Technology on seed production and culture of orange spotted grouper - A breakthrough for Indian mariculture

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Mariculture represents an immense opportunity for food production but remains a largely untapped farming activity. At present, the contribution of marine/coastal aquaculture production to the India's production (2014) is around 10% (0.49 million tonnes), in which crustaceans, finfishes and molluscs contributes 0.386 (7.9%), 0.09 (1.84%) and 0.014 (0.29%) million tonnes (t) respectively. The major reason for the low production levels in this sector is that unlike the freshwater sector, the contribution by the finfish is meagre and mainly limited to shellfish such as shrimps and prawns. The contribution of marine finfishes to the world culture fish production is around 8.5% but the revenue generation is more than 25% of the total revenue. While the current marine finfish production trends around the world shows positive trend, in India, significant growth in culture production of the marine finfish has not yet been achieved.

In India, the population growth is projected to be around 1.65 billion by 2050 and hence, nearly 10.5 million t of marine fishes to meet the food requirement is estimated. To achieve the huge fish production, significant contribution through mariculture/coastal aquaculture is essential as wild caught marine fish production of 3.5-4.0 million t reported for the last few years, has stagnated. Achieving the huge production through mariculture would be possible by species diversification using marine finfish species. Therefore, the seed production technology developed for the high value marine finfish, orange spotted grouper would definitely lead to enhancement in the fish production. It would also present enormous scope for foreign exchange earnings and food security of the country in future and also improve the livelihood of the fishermen.

In spite of having huge potential for mariculture India is still at the initial stage in marine finfish production. Unlike shrimps, the marine finfish culture has not been taken up in a big way due to several problems associated with marine finfish culture and mainly due to unavailability of fish seeds. Breeding and seed production of marine finfishes of high value have been expanding in recent years internationally. In India the development of hatchery technology for commercial level seed production of marine finfishes is still in its infancy, except for the Asian seabass (*Lates calcarifer)*, cobia (*Rachycentron canadum*) and snub nose pompano (*Trachinotus blochii*). Hence, research and development needs to be focused in evolving technologies for the seed production and farming of other highly valued marine food fishes.

Orange spotted grouper is highly prized in the world Live Reef Food Fish (LRFF) trade with several traits like fast growth, hardy in nature with tolerance to range of water salinities and high market value. Grouper culture was first introduced in the early 1970s in Singapore, Malaysia, Hong Kong, Thailand and Taiwan and is now practiced throughout south-east Asia with more than 20 species. Grouper culture was initially started using wild caught seeds (fry and fingerlings). Attempts at seed production of grouper started by 1990s and thereafter seed production technology was developed for some of the species. Research on breeding and seed production of orange-spotted

grouper (*E. coioides* reported as *E. tauvina* or *E. suillus* in earlier publications) was started during 1970s. Natural and unstimulated spawning of this species in tank using spawners caught from wild was reported (Hussain *et al.*, 1975 Kuwait Institute for Scientific Research, Kuwait, 12 pp.). Successful induced spawning of the species in cage reared broodstock was also reported (Chen *et al.*, 1977 *Singapore J. Pri. Ind*, *5*: 1-21). Research continued and by 1999, groupers were cultured in many south-east Asian countries, including Indonesia, Malaysia, Philippines, Taiwan, Thailand, Hong Kong, south-east China and Vietnam, as well as other parts of the tropics in the south-eastern USA. More recently, other countries such as Sri Lanka, Saudi Arabia, South Korea and Australia have joined them. The establishment of hatchery seed production technology has helped in increasing orange spotted grouper aquaculture and the aquaculture production of the species has enhanced from 75 t in 2004 to 570 t in 2014. This fish fetches an average price of ₹ 1400 to 1500/kg in Live Reef Food Fish (LRFF) trade around the world, especially in Hong Kong. Envisaging the prospects of orange spotted grouper farming in India, broodstock development was initiated at the Visakhapatnam Regional Centre of the ICAR-Central Marine Fisheries Research Institute in 2010 and the first successful induced breeding and seed production was achieved during April 2013. With further efforts, the mass scale seed production with higher survival rates during larval rearing was achieved in October 2016.

Broodstock development and egg production

Dependable quantity of quality seeds of grouper from hatcheries is key to establishing reliable and sustainable grouper culture. The major bottlenecks in the development of commercial aquaculture are the control of reproductive processes of fish in captivity and production of biosecure and quality-certified fry. Broodstock management usually includes collection, selection and domestication of brooders as well as control of maturation, spawning and egg collection.

The broodstock at Visakhapatnam was developed in Re-circulatory Aquaculture System (RAS) of 8 m diameter and 2.5 m depth tank (Ranjan *et al.*, 2017 *Aquaculture Research* 48, 5864-5873). The wild collected adult female fish of 2-2.5 kg weight were stocked in November 2014. These were obtained from the commercial hook and line operations and transported live into 300 l tanks in the mariculture hatchery. After arrival at the hatchery, the fishes were relieved of barotrauma stress through specific procedures and then treated with 200 ppm formalin for 30 minutes followed by a freshwater dip for 5 minutes. After prophylactic treatment, fish with normal body shape, good colouration, devoid of any skeletal abnormalities and injuries were selected and stocked in an RAS tank. The sexes were identified by live ovarian biopsy using flexible catheter of 1 mm inner and 2 mm outer diameter and found that all collected fishes were females. After 15 days of stocking, a total of 15 fish were randomly selected for sex reversal from female to male and were implanted with a combination of 17$\propto$ methyl testosterone (MT) and letrozole at the rate of 5 mg and 0.2 mg per kg body weight respectively (Ranjan *et al.*, 2015 *Aquaculture Research* 46 (9), 2065-2072). The remaining 15 fish were left without any implantation for ovarian development. Thereafter, the females were cannulated every month to assess the diameter of the intra-ovarian eggs.

The fishes were continually fed fresh squid twice (at 0900 and 1530 hours) in a day till satiation. The fresh feed was given piece by piece enabling the fish to feed on the pieces in the water column itself before the feed fell to the bottom of the tank. Moreover, vitamin A (25,000 IU, USV limited, Nani Daman, India), vitamin B-complex (Pfizer, India), vitamin C (500 mg; Abbott Healthcare Pvt. Ltd., Thane, Maharashtra, India), vitamin E (400 mg) (Merck, Goa, India) and vitamin-mineral mix (Agrimin Forte, Virbac Animal Health India Pvt. Ltd., Mumbai, Maharashtra, India) were supplemented twice a week along with the feed to avoid any possible nutritional deficiencies in their diet. The excess feed was removed from the bottom of the

tank after 30 minutes. After 3 months of stocking in the tank, females underwent final oocyte maturation with 65.53% of the ova having more than 400 μm size. The hormone implanted female fish got sex reversed to male and were found to be oozing after 2 months of implantation. The natural spawning of orange spotted grouper started from February 2015. The egg collection net of 500 μm was fixed in egg collecting chamber connected to broodstock tank. Early next day eggs were collected by passing the surface water through the egg collection chamber. These eggs were treated in 20 ppm iodine solution for 10 minutes and stocked in aquarium for incubation. The eggs hatched after 18-22 hours (h) of incubation at a temperature range of 28-30 ºC (Figs. 1 & 2). A total of 47.23 million of eggs were produced with an overall fertilization and hatching rate of 80.44 ± 0.56% and 85.79 ± 0.50%, respectively, without any hormonal intervention during the spawning. The use of an RAS ensured year-round natural spawning of orange-spotted grouper. The newly hatched larvae measured 1.2-1.6 mm in total length. The mouth opening was formed after 52-56 hours at 28-30 °C at a length of around 1.8-2.0 mm.

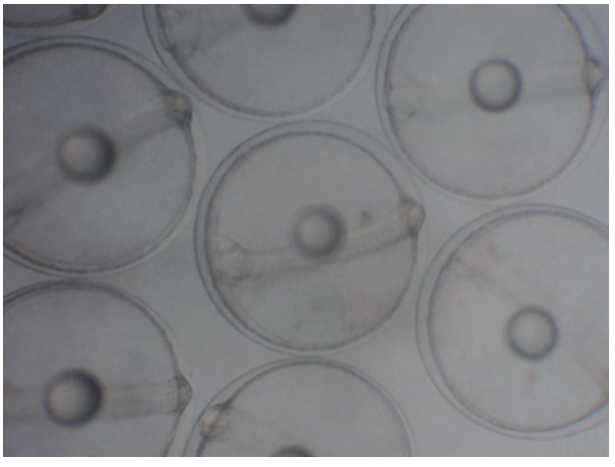

Fig. 1. Initiation of hatching process of fertilized eggs of grouper

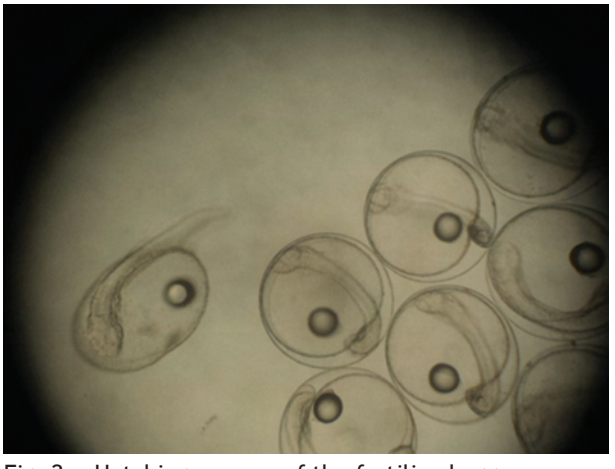

Fig. 2. Hatching process of the fertilized eggs

Larviculture and seed production

The newly hatched larvae of orange spotted grouper measure 1.2-1.4 mm total length. The mouth opens between 2–3 days after hatching (around 60 hours) and the yolk is completely absorbed by 3rd-4th DPH (Days Post Hatching). At the time of mouth opening, mouth gape was 120 μ which increased to 180 μ after 10-12 hours. The stomach and eyes becomes pigmented on 3rd DPH. From 4th to 6th DPH, there are no major morphological changes, but pigmentation around the stomach increases. At 8th DPH, the buds of the dorsal and pectoral spines appear and by 10th-25th DPH, most of the orange spotted grouper larvae have elongated dorsal and pelvic spines typical of serranids larvae. When the larvae are reared at high densities, they often get entangled because of these spines. This leads to high mortalities between 10th and 25th DPH. After 25th DPH, body pigmentation increases and the larvae appear darker in colour. The dorsal and pectoral spines begin to recede. Orange spotted grouper larvae show drastic changes in their shape as they grow from the newly hatched larva to the juvenile stage, just like in other serranids. The larvae before metamorphosis to the juvenile stage are highly sensitive to environmental conditions and substantial mortality occurs even due to minor stresses. Orange spotted grouper larvae metamorphose to juveniles at about 33rd- 37th DPH, but this can be delayed because of low water temperatures and poor nutrition. After metamorphosis, cannibalism starts with larger larvae attacking the smaller ones. As the orange spotted grouper larvae are highly sensitive, careful management is required throughout the larval-rearing phase. Standard methods for larval rearing are to be applied. Artificial lighting from hatching day (= day 0) was provided as follows: at day 3 when

larvae start feeding, from 07:00 to 24:00; at days 4 and 5, continuous; at day 6, from 07:00 to 22:00; at day 7, 07:00 to 20:00. Natural lighting is provided from day 8 onwards. Aeration is to be provided in a 'grid' pattern to ensure even mixing of the water in the tanks and to ensure proper maintenance of dissolved oxygen levels throughout the tank (Sugama *et al.*, 2001 *Manual for the seed production of Humpback grouper, Cromileptes altivelis*. Gondol Research Institute for Mariculture and Others. 37 pp.). Airstones are placed in each corner of the tank to prevent stagnation. Aeration is mild during the early stages of larval rearing to avoid physical damage to the larvae. However, it is increased gradually with progression of the larval-rearing cycle when the larvae become more robust. The sea water used in larval-rearing tanks is pre-treated through sand filter to remove particulate matter and is then ozone (0.1 ppm) sterilized to eliminate pathogens. The recommended initial stocking density for orange spotted grouper is 10 larvae/litre (l). Oil is generally added to form a thin film on the water surface (around 0.2 ml/m^2) during 1st-3rd DPH for preventing surface aggregation mortality in early-stage grouper larvae.

Larviculture protocols were developed by appropriate management of live feeds in suitable quantities and also taking into consideration the nutritional requirements of the larvae. The larvae are stocked in FRP tanks of 2 t capacity for larviculture (Fig. 4). The intensive larviculture tanks are provided with green water (*Nannochloropsis* sp. and *Isochrysis* sp. 3:1 ratio) at a density of about 1 x 10^5 cells/ml and screened rotifer with 80 μm enriched with ALGAMAC at a density of 5-10 numbers/ml from 3 to 5 DPH. The copepod nauplii are added @ 2 numbers/ml. The critical stage for the larvae is 3 to 5 DPH when they shift to exogenous feeding from yolk sac feeding. The larvae are fed with rotifer screened with 100 μm from 6th DPH onwards at a density of 10-15 numbers/ml, which is gradually increased to 20 numbers/ml from 11th to 18th DPH. Rotifer density gradually decreases with increase in the rate of rotifer consumption by the larvae and eventually by 30th DPH, the rotifers disappear. Freshly hatched out *Artemia* nauplii are fed at density of 1 individual/ml from 15th DPH and their size increasing with advancing in rearing period. Adult copepods are fed during 16th-20th DPH in larval rearing. Weaning of grouper larvae with artificial diets starts from 15th DPH. Artificial diet with a particle size of 100-150 μm is used initially. The formulated feed is sprinkled onto the surface of the water in small amounts frequently throughout the day. Formulated feed is added in small amounts so that the feed is consumed within 5 or 10 minutes, as excess feed should not be allowed to accumulate on the bottom of the tank where it get decomposed and degrade water quality. The size of particulate feed is increased to 400–800 μm from 30th-45th DPH. In addition, minced fresh fish meat is fed from 30th DPH.

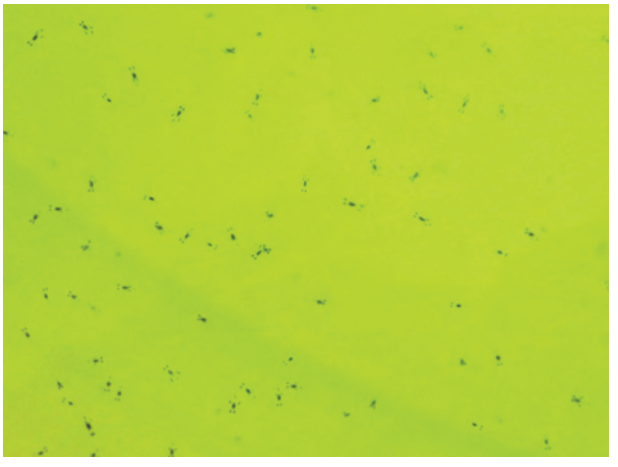

Fig. 3. Larvae of orange spotted grouper reared in green water system

Larval-rearing tanks are maintained statically until 11th DPH, and then from 12th DPH, 5-10% of water exchange per day is required to maintain the rearing water quality. Water exchange is increased to 20% per day, when both rotifers and copepod are being fed together (15th-20th DPH). Water exchange gradually increases to 50% per day from 25th DPH, and is 100% per day from 35th DPH. Bottom siphoning of the tank is started on 8th DPH. From 12th DPH, faeces, dead larvae and uneaten food accumulate on the tank bottom which should be siphoned out at least once daily for maintaining water quality.

A survival of more than 12 % is achieved during the larval rearing. From 35 DPH, grading of larvae is started. The shooters are fed exclusively with

the artificial feed of size 500-800 μ. The grading is performed weekly for at least 2-3 weeks to segregate bigger juveniles from smaller to avoid cannibalism. The juveniles measuring 2.5-3.0 cm length (0.4g) (Fig. 4) are ready for stocking in hapas, cement tank or in pond based hapa for nursery rearing for another two to three months.

Fig. 4. Juveniles ready for nursery rearing

Nursery rearing

The nursery rearing of orange spotted grouper is standardized with different feed and culture conditions. Nursery rearing of grouper comprises of two phases. In the first phase, 2.5-3.0 cm (0.4g) fry are cultured in tank for 2 weeks till they accept artificial feeds fully, by which time they reach upto 5-6 cm. The fry during this period is reared in 1 t capacity tank @ 1 number per litre. They are fed on artificial diet and *Artemia* for completely weaning the larvae to artificial feed. Artificial feed containing 45 % protein and 10 % fat is used during this period. Grading is performed every 5 days to grade the larvae according to size. The second phase consists of growing 5-6 cm size to 10-15 cm for stocking in cage as well as in pond. During this period, the fingerlings are reared either in pond based *hapa* (2 mm mesh size), flow through cement tank (Fig. 5) or in re-circulatory system. Pellet feed with different protein levels and minced fish meat are used as feed, among which pellet feed with 45% protein exhibits the highest growth. The rearing system is also found to influence the growth rate, where highest average daily weight gain of 0.59g per day is observed in RAS, followed by 0.4g per day in pond and 0.26g per day in cement tanks. In all the rearing systems, fingerlings of 2-3 g stocked grew to an average size of 15-20 g after a month when fed with pellets containing 45% protein. The fingerlings produced have been distributed to private entrepreneurs in Andhra Pradesh, Tamil Nadu, Kerala and Karnataka.

Fig. 5. Fingerlings reared in *hapa* in cement tank

Grow out culture

Advanced fingerlings are stocked in 6m diameter floating HDPE cage for grow-out. The fishes are maintained here for one year during which different mesh sizes are used at different period of the culture depending on the size of the fish. Advanced fingerlings of approximately 10 cm (15g) are an ideal size for stocking either in cages or ponds for culture. These can reach around 1 kg after 10-11 months of culture in the cages (Fig. 6). Initially the fishes are stocked in cage with 1 cm mesh size net. On reaching 100 g size, fishes are transferred to cages with 2

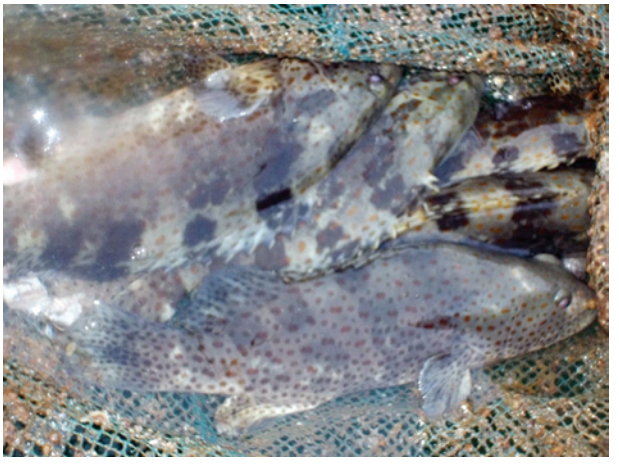

Fig. 6. Grow-out system in cages

cm mesh size nets and finally on reaching 500 g size, they are stocked in 5-6 cm meshed net cages till harvest. During grow-out, low valued fish (sardine, scads, tilapia, etc) is considered as good feed. Additionally, pelleted feed with 40% protein content is also used for grow-out. Net exchange is performed once in 30-45 days for proper management. Based on experience obtained from different trials, fish stocked at 15 g grew to 250g, 500g and 1000g after 6 months, 8 months and 12 months of rearing in cages. The stocking density of 15-20/m^3 is found to be optimum for cage reared grouper.