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BROODSTOCK DEVELOPMENT OF GROUPERS

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ABSTRACT

The major constraint in grouper farming is the shortage and irregular availability of fingerlings from the wild at the right time. For a regular supply of sufficient quantity of hatchery-produced seeds, healthy broodstock is highly essential. Many of the culturable species of groupers are protogynous hermaphrodites. They mature initially as females and later become males; in nature this transformation would take a very long time. Broodstock can either be caught from the wild or can be developed by rearing fingerlings in captivity. The groupers used for spawning in the present study were developed by rearing fingerlings in the size range 90 to 200mm taken from the wild. Initially these were fed @10% of their body weight, and in the second and subsequent years only 5% and 2% respectively. The fish matured as females at the age of 2+ at about 450-500mm standard length. These were developed into healthy female broodstock by giving them feed enriched with vitamins and minerals. Simultaneously, a few of these were administered the male hormone methyl testosterone (MT) for sex reversal to males. Mature spermiating males were developed by this technique. The male and female broodstock thus developed spawned spontaneously when released together in the same 5ton FRP tanks, in re-circulating sea water system.

INTRODUCTION

Groupers are much relished and highly priced marine food fish in many tropical and subtropical countries. But for the high sensitivity and fragility of larvae of many of the species, serranids generally have characteristics like, fast growth, good feed conversion rate and high adaptability in different culture systems, which are favourable for culture. Development of grouper culture is one of the most important aquaculture targets in the tropics. The non-availability of sufficient quantity of seeds from the natural grounds at the right time for farming purpose is the major constraint in the farming of groupers. Studies on the reproductive biology and attempts on breeding and hatchery production of seeds of groupers are progressing actively in many countries world around.

In many southeast Asian countries, culture of groupers followed the decline of shrimp farming industry and since 1990 many shrimp farmers are forced to stop operation and began to culture sea bass and groupers in these ponds. Due to the rapid growth rate and good profit, groupers became the most important fish for culture and the farming area for groupers increased by three fold from 1987 to 1997 in this region.

Although research on breeding of groupers is going on for more than 20 years, the first success in mass propagation of grouper seeds was achieved in 1988 at Tamano sea farming association in Japan, which produced over 100,000 fry of *Epinephelus akaara* (Fugunaka *et al.,* 1990). Several attempts have been reported on spawning of quite a number of species of groupers. Natural spawning of E. *tauvina* was obtained in tanks by Hussain and Higuchi (1980) in Saudi Arabia and

Mathew *et al.* (2002) in India and E. *polyphekadion* in Saudi Arabia (James *et al.,* 1997). Toledo *et al.* (1993) and (Lim *et al.*, 1990) have reported the natural spawning of *E. suillus* and E. *fuscoguttatus* in captivity in a concrete tank and also in a floating net cage. In Singapore, although initial success obtained was in induced spawning, considerable progress has been made in achieving spontaneous spawning of *E. malabaricus*, *E. akara* (Chen *et al.,* 1977). Tucker (1994) in his review on spawning of captive serranid fishes states that many serranids will reproduce voluntarily if they are well nourished and protected from stress - mainly crowding, low water quality and disturbance.

Fishes belonging to the family Serranidae are protogynous hermaphrodites, initially maturing as females but transforming into males when they grow bigger and older (Tan and Tan, 1974). In the initial experiments in Singapore, sex reversal in groupers was effected by oral administration of methyl testosterone (MT) inserted in capsules, which were in turn incorporated into the feed of the brooders (Chen *et al.,* 1977, Chao and Chow, 1990). They found that the most effective form of treatment was MT liquid in silastic capsule, where all the treated fish got transformed to functional males. The MT silastic capsule was implanted into the abdominal cavity of the brooder, by making an incision of about 0.5 to 0.7cm, 3 to 4 cm anterior to the cloaca.

Disease free healthy broodstock is the most important pre-requisite in the successful production of seeds of any finfish or shellfish in a hatchery. The present paper attempts at elucidating the brood stock development and maintenance, preparation, etc. of *E. tauvina* reared from fingerlings caught from wild, in captivity in sea water recirculating system in 5 ton tanks. This is the first attempt on broodstock development and natural spawning of *E. tauvina* in captive condition in India.

BROODSTOCK DEVELOPMENT AND MAINTENANCE

The grouper breeding season in the wild is during October to March every year. In wild catches almost all groupers are females, the wild population of males of the greasy grouper *E. tauvina* in the Arabian Gulf waters was only 0.17% (Sayed and Abdel-Bary, 1999).

The availability of high quality mature spawners of both the sexes in sufficient numbers and in good condition is of primary concern in broodstock development and maintenance. This involves development of male brooders and improvement of the quality of female brooders.

Broodstock can be developed from large adult fishes caught from the sea using fish trap or vertical hand line, brought to the shore, and kept in net cages (5x5x3m) with stocking density less than 80 fish per net. They are fed at 2-5% body weight daily. To ensure effective water exchange, the holding nets are replaced monthly to remove silt and fouling organisms.

When there are limitations of getting wild adults, and difficulties in keeping large groupers alive, it is necessary to develop broodstock by rearing juveniles to adults. For breeding purposes, some of the female groupers need to be artificially transformed to sexual males.

Rearing can be carried out in floating net cages (5x5x3m) anchored within sea enclosures where the water salinity is around 28-34ppt throughout the year, or in earthern ponds or in indoor tanks as in the present study. This method enables the history of the broodfishes to be traced. On attaining a mean individual weight of 1kg, the fish were stocked in brooder cages of 50mm mesh size at a density of 10kg/m^2. Feeding at this stage was with trash fish at 5% body weight. Further when the fishes have grown into the second year, the brooders are usually fed at 2.5% of body weight. The fishes can then be transferred to 150 ton round shaped concrete tanks or ponds as in Taiwan. Induce maturation can then be given using HCG and/or LHRH-a for egg development and ovulation with a typical dose of 500-1000 IU/kg body weight. Usually two injections are required and ovulation occurs within 36-50hrs, depending mainly on the species, initial oocyte stage and temperature. The eggs can then be artificially fertilized with male spawner, which is caught from the sea or can be left in the ponds or released into the tanks along with the hormonally transformed males for natural spawning.

In some of the southeast Asian countries due to scarcity in local availability, grouper spawners of high economic value such as *Cromileptis altivelis*, or large size spawners of *E. lanceolatus* are imported from other countries.

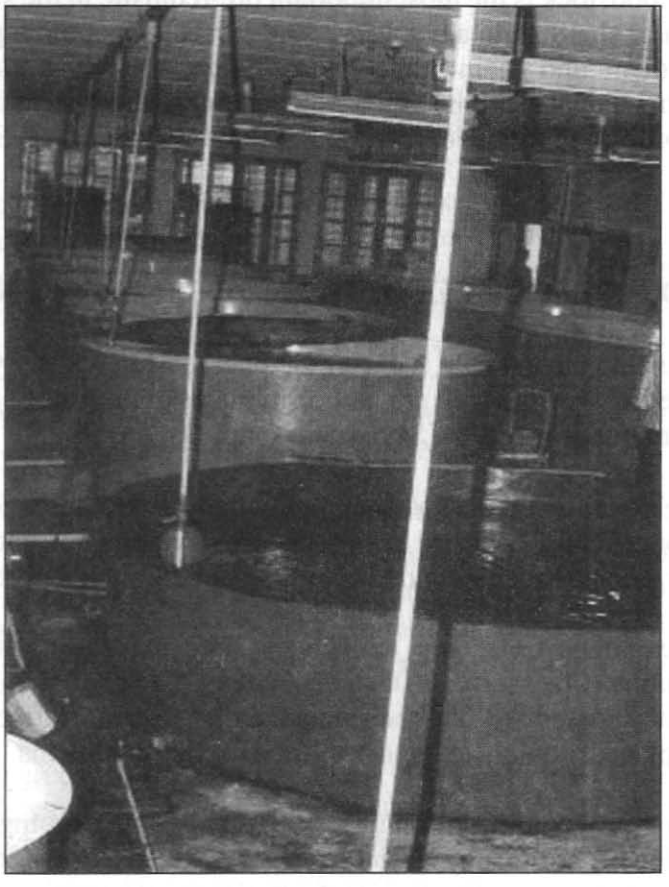

Fig.1 Grouper broodstock development facility at CMFRI

At the Cochin Fisheries Harbour Laboratory of CMFRI, where the grouper breeding experiments were carried out, broodstock of groupers *E. tauvina* and E. *malabaricus* were developed by rearing the fingerlings in indoor tanks under controlled conditions. The grouper fingerlings collected off Tuticorin were transported in oxygenated bags and stocked in 5-ton capacity FRP tanks in recirculating seawater and *in situ* biofilters (2 to 3 numbers) at the onshore rearing facility at Cochin (Fig. 1). These tanks made of FRP for rearing the groupers are cylindro-conical in shape, with smooth interior and sea-blue in colour, Initial stocking density of fingerlings of size 90-200mm was at a rate of 4 nos /m^2. The fingerlings were fed with small sciaenids, nemipterids, goatfishes and small cephalopods, taken from trawl catches, twice a day at an average of 10% of body weight, in the initial stages; after one year the fishes were fed at a rate of 4-5 % which in the second and subsequent years was 2% of body weight.

Throughout the period of broodstock rearing the hydrographic parameters of the sea water in the tanks were maintained at salinity: 30 ±2 ppt, temperature: 26 to 29°c and DO: 4-5mg /L. The pH was maintained between 7 and 8.3, and ammonia nil; phosphate was kept below the tolerance limit at <60µg atom /L, and nitrite at 0µg atom/L. (Fig.2). The biofilters served for removal of nitrogenous wastes from the metabolites and for recirculating the water.

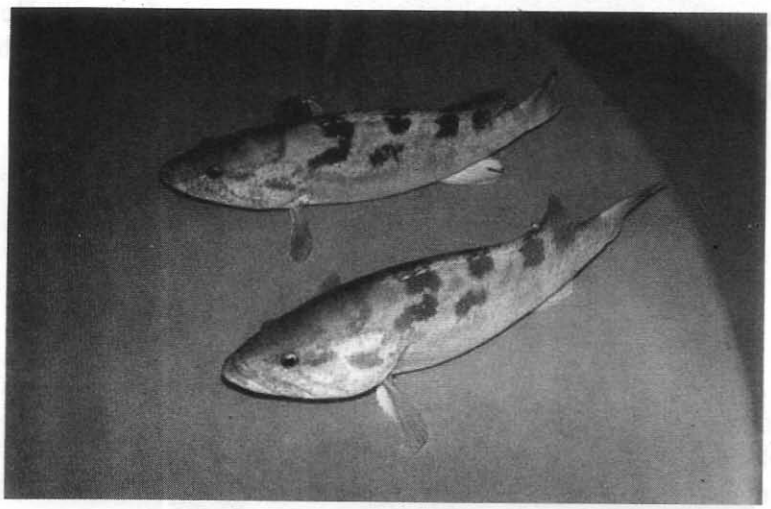

Fig.2 Water quality and growth of broostock in the culture system

Fishes were periodically examined for gonadial condition through biopsy. Care was taken to ensure that the fishes remained free of pathogens. They were treated (dip or bath) with 10-20 ppm furacin (9.3% nitrofurazone), for controlling bacterial infection and 100-ppm formalin for other ectoparasitic infections.

Experiments carried out at the Fisheries Harbour Laboratory of CMFRI, Cochin on the effect of photoperiod on the gonadial development of groupers showed that by subjecting the pre adult fishes to reduced light for a period of 2 months just at the beginning of post monsoon season enhanced gonad growth and maturation compared to the control.

Hormone induced acceleration of sex reversal

Holding large males under captivity for breeding is difficult and hence hormonally transformed males are used here for breeding purpose. Synthetic androgens such as 17α methyl testosterone (MT), mibolerone are generally used to stimulate sex reversal in groupers. In the greasy grouper *E.tauvina*, and the malabar grouper *E.malabaricus* natural males were generally not obtained from the wild along our coast. Spawning of these were resorted to by transforming female fishes to males hormonally. Experiments were carried out to identify suitable methods of administration of the hormone, like intra muscular injections, hormone pellet implantation and oral administration using different hormone preparations. Oral administration through feed was found to be more suitable in our condition as the other methods entail the fishes to be netted out and handled, which was found stressful to the brooders.

Female fishes of 2+ age, weighing 3.2 to 4.3 kg and measuring 54 to 61cm total length were selected and the male hormone 17α methyl testosterone was administered orally, by making pellets using cholestrol and gum acacia, through trash fish, at an average dose of 3mg/kg body weight. These fishes were examined periodically for the presence of milt. Oral administration of 17α methyl testosterone for a period of 2 months transformed the female into spermiating male. A gentle pressure on the abdomen of this spermiating male yielded milt. Development of this technique of hormonal sex transformation enabled release of spawners of both the sexes into the same tank resulting in natural spawning of these species of groupers (Fig. 3).

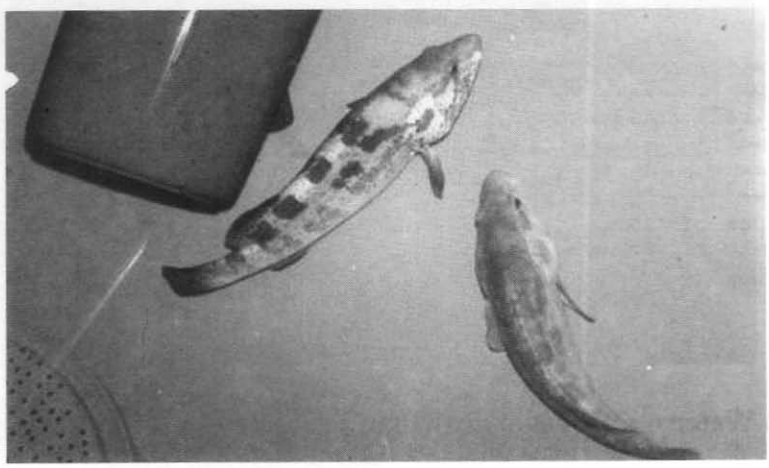

Fig.3 Broodstock of *E.tauvina*

Enrichment of broodstock diet and broodstock preparation

The brood fishes were normally fed trash fish including squid meat or other cephalopod meat at 2-2.5% body weight daily at about 10 a.m, supplemented with commercially available highly unsaturated fatty acid boosters in order to enhance the dietary essential fatty acids (EFA). Prior to spawning the broodstock were also fed Vitamins A, E and ascorbic acid twice a week by

was observed that the dietary enrichment of the broodstock has a significant effect on the enhancement in certain characteristics of egg quality, egg hatching rate and larval survival.

Potential female spawners were selected on the basis of egg size and yolk deposition. Ovarian biopsies were carried out by inserting surgical polyethylene cannula with inside diameter slightly larger than ovulated egg diameter, taken from females. Those with high percentage of stage IV oocytes having average diameter of 380-420μm were selected. Spawning success was more with fish having oocytes in fully yolked to early hydration stage. The ratio of male and female released in the spawning tank was 1:1 to 1:2. Hydration was indicated by noticeable swelling of the abdomen of the female brooders, and the ovulated egg mass could be seen protruding through the genital opening (Fig. 4).

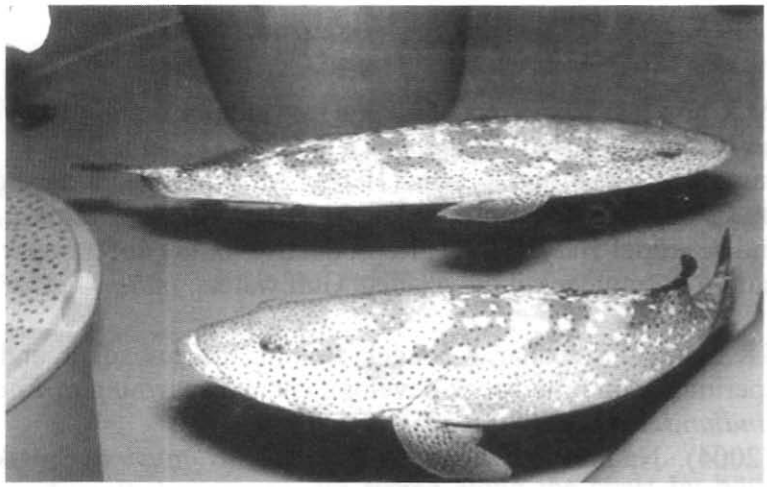

Fig.4 Broodstock of *E malabaricus*

Fully mature spawners normally spawn during 1600-2300 hrs, though a few species like *E.akaara* and *C.striatus* spawn in the morning (Tucker, 1994). In captivity many serranids spawn voluntarily under stressfree and well-nourished conditions. Mature broodstock of *E.tauvina* spawned naturally without any hormonal inducement during almost all the months of the year, during its natural spawning season as well as non-spawning season (Grace Mathew, in press).

CONCLUSION

Many species of serranids are good candidates for aquaculture due to their high meat value, good feed conversion rate and fast growth rate. Sedentary species like *E. fuscoguttatus, E.malabaricus, E.tauvina, E. striatus, E.polyphekadion* and *E.guttatus* adapt well to captivity and tolerate handling (Tucker, 1994). Broodstock of most of these species developed under captivity are capable of spawning naturally if they are provided with good nourishment, grown in good quality water, and protected from stress, disease and disturbance. At the F.H.L. of CMFRI, where high water quality was maintained in the brooder tanks, broodstock were well nourished

with enriched diet, free from disease and disturbances it was possible to spawn *E.tauvina* 15 times, during the period from October 1998 to December 2000, in all the months of the year.

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REFERENCES

Chao , T.M., and M. Chow, (1990). Effect of methyl testosterone on gonadal development of *Epinephelus tauvina* (Forskal). *Singapore J. Pri. Ind.* 18: 1-14.

Chen, F. Y., M. Chow, T.M. Chao, and R. Lim, (1977). Artificial spawning and larval rearing of the grouper, *Epinephelus tauvina* (Forskal) in Singapore. *Singapore J. Pri. Ind.* **5**: 1-21.

El Sayed, A.M., and K. Abdel-Bary, (1999) . Reproductive biology of grouper fish *Epinephelus tauvina*(Family Serranidae) in the Arabian Gulf waters. *Indian J. Mar . Science.* Vol:**28** pp89-91.

Grace Mathew, N.K.Sanil, N.Sreedhar, K.S.Leela Bhai, L.R.Kambadkar, and N.Palaniswamy, (2002). Experiments on broodstock development and spawning of *Epinephelus tauvina* (Forskal) . *Indian J. Fish.*, **49**(2) pp.135-139.

Grace Mathew, (2004). Natural spawning of *Epinephelus tauvina* (Forskal) in captivity. (in press).

Hussain, N. A., and M. Higuchi, (1980). Larval rearing and development of the brown spotted grouper, *Epinephelus tauvina* (Forskal). *Aquaculture* . **19**: 339-350.

James, C.M., S.A. Al-Thobaiti, B.M. Raseem, and M.H. Carlos, (1997). Breeding and larval rearing of the camouflage grouper *Epinephelus polyphekadion* (Bleeker), in the hypersaline waters of the Red sea coast of Saudi Arabia. *Aquaculture Research.* **28**: 671-681.

Lam,T.I., (1983). Environmental influence on gonadal activities in fish. In *Fish Physiology,* Vol. IX, *Reproduction part B, Behaviour and fertility control* (edited by W.S. Hoar, D.J.Randall and E.M. Donaldson.) pp 187-237. West View Press, Boulder, Colorado, USA.

Lim, L.C., (1993). Larviculture of the greasy grouper *E. tauvina* (F.) and the brown- marbled grouper *E.fuscoguttatus* (F.) in Singapore. *Jour. Worl. Aquacult. Soc.* **24**:262-274.

Lim, I. C., T.M. Chao, and L.T. Khoo, (1990). Observations on the breeding of brown- marbled grouper, *Epinephelus fuscoguttatus* (Forskal). *Singapore J. Prim. Ind.* **18**: 66-84.

Toledo, J.D., Akinasa Nagai, and J. Danillo, (1993). Successive spawning of grouper, *Epinephelus suillus* (Valenciennes) in a tank and a floating net cage. *Aquaculture.* **115**: 361-367.

Tucker J. W.,(1994). Spawning of captive Serranid fishes: A review. *Jour. Worl. Aquacult. Soc.* **25**(3): 345-359.