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Larval rearing trials of the honeycomb grouper *Epinephelus merra* Bloch under laboratory conditions

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ABSTRACT

Groupers being economically important food fishes are experimented widely for controlled breeding world over. In India, attempts were made on few species of the genus *Epinephelus* such as *E. tauvina*, *E. malabaricus* and *E. polyphekadion* at the Central Marine Fisheries Research Institute and limited success was achieved. The present paper discusses on larval rearing trials of the honeycomb grouper *E. merra* up to juvenile stage. Larvae measuring 1.3 – 1.6 mm obtained from the captive spawning of broodstock of *E. merra* were used for the larval rearing studies. The feeding protocol, water exchange and larval rearing methods adopted are detailed. The larval mouth opening appeared on day 3 post-hatch. The larvae gradually metamorphosed into juvenile by day 60 and attained a size of 45 mm. The possible reasons for initial mortality, the advantage of HUFA rich feeding and effect of large volume of rearing tanks on the growth and survival of the larvae are discussed.

Keywords: Broodstock, *Epinephelus merra*, Honeycomb grouper, Larval rearing

Introduction

The honeycomb grouper *Epinephelus merra* is a small sized coral reef fish species inhabiting patchy reefs of shallow lagoons and protected water bodies. They are found at depths of less than 20 m. The species is considered as a good table fish with demand in both domestic and international markets. Expansion of aquaculture of marine finfishes in India is hindered by the lack of seeds for stocking. As far as the development of hatchery/breeding technique for marine finishes in India is concerned, marine fish species such as the grey mullet, *Mugil cephalus* (Krishnan *et al*., 1996; Abraham *et al*., 1999), *Liza macrolepis* (Sebastian *et al*., 1975), seabass *Lates calcarifer* (Thirunavukkarasu *et al*., 2001) and grouper *Epinephelus tauvina* (Mathew, 2002) have been attempted. Kailasam *et al*., (2001, 2002) have reported on the larval rearing of *L. calcarifer*. Successful hatchery and larval rearing techniques of marine ornamental fishes have also been reported from India (Ignatius *et al*., 2001; Gopakumar *et al*., 2009).

The two most important problems encountered in the larval rearing of groupers are that they have small mouth size at full opening and limited yolk reserves at the time of hatching (Kohno *et al*., 1990; Doi *et al*., 1991). Larval rearing depends on the availability of appropriate sized and nutritionally adequate food for the larvae. Successful broodstock development and natural spawning of this species have been reported (Jagadis *et al*., 2007). Previous experiments on the spawning and larval rearing of *E. merra* gave inconsistent results (Anon., 2003). Successful larval rearing of various species of groupers such as *Epinephelus salmoides* (Kungvankij *et al*., 1986; Salem Al-Thobaity and Charles, 1996); *Epinephelus tauvina* (Chen *et al.,* 1977' Hussain and Higuchi, 1980; Lim, 1993, Mathew *et. al*., 2002), *Epinephelus suillus*, (Duray *et al*., 1997), *Epinephelus polyphekadion,* (James *et.al*., 1977), leopard grouper (Gracia-Lopez *et al*., 2005), *E. fuscoguttatus* (Lim, 1993), *Epinephelus tukula* (Yeh *et al*., 2003) have been achieved and reported.

The objective of the present study was to standardise the rearing technique of *E. merra* larvae produced from captive bred broodstock**.**

Materials and methods

Broodstock and spawning

Broodstock of *E. merra*, in the size range of 400 mm/ 700 g were maintained in 5 t circular FRP tanks with recirculating water system, in the ratio of 1 male: 3 females. These fishes were reared in captivity for a period of 8 months prior to shifting to broodstock tanks. Fishes were fed with freshly collected marine trash fish and occasionally cephalopods @ 10% of the bodyweight on alternate days. To improve the nutritional quality of the feed, cod-liver oil capsules and vitamin capsules were kept in the visceral cavity of the trash fishes and fed to the broodstock fishes.

The spawning of fishes was observed during September – October. Each spawning occured during late night hours and lasted for 2-4 days before or after full/new moon. A total of 3,28,000 eggs were obtained from 6 spawnings during this period. The average fertilization and hatching rate recorded were 63% and 75%respectively. The buoyant fertilized eggs were collected next day morning using a collecting net of suitable mesh size and incubated in 500 l circular FRP tanks. The mean size of the egg was 720 µ and the fertilized eggs hatched out into larvae after 24-27 h of incubation. Newly hatched larvae measuring 1.4 - 1.6 mm were collected and used for the larval rearing trials.

Culture of natural food organisms

The green algae *Chlorella* spp. were cultured in 1tonne FRP tanks following the method detailed by Gopinathan (1982). The rotifer *Brachionus rotundiformis* was cultured in 1 t FRP tanks using green water. Adult copepods for the experiments were collected by sieving raw seawater and cultured following the batch culture method described by Stottrup and Norsket (1997).

Experiments on larval rearing

Larval rearing in low volume rectangular FRP tanks

In this experiment, rectangular FRP tanks of 250 l and 1000 l capacity were used. These tanks were set up in outdoor finfish hatchery having translucent roofing for sufficient light for the development of algal culture. These tanks were filled with filtered seawater upto 80% of its volume and continuous aeration was provided through an oil free twin lobe air blower. In order to reduce the light intensity on the larval rearing tanks, partial shading was provided to the tanks using polyethylene garden nets. The larvae collected from the hatching tanks (1.4-1.6 mm) were stocked at a density of 25 nos. l^{-1} in both the culture tanks. On alternate days, 20% of the water was exchanged up to the 5th day, 30% up to 9th day and 50% till 15th day. From day 15 onwards, the same level of water exchange was done on daily basis by draining the rearing tank using a 300 µ mesh drainers and then slowly replacing with fresh seawater and required quantity of green water to maintain good water quality and algal cell concentration ($2\text{-}3x10^5$ ml^{-1}) throughout the rearing period. Rotifers filtered through 150 µ sieve were added to the rearing tanks to maintain a rotifer density of 15-20 nos. l^{-1} in the tank.

Larval rearing in large volume circular FRP tanks

For this experiment, circular FRP tanks of 5 t (2 m dia) capacity were used. Rearing tanks were filled with filtered seawater two days prior to the stocking of larvae. Green algae *Chlorella* was introduced in order to condition the water and also as feed for the rotifers. Larval stocking density used was 25 nos. l^{-1} of water. The feeding protocol for the first feeding (3-5 days) of larvae consisted of rotifers sieved through a 150 µ sieve at concentration of 10-20 nos. l^{-1}. From day 5 onwards copepod nauplii (assorted species) were added to the larval rearing tanks at a density of 10-15 nos. l^{-1}. Feeding with adult copepods and *Artemia* nauplii was started after 24 days of rearing. After 40 days of rearing, the larvae were also fed with minced fish meat. As the tank volume was large, 20% water exchange was provided on alternate days till the end of the rearing period. The water quality parameters of all the rearing tanks were monitored by fortnightly samplings

Results

Natural food organisms

Nanochloropsis and *Chlorella* spp. attained a cell density of 2 million cells ml^{-1} within 3 days of inoculation and was suitable for green water preparation. Rotifers (*Brachionus* spp.) stocked attained a concentration of 200-220 nos. ml^{-1} in a period of 3-4 days. The young ones dominated the culture during the exponential growth phase. Copepod cultures stocked in green water (*Nannochloropsis* and *Chlorella* spp at 0.1 lakhs cells ml^{-1}) was ready for harvest after 10 days of culture.

Water quality of the larval rearing tanks

Water quality parameters recorded in different larval rearing tanks are presented in Table 1.

Larval rearing in low volume tanks

The larvae stocked in 250 l FRP tanks encountered about 50% mortality on the 3rd day post-hatch, when all the larvae had developed mouth opening. On day 5, pectoral fins developed and the larvae measured 2.5 mm (n=50). Subsequently, the larvae developed prominent dorsal, pelvic and pectoral fins. Continued mortality of the larvae stocked was evident throughout the rearing period. At the end of the 20 days rearing, a survival of 1% of the initial stocking was observed and the larvae attained 6 mm body length. However, these larvae did not survive and total mortality was noticed on day 21 (Table 2).

Table 1. Water quality parameters in the larval rearing tanks

Trial. no/Tank size	Temp. ^oC	pH	Salinity (ppt)	DO_2 (ml l^{-1})	NH_3 (mg l^{-1})
Trial 1; 250 l	27.5 – 28.8	7.9 - 8.4	32.6 – 34.2	4.9 – 5.3	0.0-1.4
Trial 2; 1000 l	28.9 – 29.5	7.9 – 8.5	33.5- 34.9	4.9 – 5.2	0.0 – 0.5
Trial 3; 5000 l	26.5 – 28.1	8.3 – 8.6	33.6 – 34.6	5.1 – 5.3	0.0 – 0.4

In 1000 l tanks, mortality to the extent of 50% was observed on 3rd day at the time of mouth opening and first feeding. Further heavy mortality (about 70%) was noticed between 5-7 days. The larvae showed similar growth as in the case of 250 l tanks and at the end of 24 days, they had grown to a size of 8.5 mm and a survival of 1% of the initial stock were noticed. Subsequently on day 24, complete mortality of larvae was observed (Table 2).

Table 2. Larval rearing trials in shallow rectangular FRP tanks

Tank size (l)	Density	Survival (%)					Remarks
		days					
	(nos. l^{-1})	01	03	05	07	30+	
250	25	100	50	2.0	2.0	0	Larvae reached 6 mm and total mortality occurred on 21st day
1000	25	100	50	2.0	1.0	0	Larvae reached 8.5 mm and total mortality occurred on 24st day

Larval rearing trials in large volume circular FRP tanks

Larvae stocked in 5000 l tanks were active and swimming by the twitching movement of the caudal fin. The mouth of the larvae was fully formed on day 3 and the larval body length was about 2.2 mm . By this time the larvae had completely exhausted its yolk reserve. From day 5 onwards, the larvae were found to accept the copepod nauplii as well as copepods and had grown to a size of 2.5 mm. Larvae were found very actively hunting the feed at this stage. The larvae grew to a size of 3.2 mm on 9th day. On day 12, the spines on the dorsal and pelvic fins developed and reached a size of 3.6 mm. On day 16, the spines were very prominent and larvae had grown to 4.7 mm and reached 6 mm on day 20. The three spines on the dorsal fin were very prominent and the second one, which is the longest, had serrations on both the sides. From day 24 onwards, *Artemia* nauplii along with copepods were found acceptable to the larvae. The colour of the transparent larvae became brownish black. The swimming pattern of the larvae also changed. It moved down to the column/ bottom of the tank and found swimming along the edges of the tanks. By day 30, the larvae resembled almost like adult fish and by day 48 it grew to a size of 28 mm. The larvae further metamorphosed into a juvenile grouper, attained its colour pattern and reachd a size of 45 mm on 60th day. The survival percentage at this stage was only 0.05% (Table 3). The general growth of the larvae is presented in Fig. 1.

Discussion

Larvae of most grouper species are small and fragile and have small mouths at first feeding. The larval period is long (35-70 days) and grouper tend to require live food longer than most marine fish that have been reared (Tucker, 1998). Rimmer (2000) while reviewing the grouper hatchery technology stated that the principal constraint is the small gape of the larvae and hence the requirement for small prey at first feed and the occurrence of heavy mortality at various stages through the larval rearing phase.

Although *Brachionus* spp. are the most suitable food organisms for fish larvae, their size restricts their use for small mouthed fish like groupers. In *E. merra* larvae also, the mouth gape is considerably small and requires small sized feeds during its larval cycle. The mortalities observed on day 3 in all the trials conducted could be attributed to the non-availability of feed at suitable size for the larvae. Improper formation of mouth and its opening also might cause poor feed intake. Addition of rotifers into the larval rearing tanks prior to stocking is always advantageous as the rotifers added into the tank prior to larval stocking would reproduce and early stages of rotifers would be available in the rearing medium when the feeding of larvae commences. To ensure the availability of suitable sized prey to larvae, feed density was maintained at 20 nos ml^{-1}. This has probably improved the availability of small sized

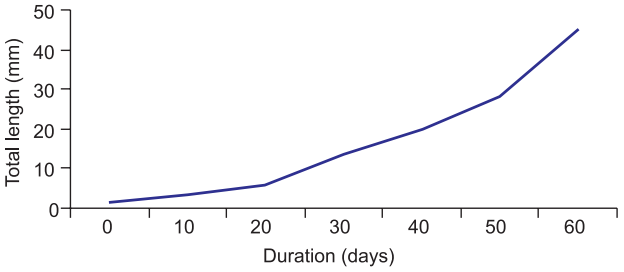

Fig. 1. Growth of larvae of *Epinephelus merra*

Table 3. Larval rearing trial in large volume circular FRP tanks

Tank size (l)	Density	Survival (%)					Remarks
		Days					
	(nos. l^{-1})	01	03	05	07	30+	* Larvae survived for more than
5000	25	100	50	2.0	1.0	0.4	60 days and transformed into juveniles

rotifers and resulted in higher feeding incidence during the time of mouth opening. In the trials, maximum mortality of larvae was observed within 12 days of rearing when fed only with rotifers. This may be due to the insufficient nutritional quality of the feed.

Studies have shown that HUFA, eicosapentaenoic acid (EPA- 20:5n-3) and dicosahexaeonic acid (DHA-22:6n-3) are essential dietary components of marine fish larvae (Webster and Lovell, 1990). Copepods, which are having a good HUFA content are used as feed along with rotifers in the larval rearing of various grouper species (Yeh *et al*., 2003). During the present study also, the addition of copepod as a feed component in the third trial along with rotifers resulted in successful growth and development of juveniles.

The improved growth and survival observed in large volume rearing tanks as compared to that of 250 and 1000 l tanks, may be attributed to the lower possibility for physical injury by hitting against the tank walls, more favourable water quality or possible development of a natural food chain as suggested by May *et al.* (1974). Duray *et al*. (1995) has reported higher survival of *E. suillus* larvae reared in 500 l than those reared in 200 or 40 l tanks and at initial stocking density of 20 larvae l^{-1} than at 30 larvae l^{-1}. Larvae are found to be planktonic until 42 days post-hatch and transformation of larvae into juvenile occurred during the period 46-70 days (Tucker, 1999). Similarly, in *E. merra* the larval transformation into juveniles was observed at around 60 days post-hatch.

The preliminary success achieved in closing the larval cycle of *E. merra* under laboratory conditions is the first report from India and it throws ample light in the endeavor for developing complete larval rearing technical package for mass production of grouper larvae.

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