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Natural spawning of honeycomb grouper *Epinephelus merra* Bloch under captive conditions

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Abstract

The paper details the observations made on the natural spawning of honeycomb grouper (*Epinephelus merra*) achieved at the Regional Centre of Central Marine Fisheries Research Institute (CMFRI), Mandapam Camp. The construction details of indoor maturation and spawning system developed for the captive maturation and natural spawning of small sized groupers are given. Sub-adults of this species were collected, maintained, developed into brood stock and transferred to indoor maturation tank for observations on natural spawning. The natural spawning occurred between August and October 2004 and corresponded to the lunar phase of each month. Number of eggs, percentage fertilization and hatching, the water quality parameters, feeding protocol adopted for sub-adults and brood stock are detailed. Disease occurrence and their control, importance of PUFA enriched feed are also discussed.

Keywords: Natural spawning, captive condition, honeycomb grouper

Introduction

Groupers of the family Serranidae are among the most commercially important fishes in the tropics. Increasing market demand for these fishes in the live reef food-fish trade has led many Southeast Asian and Pacific countries to focus attention on increasing the production by exploitation from the wild and through aquaculture (Sadovy *et al.*, 2003). Grouper aquaculture remains heavily dependant on the capture and grow out of juveniles caught from wild. Around 70-85% of cultured groupers are grown from wild caught fry (Sadovy *et al.*, 2003). Limited availability of fingerlings is one of the most important constraints to the sustainable development of this industry (Rimmer, 2004).

Forty species are reported from Indian waters. A few such as *Epinephelus tauvina* and *E. malabaricus* are considered as potential species for aquaculture in our country. (Nammalwar *et al.*, 1997). Experimental culture of groupers was initiated during 1985 and 1992 by CMFRI (James *et al.*, 1985; Hamsa and Kasim, 1992; CMFRI, 1999). CMFRI was also successful in brood stock development, sex inversion and captive spawning of *E. tauvina* and *E. malabaricus* (Pillai *et al.*, 2002), embryonic and larval development of honeycomb grouper *E. merra* (Jagadis *et al.*, 2006). The present paper discusses the results of spawning of honeycomb grouper *E.merra* under indoor maturation system developed at the Regional Centre of Central Marine Fisheries Research Institute, Mandapam Camp.

Materials and methods

Collection and stocking of sub-adults: Sub-adults of *E. merra* were collected live from the trap catches of various landing centres along Gulf of Mannar mainly from Mandapam and Keelakarai. They were transported to finfish hatchery at RC of CMFRI in well aerated plastic bins of 100 l capacity. The transportation duration varied between 1to 5 hrs. On arrival at the hatchery, the fishes were given prophylactic treatment with malachite green (0.05mg/l) and quarantined in outdoor RCC tanks.

Two outdoor rectangular cement tanks, each of seven ton capacity, were used for acclimatization and maintenance of this wild stock comprising of juveniles and sub-adults. The tank was provided with hideouts for these fishes to take shelter and also partial cover for avoiding direct sun light. The growth and weight gains were observed before stocking into experimental spawning tanks. Five ton filtered seawater was maintained and the water depth in the tank was 1.0m. A total of 46 fishes having an average size of 273 mm and weight 340g were stocked in these tanks for developing the brood stock. Water quality parameters were monitored regularly. Freshly collected trash fish was fed at the rate of 10% of the total

weight of the stocked fishes on alternate days. Cleaned whole squids were given as supplementary feed twice a week and 50% water was exchanged every alternate day. Rearing tank was cleaned once in a week for removing the algal growth in the bottom. The stocked fishes were measured once in a month for recording their growth and survival.

Indoor maturation system: Five ton circular FRP tanks (dimension 145 cm dia. x 120 cm h.) were used to construct indoor maturation and spawning tanks. A set of three such systems was constructed and used for maturation and spawning experiments. The water was recirculated through airlift system made by constructing a square frame with 3" perforated PVC pipe wound with fine mesh velon netting (500µ) and with four vertical stand pipes fitted in corners of the square frame. An airline was inserted to each of the standpipe. Over the frame, coral rubbles (20-25mm) were spread compact to a height of about 15cm and on top of it a layer of 5-10cm coarse coral rubbles were provided. A velon net screen having a mesh size of 1.0mm was provided between the two layers. Water volume was maintained uniformly at 4.0 ton in each of the tank. The airlift was effected by a twin lobe oil free air blower on continuous basis. Airlift of water was so adjusted that an overturn of 400 percent of water was effected daily. The tank was covered partially with black cloth to curtail over exposure of light.

Brood stock maintenance and spawning: In each maturation and spawning system, four healthy fishes were stocked. The fishes with size/weight range from 290 to 410mm/350 to 750g were segregated from the outdoor RCC tanks and transferred to each of the indoor maturation and spawning tanks. The water quality parameters such as temperature, salinity, dissolved oxygen, *p*H and ammonia were monitored at monthly intervals. 100% water exchange was given in the indoor maturation and spawning tanks once in three months. The general health of the stocked fishes was monitored and prophylactic treatments were provided as and when required. Under indoor maturation system, the brood stocks were fed primarily with marine trash fishes on alternate days at the rate of 10% of their body weight. Freshly collected marine trash fishes were cleaned thoroughly before feeding. In addition to trash fishes, squids were given as supplementary brood stock diet at 10% of body weight twice in a week. To improve the nutritional quality of the feed given to the brood stock, cod liver oil and vitamin capsules were kept in the visceral cavity of trash fishes and fed individually so that all the spawners in the maturation and spawning tank were equally provided with sufficient polyunsaturated fatty acid rich feed.

Results

Year round availability of *E. merra* sub-adults were noticed in the landing centres of Gulf of Mannar. The maximum availability was during March and July. Mortality of fishes during transportation was found to be nil during the study period.

Growth and survival of sub adults: There was no wide fluctuation in the water quality parameters. (Table.1). During the 7 month study, on the growth of sub adults in outdoor tanks, fishes had grown to 305mm in mean length and 430g in average weight from the initial men sizes (Table 2). The specific growth rate recorded was 3.35g/month.

Growth and maturation: Three experiments were conducted to ascertain the efficiency of the indoor maturation system. The details of the water quality parameters, growth and maturation of the fishes have been monitored (Table 3, 4, 5)

Trial 1: In the first trial (Tank-1) the four fishes that

Table 1. *Hydrographical parameters in the outdoor RCC acclimatization and maintenance tanks*

Tank No.	Temperature (° C)	*p*H	Salinity (ppt)	DO_2 (ml/l)
1	28.9-30.5 (29.6)	7.9-8.5 (8.2)	32.0-33.8 (33.4)	4.96-5.41 (5.13)
2	29.1-30.7 (29.9)	7.9-8.4 (8.2)	32.3-33.7 (32.9)	4.97-5.36 (5.20)

Table 2. *Growth and development of* E. merra *in outdoor RCC acclimatization and maintenance tanks*

No. of fishes stocked	Total length range (mm)			Weight range (g)			Duration (month)
	Initial	Final	Growth increment	Initial	Final	Growth increment	
46	170-375	200-410	32	50-630	110-750	90	7

Table 3. *Hydrographical parameters in the indoor maturation and spawning tanks*

Tank No.	Temp.°C	*p*H	Salinity (ppt)	DO_2 (ml/l)	NH_3 (mg/l)
1	27.0-28.3 (27.6)	7.97-8.41 (8.19)	32.2-34.1 (32.9)	4.85-5.29 (5.07)	0.0-1.2 (0.2)
2	27.1-28.3 (27.9)	7.99-8.41 (8.22)	32.3-33.7 (32.9)	4.97-5.36 (5.20)	0.0-1.4 (0.3)
3	26.5-28.0 (27.0)	8.29-8.61 (8.27)	33.3-34.6 (34.1)	5.05-5.25 (5.15)	0.0-0.4 (0.1)

Table 4. *Development of brood stock of* E. merra *in indoor maturation and spawning tanks*

Tank No.	No. of fishes	Total (mm) range			Wt.(g) range			Duration (month)
		Initial	Final	Growth incre.	Initial	Final	Growth incre.	
1	4	340-410	370-430	25	450-750	550-910	133	7
2	4	300-350	340-370	30	400-560	480-640	120	7
3	4	290-320	310-340	20	350-460	390-500	40	3

Table 5. *Results of spawning experiments on* E. merra *under indoor maturation system*

Tank No.	Spawning period (days)	Date/Lunar phase		Fertilisation Y/N	Total eggs	Egg size (μ)	Rate of fertilisation	Rate of hatching	Time of hatching (hrs)	Larvae size (mm)
1	1	30.08.04	o	No	47800	710	-	-	-	-
	2	31.08.04	o	No	113600	720	-	-	-	-
	1	29.09.04	o	Yes	22000	710	70	80	26	1.5
	2	30.09.04	o	Yes	180000	720	80	80	27	1.5
	3	01.10.04	o	Yes	126000	730	80	90	24	1.6
	1	26.10.04	o	Yes	18000	720	50	60	25	1.5
	2	27.10.04	o	Yes	36500	730	70	80	24	1.6
2	1	28.09.04	o	Yes	28000	730	60	70	24	1.4
	2	29.09.04	o	Yes	42700	720	60	70	25	1.5
	3	30.09.04	o	Yes	85000	710	70	80	27	1.5
	4	01.10.04	o	Yes	63200	720	50	80	26	1.5
	1	13.10.04	•	Yes	34500	730	50	80	25	1.6
	2	14.10.04	•	Yes	20000	710	60	80	28	1.5
	1	26.10.04	o	Yes	47000	730	60	70	26	1.4
	2	27.10.04	•	Yes	23500	720	60	60	24	1.5

were stocked had grown to a cumulative mean size/weight of 400mm/730g from the initial cumulative mean size/ weight of 375mm/600 g after a period of 7 months. The growth/weight increase recorded in this experiment for the period was 25mm/130g. The average monthly growth rate/weight increase worked out to 3.6mm/19g.

The fishes stocked in this tank matured and spawned thrice during the study period (Sep-Oct.'04). Each spawning lasted for 2-3 days before or after full moon. Each time the spawning occurred during night (around 20.30 hrs) and continued for 2-4 days. The buoyant fertilized eggs were collected next day morning using collecting nets of suitable mesh size. The number of viable eggs obtained in the various spawning ranged from 20,000 to 85,000. In all the spawning 50-70% fertilization rate was observed. The fertilized egg size ranged from 710-730μ and the rate of hatching varied from 60-80%. The incubation period varied from 24 to 27 hours.

Trial 2: In the second trial (Tank-2) four fishes were stocked. They had grown to a cumulative mean size/ weight of 355mm/560g from the initial cumulative mean size/weight of 325mm/480g after a period of 7 months of maintenance. The growth/weight increase recorded in this experiment for the period was 30mm/120g. An average monthly growth rate of 4.3mm/17g was observed.

In this trial also the fishes spawned three times corresponding to the lunar cycle. Each spawning lasted between 2 – 4 days and occurred during night hours

(around 20.30 hrs). In this trial, one spawning for a period of 2 days was also observed during the new moon phase. The fishes maintained in this tank matured and spawned three times during the period (Aug-Oct. '04). On two occasions viable eggs were produced, numbering from 18,000 to 1,80,000. (size range 710-730µ). The rate of fertilization ranged from 50-80% and the hatching was between 60-90%. The incubation period varied from 24 to 28 hours.

Trial 3: In the third trial (Tank - 3) also four fishes were stocked. Even though the conditions were similar to the other two trials these fishes failed to spawn naturally. They had grown to a mean size/weight of 325mm/445g from the initial mean size/weight of 305mm/405g after a period of 3 months of maintenance. The growth/weight increase recorded in this experiment for the period was 20mm/40g. A monthly growth rate of 6.6mm/13.3g was observed.

Discussion

Captive breeding and spawning in most of the groupers are difficult since they are protogynous in nature and males are available only after certain years of age *i.e.*, when they grow and reverse sex to male. Hence, the availability of milting males at the time of requirement is always difficult. Successful attempts at induced sex change by administering androgenic hormones have been recently reported by Pillai *et al.* (2002), Glamuzina *et al.* (1998) and Roberts and Schllieder (1983). Many of the grouper species have been induced to ovulate at required time with certain degree of success (Kungvankij *et al.,* 1986; Al-Thobaity and James, 1996; and Chen *et al.* (1977). The size at sex change of *E. merra* is reported to be around 200mm TL, occurrence of both sexes in the wild is common in contrast to that observed in large sized grouper species. Hence, no hormonal manipulation of sexes was required for this species.

The first natural spawning of captive grouper *E. coioides* (= *E. suillus*) in Philippines was reported in 50 t cement tank (Toledo *et al.,* 1993) lasting 3 days before or after the last quarter of moon. Tucker (1999) stated that voluntary spawning of captive groupers has occurred mostly with well fed, uncrowded fish during the natural spawning seasons under conditions of ambient temperature and partial or total natural light. This observation has been proved to be similar as in the present study. *E. merra* spontaneously spawned in the indoor maturation system after a period of maintenance of not less than 7 months in ambient conditions. In the present trials, this exhibited spawning activity corresponding to full moon and new moon phases was similar to the observations of Tucker (1999).

Out of the three experiments, success in breeding was achieved in two experiments (67%). The spawning duration varied from 2 to 4 days and egg size ranged from 710 to 730 μ and always coincided with lunar periodicity (Full moon phase) and once in a month. In one occasion (October spawning), there was spawning during both the lunar phases. The spawning also invariably occurred in most cases during night.

The eggs of honeycomb grouper *E. merra* are single, non-adhesive and buoyant at normal salinities. Nassau grouper (*E. striatus*), having a body weight of 6 kg, spawned naturally/induced ovulation produced 3.3 million eggs in a spawning period that lasted for 1-4 days. Spawning of *E. tauvina* was reported to occur continuously for four days in captivity and the number of eggs released in each spawning varied from 0.25 to 2.0 million (Pillai *et al.,* 2002). In the present trials of spawning of *E. merra*, the spawning period lasted from 2-4 days and a total of 3.28 lakhs eggs were produced by fishes weighing around 1.0 kg body weight. It seems that the number of eggs produced is directly related to its body weight. A fertilization rate of 85-86% in the Nassau grouper is reported while in the current trials on *E. merra* it ranged from 50-80%. The eggs hatched out within 24-28 hours of incubation that was longer compared to that of *E. polyphekadion*. The average size of the larvae was 1.5mm. These observations compared very well to that of Al-Thobaity and James (1996) for *E. polyphekadion* in Saudi Arabian waters.

The average fertilization and hatching rates were 63% and 75% respectively. This is a good indication of the suitability and efficiency of the indoor maturation and spawning system developed for this experiment, which effects 400% of overturn of water and almost negligible level of NH_3. Al-Thobaity and James (1996) recommended 500% water exchange for the indoor maturation and spawning of *E. polyphekadion* and *E. fuscoguttatus.*

Supranee Chinabut (1996) while discussing the diseases in grouper stated that problems in nutrition and environmental stress indirectly would lead to diseases. Disease occurrence in the stocked brood fishes in the indoor maturation and spawning system was minimal. Mortality of the brood stocks in the system was nil. Thus the indoor maturation and spawning system developed in the present experiment has proved good for keeping the ambience of water quality for this type of smaller sized grouper species.

In the third experiment, spawning could not be achieved even though comparable growth and weight increase to the other set of spawners was noticed. One obvious reason that could be attributed for the failure in

the third trial is the relatively shorter period of maintenance (3months) compared to 7 months in the other sets. Hence, insufficiency in PUFA supplemented diet (in the form of cod liver oil) has led to failure in maturation and spawning. Al-Thobaity (1996) has fed the brooders of *E. polyphekadion* and *E. fuscoguttatus* with cod liver oil injected sardines to satiation. Quinitio *et al.* (1996) has enriched the nutritional value of trash fishes used as feed for grouper with commercial HUFA (SELCO) or cod liver oil. Toledo *et al.* (1993) has mentioned that nutritional deficiency could be one of the reasons for having inconsistent quality of spawns of *E. coioides*. It may also be derived from the present experiment that a minimum of 7 months maintenance is essential for maturation and successful spawning in this species.

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