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Spontaneous spawning of *Epinephelus tauvina* (Forskal) in captivity

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Abstract

This paper describes natural spawning of the greasy grouper *Epinephelus tauvina* in captivity in recirculating sea water system under controlled conditions. The broodstock was developed in the culture system by rearing the wild fingerlings (lengh range: 90 to 200 mm). Females matured within two years. *E. tauvina* being a protogynous hermaphrodite, male spawners were developed through hormonal sex inversion of females. Natural spawning occurred in almost all the months of the year; 9 times during October 1998 to July 1999 and 9 times during the period from October 1999 to December 2000. On all occasions, spawning occurred between 1500 and 2000 hrs and continued for 3 days. The same pair of male and female spawned continuously 4-6 times in a year. Single female released, on an average, 50,000 eggs/kg body weight on each day of spawning. Spawning was mostly related to lunar cycle, coinciding with the last or first quarter of lunar cycle. Fertilization rate obtained in the present study varied from 60 to 99%. Egg diameter ranged from 0.720 to 0.910 mm, with a single oil globule. The eggs hatched out in 22 – 23 hours. Hatching rate ranged from 45% to 80%. Newly hatched larvae measured 1.74 mm.

Keywords: Natural spawning, grouper, *Epinephelus tauvina*, in captivity

Introduction

Groupers have white, tender and tasty meat that make them the much relished and highly priced marine food fish in many tropical and subtropical countries. But for the high sensitivity and fragility of larvae, many of the species of serranids generally have characteristics which are favourable for culture. Many species are suitable for mariculture because of fast growth, good feed conversion rate and high adaptability in different culture systems. Development of grouper culture is one of the most important targets in the tropics. High demand for these fishes in the international markets has led to indiscriminate fishing resulting in the depletion of the natural stocks to an alarming level.

Added to this is the non-availability of sufficient quantity of seeds from the natural grounds at the right time for farming purposes. Attempts on breeding and hatchery production of seeds of groupers are progressing actively in many countries.

Several attempts have hitherto been made on spawning of several species of groupers. Artificial spawning and larval rearing of *Epinephelus tauvina* was carried out by Chen *et al.* (1977) in Singapore, while natural spawning of the same species was observed by Hussain and Higuchi (1980) in Saudi Arabia. Natural spawning of *E. akaara* was observed in tanks (Ukawa *et al.,* 1966). Toledo *et al.* (1993) have reported natural spawning of *E. suillus* in captivity in a concrete tank and also in a floating net cage. In Singapore, although initial success was obtained in induced spawning, considerable progress has been made in achieving spontaneous spawning of *E. malabaricus* and *E. akara (*Chen *et al.,* 1977). Natural spawning of *E. fuscoguttatus* (Lim *et al*., 1990) and *E. polyphekadion* (James *et al.,* 1997) was obtained in tanks. Tucker (1994) in his review on spawning by 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. The present paper elucidates the natural spawning, egg

production, egg quality etc. of *E. tauvina* broodstock reared from wild caught fingerlings in captivity in sea water recirculating system in 5 ton tanks. This is the first attempt on natural spawning of *E. tauvina* in captive condition in India.

Material and Methods

Broodstock and Spawning: Natural spawning experiments of the greasy grouper *E. tauvina* were carried out during October 1998 to July 1999 and again from October 1999 to December 2000, at the Fisheries Harbour Laboratory of the Central Marine Fisheries Research Institute at Cochin. The spawners were developed by growing fingerlings caught from the wild in cylindro-conical 5 ton FRP tanks of light blue interior colour, in re-circulating sea water and were maintained in the same tanks indoors in sea water of salinity 32 ppt, dissolved oxygen >4.0 ppm and ammonia N-level 0.01- 0.02 ppm. Temperature in the system ranged from 26-29º C and pH was maintained between 7.8 and 8.3. The height of water column in the tanks was restricted to 1.1 m. Light intensity in the spawning tanks varied between 350-400 lux.

Female spawners measured 585.4 ± 2.8 mm to 720.2 ± 1.8 mm in total length and 3798.6 ± 2.4 g to 6202.4± 3.4g in body weight; males of 538.7 ± 4.8 mm and 721.2 ± 3.9 mm total length and body weight 3247.7 ± 4.9 g and 7098.3 ± 2.8 g . Broodstock were fed on trash fish including small squids, cuttlefish and octopus, supplemented with Vit. E, Vit. B12, ascorbic acid and sea cod for providing essential enrichment of 20:5w3 eicosapentaenoic acid (EPA), 22:6w3 docosahexaenoic acid (DHA) and polyunsaturated fatty acids. Feeding was *ad libitum* at around 10 am daily. The remains of feed and faecal matter were siphoned out and the water lost was replaced with fresh seawater. Gonadal maturity of females was monitored by inserting a cannula of inner diameter 1.5 mm through the urinogenital opening and biopsy examination of gonads was carried out. A mature female was identified when vitellogenic eggs were obtained on biopsy. A gentle pressure on the abdomen of mature males showed presence of milt.

Male spawners were developed through hormonal sex inversion of females. The male hormone 17 a methyl testosterone was made into pellets using cholesterol and gum acacia, implanted into trash fish and fed. Hormone was administered orally at an average dose of 3 mg/kg body weight and the fishes were examined periodically for the presence of milt. Stocking density was restricted to 1 male : 1 female or 1male : 2 females. Photoperiod regime followed was 8L:16 D for gonad development.

The spawning pair developed a peculiar colouration, and their spawning behavior was observed consecutively for 2 to 3 days.

Quality of eggs and egg production: Transparent, buoyant eggs were observed in the tanks after the spawning activity took place. Aliquot samples were examined periodically under a compound microscope for recording the egg diameter, oil globule size, fertilization rate and further embryonic development. Total number of eggs was estimated by counting three replicate samples from the tank water. All the viable buoyant eggs were siphoned out from the tanks on the following morning at about 0800 hrs after the embryo has almost reached eyed stage. The buoyant eggs were collected using 400 mm mesh plankton sieve, washed with clean seawater in order to remove dirt and debris attached to them and incubated at ambient temperature in 300-500 L capacity fiberglass tanks, at a stocking density of 250-300 eggs L^{-1}. Incubation tanks were provided with moderate aeration. Unfertilized opaque eggs found sunken to the bottom of the tank were collected, numbers recorded and discarded.

Results

***Spawning*:** From 18 instances of natural spawning during the experimental period, 9.2 million eggs were collected, with an average fertilization rate of 90%. Natural spawning of *E. tauvina* occurred 9 times during October 1998 to July 1999 and 9 times during October 1999 to December 2000. On every occasion, the spawning run by a single pair lasted for 2 or 3 days. At the time of spawning the male developed a whitish pale colour while the female was greenish brown above and white below. Actual spawning activity started from 3 pm and continued up to 8 pm. Prior to spawning, the spawning pair swam together, contacting each other

(Fig.1). The pair ascended rapidly in the water column with anterior part of their bodies exposed and released the gametes while dashing into the water.

Fig. 1. Pre-spawning behavior of the spawning pair

Captive spawning of *E. tauvina* in the indoor 5 ton FRP tank in re-circulating seawater occurred for the first time on 29th October 1998, four days before full moon and continued on the next day. The male to female ratio was 1:1; the female measured 591.7 ± 2.5 mm in total length and weighed 3848.6 ± 3.3 g. The male fish was of total length 543.5 ± 3.7 mm and weight 3247.7 ± 3.2 g. Subsequent spawning by the same pair in December coincided with the new moon day. In the present study, on most occasions there was a close correlation of spontaneous spawning with the lunar phase. Spawning occurred either during the last phase of the lunar cycle (41.6%) or on days of full moon (25%) or new moon (33.3%). The same pair of spawners spawned on 16th May 1999 which was a new moon day; after a gap of four days again they started spawning in the last quarter moon phase and continued up to 25th May. Spontaneous spawning, which occurred in June as well as December, coincided with the full moon phase and the last quarter before full moon respectively. However, there was no significant correlation between the number of eggs released and lunar periodicity on any of the occasions. A single female of 619.6 ± 3.8 mm total length and 4878.2 ± 2.6 g weight consistently released eggs during May, June and July 1999.

Egg production and quality of eggs: Egg production ranged from 0.29 million to 0.5 million (Fig. 2). The females released an average of 50,428 eggs /kg body weight on each day of spawning. Eggs were transparent and spherical with a smooth chorion. Ova diameter ranged from 880 mm to 910 mm, with a single oil globule, measuring 190 mm (Fig. 3). Good quality eggs possessed a single large

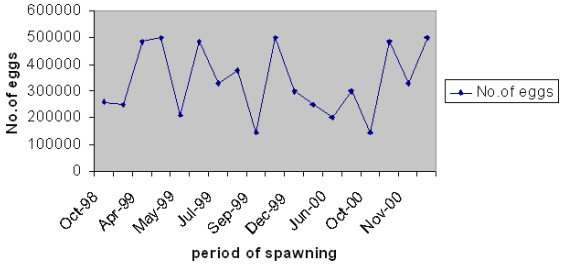

Fig. 2. Production of eggs during natural spawning

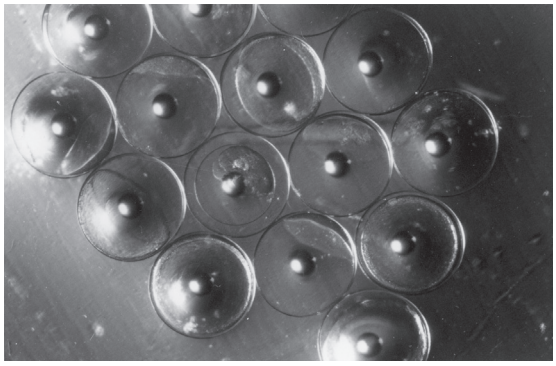

Fig. 3. Fertilized eggs of *E. tauvina*

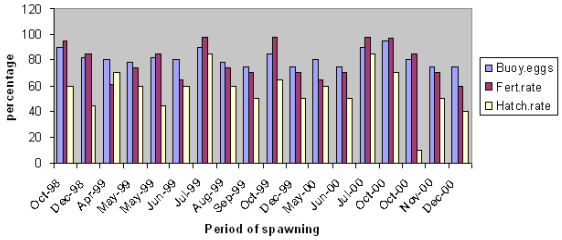

Fig. 4. Quality of eggs produced during natural spawning of *E. tauvina*

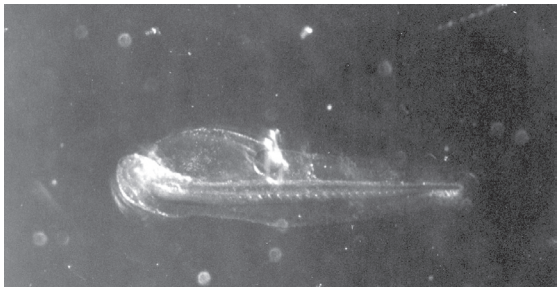

Fig. 5. Newly hatched larva of *E. tauvina*

oil globule. Fertilization rate obtained in the present study was 85-99%. During all the spawning runs, the eggs released were of good quality in terms of buoyancy and fertilization rate (Fig. 4). The fertilized buoyant eggs hatched out in 22 to 23 hrs after spawning. Newly hatched larvae measured 1.70 to 1.75 mm in length, with an oval yolk sac and a single oil droplet situated at the rear of the yolk sac (Fig. 5). Rate of hatching varied from 45% to 80%. Though larval rearing was attempted on most of the occasions, larval survival could not be achieved beyond thirteen days.

Discussion

This study shows that the greasy grouper is capable of spawning naturally under captive conditions as already reported by Hussain and Higuchi (1980). The earlier reports on natural spawning were either in ponds or in tanks of very large capacity. Toledo *et al*. (1993) reported year round spontaneous spawning of the grouper *E. suillus*. Shapiro (1987) reported that most species of groupers exhibited some lunar periodicity of spawning which is evidently seen in the case of the greasy grouper *E. tauvina* in the present set of experiments. In many species of groupers like *Epinephelus fuscoguttatus, 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 sub-tropical species of fishes, peak spawning activity is often associated with lunar cycle, rainfall or floods.

Okamura *et al.* (2002), reporting on the low fertilization rate obtained in the case of *E. akaara* cultured in tanks, states that this is probably be due to poor nutritional value of broodstock feed or other reasons like stress under high population densities in the tanks. In the present study, although hormonally sex - inverted males were used, fairly high rate of fertilization and hatching rate were obtained, which reflects the health of the spawners, mainly by adopting a low stocking density and good management procedures. Hamamoto *et al.* (1986) and Manabe and Kasuga (1988) reported spawning behaviour of malabar grouper *E. malabaricus* and *E. moara* in aquarium tanks of 2 m depth where the spawning pairs swam at the surface with their heads out of water prior to spawning and fertilization rate obtained was under 5% which could be due to the limited space in the tank.

Egg fertilization rate (up to 99%), and hatching rate (up to 80%) obtained in the present investigations are much higher than those obtained for the same species in Kuwait, where fertilization rate was only 9% and hatching rate 24% (Hussain and Higuchi, 1980). 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) 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. tauvina* 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 mm in size. There is also a significant correlation between the egg diameter and hatching rate. Lam (1983) and Chao and Chow (1990) have pointed out that high levels of D H A (Docosahexaenoic acid) and E PA (Eicosapentaenoic acid) are essential in the broodstock diet to obtain good quality eggs as well as to enhance larval survival in *E. tauvina*. Tucker (1994), reviewing spawning of captive serranids, states that egg quality could vary with the type of spawning, condition of broodstock, spawning season, and size and age of the female. Good nutrition, especially proper quality and quantity of fat ingested by females are important to ensure high quality of eggs. In general, eggs obtained from natural spawning are bigger in size and better in quality than those obtained through induced ovulation. Viability of artificially spawned eggs is also likely to fluctuate.

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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.

Hamamoto, S., S. Manabe, A. Kasuga and K. Nosaka. 1986. Spawning behaviour and early life history of the malabar grouper *Epinephelus salmoides* (LACEPEDE) in an aquarium. *Saibai Giken,* 15: 143 - 155.

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

Hussain, N. A., M. Saif and M. Ukawa. 1975. On the culture of *Epinephelus tauvina* (Forskal). Kuwait Institute of Scientific Research, Kuwait.

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. *Aquac. Res.,* 28: 671 - 681.

Lam, T. I. 1983. Environmental influence on gonadal activities in fish. *In*: W. S. Hoar, D. J. Randall and E. M. Donaldson (Eds.) *Fish Physiology, Reproduction part B, Behaviour and Fertility Control.* Vol. IX, West view press, Boulder, Colorado, USA, p. 187 - 237.

Lim, L. C. 1993. Larviculture of the greasy grouper *E. tauvina* (F.) and the brown- marbled grouper *E. fuscoguttatus* (F.) in Singapore. *J. World 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.

Manabe, S. and A. Kasuga. 1988. Spawning behaviour and early life history of *Epinephelus moara* in an aquarium. *J. Jpn. Assoc. Zool. Gard. Aquariums,* 30: 16 - 24.

Okumura, S., K. Okamoto, R. Oomori and A. Nakasono. 2002. Spawning behaviour and artificial fertilization in captive reared red spotted grouper, *Epinephelus akaara*. *Aquaculture,* 206: 165 - 173.

Shapiro, D. Y. 1987. Reproduction in groupers. *In*: J. J. Polovina and S. Ralston (Eds.) *Tropical Snappers and Groupers: Biology and Fisheries Management*. Westview Press, Boulder, Co., p. 295 - 327.

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. *J. World Aquacult. Soc.,* 25(3): 345 - 359.

Ukawa, M., M. Higuchi and S. Mito. 1966. Spawning habits and early life history of a serranid fish, *Epinephelus akaara* (Temminck and Schlegel). *Jpn. J. Ichthyol*., 13: 156 – 161.

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