiber glass tanks (300-1,900 l) and placed into 1,000-30,000 l fiber glass spawning tanks. Fish were handled carefully to avoid surface abrasions which could result in infections. External parasites were removed with a 25-50 ppm bath of malachite green and Formalin.[3] After this treatment the fish were placed in spawning tanks. Details of the seawater holding facilities are described by Arnold et al. (1976).

Photoperiod and temperature data were gathered from U.S. Coast Guard and U.S. Weather Service records for Port Aransas, Tex. By comparing larval collections or adult spawning records of

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redfish (Simons and Breuer 1962), southern flounder (Stokes 1975), spotted trout (Miles 1950), and red snapper (Mosely 1966), the photoperiods and temperatures for the natural spawning seasons were determined. The laboratory photoperiod and temperature regimes for induced spawning of these four species were based on these meteorological and hydrological records.

Adults of the three species collected began feeding from 1 to 14 d after introduction to the spawning tank. Frozen penaeid shrimp and mullet were fed ad libitum at 1.6% of the adult body weight during 1973-76; but, after 1976 only shrimp was fed and mullet was discontinued because a suspected bacteria carried by mullet caused mortality in redfish. Immature red snapper fed almost immediately after collection on frozen penaeid shrimp and sciaenid fish at 5% body weight. Red snapper diets were reduced proportionately over the 12-mo period to ad libitum feedings of about 1.6% of the adult body weight.

Loadings of adult fish per volume filter box have not exceeded 60 kg (0.137 g/cm^3), except 6 adult redfish (70 kg) were maintained successfully by the addition of a second filter box and air lift. The mean loadings of filters and number of fish for red drum was 0.080 g/cm^3 (6 fish), spotted seatrout 0.044 g/cm^3 (12 fish), flounder 0.025 g/cm^3 (12 fish), and red snapper 0.037 g/cm^3 (12 fish). The ratio of males to females was 1:1 in most of our experiments. Flow per unit weight ranged from 1.5 to 2.3 l/kg fish per min which was much lower than reported for freshwater trout in closed systems (Fyock 1977).

Eggs of the four species were buoyant and were collected from the filter box surface (Arnold et al. 1976). Mean egg size and larval hatching times were similar for the three pelagic species, but the flounder had a much longer larval developmental period with much lower hatch success (Table 1). Larval total length at hatch was similar for all species.

Until 1974, water quality was maintained by weekly flushings with fresh seawater and weekly vacuuming of the tanks. In 1974, a program of daily sampling for ammonia, nitrite, and nitrate was initiated to match feeding rates with these water quality parameters. When 0.5 mg/l NH_3, 0.9 mg/l NO_2, or 30.0 mg/l NO_3 was reached, ad libitum feeding rates were reduced for a few days or fresh seawater was added. But, if 3% body weight feeding rates were not exceeded and tanks vacuumed weekly, these concentrations were not exceeded. Uneaten food was immediately removed from spawning tanks.

Laboratory temperatures and photoperiods were set to approximate those at collection near Port Aransas. Depending upon the acclimation process and weight regaining process, a period of 15-40 d was spent in this first laboratory regime before fish were changed into a laboratory season. Once fish had been moved to a laboratory season, independence from the natural environment was completed.

RESULTS

The initial spawning experiments on these four species were patterned on the natural water temperature and photoperiods during each of the four seasons; however it can be noted that spawning started at $<$ 365 d in the red drum (317 d) and spotted seatrout (253 d). Southern flounder were in the primary stages of gonad maturation at capture and laboratory cycling was set to lead these 12 fish into their natural spawning season over a 131-d period. Juvenile red snapper were maintained for 2 yr from capture to maturity. Nine spawns were recorded over a 45-d period.

Red snapper are regarded as an offshore Gulf species that frequents reef structures, unlike the other three euryhaline species. Two hundred and thirty-one days after being placed in the brood tank the first of seven spawns over a 40-d period occurred (Arnold et al. 1978). Spawning was observed only one time. Egg release occurred (during this observation) at the tank surface in the evening and involved one female and three males. No bodily contact or butting by males was observed. A total of 2.8 $\times$ 10^4 eggs were produced with an average of 1.5-2.0 $\times$ 10^3 eggs/kg in these first year spawning fish. It is believed that this *Lutjanus* species may be a multiple or a continuous spawner but a malfunction in the environmental control system of 4 d interrupted the laboratory spawning regime.

Red drum were maintained for 268 d when the first spawning behavior was noted and 49 d later, the first spawning occurred (Arnold et al. 1977). Sexual dimorphism in this species is not distinct, but initial spawning behavior and drumming and butting by males permitted sexual identification of the three males. At spawning, males took on a dorsal blue-black and ventral silvery-white coloration. Females were butted and pushed to the water surface where eggs were released. Spawning was a social behavior with males and females participating en toto; only at the egg release near laboratory dusk did spawning become a sole female

Table 1.—Spawning experiment data and relationships for spotted seatrout, red drum, southern flounder, and red snapper induced to spawn by temperature and photoperiod cycling.

Item	Spotted seatrout	Red drum	Southern flounder	Red snapper
Date test started	8/6/73	10/1/74	8/13/76	9/16/77
Date of first spawn	4/18/74	8/14/75	12/21/76	5/7/78
Date of final spawn	7/30/76	11/3/75	1/3/77	6/12/78
Number of spawns	82	52	13	7
Number of eggs/spawn	5.8 $\times$ 10^4	1.5 $\times$ 10^6	5.0 $\times$ 10^3	2.5 $\times$ 10^3
Photoperiod (h L-h D)	15-9	12-12	9-15	15-9
Temperature range (°C)	23-26	22-26	16.5-17.5	23-25
Salinity range ($^0/_{00}$)	25-30	26-30	25-30	31-34
Mean egg size (mm)	0.77 mm	0.95	0.93	0.80
Number of oil droplets	1	1	1	1
Percent fertilization rate	99	> 99	30-50	> 90.0
Hours to hatch	15 h @ 27°C 21 h @ 23°C	19-20 h @ 24°C	61-71 h @ 24°C	20 h @ 24°C
Percent hatch rate	95-99	94-99	6-35	> 95
Total length larvae @ hatch (mm)	1.3-1.6	1.7-1.9	1.3-1.5	1.7-1.9

and multiple male activity. No aggressive behavior by either sex was noted. A total of 52 spawns over 76 d produced 60×10^6 eggs (Table 1). From spawn 41 to the end, egg number decreased from about 1.5×10^6 to 5.0×10^4 eggs per spawn delineating the end of the spawning season. The average per female was 1.8×10^6 eggs/kg. Whether spawning ceased as a response to a lack of eggs in the ovaries, or some endogenous rhythm, was not ascertained. Red drum are classified as multiple spawners within their spawning season.

Southern flounder were collected at the beginning of the winter spawning run to the Gulf. Males were distinguished easily from large females by their smaller size and their movement to Gulf waters before females. Six females placed in the spawning tank began to hydrate 21-28 d prior to spawning. At this time, males began to follow the females around the perimeter of the tanks. Several days before spawning, males positioned their heads near the females vent. Spawning at midday occurred at the surface between one pair of fish. No aggression or competition for a female, or between any fish, was noted. A total of 13 spawns by the three largest females produced a total of 1.2×10^5 eggs. The average varied from 1.0 to 1.5×10^3 eggs/kg. At the end of spawning, all females were hydrated indicating the presence of eggs in the ovaries. No explanation for this cessation of spawning was found. Southern flounder are multiple spawners within a set spawning season, but to a lesser degree than red drum.

Spotted seatrout placed in brood tanks spawned 30 d after the laboratory spawning period was initiated and spawned 82 times over the next 380 d. Sexual dimorphism in this species is not pronounced but generally females tend to be larger. Sexual identification of the six males was made at spawning time by the presence of a dark black stripe 1-3 cm wide which was superimposed upon the lateral line system. At laboratory spawning time (dusk) as the social courtship intensified, the coloration of the male stripe also intensified. Spawning occurred at the surface with a female and several males. Eighty-two spawns over a 380-d period at 23°-26°C produced approximately 7.1×10^7 eggs. Our experiment ceased because laboratory conditions were changed. Whether spawning would have continued is unknown.

These initial results suggest that natural spawning of marine fish is indeed possible and that prolonged spawning of some species may provide a continuous supply of eggs and larvae for aquaculture.

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