METHODS AND TECHNIQUES FOR SPAWNING AND REARING SPOTTED SEATROUT IN THE LABORATORY

by
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ABSTRACT

Spotted seatrout were maintained in an indoor tank (6 x 3.3 x 1.5m). Temperature and light were adjusted to simulate the seasons. When the light regime equaled 15 hr light and 9 hr dark and the temperature was 26 C, the spotted seatrout began to spawn. They continued to spawn during each of 13 consecutive months for a total of 82 spawns. Eggs were collected from the filter box with glass beakers and placed in 74-liter aquaria. The eggs hatched after 18 hr. Newly hatched seatrout were fed rotifers (Brachionis plicatilis) *and brine shrimp* (Artemia *sp.*).

Descriptions and illustrations are provided of the tanks, aquaria, lighting equipment, collecting gear, and plumbing. Procedures and methods for collecting adults, for including them to spawn, for collecting the eggs, for rearing the larvae, and for growing food for the larvae are described in detail.

The spotted seatrout (*Cynoscion nebulosus*) is one of the most important game and commercial fishes in the Gulf of Mexico. It is the most important game fish (Deuel 1973) and the seventh most important commercial fish (U.S. Department of Commerce 1975) by weight of landings in the Gulf. Information concerning the life history of seatrout has been presented by Welsh and Breder (1923), Hildebrand and Schroeder (1928), Pearson (1929), and Hildebrand and Cable (1934). More recent studies have been conducted by Gunter (1945), Miles (1950, 1951), Moody (1950), Tabb (1958, 1960, 1961), Klima and Tabb (1959), Moffett (1961), Stewart (1961), Iversen and Moffett (1962), Iversen and Tabb (1962), and Seagle (1969).

Cynoscion nebulosus ranges from New York to Mexico, but is rare north of Delaware Bay (Welsh and Breder 1923). It is a euryhaline species, inhabiting estuarine systems characterized by the predominance of marine grasses. In these areas, the spotted seatrout is the most important predator (Tabb 1958). Seagle (1969) showed that in Texas, a diet change occurred as seatrout grew larger. Small trout (130-225 mm standard length) fed primarily on invertebrates, but their dependence on fishes as food increased as they grew until, between 450 and 610 mm in length, they fed almost exclusively on fishes.

Temperature is an important environmental factor for seatrout. Seatrout remain on grass flats in some areas of southern Florida throughout the year in water temperatures from 15-27 C (Tabb 1958). In areas where water temperatures drop below this level, seatrout may remain near the flats in canals where water depth is sufficient to escape colder surface temperatures. Seatrout may acclimate gradually to temperatures from 4-33 C and feed actively and live in these ranges (Simmons 1957). Water temperatures are avoided in many Texas bays by a late summer and early fall movement of adult fish into the nearby Gulf of Mexico (Miles 1950). Large kills can occur when seatrout in shallow bays are subjected to sudden temperature drops associated with the passage of winter cold fronts. Tabb (1958) states that fish exposed to water temperatures of 9 C for 12 hr may not recover and that exposure to 7.2 C for 24 hr is lethal.

As a euryhaline species, the spotted seatrout is affected more by changes in temperature than by variation in salinity (Tabb 1958). Simmons (1957) reports catches of spotted seatrout in 75‰ salinity; while Gunter (1963), Gunter and Hall (1965) and Perret (1971) report trout taken in salinities near 0.2‰. Clearly, adult seatrout can adapt to situations that are nearly freshwater.

Salinity and temperature conditions suitable for spawning in Florida have been reported to be in the ranges of 30-35‰, and 25-28 C (Tabb 1958). In Texas these conditions occur in early April and continue through October, with the greatest spawning activity in May, June, and July (Miles 1951). Tabb (1961) states that trout prefer deep channels (2.5-4.5 m)

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immediately adjacent to shallow grass flats to spawn, and that young fish move to the grass flats as juveniles. Young trout spend their early lives in grass flats where they feed on plankton and later on immature stages of several species of shrimp and small fishes. Schooling behavior begins by the age of 6 or 8 weeks and continues until the age of 5 or 6 years, when they become semi-solitary in habit (Tabb 1966).

An important aspect of spotted seatrout biology is its non-migratory behavior. Tagging studies in Florida by Moffett (1961), and Iversen and Tabb (1962) have shown that tagged fish seldom moved more than 48 km and very few individuals migrated to adjacent estuaries. Miles (1950) reported that 80 km was the greatest distance traveled by a tagged trout in Texas. *C. nebulosus* populations are apparently dependent upon local conditions for survival.

Although much information on various aspects of the adult seatrout is available, little is known of the early stages, stages during which this fish is most vulnerable to alterations of its environment. Because larvae and juveniles are inhabitants of estuarine areas, and because estuarine habitats in the Gulf are being subjected to environmental alterations (Lindall 1973; Lindall and Trent 1975), the need is great to learn the tolerances and physiological reactions of the various early life stages of this important species to various ecological factors and pollutants.

In this regard, we develop methods and techniques to maintain adult seatrout in captivity, to induce them to spawn repeatedly, and to culture the young in order to have eggs, larvae, and juveniles of known history for experimental purposes. Our methods and techniques are presented herein.

SPAWNING TANK

The seawater system at the Port Aransas Laboratory consists of a Chemtrol[3] pump with a 5-hp motor, 15.2-cm PVC pipe, and a 113,550-liter storage tank on the roof of the laboratory. The pump is next to the ship channel, and seawater is pumped approximately 100 m to the storage tank. The unfiltered seawater flows by gravity from the storage tank to the spawning tank inside the laboratory.

The spawning tank measures 6 x 3.3 x 1.5 m and has a volume of approximately 30,000 liters. It is made of fiberglass and is strengthened with four double 5- x 10-cm boards embedded in the fiberglass running horizontally along each side near the bottom and two single 5- x 10-cm boards at the top. Seawater is removed from the spawning tank by a

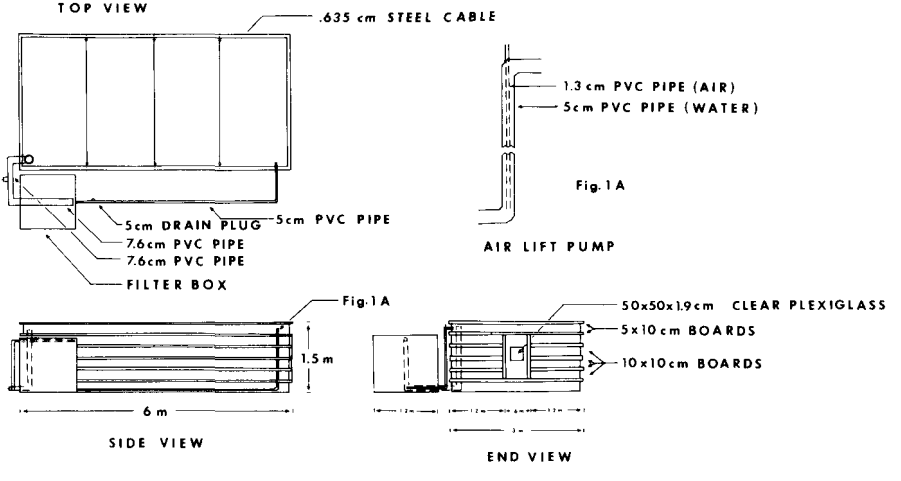

Figure 1. Diagram of spawning tank, filter box, and airlift pump.

standpipe of 7.6-cm PVC with 6.2-mm holes drilled through the bottom. Seawater flows through these holes from the bottom of the spawning tank, while the open upper end of the pipe drains water off the surface (Fig. 1). Wire cables attached across the top of the tank prevent excessive bulging when full (Fig. 2). Seawater is strained through a 505u plankton net to remove large organisms and materials before entering the tank.

Figure 2. Wire cables across top of spawning tank.

A filter box, 1.2 x 1.2 x 1.2 m, constructed of 1.9-cm, fiberglass marine plywood is used to remove waste materials. The filter box is 30-40 cm lower than the water level in the spawning tank, and therefore seawater flows into the box by gravity. The water is distributed across the surface of the filter box by a horizontal 7.6-cm PVC pipe with holes set in two rows at 45° from the bottom (Fig. 3). The effluent pipe lies just off the bottom of the filter box. It is a 7.6-cm PVC pipe with four lengths of capped 3.8-cm PVC pipe set perpendicularly through it, with 0.6-cm holes in two rows at 45° from the bottom (Fig. 4). This provides an evenly spaced configuration for picking up the filtered seawater. Filtering material consists of chert gravel poured over the effluent pipes to a depth of 30.0 cm; a 2.5-cm layer of crushed oyster shell on top of this; then a 5.0-cm layer of coarse (#2) sandblasting sand on top of the shell (Fig. 4).

The water from the filter box is returned by an airlift pump into the opposite end of the spawning tank (Fig. 1a). If a power failure occurs, the compressor operating the airlift will cease operation. The spawning tank will continue to drain into the filter box, and it will overflow until the two reach the same level. At this point the spawning tank would still be over half full, and fish should survive until the power can be restored.

The temperature of the spawning tank water is regulated by the laboratory heating and air-conditioning system. The photoperiod is controlled by three time clocks which regulate three rows of fluorescent lights. The 40-watt "cool white" bulbs are turned on within a 5-minute period and off in the same time period.

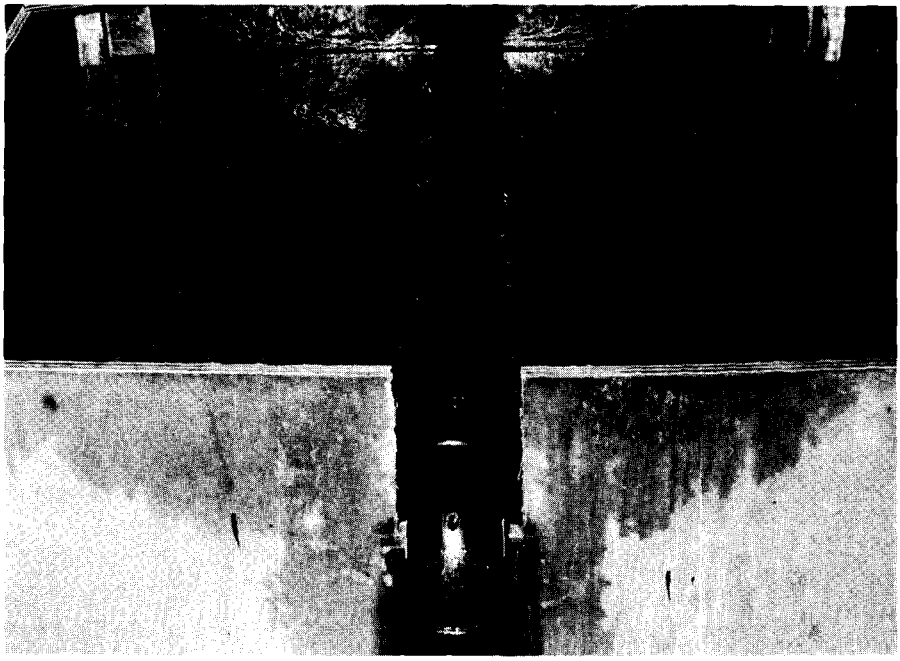

Figure 3. Water flowing from header into filter box.

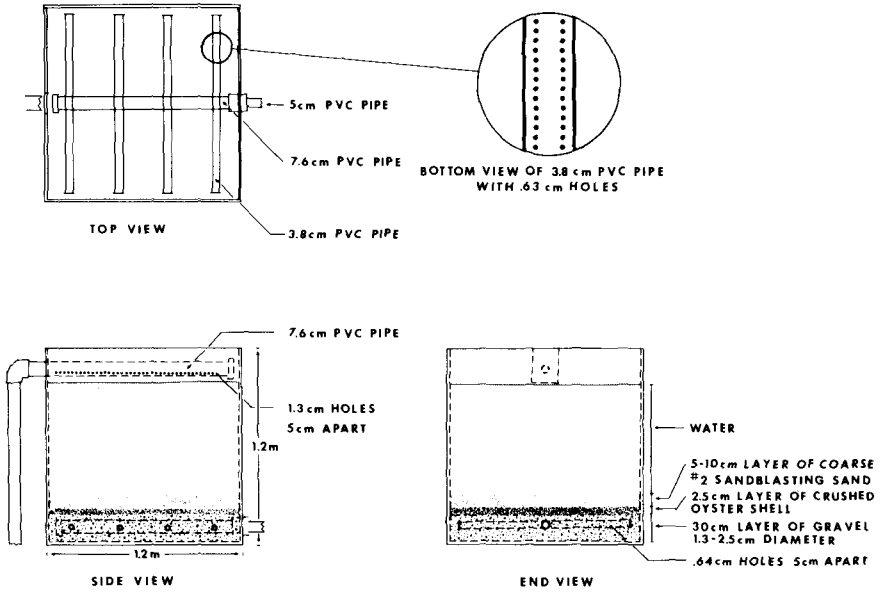

Figure 4. Details of the filter and filter box.

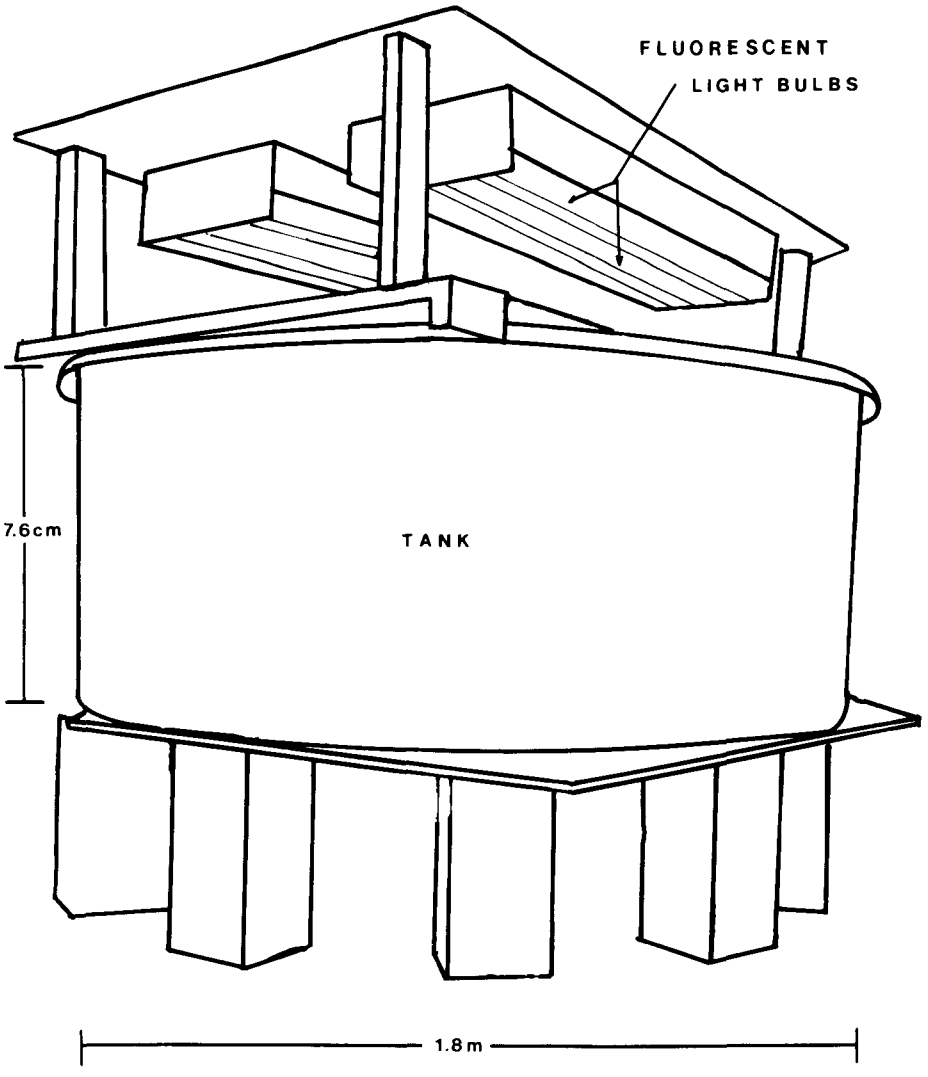

Figure 5. Algal culture tank with lights.

No metallic materials are permitted to contact the seawater in the filtering system or tank, as they may deteriorate and release substances that are toxic to fish. Sealants are of the type manufactured for use in aquaria.

CARE OF BROOD FISH

Methods of capture and handling of brood fish prior to release in the spawning tank are crucial factors in the survival of the fish. Since *Cynoscion nebulosus* is relatively sensitive to handling, potential brood fish are collected with hook and line, and care is taken to avoid any handling of the fish that might loosen scales or remove their protective mucus.

A period of adjustment occurs when the fish are introduced into the spawning tank. They usually begin to feed 2 to 6 days after introduction. At first, live penaeid shrimp (*Penaeus* sp.), live mullet (*Mugil cephalus*), and live pinfish (*Lagodon rhomboides*) are placed in the tank as food. Injured food fish are used, because they swim erratically and eventually die if not eaten, and thus, do not become established in the tank.

Once the fish have begun to feed on the live food, dead food is introduced into their diet. Frozen penaeid shrimp is the main diet, but mullet and squid are used as supplementary foods. Fish are fed once daily at 3-4% body weight. Feeding behavior varies, but generally the fish are active feeders, taking food before it reaches the bottom of the tank. Fish are fed until they no longer take food, and several pieces have accumulated on the bottom. They will continue to feed, although at a slower rate, on these food items.

After the fish have begun feeding regularly, maintenance of their spawning tank consists of vacuuming the bottom with a siphon every 2 weeks. At first, food buildup on the bottom is heavy. This is kept to a minimum by using small food portions and vacuuming frequently. When correct amounts of food are used, only fecal material will accumulate on the bottom. After every vacuuming, seawater is added to raise the water level to the correct height.

PRODUCTION OF ALGAE

Algae are necessary in the culture of larval fish to maintain water quality by utilizing larval metabolic products and to provide a food source for rotifers. When rotifers are to be cultured, algae must be maintained simultaneously in equal, or larger, masses to provide rotifers with a dependable food supply.

The National Marine Fisheries Service Laboratories at Port Aransas and Galveston have selected *Tetraselmis chuii* as food for rotifers and larval crustaceans. This flagellated green alga was found in East Lagoon, Galveston Bay; a synopsis of the culture of this alga was given by Griffith et al. (1973).

Tanks, Air Supply and Nutrient Flow Patterns

Selection of containers for use in the culture of *T. chuii* has to be made with some care if large crops of algae are to be produced. Fiberglass, white-gel-coated tanks 1.8 m in diameter and 76.2-cm deep are used (Fig. 5). A depth of 71 cm of water in this tank provides a volume of approximately 1,178 liters. An even distribution of nutrients is maintained with an airlift pump which provides a continual flow of nutrients from the bottom to the surface of the tank.

Light Supply

Continuous illumination of the tank surface is necessary for successful production of *T. chuii*. Two 122-cm, 120-V, light strips holding four "cool-white" 40-watt fluorescent bulbs provide necessary illumination for rapid algal growth and reproduction. Bulbs and light strips are positioned approximately 60 cm above the water surface by a simple wooden frame.

Tank Preparation and Initial Innoculation

Each tank is thoroughly cleaned and disinfected before algal culture is attempted. If the tank has been previously utilized for other purposes, the interior surface is washed and scrubbed several times with chlorinated tap water at a temperature of 60 C.

Water is filtered through a double thickness of 80-grade Nytex to eliminate potentially harmful organisms and other materials from entering the tank. When full, the culture tank is allowed to sit for 2 days to permit the water temperature to stabilize. We have found the optimum temperature for growth of *T. chuii* to be 24 ± 2 C. Culture room temperature is satisfactorily maintained by a heating and air-conditioning system. Optimum salinity for *T. chuii* growth has been approximately 28‰ (±3‰). Griffith et al. (1973) report good cultures of *T. chuii* with a range of 16-36‰. The pH of the culture tank is maintained between 7.5 and 8.0. Water in the storage tank has remained in the pH range of 7.8 to 8.0, and adjustment of new culture water has not been necessary. Griffith et al. (1973) suggest adding 400 ml of Tris buffer per 100 liters of culture water adjusted to a pH of 8.3 with

concentrated hydrochloric acid. Control of pH is important in algal tanks for optimal growth.

After the tank has been set up, and the chemical and physical parameters adjusted, the initial innoculation is made. For the 1,178-liter tank, 100 liters of algal stock is used at a concentration of 100,000-130,000 cells/ml. Griffith et al. (1973) suggest an additional 100 liters be added each day for the next 2 days to insure success of culture organism. If the alga is not growing after two or three innoculations, the tank is drained and restarted.

Nutrients are provided as a mixture of ammonium sulphate (500 g) and superphosphate (500 g). The mixture is added to the new culture tank before the algal innoculate is introduced. It is put into solution by slowly stirring the contents of the tank.

Three to four days after the algal innoculate is added, the algal population should be approaching a maximum density of 132,000 cells/ml.

Algal Maintenance

Peak algal concentrations and growth are difficult to maintain for an indefinite period of time. To obtain consistent, maximum daily yields of algae and to prevent sudden population declines, the population should not be allowed to reach the maximum capacity of the culture tank. This pre-asymptotic level of algal cells is maintained by: (1) draining one-half to two-thirds of the entire tank volume each day; and (2) refilling the tank with filtered seawater. The refilling process renews the original volume of the tank and decreases the density of cells. Populations of *T. chuii* have been maintained for as long as 30 days at pre-symptotic levels in our tank using this method.

Fertilization of the culture tank should be done once a week with 100 g of the combination fertilizer.

Contamination

Accidental introduction of rotifers may cause a rapid decline in the algal population. Contamination with rotifers can be avoided in three ways: (1) by placing the algal tanks 30 to 34 cm above rotifer tanks; (2) by using "algae only" containers and hoses to grow and transfer the algae; and, (3) by filtering all water used to refill the algal culture tank through 80-grade Nytex.

If contamination occurs, a Nytex bag over the airlift pump can catch unwanted organisms. The bag is continually monitored and cleaned to eliminate the fouling organisms. If the "bloom" of intruding organisms is greater than the bag can efficiently eliminate, the entire volume is drained and the culturing procedure is started anew.

PRODUCTION OF ROTIFERS

The original culture of the rotifer, *Brachionis plicatilis,* was obtained from the National Marine Fisheries Service Laboratory at La Jolla, California, where this organism was being cultured as food for larval anchovy by Theilacker and McMaster (1971).

Rotifer Tanks

Large fiberglass tanks, approximately 1.8-m diameter and 76.2 cm in depth, are used to maintain rotifer populations. Tanks, when filled to a depth of 71 cm, hold 1,178 liters of water that sustain populations of rotifers in densities up to 200/ml. Each rotifer tank has been placed below the level of the algal tanks, to prevent the introduction of rotifers into the algal culture.

Air Supply and Nutrient Flow Patterns

Two airlift pumps of 5-cm polyvinyl chloride (PVC) pipe are set up at the opposite sides of the culture tanks to circulate the water. The airlift pipes are of the same construction and operate on the same principle as those of the algal tanks.

Light Supply

Lighting provided to the rotifer population is the same type and intensity as for the algal culture tanks.

Tank Preparation and Innoculation

All tanks utilized in the culture of *B. plicatilis* are cleaned as recommended for algal tanks. Water is passed through a double layer of 80-grade Nytex to filter out unwanted organisms. The tank is filled to one-half its capacity with filtered water and the final one-half from *T. chuii* culture tanks. Salinity adjustment has not been necessary for *B. plicatilis*. We have found that it grows and reproduces successfully in salinities from 26 to 32°/₀₀. Theilacker and McMaster (1971) report satisfactory growth of this rotifer in salinities of 25-35°/₀₀ when food is adequate.

After water and alga have been added to the new tank and the temperature reaches 24 C (± 2), approximately 3 liters of water containing rotifers (50/ml) are added. This can be taken from cultures of rotifers set aside for this purpose, or concentrated from an existing rotifer tank by using a siphon hose and 80-grade Nytex.

As the population of rotifers increases, algal counts will decrease and the culture water will turn from a light green to a clear, yellow color.

Populations of rotifers with sustained concentrations of 100-150 ml are desirable for larval fish food. These concentrations can be maintained by: (1) daily drawdowns of one-third to one-half of the culture tank; and, (2) replacement by the addition of algal culture water.

SPAWNING OF SPOTTED SEATROUT

Collection of eggs by surface-net tows, catching "running ripe" fish, artificially fertilizing the eggs, or inducing spawning of "wild" fish with hormone injections are often unreliable. The disadvantage to the latter approach is that the brood fish are often injured or killed as a result of handling and injections. The best method used at this laboratory has been to induce spawning by manipulating photoperiod and temperature. This method may be used repeatedly with the same fish. The correct photoperiod and temperature must be determined for each prospective species.

Spawning of spotted seatrout at the Port Aransas Laboratory was induced as follows. Seatrout were collected in August 1973 by hook and line. After installation of the closed seawater system in December 1973, controlled winter conditions were set by temperature and photoperiod control. In February 1974, conditions were changed to simulate summer (Table 1). The photoperiod was set at 15 hr of light and 9 hr of dark. The temperature was gradually increased and by mid-March was up to 26 C. About 1 month later (April 18) the seatrout spawned. The photoperiod and temperature (26 ± C) remained constant and the seatrout spawned 82 times in the following 13 consecutive months.

The salinity in the spawning tank was not controlled, but did not vary more than 5°/₀₀. The spotted seatrout were not adversely affected by salinities in this range (25-30°/₀₀).

Table 1. Photoperiod and temperature regime used to induce spawning in spotted seatrout in 1973 and 1974.

	Temperature (C)		*Photoperiod (Hr)*	
Month	*Average*	*Range*	*Light*	*Dark*
August	29	28-30	15	9
September	28	28-29	15	9
October	26	25-27	15	9
November	25	23-26	15	9
December	17.8	16-25	9	15
January	14.2	13-16	9	15
February	18.7	14-22	15	9
March	25.2	23-26.5	15	9
April*	26	25.5-26	15	9
May	26	24-27	15	9

*Seatrout first spawned 4/18/74.

Our observations agree with Pearson's (1929) supposition that the eggs of *C. nebulosus* are bouyant. Miles (1950) suggested that *C. nebulosus* eggs were probably demersal. Tabb (1966) stated that the eggs sink to the bottom and hatching occurs in bottom vegetation and debris. Although the design of our filter box and tank allows only for the collection of pelagic eggs, we feel that eggs which sank were either dead or had not been fertilized. Bouyant eggs collected in the filter box after each spawn were 99% fertilized.

EGG HANDLING

Two methods have been used to remove bouyant eggs from the filter box. Approximately 75% of the seatrout eggs can be collected by using a wide-mouth 100-ml glass beaker and dipping eggs out with filter-box water. This method is preferred since few, if any, eggs are lost as a result of coming into contact with the dipping apparatus. The remaining 20 to 25% of the eggs can be gathered by slowly moving a small mesh dip net in a zig-zag pattern just under the water surface. Some eggs may be damaged by the net, so it is best to collect as many eggs as possible with the 100-ml beaker.

LARVAL REARING

Two types of rearing containers have been used indoors with almost equal success; glass aquaria (55-111 liters) and circular, 185-liter fiberglass tanks (99-cm diameter). Aquaria are filled with algae and rotifer culture water, 1 to 2 days before introduction of larvae. Algae aids in control of water quality by utilizing larval metabolic waste and provides a food base for rotifers that will become the initial food supply for larval fish. To insure algal growth, two "cool-white" fluorescent light bulbs (40 watts each) are placed above each aquarium for a constant light source.

Larval stocking densities should not exceed 5/liter. Densities greater than this result in slow growth and a low survival rate. Houde (1975) concluded from his studies that initial stocking densities should not exceed 8 eggs/liter.

Larvae should not be moved with a net if at all possible. Nets may cause physical damage and death to many of the larvae. Larvae tend to clump in the corners and along the sides, where large numbers may be removed by dipping with a 100-ml beaker.

Outdoor (14,000 liters) tanks at the Port Aransas Laboratory have been stocked with 1 to 2 fish/liter. We have obtained survival of 75 to 80% for 3-day-old larvae and 60 to 65% for 10-day-old larvae with the maintenance of a good population of rotifers (20/ml) and algae (50-75,000/ml).

Size of Food

As larvae grow, they require larger food items. Thus, a culture facility should provide a steadily increasing size selection of food. If the food supply is less than optimal, growth slows and mortality occurs. The initial food organisms should be less than 75μ in size. The seatrout larvae (second day after hatching) are fed *B. plicatilis* at a rate of at least 20/ml of water. This rate is maintained until the 5th day after hatching, then rotifers and brine shrimp nauplii (3-5/ml) are both fed to the larvae. This combination is fed until the larvae are 8 days old; then brine shrimp nauplii are used as the only food source. *B. plicatilis* and nauplii of *Artemia* sp. supply nutritional needs of larvae until the 13th or 14th day, when large numbers of fish begin to die. Apparently, the mortality at this age is due to nutrient deficiencies. Mortality of over-14-day-old fish can be greatly reduced if the *Artemia* sp. nauplii are allowed to feed on algae for at least 1 day before being offered as food. *Artemia* nauplii that have fed upon algae can be used to rear trout to 30 days with a survival of 30%.

Feeding Behavior

Newly hatched larval trout assume a head-down position until the yolk-sac is absorbed, which occurs 72-80 hr at 24 C. From the 3rd to 7th day, larvae begin to disperse throughout the water column and begin feeding on prey that are within a radius of 1-2 body lengths (2 mm). At 7-14 days larvae are capable of swimming through the water column and stalking food organisms. When a prey organism is sighted, the larva slowly closes the distance to less than one body length and lunges forward to capture the prey.

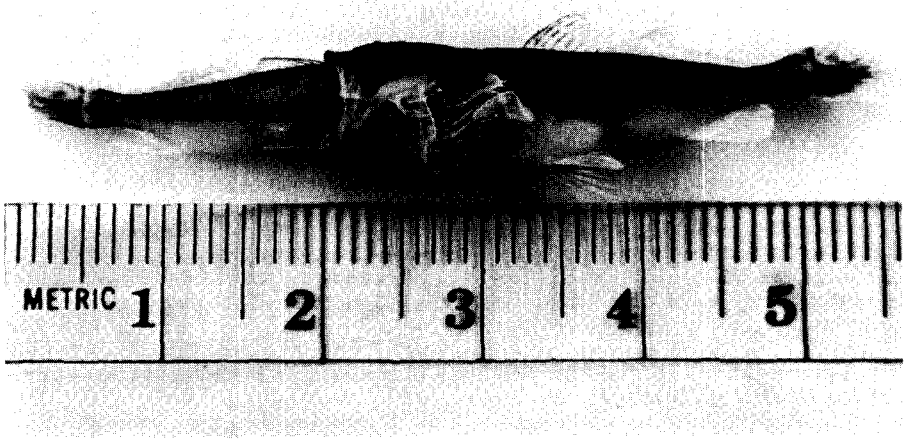

Figure 6. Suffocation of young seatrout (*Cynoscion nebulosus*) caused by attempted cannibalism.

Cannibalism occurred initially at an age of 10 days. Larger fish usually fed on smaller ones, but on many occasions seatrout attempted to eat fish of near equal size. When fish took large prey tail first, the prey usually escaped if it was too large to be swallowed. When seatrout took its prey head first, death by suffocation for both prey and predator usually occurred (Fig. 6) unless the prey was small enough to be swallowed or ejected. As the fish grew larger, they became more selective and chose smaller individuals which could be swallowed. Often seatrout would strike others and fatally injure them.

In one aquarium, 500 larvae were reduced to 8 fish within 30 days, primarily, we believe, from cannibalism. In a similar case, Schumann (in Bardach 1968) attributed to cannibalism a 50% reduction in the number of larval Pacific mackerel, *Scomber japonicus*, in the first 9 days after hatching.

Cannibalism in larval and juvenile seatrout held in aquaria may be caused by a number of factors. It may be due to being held in an unnatural situation, to an insufficient supply of food of proper size, or to an intraspecific predation which may occur in wild larval and juvenile seatrout.

SUMMARY AND CONCLUSION

Methods and techniques described may be used to obtain natural spawns from adult seatrout and to rear the larvae. Eggs from natural spawns are approximately 99% fertilized. Rearing of the larvae to an age of 3 days with a 75-80% survival and to 30 days with a 30% survival have been achieved. Low percent survival from larvae to juveniles is caused by cannibalism and lack of proper food supply.

[1] The use of tradenames in this publication does not imply endorsement of commercial products.

Mass rearing of rotifers and alga is necessary for rearing larvae. Rotifers provide an initial larval food supply for 5-6 days after hatching and are replaced by brine shrimp nauplii. Brine shrimp nauplii appear to have more nutrient value for larval fish if permitted to feed on alga for at least 24 hours.

Cannibalism and lack of proper food appear to be the major problems in the mass production of seatrout.

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