CMFRI

Course Manual

Winter School on

Recent Advances in Breeding and Larviculture of Marine Finfish and Shellfish

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BROODSTOCK DEVELOPMENT AND BREEDING OF GROUPERS

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Introduction

Groupers belonging to the family Serranidae, distributed in the tropical and subtropical seas of the Indo-west Pacific have very high potential as candidate species for aquaculture and have high market value especially in south East Asian countries, Middle East and the Polynesian countries. Groupers are classified in 14 genera of the subfamily Epinephelinae, which comprises at least half the approximately 449 species in the family Serranidae. Throughout most tropic and temperate marine regions, serranids are also kept in aquariums. Maximum size ranges from 12 cm for the Pacific creole-fish (*Paranthias colonus*) to more than 13 feet (4 m) and e"440 kg for the king grouper or brindle bass (*Epinephelus lanceolatus*). Several grouper species have been raised on a commercial scale (mainly in Japan, Taiwan, Hong Kong, Southeast Asia and the Middle East), but mostly by growing out captured wild juveniles. The major constraint to the development of grouper aquaculture is the shortage and uncertain supply of fingerlings from the wild. Hatchery production has increased in recent years (for example, in Japan, Taiwan and Kuwait). Major farmed Asian species are red spotted grouper (*E. akaara*), orange-spotted grouper (*E. coioides*), brown marbled grouper *(E. fuscoguttatus*), Malabar grouper (*E. malabaricus*), camouflage grouper (*E. polyphekadion)*, and greasy grouper (*E. tauvina*). King grouper, polka dot grouper (*Cromileptis altivelis*), and coral-trouts (*Plectropomus spp.)* are raised in some areas. The Chinese perch (*Siniperca chuatsi*) is mostly raised from brackishwater ponds in China. For farming in the south-eastern U.S. and Caribbean, Nassau groupers (*E. striatus*), gag groupers (*Mycteroperca microlepis*), black groupers (*M. bonaci*), and jewfish (*E. itajara*) seem to have good potential. Juveniles and adults of some grouper species live in coastal waters and estuaries, but others prefer the cleaner waters of off-shore reefs. Eggs are single, non- adhesive, and buoyant at normal salinities. Larvae of most species spend at least their first few weeks drifting with the oceanic plankton. As they become juveniles, groupers settle in shallow water where they can find hiding places. Until a few inches long, they hide almost constantly. As they increase in size they become bolder and move to deeper water, but most species continue to stay near small caves for security. Wild grouper larvae at first eat copepods and other small zooplankton, then larger crustaceans such as amphipods and mysid shrimp. Wild juveniles and adults eat mainly fish, crabs, shrimp, mantis shrimp, lobsters and molluscs.

Most groupers that have been studied will mature within 2 to 6 years. Many serranids are protogynous hermaphrodites (i.e., most individuals mature first as females and some of them become males later). Some of those species, as a rule, change from female to male as they grow older; others might change only if there is a shortage of males. In nature, many groupers spawn in large aggregations (hundreds to thousands of fish). Gangs spawn in harems, with a sex ratio often near 1 male:10 females. Small serranids often spawn in pairs without aggregating. A few of the small species are simultaneous hermaphrodites (male and female at the same time), but self-fertilization seems to be rare.

Broodfish and breeding

The pre-requisite in any successful hatchery operation is the availability of good number of healthy male and female broodstock of the candidate species to obtain healthy seeds. Research on broodstock development and breeding techniques of all cultivable food fish has been progressing intensively in many laboratories world over. Broodstock for hatchery production of seed can be developed by acclimatizing adults or juvenile collected from the wild using traps or hook and line from rocky and coralline grounds of depth 150-200m. Most groupers studied have adapted quickly to captivity. Depending on species and capture depth, the gas bladder might expand too much for the fish to recover on its own, so that it might float. Considering the difficulties in obtaining large groupers from the deeper

untrawlable seas and the problems related to sudden change in pressure, stress and injuries, it is imperative to develop a broodstock starting from fingerlings and rear them in the hatchery system for seed production.

Epinephelus tauvina and *E.malabaricus*, two fast growing species, occur in the rocky and coralline regions along both coasts of India, and juveniles often occur along the coastal and near shore regions and mouths of estuaries. In India, concerted efforts on grouper aquaculture and hatchery development were initiated only during 1996. As a part of the development of a hatchery system, healthy broodstocks of these two species were developed at the CMFRI Cochin, in 5 t capacity FRP tanks under onshore, indoor and controlled conditions in recirculating sea water. Since groupers are well known for their protogynous hermaphroditism, it is necessary to develop female brooders and viable male broodstock (either by hormonal sex transformation or from the adult males from the wild) and maintain them. A highly efficient system has been developed using recirculating sea water for developing female as well as male broodstock of groupers indoors in a completely controlled environment. Broodfish always should be handled as gently (with soft nets, plastic bags or hands, not towels) and infrequently as possible, kept in well-oxygenated water, and fed well if kept for more than a few days. The broodstock developed and maintained in this system have repeatedly spawned producing high quality, viable, buoyant and fertilized eggs.

In the south east Asian countries, middle east and the Polynesian islands, where grouper hatcheries have been established, the broodstock are developed in near shore net cages of size 5x5x3m where they are subject to vagaries of nature. The broodstock are then transferred from the net cages into the onshore tanks for spawning either spontaneously or if necessary through inducement. For obtaining the males, sex transformation experiments were also carried out keeping the fishes in separate net cages. In general, fish selected for broodstock should be fast growing, lively fish, among the largest and strongest members of their age group and free from parasites and diseases.

Determination of maturity of spawners

Assessment of gonadal maturity is a major difficulty in the artificial propagation of finfish. To date, the commonly used method involves *in vivo* sampling of gametes. Gametes are removed from either an anaesthetized or unanaesthetised fish using a polyethylene cannula. The inner diameter of the cannula to be used varies with the size of eggs to be sampled. The cannula is inserted 4-15cm into the ovary or testis, and gametes are drawn in to the cannula by aspiration as the cannula is withdrawn. The distance to which the cannula is inserted varies with the length of the ovary or testis. Samples from the middle portion, especially of the ovary are generally considered the most representative. Eggs collected are removed into a Petri dish, preserved in 1% formalin in 0.9%NaCl. The average egg diameter is determined from a batch of 50-100 by using a micrometer and development stage assessed under microscope. The milt collected is removed from the cannula by blowing into a clean Petri dish. A small portion of this is mixed with a drop of sea water and examined immediately under microscope. Sperm motility and viability are then assessed.

Factors affecting gonad development:

1. Nutrition

Many a studies have shown that reproduction and egg quality are greatly influenced by nutritional value of the diets. Eggs produced by females fed Antarctic krill and formulated feed containing cuttle fish meal consisted of good buoyant eggs with high hatching rate, leading to high production of viable larvae.

2. Photoperiod

One of the factor considered to be of great importance to inducement of sexual maturation and spawning is photoperiod. Its manipulation is employed to alter normal reproduction of many cultured species like rabbitfish, grouper, rainbow trout, etc.

3. Temperature

Water temperature is an important factor which influences the maturation and spawning of fish. In some species, functional maturity and time of spawning are controlled by temperature.

4. Salinity

Some species of fish migrate from the marine to the freshwater environment in order to spawn, while others migrate from freshwater to marine environment to complete their reproductive cycle. Many mullets, seabass, etc. do not reproduce spontaneously in fresh water.

5. Other environmental factors

In addition to photoperiod, temperature and salinity other environmental factors which affect the maturation and spawning of broodstock include rainfall, lunar periodicity, tidal magnitude, stress, sex ratios, stocking density, human disturbance, dissolved oxygen, social behaviour of fish, pollution, etc.

Selection of spawners

The selection of spawners from the broodstock should be done months before the beginning of spawning to allow ample time for fish to be conditioned to environment and diet controls. Spawners are selected based on the following criteria: a) fish should be active, b) fins and scales should be intact, c) fish should be free from disease and parasites, d) fish should be free from injury or wounds, e) males and females of similar size preferred. The spawners transferred to the pre-spawning tanks at a ratio of 1male:1female. Immediately after stocking in the pre-spawning tank, feeding is reduced from 5% to 1% of the total body weight and the fish are fed only once a day. This is to prevent them from becoming fat, which could result in poor gonadal development. Water in the spawning tank should be maintained in good condition.

Voluntary spawning of captive groupers has occurred mostly with well-fed, uncrowded fish during the natural spawning season under conditions of ambient temperature and partial or total natural light. Day length seems to be a less important stimulus for spawning than temperature. At least 27 serranid species have spawned voluntarily in captivity, with groupers spawning in 1 to 21,200 m^3 tanks or ponds and 26 to 75m^3 cages. Eggs are collected in strainers or with soft, fine dip nets. Some species spawn near certain moon phases and others spawn any day of the lunar month. With good timing, groupers can be caught from wild just before spawning and held in tanks or cages for up to a few days until they ovulate naturally. The eggs can be stripped, or rarely the fish are left in the tank so that voluntary or accidental fertilization can occur if the males are running ripe.

Groupers can be conditioned to spawn during any month, mainly by temperature cycling (raising or lowering temperature to the spawning range of 24 to 27°C). Males begin approaching females several days before spawning begins. On a spawning day, males turn bicoloured (black above and white below) early in the afternoon, and the females become bicoloured late in the day at the time of ovulation, just before spawning occurs. Hormone induced ovulation of ripe wild or captive groupers generally is reliable. At least 31 serranid species have been induced to ovulate. Typically, a female with fully yolked oocytes (developing eggs) is given one to three injections of 227 to 454 IU human chorionic gonadotropin per pound of body weight (500 to 1,000 IU/kg). Ovulation (the release of mature eggs into the center of the ovaries) occurs within 24 to 72 hours (usually 36 to 50 hours) after the first injection. Similar results have been obtained for injections of 4.5 to 22.7 µg gonadotropin-releasing hormone analog (GnRHa) per pound of body weight (10 to 50 µg/kg). GnRHa implants also have worked. If newly caught brood fish are used, the hormone should be administered as soon after capture as possible to limit the effects of stress on oocyte development. For six grouper species with egg diameters of 800 to 1,000 µm, the minimum effective oocyte diameter (seen in biopsy samples) before injection was in the range 41 to 61 percent of the fertilized egg diameter; ovarian biopsies are not necessary if external characteristics can be relied upon as indicators of oocyte development. Females are handled as little as possible, but are monitored closely for swollen abdomen, protruding genital papilla, stretching of the membrane holding eggs in, and spawning coloration. They are checked more often (e.g., once an hour) just before the predicted time of ovulation. For groupers, the time from ovulation to over ripeness (deterioration of eggs) is only 1 to 2 hours at 26°C. When ovulation has occurred, milt is stripped from one or more males and collected in 3-cc hypodermic syringes (without needle). Eggs are then stripped from the female into a beaker, milt is added, filtered water is added, and the mixture is stirred. After 3 to 5 minutes, the eggs are transferred to a larger container and washed several times by

water exchange or repeated transfer. Transparency, buoyancy, roundness, normal egg size, size uniformity, lack of stickiness, possession of a single oil globule, and normal oil globule size are initial signs of quality. High fertilization rate, normal cell division, high hatching rate, and successful first feeding are subsequent signs.

The indoor broodstock development system established at the CMFRI, Kochi, enabled a 24h close observation and monitoring of the brood fishes. The recirculating system in the broodstock tanks enables minimum fluctuations in the physico-chemical parameters in the culture medium unlike in other systems. The pheromones secreted by reproductively mature females released in the water have a significant effect on both retention of maleness as well as in the quantity of milt production in the male. Socialization of the female and male spawners in the same tank resulted in retention of maleness even after cessation of hormone administration resulting in fertilization rate of upto 100% on all occasions. Though the spawning season for the groupers in the Indian Ocean region is reported to be from April to November, in recirculting sea water system, they spawned throughout the year, during all the months of the year. Spawning takes place consecutively for three days. The fecundity obtained for each female in this system during each spawning day ranged from 0.05million to 0.1million healthy, buoyant and viable eggs.

The eggs are incubated under very clean, constant, optimal conditions. Often they are put in conical tanks, which facilitate the removal of any sunken dead eggs. Usually eggs are transferred to rearing tanks just before hatching, or larvae are transferred just before first feeding. However, it is best not to handle grouper larvae. With fresh milt and good water, the fertilization rate was 85 to 86%. Hormone injection was not always necessary because female groupers that were about to ovulate could be identified during the early afternoon. If females were held with running-ripe males, the eggs usually were 100% fertilized in he holding tanks.

The fertilized buoyant eggs hatched out in 22 to 23 hrs after spawning. Known egg diameters range from 0.8 to 1.0 mm, and total length of hatchlings is 1.6 to 2.3 mm. Larvae of most grouper species are small and fragile and have small mouths at first feeding. Yolk and oil, which nourish the early larva until after feeding begins, tend to be exhausted quickly (2 to 5 days). Typically, the larval period is long (35 to 70 days), and groupers tend to require live food longer than most marine fish that have been reared. Commercial scale Asian hatcheries have raised large batches of juveniles with survival as high as 34 percent from the hatchling stage. The best survival has occurred in larger tanks under partial sunlight. Species with small mouths need small rotifers, trochophores (oyster or clam larvae), copepods or other zooplankton at first feeding. Larvae of some species seem to be especially sensitive to noises (such as bumping of their tanks), which induce rapid and frantic swimming.

Providing the correct amount of turbulence in larval tanks is critical. With too little turbulence, the water stratifies (may be thermally), and zooplankton and fish can aggregate dangerously because they are attracted to bright areas of the tank. This can result in oxygen depletion, frequent collisions, and feeding difficulty. With too much turbulence, the fish are battered. Larvae stressed by fright, strong current, toxins, pathogens or malnourishment might appear exhausted or stunned, swim erratically, drift with the current, and/or not feed well. Early grouper larvae, especially when stressed, sometimes exude a large amount of mucus, which can cause them to stick to each other, to the surface film, or to solid objects. Gorging on *Artemia* (brine shrimp) is another source of mortality for mid-stage larvae, and cannibalism among early juveniles is a potential problem. Gorging can be minimized by adding the *Artemia* in small amounts and by feeding rotifers and copepods for at least several days after *Artemia* are started. Cannibalism can be minimized by feeding the fish well, weaning them as soon as possible, and removing extra large fish regularly (grading). Larvae of most species are fragile, and reported survival from eggs to juveniles often has been 1 percent or less.