A Review of Grouper (*Epinephelus suillus*) Fry Production Research in Malaysia

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Abstract

Research on grouper (*Epinephelus suillus*) fry production in captivity has been carried out in Malaysia since 1986 at Tanjung Demong Marine Finfish Production and Research Centre (TDMFPRC) but the breakthrough was only achieved four years later in 1990. Eggs were obtained through natural and induced spawning in tanks. Natural spawning of grouper in captivity seldom occurred and was unpredictable. However induced spawnings were successfully carried out by injecting human chorionic gonadotropin (hCG) intramuscularly at a dose of 500-1000 IU/kg fish.

The results from several trials on larval rearing conducted since 1989 until recently showed that larvae obtained from natural spawnings survived longer with some reaching the juvenile stage. The highest recorded survival rate of 43 days posthatch (32.5 mm total length) was 12.1% at 28-32 °C water temperature. On the other hand, 100% mortality usually occurred in larvae obtained from induced spawning 7 days after hatching.

The major constraints of grouper fry production in Malaysia are lack of male spawners, inconsistent and unpredictable natural spawning, small quantity of eggs released every spawning day, poor fertilization and hatching rate, weak hatchlings, and high mortality rate at the early stages of larval development. The latter is probably due mainly to problems on initial feeding.

Introduction

The grouper (*Epinephelus suillus*) locally known as "Kerapu" is a popular marine food fish which commands a high price among the cultured fishes in Malaysia. *Epinephelus tauvina, E. malabaricus,* and *E. salmoides* are other large-sized groupers of the subfamily Epinephelinae which are also important aquaculture commodities in the Indo-Pacific region. Morphologically, these four species are very similar and difficult to distinguish. Doi et al. (1991) summarized some of the distinguishing external features of these species such as type of scales, number of lateral line scales, size of anterior and posterior nostrils, type and color of spots and the teeth rows on the mid-lateral part of the lower jaw.

In: Marte CL, Quinitio GF, Emata AC (Eds.). 1996. Proceedings of the Seminar-Workshop on Breeding and Seed Production of Cultured Finfishes in the Philippines. 4-5 May 1993, Tigbauan, Iloilo, Philippines. SEAFDEC/AQD, Tigbauan, Iloilo, Philippines. p. 140-149.

Previous studies that have been conducted aimed at improving large-scale production of grouper fry and fingerlings all over the region as reported by Chen et al. (1977), Hussain and Higuchi (1979), Teng et al. (1980), Kungvankij et al. (1986), Predalumpaburt and Tanvilai (1988) and Doi et al. (1991). However, mass production of grouper fry is still at the experimental stage and solutions to the problems identified are yet to be found. Kohno et al. (1992) emphasized that characteristics of grouper larvae such as small larva at hatching and long duration to metamorphosis are biological disadvantages compared to other cultured fish species in the region such as sea bass, milkfish, and siganids which could be the major causes of heavy mortalities in the early larval stages. Further, he postulated that variable egg quality was another factor which may contribute to fluctuation in survival although its effects are difficult to clarify.

This paper summarizes the results of grouper fry production research carried out at the Marine Finfish Production and Research Centre, Tanjung Demong, Terengganu, Malaysia (TDMFPRC).

Broodstock

The first batch of 29 grouper spawners of approximately 8-9 years old were transferred from floating net cages in Setiu Lagoon into 150-ton broodstock tanks at TDMFPRC in February 1986. All died two weeks later due to heavy infection from white spot disease on eyes, gills, and body. Another batch of 25 spawners were brought in from the same source about a year later. In the following year, a few pond-reared grouper spawners from Gelang Patah Brackishwater Aquaculture Research Centre, Johor were added to replace spawners that died due to excessive handling during induced spawning trials. Majority of the grouper broodstock reared at TDMFPRC were identified as *E. suillus,* some were *E. malabaricus* but none was identified as *E. tauvina* (Doi et al. 1991).

According to Tseng and Poon (1983), most *Epinephelus* species are hermaphrodites. They claimed that three years old adult individuals are female, but at four years or older they may change to male without any distinct morphological changes. Tan and Tan (1974) reported that the minimum size of wild female *E. tauvina* is 450-500 mm standard length (SL) compared to 700 mm SL or more than 11 kg in body weight (BW) for male fish with ripe testes. The fish also matures as female almost at the same size in captivity at 2 years (Chen et al. 1977).

Wild male groupers are scarce and found only in deep seas (Kungvankij et al. 1986). Male groupers are also scarce among captive broodstock. For example, at TDMFPRC only three males were observed out of 25 fish of 59-89 cm TL or 2.2-14.5 kg BW in November 1988. The rest were females except seven whose sex were

not confirmed. Two years later, five males were detected among the 24 fish (70-85 cm TL, 6-12 kg BW). In February 1992, only three males were detected out of 22 spawners (Table 1).

Table 1. Body weight and sex of grouper *E. suillus* reared in 150-t broodstock tank at TDMFPRC based on February 1992 sampling.

Fish No.	Body Weight (kg)	Sex
1	10.5	Male
2	10.5	Male
3	9.0	Male
4	10.0	Female
5	11.0	Female
6	9.0	Female
7	13.0	Female
8	8.0	Female
9	9.0	Female
10	7.0	Female
11	8.0	Female
12	10.0	Female
13	8.0	Not Confirmed
14	8.0	Not Confirmed
15	13.0	Not Confirmed
16	14.5	Female
17	8.0	Female
18	6.0	Female
19	9.5	Female
20	8.0	Female
21	7.0	Female
22	9.0	Female

In order to balance the male and the female ratio, sex inversion trials were conducted twice (in 1989 and 1992) using methyltestosterone (MT) following the method described by Chen et al. (1977). However, both trials were not successful and the experiments were terminated half way. The fish refused to take the MT treated feed given after the first feeding.

Egg Production

Natural spawning of grouper, *E. suillus,* at TDMFPRC can be considered a rare occurrence. It was first observed in 1986, one week after the first batch of spawners were transferred from the floating net cages into the broodstock tank. The second natural spawning was observed also in the same year (9 July 1986) in floating net cages at Setiu Lagoon, Terengganu where the rest of the spawners were reared. In the former case, fertilization of spawned eggs was very poor and only a few hatched while in the latter case, no fertilization was observed. No natural spawning was observed in the next three years.

The next natural spawning of grouper *E. suillus* in captivity were observed in 1989 (Doi et al. 1991). It occurred immediately after the males and females were brought together again after 2-3 months of separation by netting wall installed for sex inversion treatments within the same broodstock tank. Ali et al. (1992) concluded that natural spawning of *E. suillus* at TDMFPRC exhibited seasonal similarity as observed in *E. tauvina* in Singapore and *E. malabaricus* in Thailand. However, the spawning of this species did not coincide with the moon phase. In sea bass *Lates calcarifer,* spawning occur throughout the year at least once or twice a month during the new moon and just after the full moon. Although the grouper spawned naturally, fertilization rate was usually less than 25% and sometimes 0% but never exceeded 80% (Doi et al. 1991). Few eggs were released every spawning day compared to the size of spawners. Table 2 gives data on the natural spawnings in 1989 and 1990.

Between 1986 and 1989, induced spawning trials were carried out using the same breeders. The first induced spawning was carried out in 1988, the second in 1990, and the rest were carried out in 1992, and in February 1993. Females with eggs having diameters of about 380-400 µm and males with running milt were chosen. Human chorionic gonadotropin (hCG) was injected intramuscularly at dosages between 500-1000 IU/kg BW to females and 250-500 IU/kg BW to males. Two or three injections at 24-h intervals were given. The female fish normally released eggs naturally 12-60 h after the final injection. In another instance, two females were injected with 0.5 ml/kg BW twice at 24-h interval. Eggs were released after 24 h but were not fertilized. The results of the induced spawning trials conducted since 1988 until recently are shown in Table 3.

Table 2. Natural spawning record of grouper at TDMFPRC (after Doi et al. 1991).

Spawning season (spawning days)		Total no. of eggs collected (million)	No. of spawns with indicated range of fertilization rates				
			<5%	5-25%	25-50%	50-75%	>75%
1st	7 May-3 Jul 1989(26 days)	3.9	16	7	1	1	1
2nd	10 Nov 1989-20 Jan 1990 (36 days)	7.9	23	7	3	3	0
3rd	3 Mar-2 Apr 1990(29 days)	13.4	2	12	10	5	0
4th	21 May-31 May 1990 (9 days)	3.0	5	3	1	0	0
Total: (100 days)		28.2	46	29	15	9	1

Larval Rearing

The first trial of grouper *E. suillus* larval rearing at TDMFPRC was conducted in May 1989. More than 20 trials have been carried out using hatchlings from the first natural spawning season. Approximately 10-30 fertilized eggs/l were stocked in 1- or 5-t tank filled with filtered seawater at ambient salinity and temperature. The tank was provided with mild aeration at 100-200 ml/min/t and 5-15 x 10^3 cell/ml *Tetraselmis* sp. was added. Rotifers screened through 100 µm-mesh plankton net was introduced to the larvae from day 1 or day 2 after hatching. The details of the *E. suillus* larval rearing methods are described by Doi et al. (1991). Of more than 20 trials conducted in 1989, only one juvenile was produced.

For the succeeding trials, almost the same methods were applied by Doi et al. (1991) except for some minor changes such as using *Chlorella* sp. at 100-300 x 10^3 cells/ml instead of *Tetraselmis,* oyster eggs or larvae for initial feeding, reducing water salinity from 31 ppt to 27-29 ppt, and feeding small size-rotifer (ss-rotifer), micro-encapsulated diet and enriched rotifer and *Artemia* as alternative or supplementary feeds during the critical period. As a result, nearly 10,000 *E. suillus* juveniles were produced in 1990. On the other hand, the result of *E. suillus* fry production trials conducted in 1992 and early 1993 using the hatchlings produced by induced spawning were very poor. Total mortality of larvae occurred 5-7 days after hatching. This could be due to poor egg quality as speculated by Kohno et al. (1992) and unavailability of ss-rotifer for the first feeding which was claimed by Doi et al.

(1991) as one of the major factors contributing to better survival of 10-day old larvae. The results of fry production trials conducted at TDMFPRC using hatchlings from natural and induced spawnings are summarized in Table 4.

Table 3. Induced spawning of groupers by hormone injection at TDMFPRC.

Trial no.	Date	Spawner TL (cm)	Spawner BW (kg)	Spawner Sex	Hormonal treatments	Results and remarks
1	30 Nov 1988	79	11.0	F	1st and 2nd injection with 500 IU hCG/kg female and 2nd 250 IU hCG/kg male	1 female released eggs by stripping 24 h after 2nd injection. Artificial fertilization was very poor at 25.6°C and 8 ppt
		78	10.0	F		
		78	9.6	F		
		73	12.0	F		
		81		M		
		84	11.0	M		
		74	6.3	M		
2	23 Sep 1990	68	5.5	F	Female 1 received 5000 IU hCG in two injections and Female 2 received 9000 IU in 4 injections at 24-48 h interval. Male received 11000 IU hCG	Female 2 released eggs naturally but were not fertilized
		63	4.6	F		
		77	9.0	M		
3	26 Feb 1992		10.0	F	3 females were injected 500 and 1000 IU/kg BW for 1st and 2nd injection at 24 h interval. Only 1 male was injected 500 IU hCG/kg BW on the 2nd day	Eggs were released naturally for 10 consecutive days. Approximately 3 million eggs were collected and egg fertilizaton ranged from 0-29%
			11.0	F		
			13.0	F		
			10.5	M		
			10.5	M		
			9.5	M		
4	27 June 1992		8.0	F	Both females were injected with "Ovaprim" at 0.5 ml/kg fish twice at 24 h interval	Eggs were naturally released 40 h after 2nd injection but were not fertilized
			10.0	F		
5	1 Feb 1993		8.0	F	4 females were injected 500, 750, and 1000 IU hCG/kg for 1st, 2nd and 3rd injection, respectively at 24 h interval	Eggs were released naturally for 20 days almost daily. About 3 million eggs were collected (0-10% were fertilized)
			11.0	F		
			10.0	F		
			10.5	F		
			10.5	M		

Table 4. Fry production trials of grouper, *Epinephelus suillus,* at TDMFPRC.

Year	No. of trials	Source of eggs	Feed combination	% Survival at termination	Remarks
1989/ 1990	>20	Natural spawning (1st & 2nd season)	*Tetraselmis* or *Chlorella* Oyster eggs/larvae *Brachionus plicatilis* Copepods	~0% (14 days)	Only 1 juvenile was produced (Doi et al, 1991)
1990	>13	Natural spawning (3rd season)	Same as above but supplemented with boiled egg yolk (<100µ) for initial feeding	0.1-4.2% (10 days)	Salinity reduced from 30 ppt (Doi et al., 1991)
1990	>5	Natural spawning (4th season)	*Chlorella* at 1-3 x 10^5 cell/ml Oyster eggs/larvae SS-rotifers Microencapsulated feed (250-400µm) Enriched rotifers and *Artemia* nauplii Copepods Minced fish	0.3-12.1% (41 to 46 days)	Survival rates were based on the number of fertilized eggs (Doi et al., 1991).
1992	4	Induced spawning	Oyster eggs/larvae Ordinary rotifers Copepods and *Artemia* nauplii	0% (5-30 days)	Only one trial reached 30 days old, the rest were terminated before day 10
1993	5	Induced spawning	Oyster eggs/larvae Ordinary rotifers *Tetraselmis*	0% (5-7 days)	Some of the larvae died before first feeding.

Recommendations

Fry production of *E. suillus,* just like other groupers of commercial importance in this region, is faced with a lot of constraints. Further research on the following areas are therefore suggested:

i. Upgrade broodstock development programs to ensure sufficient and continuous supply of spawners especially males which are scarce and takes a longer time to mature.

ii. Improve techniques of sex inversion to ensure that some of the existing females can be changed to males. Pellet implantation and osmotic pump implantation techniques as reported by Nacario (1986) for induced spawning of sea bass (*Lates calcarifer*) could be an alternative method for grouper sex inversion.

iii. Encourage natural spawning through environmental manipulation and enhance egg quality using a special formulation or natural diet. For instance, a relatively high fertilization rate and increased numbers of naturally spawned eggs were reported by Doi et al. (1991) after vitamin-mix was given daily to the spawners.

iv. Production of smaller size rotifers of different strain or species for the first feeding grouper larvae. Adult ss-rotifer (155±20µm and 124±14 µm lorica length and width, respectively) and ordinary rotifer (217±32 µm and 145±22µm lorica length and width, respectively) may be too big compared to mouth opening (vertical opening) of 3 day old *E. suillus* which is approximately 150-180µm (Doi et al. 1991). Pechmanee and Chungyampin (1988) showed that for 2-10 days old red snapper (*Lutjanus argentimaculatus*) only s-type rotifer with less than 44% lorica width/mouth size were consumed. Preliminary culture trials of *Colurella adriatica* a freshwater rotifer of the family Colurellidae as a potential feed for grouper and snapper larvae have been conducted (Doi et al. unpublished) and recent observations by Nik Razali Nik Lah (personal communication) indicate that this species was more favorable to *E. suillus* larvae during 60-166 h after hatching compared to ss-rotifer.

v. The period of endogenous feeding may be delayed by reducing the rate of yolk/oil globule absorption which could be done by lowering salinity. A study showed that *E. tauvina* larval development was normal at salinity conditions between 40-100 ppt seawater and the rate of yolk absorption appeared to decrease proportionally with the

decrease of salinity above 30 ppt seawater. Thus, the food supply to the larvae may be delayed for 20-48 h. Yolk absorption occurs within 72 h in larvae reared under ambient seawater conditions (Akatsu et al. 1980).

Acknowledgments

I wish to thank the Director-General of Fisheries, Malaysia, Y. Bhg. Dato Shahrom b. Hj. Abdul Majid for his permission to present this paper; Mr. Tan Cheng Kiat, Director of Research and Planning Division, Department of Fisheries, Malaysia for his encouragement; Mr. Ali Awang, Head of Station, Marine Finfish Production and Research Centre, Tanjung Demong, 22200 Besut, Terengganu, Malaysia for his critical comments on the manuscript and those who were involved directly or indirectly with the completion of this paper.

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