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Hatchery seed production and cage farming of Tiger grouper *Epinephelus fuscoguttatus* (Forsskal 1775) in Andaman and Nicobar Islands, India

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Grouper fishes have been considered as commercially important candidate species for aquaculture. Among them, the Tiger grouper (*Epinepheleus fuscoguttatus*) is one of the most relished finfish that demands an excellent price in the fish markets of Southeast Asia. Rajiv Gandhi Centre for Aquaculture (RGCA), has launched a project on breeding, seed production and grow out farming of grouper at Andaman & Nicobar Islands (ANI). The project developed technology for breeding and grow-out farming of Tiger grouper, which is widely distributed in Andamans and also form a candidate species for captive breeding and sea cage culture. Its wild catches are extremely limited and insufficient to meet the huge market demands. Hence, development of a standard technology for the seed production of Tiger grouper is an imperative to boost the country's export revenue. The paper presented here, is one such initiative by RGCA on seed production and cage culture of *E. fuscoguttatus*.

[**Keywords**: Andaman and Nicobar Island, Cage culture, Captive breeding, *Epinephelus fuscoguttatus*, Larval rearing, Tiger grouper]

Introduction

Grouper fishes are commonly found in the coral reef area and broadly disseminated in the tropical and subtropical seas, particularly in Indo-Pacific region[1]. Groupers consists of 115 species belonging to subfamily of Epinephelinae with 22 genera and one among the five subfamilies of family Serranidae[2,3]. Since 1993, the grouper fish aquaculture production was rapidly increased at 10-77 % per year[3]. This extreme growth is observed mainly due to the constant demand and market potential for the high value fishes which are sold alive in the Hong Kong and China. The fish has immense potential in the trade market and also well accepted in the domestic market[1]. Globally, the fish has been the focus of research and development areas which focuses to enhance its production through various aquaculture technologies. It was estimated that, around 95,000 tonnes of cultured groupers were produced worth of USD 550 million in the year 2011. A major part of grouper production was contributed from Asian countries mainly by China, and subsequently by Taiwan, Indonesia and Malaysia[4]. The estimated market for the live grouper in Hong Kong and China is about 15,000 to 20,000 tonnes per annum[5]. Groupers are generally grown in net cages and some species are also grown in earthen ponds[6].

There is vast scope for grouper aquaculture in the Andaman and Nicobar Islands (ANI). Availability of several species of fishes in Andaman waters that are amenable to aquaculture had been reported[7]. Altogether, 43 species of grouper had been reported from ANI[8]. Further, the Andaman Islands provide a relatively pristine environment suitable for small scale cage culture of groupers.

Notably, the Tiger grouper is abundantly available in the waters around ANI. It has a standard price in the live reef fish trade and attains market size (around 500 gm) in 6 – 7 months. Considering its market potential and faster growth rate, this species was selected for breeding and seed production in the present study.

Materials and Methods

Development of open sea cage facility to condition wild caught Tiger grouper for the breeding programme

The High Density Polyethylene (HDPE) cage rafts of twenty one numbers with dimension 3 m L x 3 m W type with a watchman shed erected near the Rutland Island, South Andaman (Lat: 11°29' N and Long: 92°40' E) to hold and condition the wild caught Tiger grouper, *Epinephelus fuscoguttatus*. In that, seven numbers of rafts were used for holding grouper brood stock and remaining 14 cage rafts were used for cage farming. In addition to this, two numbers of wooden working platforms each of 5 m L x 5 m W dimension with an aluminium roof top were also fabricated. Appropriate mesh size and suitable net material were used based on the size of the fish attained (Table 1).

Net cages for brood stock maintenance

The nylon knotless double layered square net cage of dimension 3 m L x 3 m W x 3 m H were used to avoid the injuries over the body surface of brood stock. The nylon cages had inner netting with 16 ply/ 24 mm KK & outer net with PE knotted 1.5 mm/ 38 mm KK. These net cages were purchased from M/s. Garware - Wall Ropes Ltd., Pune, India.

Collection of brood stock from the wild

Tiger grouper brood stocks were collected with the help of local fishermen by hook and line gear in the region of South Andaman Island and the brood stocks were carried to open sea cage facility in 200 L of fibreglass tank filled with seawater using a mechanized boat with 100 % water exchange per hour. For quarantine, collected breeders were kept in the separate cages and then treated with 200 ppm formalin for 1 hour in order to eliminate the occurrence of parasites before being stocked into the respective brood stock cages. Only healthy fish with body weight 5 – 9 kg, free from major injuries were used for stocking. The brood stocks were fed with frozen fishes thawed prior to feeding. The fish were fed every second day on trash fish mainly comprising mackerel and sardines. Besides, squid or cuttle fishes were also fed once in a week. The trash fishes were collected depending upon the availability and due to the high nutritional value, squid or cuttle fish were fed to improve gonadal development.

Maintenance of grouper brood stock in net cages

The cages were covered on the top by shade cloth nets to avoid the light penetration. The cage nets were replaced by the washed nets by monthly or whenever required to minimize biofouling. The cages were periodically checked for any damages and also to assess the behaviour of the fish and as well as to check the presence of parasites or epibionts on the fish. Dip treatments in freshwater was also provided once in month to reduce the parasite load on the fish. The anchor ropes were regularly cleaned at every fortnight in order to remove attachment of macrophytes and barnacles, if any. In parallel, new anchors, 25 mm polypropylene (PP) ropes for net cages were kept ready in the cage site as reserve during adverse weather conditions. Water quality data including pH, temperature, and salinity were recorded daily at 06: 00 hrs from the sea cage site. Sex of the stocked fish and egg developmental stages were determined by Cannulation method. All brood stocks were tagged by Radio Frequency Information Device (RFID) tags for tracking the performance of individual fish.

Grouper hatchery facility

A private hatchery was taken on lease by RGCA and modified to suit our requirements to produce Tiger grouper fingerlings. The hatchery was equipped with 8 numbers of 4 ton concrete larval rearing tanks (8 nos) and a live feed culture unit. Different species of micro algae, *viz*; *Nannochloropsis* sp., *Isochrysis* sp., and *Tetraselmis* sp. and a variety of rotifer strains SS, S and LS were cultured in this section. A mini unit for Calanoid copepod production was also in place to produce copepod nauplii to feed the initial stages of grouper larvae.

Spawning and hatching

For breeding 3: 1 ratio (female: male) of Tiger groupers were used for natural spawning in the cages. The size of the female used were 4.3 ± 0.61 kg with 70.5 ± 1.6 cm length and male was 7.1 ± 0.84 kg with

Table 1 — Specifications of cage material and mesh size used in the study

Sl. No	Fish Length	Mesh size	Net cage size	Cage material
01	5 cm to 10 cm	12 mm KK	Square cage 3 m Length x 3 m width x 3.5 m deep	Preferably nylon knotless.
02	10 cm -20 cm	16 mm KK	Square cage 3 m Length x 3 m width x 3.5 m deep	Preferably nylon knotless.
03	20 cm and above	25 mm KK	Square cage 3 m Length x 3 m width x 3.5 m deep	Preferably nylon knotless.

150.2 ± 5.2 cm length. The cages were provided with 400 µm happa net to facilitate egg collection. Then the collected eggs were immediately brought to the hatchery and were subjected to the ozone treatment @ 1 mg/L for 60 seconds before stocking in the cement tanks. The eggs were hatched out after 18 hours. The water quality parameters are mentioned in Table 2.

Feeding regimes in larval and grow outs

Feeding begins within 48-72 hours for the newly hatched larvae and metamorphosis starts after 35 days from the day of hatching. After attaining 3 cm size, the fry were reared in the nursery tanks and then reared in the floating net cages up to the length of 10 cm size. Thereafter, they were reared in the cages to reach market size of 700 g to 1 Kg. The feeding strategy followed was a slightly modified protocol[6,9]. Live feeds such as microalgae, rotifers and *Artemia* were used for larval rearing. Feeding regimes of larval and grow out culture is furnished in Tables 3 – 5.

Initially, the fish larvae were fed on calanoid copepod nauplii along with enriched SS rotifer *Brachionus rotundiformis* for the first five days. The rotifers are enriched with Algamac 3050 before feeding. Subsequently, S & L rotifers were fed to the larvae and thereafter Algamac 3050 enriched *Artemia* supplemented with skretting microencapsulated artificial feed was used for larvae culture. On attaining the size of 1 – 1.2 cm TL (fry stage), the fish were graded twice a week or whenever significant size variation was observed. The fry on attainment of 2.5 to 5 cm size were shifted to nursery tanks and were further reared to 10-15 cm TL which is the ideal size for stocking in floating net cages for grow–out farming.

Results and Discussion

The cage site was located about 3 km off Chidiyatapu by sea and 5 km away from the hatchery which is situated at Kodiyaghat. The Tiger grouper is a ‘protogynous hermaphrodite,' where they mature as

Table 2 — Physico-chemical parameter for larval rearing of Tiger grouper

Parameters	Recommended
Temperature	28-30 °C
Salinity	32-34 ppt
Light	500-700 lux
Photoperiod	Natural
Aeration	0.62-1.25 mL/min/L
Dissolved oxygen	80-100 % saturation
Ammonia ($(NH_3$ –N)	< 0.1 ppm
Nitrite (NO_2 –N)	< 0.1 ppm

Table 4 — Nursery feeding regimes of Tiger grouper

Fish length (mm)	Pellet size (mm)	Number of feeding times/ day
10 - 25	0.8-1	6-8
25-30	1.2-2	4-6
30-35	2.0	4-5
35-45	2.5-3.5	4-5
45-55	3.5-4	4
55-75	4.5-5	3-4
75-100	5-6	2-3

Table 5 — Grow out culture feeding regimes of Tiger grouper

Fish size (g)	Daily feeding rate (% ABW)	Number of feeding times/ day
50-100	2.0 – 2.5	2
100-200	1.5-2.0	2
200-300	1.2-1.5	2
300-400	1.0-1.2	1-2
400-500	1.0	1-2
>500	0.8-1.0	1

Table 3 — Feeding regimes for larval rearing of Tiger grouper

Larval stage (DAH)	Microalgae	Copepod	Rotifer	*Artemia*	Artificial diet	Water exchange	Flow through	Siphoning
Yolk sac stage (DAH 1-3)	-	-	-	-	-	-	-	-
Early feeding stage (DAH 3-6 day)	300-500 x 10^3 cells/ml	2-3 ind/ml	5-7 ind/ml SS	-	-	-	-	-
Swim bladder inflation (DAH 6-9 day)	300-500 x 10^3 cells/ml	-	8-10 ind/ml S	-	200-400 µm	10 %/day	-	1 time/day
Long spin (DAH 9-14)	300-500 x 10^3 cells/ml	-	±15 ind/ml LS	-	200-400 µm	20 %/day	-	1 time/day
Free swimming (DAH 14-21)	300-500 x 10^3 cells/ml	-	±15 ind/ml LS	0.2-0.5 ind/ml	200-400 µm	20 %/day	-	1 time/day
Metamorphosis (DAH 21- 35)	-	-	-	0.2-0.5 ind/ml	400-800 µm	50 %/day	50 %	2 time/day
Juvenile (DAH 35-45)	-	-	-	0.2-0.5 ind/ml	400-800 µm	-	100 %	2 time/day

female initially and then change its sex as male at a later stage[10]. In our personal observation it was recorded that the mature female caught from the wild was between 3 kg to 6 kg and males are above 7.5 kg (data not shown). A reduction or complete stoppage in feed intake by the brood stock was observed on three days before during the new moon period along with changes in the peculiar colouring pattern. The 3 L x 3 W x 3.5 m happa net with 400 micron mesh was fixed inside the net cage i.e. placed in between the two net cages. The happa net was lifted during evening and lowered during morning so as to allow free flow of water. Usually the spawning takes place between 10 PM to 11.30 PM. The eggs that floated on the water surface were collected at the initial neurula stage (8 – 10 hours after spawning) and stocked in a 500 litre FRP tank kept on the wooden working platform provided with mild aeration. The collected eggs were packed in an oxygenated polyethylene bags with 6 L of sea water. The eggs were packed @ 1000 eggs per L of seawater and then transported to the hatchery facility, Kodiyaghat. The duration of transportation of eggs from cage site to hatchery was about 25 minutes. Fecundity of the Tiger grouper was found between 0.8 – 4.0 million eggs. However, the fecundity rate may vary depending on the size of the brooders, physico-chemical parameters, feeding rate and genetic defects etc.[11,12] .

The Tiger grouper eggs were shifted to the hatchery facility and stocked in one tonne FRP tank to allow settlement of the damaged eggs. The floating eggs were carefully removed and transferred into a 100 litre FRP tank for estimation. The eggs were then treated with 1 mg/L of ozone for 60 seconds[13,14] and then stocked at a rate of 10 eggs per litre in the larval rearing tanks. Hatching took place in larval rearing tank after 18 hours of spawning. The un-hatched eggs settled at the tank bottom were then siphoned out. The newly hatched larvae possessed undifferentiated jaws and suspends in the water column. Grouper larvae have relatively small body and mouth size at the first feeding stage; hence the small size live feeds are very essential for the successful larval rearing.

In the present study, it was observed that the mouth of larvae opened on the third day of hatching. The larvae were fed with copepod nauplii at the rate of 2 to 3 individuals per ml for the first 3-6 days along with SS rotifers *Brachionus rotundiformis* of size 95 micron which were further screened to harvest only neonates and small adults for first feeding. The rotifers were enriched with Algamac 3050 (Nutritionally balanced with amino acids, vitamins & minerals, high percentage of lipid and omega-3 DHA) for 6 hours before feeding to larvae. The swim bladder inflated between 6-8th day and the larvae engulfed air from water surface to inflate their swim bladder. During this stage enriched S type rotifers of above 160 micron size were fed to the larvae. The second dorsal and pelvic fin spine attained maximum length at day 14 and at this stage the larvae were highly sensitive to mechanical stress. LS type rotifer *Brachionus plicatilis* of size above 239 micron were fed to the larvae after enrichment. On 18th day onwards enriched *Artemia* were fed to the larvae along with microencapsulated feed. On the 21st day unpaired fins separated and the caudal peduncle was clearly observed. The larvae started swimming against the water current. On 35th day, dark spots developed on the body surface and the elongated fin spines almost regressed to the size of other spines. On 60th day, the larvae attained juvenile stage and developed chasing feeding habit and became more cannibalistic. Grading was done on a regular interval at this stage to avoid cannibalism and to improve growth and survival. Larval stage of Tiger grouper is given in Figure 1. During the course of larval rearing, water was exchanged as per standard protocols and the water quality measures such as DO, ammonia, nitrite, pH, salinity and temperature were maintained to the permissible level (Table 2). The first successful production of Tiger grouper (first time in India) was achieved with a survival rate of 0.4 % by Rajiv Gandhi Centre for Aquaculture. Subsequently, the survival rate had been increased up to 1.35 % with the proper enrichment protocols followed for live food using HUFA enrichment products. However, the highest survival of 5.5 % was achieved by the application of probiotics and appropriate modification in Standard Operating Procedure (SOP) for larval rearing.

Grow–out culture of Tiger grouper was done in floating net cages with different mesh sizes. Initially the 10 cm size Tiger grouper fingerlings were stocked in the net cages and covered with shading cloth. The stocking was done in the early morning at 5 AM to reduce the temperature stress on the fingerlings. Initially, the stocking density was maintained @ 15 pcs/cubic meter based on the site conditions. Slow sinking pellet feed was given to the fishes based on the bio mass. Feeding was done twice daily until the

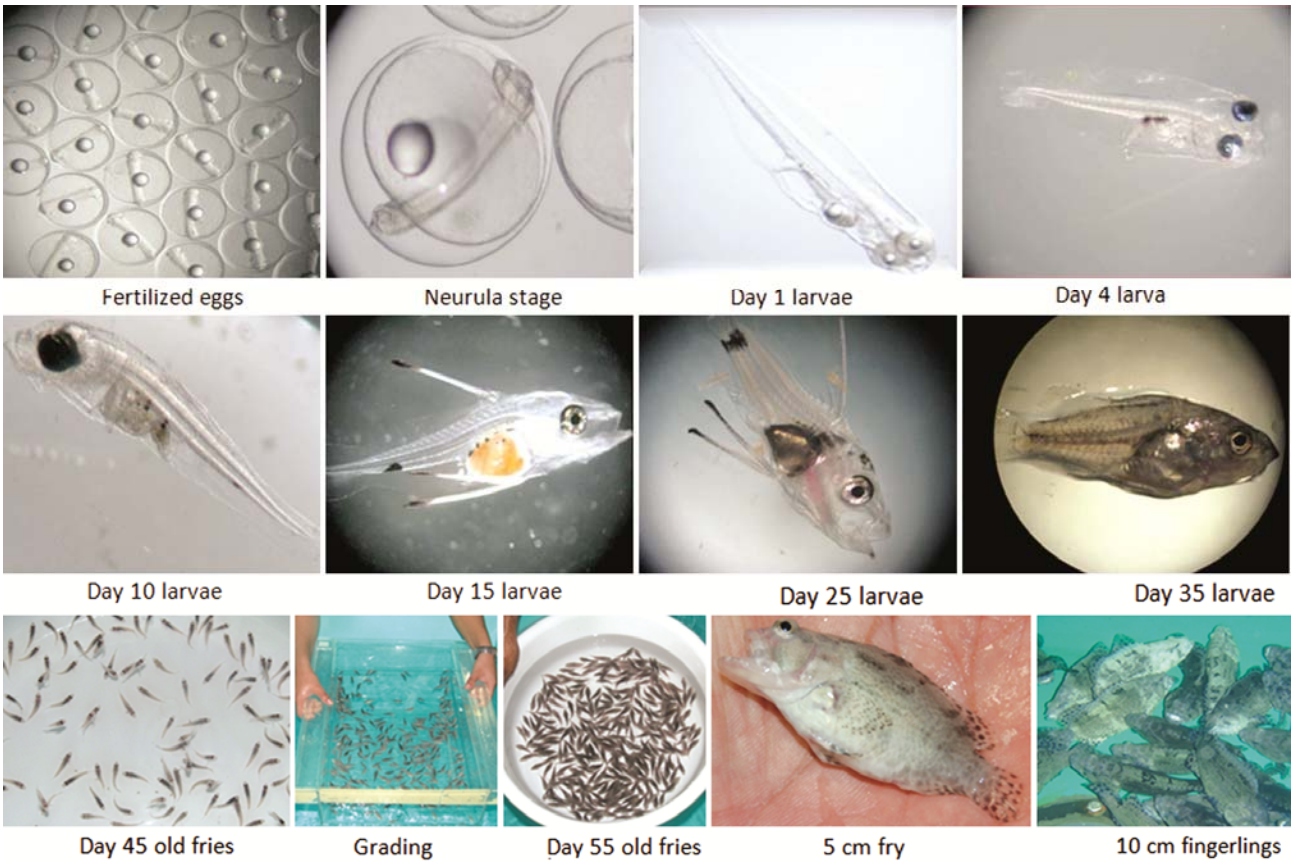

Fig. 1 — Larval stages of Tiger grouper

individuals attained 300 gm size and after 300 gm size, feeding was done only in the evening. Feed conversion ratio (FCR) of the grow-out culture was 1.8:1. The stocking density of the fishes was reduced based on the fish size. At every fortnight size grading was carried out to prevent stunted growth. Fresh water bath treatment was provided while grading the fingerlings to reduce attachment of parasites. The major parasite encountered during the culture period was *Benedenia epinepheli* mostly in summer months of February – May in Andamans. It is a skin fluke which are external parasitic flatworms of about 2 – 6 mm long. It affects mostly to the body surface and eyes. The affected fishes displayed lethargic swimming, loss of appetite and secretion of mucous from the damaged gills. A bath treatment of 200 ppm hydrogen peroxide for one hour in alternative days was very effective to control these parasites. Occasionally *Diplectamum* sp. a gill parasite was also encountered during the culture period. However, it is believed that the improvements in grow-out diet would be helpful to break the major constrains for the sustained grouper aquaculture[15].

Deformities in head and jaw region were reported in the hatchery production 'runs' of *Epinephelus* species[16,17]. In this study, most deformities were seen on the head region which included: deformed skull and/or anterior vertebrae that resulted in antero-dorsal flexure in the head; absence or deformed opercular structures; and deformed jaws (Fig. 2). While some deformities such as head flexure and missing opercula were also visible in small (1 – 2 g) fish; others, such as twisted jaws were not apparent until the fish attain 150 g body weight, which may indicate a later onset. In this study, the incidence of deformed fishes ranged between 12 to 38 %. However, the percentage of deformity was very less when compared to the study conducted by Song *et al.*[18] who reported 50 % of deformities in the cultured *E. Bruneus*. Similarly, Nagano *et al.*[19] recorded an occurence of 98 % of deformities in *E. Septemfasciatus* larvae. The findings in the above referred studies indicated that the deformities percentage in Tiger grouper during the present study was comparatively less than that reported from the other hatchery reared species of *Epinephelus*. The overview of HDPE cage rafts and

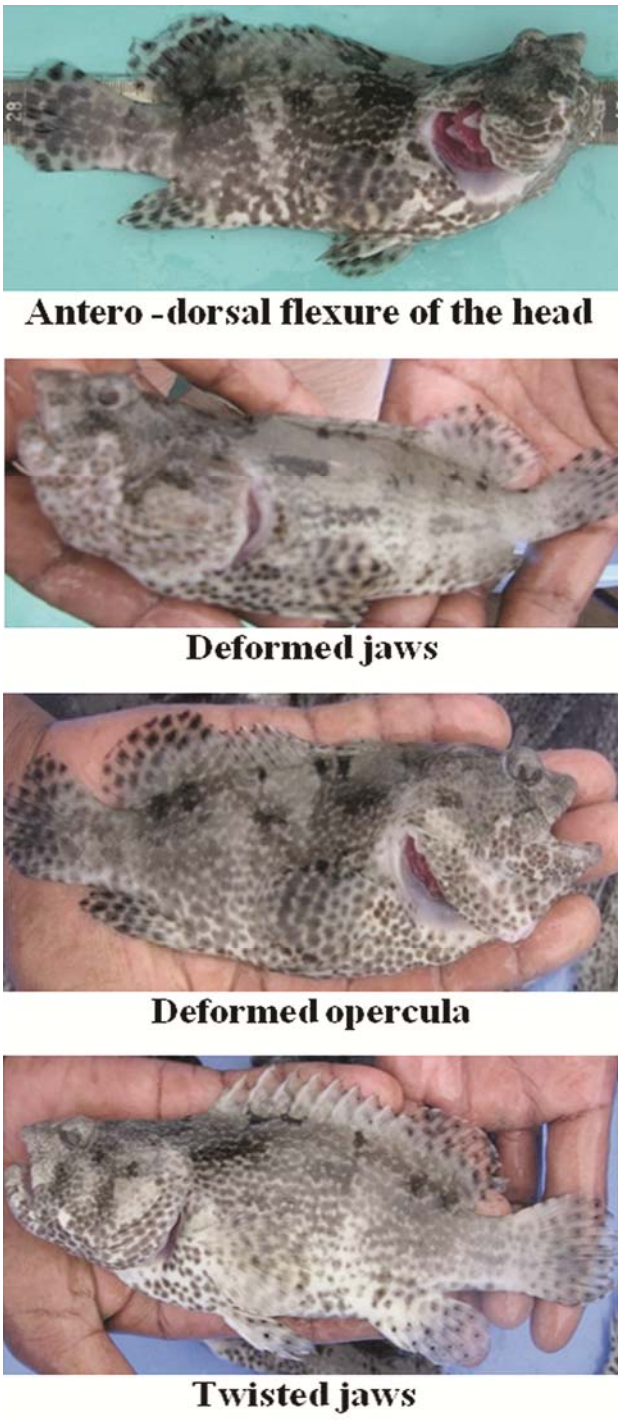

Fig. 2 — Deformities observed in Tiger grouper during study

nets is shown in Figure 3 and the growth trend of cultured groupers is presented in Figure 4. In this study, the average survival percentage of trial farming conducted in the floating net cages was 49.5 % and the maximum survival percentage was 63 % (Table 6). A total of 3,676 kg of market sized Tiger

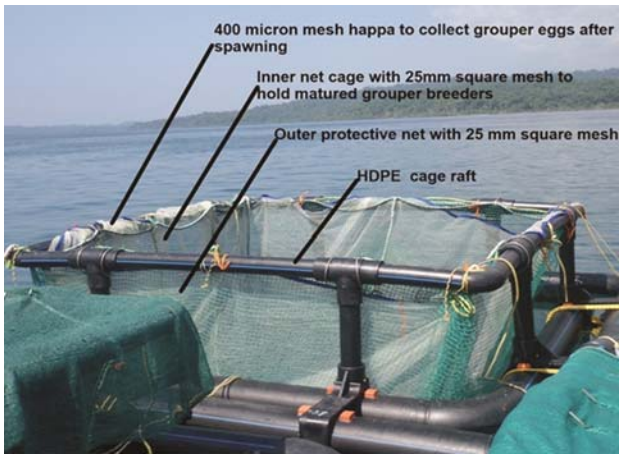

Fig. 3 — Overview of HDPE cage rafts and nets used for the present study

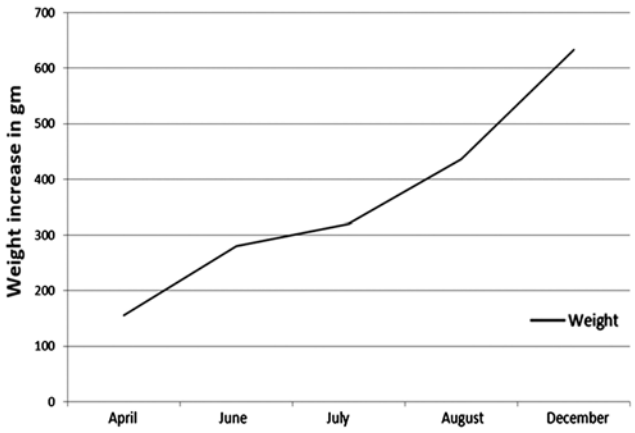

Fig. 4 — Growth trends of Tiger grouper during the culture period in the sea cages

Table 6 — Details of survival percentage of trial farming conducted in floating net cages

Trial	Nos. of tiger grouper fingerlings stocked	% of survival
First	1667	40 %
Second	3153	50 %
Third	3200	45 %
Fourth	5956	63 %
Average Survival		49.5 %

groupers of 500 to 600 g in chilled condition was sold from the hatchery produced fingerlings.

In Andamans, the North East winds develops cyclone that hits directly on the sea cage station during the month of November and December. This causes high fish mortality mainly due to the rough sea condition and high-water turbidity. However, the cage is being kept on the same station as the existing site is

easily accessible *via* road and for other communications. In order to avoid the bad weather conditions, the stocking of fingerlings was mostly done during the month of January and were harvested before November. There are many naturally protected locations available for cage culture in Andamans and these areas could be developed for cage culture through technology transfer programme of RGCA in future.

Hong Kong is the niche market for live grouper fish in the world. The market price for Tiger grouper remains steady at the rate of US$ 30 to 35 per kg (Fish Information & Services; https://www.fis.com/). The live grouper fish can be exported *via* live fish carrier vessel or/by air. The live fish carrier vessel requires at least 5 tonne of fish to transport by sea whereas any quantity of live fish can be transported by air. The Andaman & Nicobar Island with the vast potential for groupers is located very near to the Southeast Asian countries and therefore, there is ample opportunity to export live groupers from these islands once international air flights commence its operation from Andamans.

Conclusion

The present study opened up new avenues as there are plenty of opportunities available for the cage farming of groupers in other parts of India like Lakshadweep Islands, Mandapam, Tuticorin and Kanyakumari. RGCA is in the process of developing technology transfer programme for small scale nursery rearing and cage farming of groupers. The grouper culture technologies developed by RGCA will be disseminated to the fishermen and to the self-help groups in association with Department of Fisheries of respective states in India.

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Conflict of interest

The authors declare no conflict of interest.

Author Contribution

All the authors have contributed equally to the article.

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