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Experiments on broodstock development and spawning of *Epinephelus tauvina* (Forskal)

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ABSTRACT

The present paper reports on broodstock development and spawning of the greasy grouper *Epinephelus tauvina* in indoor 5 ton capacity FRP tanks in recirculating sea water system, under controlled conditions. The broodstock was developed in the culture system by rearing the wild fingerlings, caught in the size range 90 to 200mm. After attaining maturity, the fishes were fed with supplementary diet for developing them into mature females. Simultaneously, a few were administered the male hormone methyltestosterone (MT) for sex inversion to males. Mature spermiating males were developed by this technique. A mature female fish weighing 3.85 kg and a single spermiating male weighing 3.25 kg have spawned spontaneously in the same indoor tank consecutively for two days from 29-10-1998 to 30-10-1998 and also from 20-12-'98 to 23-12-'98, producing approximately 2,50,000 eggs in each spawning. On both the occasions, the same hormonally sex inverted male has spermiated. Fertilization rate was over 90%. Eggs measured 0.920 mm with a single oil globule. The eggs hatched out at 23 hours. Hatching rate observed in the first spawning was 60% and in the second instance, 45%. Newly hatched larvae measured 1.74mm. After 68 hours the larval mouth has opened and feeding has started.

Introduction

Groupers are highly priced marine foodfish in many tropical and subtropical countries. They are protogynous hermaphrodites, reversing their sex from females to functional males at an older age. According to Tan and Tan (1974), *Epinephelus tauvina* first matures as female at 45-50 cm and 2.5 to 3.0 kg; beyond 72 cm (10 kg) all are males, with transitional gonads occurring in specimens of size 62-70 cm. Males are larger in size, fewer in number and occur in deep seas. Holding large males under captivity for breeding is difficult and hence hormonally transformed males are often used. Studies carried out by Chen *et al.* (1977), Chao and Chow (1990) revealed that the male hormone, methyl testosterone administered orally and through pellet implantation in varying doses could transform mature females as well as immature fishes into functional males. Mature *E. tauvina*, caught from the wild were induced to spawn in Kuwait (Hussain and Higuchi, 1980). Induced spawning of *E. tauvina* and sex reversal to males have been achieved in Singapore (Chen *et al.*, 1977); although here initial

success obtained was in induced spawning, considerable progress has been made in achieving spontaneous spawning. Natural spawning of *E. malabaricus*, *E. akara* and *E. polyphekadion* (James *et al.*, 1997), was obtained in tanks and for *E. fuscoguttatus* (Lim *et al.*, 1990) and *E.suillus* (Toledo *et al.*, 1992) in floating net cages. Hormone-induced spawning in net cages and larval rearing of *E. tauvina* was reported by Chen *et al.* (1997), so also of *E. salmoides* (Kunjuvankiu *et al.*, 1986) and of *E. fuscoguttatus* (Kohno *et al.*, 1990). The present paper presents the results of experiments conducted on broodstock development and spontaneous spawning of *E. tauvina* in onshore controlled conditions.

Materials and methods

Broodstock Development

Grouper fingerlings collected from Tuticorin were transported in oxygenated bags and stocked in 5-ton capacity FRP tanks in recirculating seawater with *in situ* biofilters (2 to 3 numbers) at an onshore rearing facility of the Central Marine Fisheries Research Institute at Cochin Fisheries Harbour (Fig. 1). The tanks, sea-blue in colour are cylindroconical in shape, with smooth interior. Initial stocking was done on 7-3-1996, at a rate of 4 nos/m^2. The fingerlings were given prophylactic treatment before stocking. Later, they were treated whenever there was an occurrence of bacterial, fungal or parasitic infection. Bacterial diseases, mainly vibriosis was frequently encountered, especially during summer. This was controlled by giving bath treatment with oxytetracyclone at a rate of 1 gm/50 liter sea water for 1 hour duration, twice a day for 4 days. The fingerlings were fed with small sciaenids, nemipterids, goatfishes and small cephalopods, twice a day at an average rate of 10% of their body weight, in the initial stages; after one year the fishes were fed at a rate of 4-5% of their body weight.

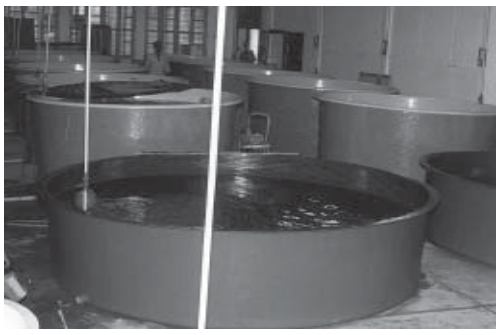

Fig.1. The indoor rearing tanks

Seawater was pumped from the adjoining Mattancherry canal at the peak high tide. Salinity was maintained between 28 and 32 ppt, temperature 26.5^0-29^{0}C, pH 7-8 and an optimum dissolved oxygen in the range of 4-4.5 ml/L. The biofilters served in filtration, removal of nitrogenous wastes from the metabolities and water recirculation.

From January 1998 onwards, the fishes were fed at the rate of 2% of the body weight; the regular feed was also enriched with cod-liver oil and vitamin E. The fishes were periodically examined for gonadial conditions 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.

Results

Sex-inversion and spontaneous spawning

One female fish weighing 3.2 kg and measuring 54 cm was selected and the male hormone, 17-α-methyl testosterone was administered orally, by making pellets using cholesterol and gum acacia, through trash fish, at an average dose of 3 mg/kg body weight, from 1-9-1998. This fish was examined periodically for milt.

A mature female of the same age, weighing 3.8 kg was released into the same tank at the same time for socialisation. Oral administration of 17-α-methyl testosterone for a period of 2 months transformed the female into a spermiating male. A gentle pressure on the abdomen of this spermiating male yielded milt. On 29-10-1998, a female weighing 3.85 kg and the above artificially sex transformed male weighing 3.2 kg have spawned spontaneously for the first time in the tank, between 1800 and 2200 hrs. Spawning occurred again on 30-10-1998, between 1700 and 2000 hrs by the same pair. The buoyant eggs were collected from the spawning tank by the overflow method using a 300 μ m mesh net. The number of viable, fertilized floating eggs and the unfertilized sunken eggs were estimated by taking an aliquot in a measuring jar and counting them. Samples of fertilized eggs were examined under a microscope and the sizes of the ova and oil globule were measured. The fertilized eggs were washed and kept in incubation tanks with filtered, UV-treated, and pre-conditioned sea water of salinity 32 ppt for hatching with mild aeration. The eggs hatched out within 22 to 23 hrs. The newly hatched larvae possessed yolk sac and a posteriorly placed, single large oil globule. These were released into 300L capacity black and blue coloured FRP tanks containing pre-treated sea water of salinity 32 ppt, at the rate of 30 nos/L. A gentle water flow was maintained in the larval rearing tanks so that a replacement of 10% of water is obtained per day. Water temperature, pH and dissolved oxygen were monitored at three hour interval.

Another spawning occurred on 20-12-1998; the same hormonally transformed male spawner mated with another female weighing 4.96 kg. Spawning activity has continued upto 23-12-1998.

Egg quality and hatching

Spontaneous spawning of *E. tauvina* in the broodstock tanks at two occations has produced on an average 0.25 million eggs. Ova diameter ranged from 880 to 920 μm, with a single oil globule, measuring 190 μm. Fertilization rate was 85-92%. The eggs produced during October and December spawnings were of good quality (Fig. 3.) Eggs from October spawnings had better fertilization and hatching rates than those produced during December. Average size of eggs obtained in the first spawning during October was 900 μm while those from the subsequent spawning in December were 880 μm.

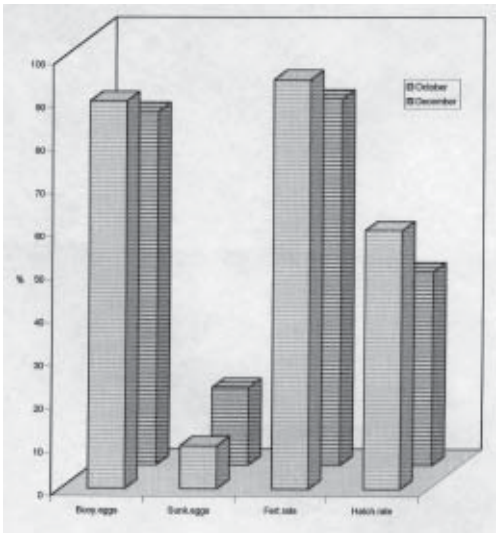

Fig.2. Egg quality, fertilization and hatching rates obtained in the experiments on *E. tauvina*

The newly hatched larvae measured 1.68 to 1.74 mm in length, transparent, characterized by a fairly large yolksac, unpigmented eyes and a single large oil globule measuring 0.19 mm, lying at the posterior end of the yolksac (Fig 3). They measured 2.64 mm in total length after 56 hours. At this stage pectoral fins developed, jaw buds appeared, gut in-

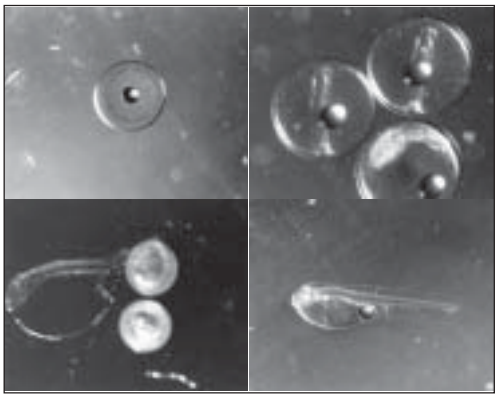

Fig.3. Fertilized eggs and newly hatched out larvae of *E. tauvina*

creased in length, oil globule and yolk sac diminished in size. At about 65 to 68 hours after hatching, the larval mouth has opened, gut showed contractions, anus opened and eyes became pigmented. At this stage, a number of melanophores have developed along the dorsal and ventral portion of the alimentary canal and the larvae have commenced feeding on minute live feed organisms.

Discussion

The androgen, methyl testosterone (MT), has been successfully used in accelerating the sex reversal process both in immature and mature female groupers. It is established that the dormant testicular tissues present along the germinal epithelium of the gonads of groupers, under the influence of the male hormone (MT), are activated to spermatogenesis (Chao and Chow, 1990). Methyl testosterone acts as an andro-inducer, accelerating sex transformation of immature as well as mature female grouper. In the present study, oral administration of 17-α-methyl testosterone for a period of 2 months transformed the fully mature female into a spermiating male, through regression of the ovarian tissues and simultaneous activation of spermatogenesis of the testicular tissues. The artificially transformed male, a two year old fish, spawned after 58 days of hormone administration, with a female of the same age. In many species of groupers like *Epinephelus fuscoguttauts, E. suillus, E. striatus, E. polyphekadion* and *E. tauvina*, a close association of spawning periodicity with lunar cycle and tidal effect has been reported by many authors. Lam (1983) suggested that in many of the tropical and subtropical species of fishes, peak spawning activity is often associated with lunar cycle, rainfall or floods. In the present investigation, a spontaneous spawning of *E. tauvina* during October and also in December occurred 3-4 days before or after the new moon phase. In Kuwait, Hussain and Higuchi (1980) reported *E. tauvina* spawning continuously for nearly 50 days in 90 m tanks while the spawning run in the present study lasted for 2 to 3 days.

Chen *et al.* (1977) reported the diameter of fertilized eggs of *E. tauvina*, as 0.90 mm and total length of the newly hatched larva as 1.70 mm., which is almost similar to that obtained in the present study. Hussain *et al.* (1975) have reported the average diameter of egg as 0.77 mm, and that the newly hatched larvae measured 1.4 to 1.5 mm. In subsequent studies, Hussain and Higuchi (1980) recorded the total length of newly hatched larvae of *E. lauvina* as 2.25 mm. In Singapore, Lim (1993), recorded the average egg diameter as 0.80 mm for *E. tauvina.* The variations observed in the size of eggs and larvae may be attributed to the condition of the broodstock, type of spawning and also to the season of spawning. It has been found that fertilized eggs of groupers vary between 700 and 960 μm in size. There is also a significant correlation between the egg diameter and the hatching rate. Lam (1983) and Chao and Chow (1990) have pointed out that high levels of D H A and E P A are essential in the broodstock diet to obtain good quality eggs as well as to enhance larval survival in *E. tauvina.* In

general, eggs obtained through induced ovulation are smaller than naturally ovulated eggs.

Fertilization rate (92%), and hatching rate (upto 65%), obtained in the present investigations are higher than that obtained for the same species in Kuwait where fertilization was only 9% and hatching rate 24% (Hussain and Higuchi, 1980). Egg quality, in terms of percentage of buoyant eggs in the present study is comparable to that obtained elsewhere for the species (Hussain and Higuchi, 1980). Larval rearing is often controlled by the availability of suitable feed.

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