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Practical Manual on Seed Production of Orange Spotted Grouper and Indian Pompano

Indian Council of Agricultural Research
Central Marine Fisheries Research Institute

Practical Manual on Seed Production of **Orange Spotted Grouper and Indian Pompano**

Ritesh Ranjan, Sekar Megarajan, Biji Xavier, Chinnibabu Bathina, Shubhadeep Ghosh, A. Gopalakrishnan

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Dr. A. Gopalakrishnan
Director
ICAR-Central Marine Fisheries Research Institute
P.B. 1603, Ernakulam North P.O.
Kochi- 682 018, Kerala, India

www.cmfri.org.in
E-mail: director@cmfri.org.in, director.cmfri@icar.gov.in
Telephone: +91 484 2394867
Fax: +91 484 2394909

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PREFACE

Mariculture is the fastest growing sub-sector of aquaculture in the world. In contrast to the global scenario, where mariculture of finfishes is a well-developed industry, in India, it is gradually emerging out from its infancy. Cage farming technology is widely recognized as the most important technology in mariculture for increasing fish production to meet the food fish demand. One important aspect hindering the rapid progress of mariculture in the country is the availability of quality seeds of high value fin fishes. However, in recent years, with success in breeding and seed production technology of several high value commercially important finfishes, thankfully due to the consistent efforts of ICAR-Central Marine Fisheries Research Institute (CMFRI), the mariculture sector of the country is poised to make a serious contribution to the fish basket of the country. Presently, quality seeds are available for cobia, Indian and silver pompano, grouper, snapper and sea-bream round the year in various hatcheries of CMFRI at multiple locations.

Marine finfish seed production has to be up-scaled for meeting the stocking demand of the cages. National Fisheries Development Board has already come forward in this regard, and financial grants were provided to several private shrimp hatcheries for conversion into marine finfish hatcheries with technological support from ICAR-CMFRI. Also, as establishment of broodbanks and maintaining broodstock is expensive for private hatcheries, ICAR-CMFRI has been continuously providing them yolk-sac larvae of marine finfish for larval rearing and subsequent nursery. However, keeping in view the huge demand of stockable sized seeds, multiple satellite hatcheries and satellite nursery rearing centres needs to be developed by the government in all maritime states.

ICAR-CMFRI has successfully developed in the recent past seed production technology for pompano (*Trachinotus mookalee* and *Trachinotus blochii*), cobia (*Rachycentron canadum*), grouper (*Epinephelus coioides*), snapper (*Lutjanus johnii*) and bream (*Lethrinus lentjan* and *Acanthopagrus berda*), for the first time in the country. Around a million seeds has been continuously and consistently produced in the last few years and have been distributed to research institutes, state government owned facilities and private enterpreneurs for nursery rearing and grow-out. However, commercial level seed production, as envisaged is possible only when seed production technology is transferred to private finfish hatcheries. Presently, for orange spotted grouper and Indian pompano, manual encompassing all aspects on seed production is lacking. The present handbook on "Hatchery management of orange spotted grouper (*Epinephelus coioides*) and Indian pompano (*Trachinotus mookalee*)" would be ideal for hatchery operators and technicians, in providing them the required technical know-how on large-scale marine seed production for orange spotted grouper and Indian pompano.

Dr. A. GOPALAKRISHNAN
DIRECTOR, ICAR-CMFRI

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Introduction

The fish requirement for domestic consumption in India is estimated to touch 15.61 million metric tons by 2030. With the present annual fish production of 12.39 million metric tons during 2018, a gap of 3.22 million metric tons will need to be bridged to meet the projected domestic fish demand (FAO, 2020). Given the limitation of marine capture fisheries sector of the country, and the modest enhancement that is likely to occur from deep sea resources along with the limited opportunities for expansion of land-based fisheries and aquaculture systems, the focus is towards expansion of mariculture. India, with 8129 km of coastline, 2.2 million km^2 of Exclusive Economic Zone, 0.5 million km^2 of continental shelf, 1.2 million ha of coastal salt affected land and 3.9 million ha of estuarine area possesses a high potential for marine finfish culture. Despite having vast potential to enhance the marine finfish production, the country is still at an early stage with respect to commercial scale marine finfish farming. However, in tune to the global scenario, there is anticipation that contribution from mariculture is poised to increase manifolds in the future.

The primary problem associated with expansion of marine finfish culture in the country is the unavailability of fish seeds in considerable quantities. Seed production technology for several commercially important finfishes (cobia, pompano, grouper, snapper and bream) has been developed and perfected by ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI). Of these, along the eastern coast, Indian pompano (*Trachinotus mookalee*) and orange spotted grouper (*Epinephelus coioides*) have exhibited considerable promise, with respect to growth and survival when cultured either in marine or coastal environments. Possessing superior market demand and higher prices, these two species are currently viewed as better options to shrimp for farming by aquafarmers in the region. In fact, the progress and success achieved with respect to seed production and culture by Visakhapatnam Regional Centre of ICAR-CMFRI have created a sense of belief, confidence and interest among many aqua-entrepreneurs from

different states and many of them, has evinced keen interest in establishing finfish hatcheries. Also presently, shrimp culture is in doldrums, due to the frequent failures of the crop. Adopting crop rotation using these finfishes, to some extent would solve the issue of diseases in shrimp industry and these fish species is suitable for crop rotation, since the shrimp pond could be used as such for the culture of these species without further modifications.

With proper technical knowledge, and a thorough understanding on various critical phases during seed production, the day is not far, when commercial level seed production would be a reality. This would lead to enhancement in the production for orange spotted grouper and Indian pompano and in-turn, would contribute significantly to food and nutritional security of the country and present enormous scope for foreign exchange earnings, thereby improving the livelihood of the fish farmers.

Grouper

Groupers belonging to the family Serranidae and sub-family Epinephelinae is composed of 15 genera and 159 species, principally distributed among the Indo-Pacific region (110 species), the East Atlantic and Mediterranean regions (14 species) and the inter-tropical American zone (35 species) (Pierre *et al*., 2007). Groupers are commercially important fish, particularly for live seafood markets in several Asian countries such as Hong Kong, China, Taiwan, Singapore and Malaysia (Johnston and Yeeting, 2006). They are highly prized for the quality of their flesh and most species fetch high market prices (Ottolenghi *et al*., 2004). The most sought-after species in the international seafood markets are usually representatives of three genera: *Epinephelus*, *Cromileptes* and *Plectropomus* (Sugama *et al*., 2012). The genus *Epinephelus* includes 98 species, of which seventy come from Indo-Pacific area, eight from East Pacific region, eleven from West Atlantic region and nine from East Atlantic and Mediterranean zones (Pierre *et al*., 2007). The genus *Cromileptes* includes only one species, *Cromileptes altivelis;* whereas genus *Plectropomus* includes seven species.

Groupers are protogynous hermaphrodites. The majority of these species live on coral reefs, but they are also found in estuaries, on rocky bottoms and less frequently in sandy or silty habitats. Some species occur in depths of 100 to 200 m; however, the majority inhabits depths less than 100 m, and juveniles are often found in tide-pools (Heemstra and Randall, 1993). They are largely piscivorous, but can also feed on crustaceans and cephalopods. Except for occasional spawning aggregations, most species are represented by solitary behaviour, resident on a particular reef for long periods of time.

Groupers are distributed all around the Indian coast and as on date, sixty nine species are reported from Indian waters; among which *Epinephelus coioides*, *E. malabaricus*, *E. fuscoguttatus*, *E. polyphekadion* and *Cromileptes altivelis* are considered as potential species for aquaculture.

Orange spotted grouper

The common name or FAO name of *Epinephelus coioides* is "orange spotted grouper". However it is known by different common names in different parts of India which are listed in Table 1.

The orange spotted grouper, *Epinephelus coioides* occurs in the western Indian Ocean from the southern Red Sea to Durban (South Africa) and east to the western Pacific where it is distributed from

Fig. 1. *Epinephelus coioides* (Orange spotted grouper)

Ryukyu Islands (Japan) to New South Wales; Oceania only to Palau in the Northern Hemisphere and Fiji in the Southern; and the eastern Mediterranean. In India, this species is distributed all along the Indian coast from Gujarat to West Bengal, including the Andaman and Nicobar Islands.

The fish is light greyish-brown dorsally, shading to whitish on sides and ventrally, with numerous small brownish orange or reddish brown spots on head and body and median fins; 5 slightly diagonal greyish-brown bars on head and body which bifurcate ventrally, the first 4 extending basally into dorsal fin; brownish orange spots on body tend to be arranged in rows parallel to dark bars, more evident on smaller fish; large dark greyish-brown blotches usually present on head, the most prominent behind the eyes and on opercle; fins whitish to light dusky with brownish orange to brown spots except distally on spinous portion of dorsal, caudal and pectoral fins. The smaller individuals of this species are often confused with *E. tauvina* and *E. malabaricus*, but can be distinguished by presence of orange spots, lack of hexagonal spots on the fins and different number of pyloric caecae.

Orange spotted grouper inhabits shallow reefs, lagoons, brackish water, over mud and rubble in depth to at least 30 m. Juveniles are commonly reported in the shallow waters of estuaries over sand, mud and gravel and among mangroves. They are eurythermal and euryhaline. It feeds mainly on fish followed by crabs, shrimps, squids, gastropods and bivalves.

Epinephelus coioides is a diandric protogynous species, where males are either derived from a juvenile phase or the transition of post spawning females. Females mature at 320 mm total length (TL) at an age of 2 years, whereas primary males mature at 242 mm TL at an age of 1 year. The sexual transition occurs at a TL of 550-750 mm at the age of 5-6 years. The major spawning period is March to June. They probably spawn during restricted periods and form aggregations for spawning after the full and new moon.

Table 1. Common names for *Epinephelus coioides in different parts of India*

Common name	Languages
Hekaru, Gobra	Marathi
Gobri, Wekhanu	Konkani
Gopra, Muni meenu	Kannada
Kalawa	Malayalam
Kalava	Tamil
Ratibonta, Bontha, Kodi punju	Telugu
Bhala	Oriya

Pompano

Pompano is a member of the jack family (Carangidae), order Perciformes and class Actinopterygii (ray-finned fishes). Several species of pompano (*Trachinotus carolinus, T. blochii* and *T. mookalee*) are well recognized as promising species for mariculture due to their attractive appearance, fast and uniform growth rate, adaptability to culture environment, acceptability to formulated feed, firm white as well as tasty meat and high market demand. The other species of interest of the genus *Trachinotus* include Atlantic permit (*T. falcatus*), palometa (*T. goodie*), longfin pompano (*T. goreensis*), derbio (*T. ovatus*) and African pompano (*Alectis cilris*). The broodstock development of Florida pompano (*Trachinotus carolinus*) and its seed production as well as farming technology has been well established in the USA during 1970s (McMaster *et al.* 2003). Artificial propagation techniques for *Trachinotus blochii* were fully developed in Taiwan during 1989 (Chang, 1993; Cheng, 1990). In the recent past, CMFRI has successfully developed and standardized the broodstock development, induction of spawning, larviculture and fingerling production of silver pompano for the first time in India (Gopakumar *et.al.,* 2012; Abdul -Nazar *et.al.,* 2012). The aquaculture of pompano is successfully established in many Asia – Pacific countries like Taiwan and Indonesia and is gaining momentum

in India. Pompano is considered one of the most desirable food fishes and it commands a significantly higher price than many other marine and freshwater species.

Indian pompano

The common name or FAO name of *Trachinotus mookalee* is "Indian pompano". However it is known by different common names in different parts of India, which are listed in Table 2. *T. mookalee* belonging to the family Carangidae (jacks and pompanos) holds immense potential for the marine finfish aquaculture sector. It is a pelagic species which inhabits shallow coastal waters (Smith-Vaniz, 1984). Indian pompano is most common in shallow coastal waters in a number of environments, including coral and rocky reefs and shore faces and tidal flats. The species has a wide salinity tolerance, as evident from the ranges from which juvenile and sub-adult fish are caught in Indian waters. The young fish eventually move to inshore reefs as they mature, before again moving to deeper outer reefs. It is distributed in western Indian Ocean from the Gulf of Oman eastward to Sri Lanka. Its range also extends to Singapore, Gulf of Thailand and Hong Kong. In India it has been reported both from the east and west coasts. Indian pompano is reported to grow to a size of 90 cm in total length (Randall, 1995) and 8.1 kg body weight (Smith-Vaniz, 1984). Under captivity, when grown in offshore cages, Indian pompano grew from 42.8 g to 969.90 g after 9 months of culture (Ranjan *et al.*, 2017).

The species predominantly takes molluscans (gastropods and bivalves) as prey; however, it supplements its diet with a varied array of other invertebrates and fish. The former includes crustaceans such as shrimps, decapods and copepods. The larger fish on reefs tend to move between reefs regularly, which is thought to be due to prey availability. Studies of different size classes of fish have revealed that their diets change with age in some locations, with the changes relating to an increased volume of fish taken. Studies at Visakhapatnam, India have shown that the diet of Indian pompano is dominated by gastropods, bivalves and crabs.

Fig. 2. *Trachinotus mookalee* (Indian pompano)

Spawning is known to occur throughout the year depending upon the temperature. Off north Andhra Pradesh, spawning season is during Feb-April. Fish aggregate in large schools prior to spawning, with pairs breaking off the main aggregation to commence spawning. Size at first maturity estimated at Visakhapatnam was 690 mm TL (approximately 3.9 kg body weight). The smallest mature female fish observed at Visakhapatnam measured 600 mm fork length (FL).

Table 2. Common names for *Trachinotus mookalee* in different parts of India

Common name	Languages
Aavoli para, Valavodu, Vellaodu	Malayalam
Mooku para	Telugu

Hatchery technology for Orange spotted grouper and Indian pompano

Considering the importance of marine finfish, initiatives have taken place in India in order to develop technology for artificial propagation of orange spotted grouper and Indian pompano. Orange spotted grouper and Indian pompano hatchery technology has been pioneered by the Regional Centre of ICAR-Central Marine Fisheries Research Institute at Visakhapatnam, Andhra Pradesh. The work on broodstock development and seed production of orange spotted grouper was started in 2009 and initial success in breeding was achieved with larval

survival of less than 1% during 2013. Subsequently, the centre has achieved in large scale seed production of orange spotted grouper (fig. 3) with an average survival rate of 12.56% and produced more than 6.0 lakh seeds since 2016. The research work on broodstock development and seed production of Indian pompano was started in 2012 and by 2013, initial success in breeding of Indian pompano was achieved with larval survival of less than 5%. Subsequently, the centre has achieved large scale seed production of Indian pompano (Fig. 4) with an average survival rate of 21% and produced more than 1 million seeds since 2016.

Fig. 3. Fingerlings of Orange spotted grouper

Hatchery design and operation

The implementation of biosecurity is essential in any hatchery to reduce the risk of incidence of diseases either in broodstock or in the larval production section. The following points need to be taken care during the hatchery operation for biosecurity reasons.

1. Separation of various functional sections of the hatchery (broodstock, live feed production such as phytoplankton and

zooplankton, larval rearing, etc.) with footbaths and hand washes at each entry and exit points

2. Access to larval rearing sections limited to required personnel only
3. Washing and disinfecting of all equipment, including water-quality monitoring equipment, nets, basins, etc. before and after use
4. Fishes brought from outside of the hatchery should be quarantined first, then allowed to enter in the existing system
5. Try to produce larvae in batches and in between batches, hatchery need to be cleaned with disinfectants and dried
6. Staff should be trained in the area of biosecurity and health management
7. Routine monitoring for pathogens and disease need to be carried out and steps for prompt diagnosis of any disease events should be under taken.

Fig. 4. Fingerlings of Indian pompano

BROODSTOCK AND SPAWNING

Broodstock

Acquisition

Initially, orange spotted grouper (Fig. 1) and Indian pompano (Fig. 2) broodstock can be acquired through collecting or purchasing wild fish. Record maintenance of collected adult fish being brought into the hatchery for broodstock development programme is important and need to be maintained for future use.

Orange spotted grouper are diandric protogynous species where males are either derived from a juvenile phase or the transition of post; spawning females (Grandcourt *et al*., 2009). However collecting males from wild is very difficult. While collecting adult orange spotted grouper at Visakhapatnam RC of CMFRI, Visakhapatnam, the smallest recorded size of mature male of orange spotted grouper from wild was 18 kg. Handling of such bigger sized male is problematic, hence collecting smaller fishes of around 2.0 kg is good for developing male broodstock by manipulating sex using different hormones and enzymes.

Adult Indian pompano of more than 3 kg can be collected from the wild by hook and line and transported to the hatchery. The sexes are separate and the maturation size is around 3.0 kg, thus the same size fish needs to be collected in more number for developing broodstock.

Pond or cage cultured fish is another source of broodstock. Cage, pond or tank-reared fish are already accustomed to culture conditions and consequently easier to develop into suitable broodstock. However, it can take 2 years to grow juvenile fish up to broodstock size, and more over inbreeding might happen when collecting fishes from culture system for developing broodstock. While selecting a fish for developing broodstock, few characteristics needs to be kept in mind as suggested by Moretti *et al*. (1999) for species such as European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*):

- Normal body shape and colour
- Absence of skeletal deformities
- Overall health status, i.e. absence of large wounds, haemorrhages, infections and parasites
- Normal behaviour, such as a good response to feed, controlled buoyancy to maintain position in the water column
- Best growth and feed conversion rate within its age group, when we are selecting from cultured one.

Transport

Generally adult fish should be collected from nearby area as far as possible. The collected fish should be transported in covered tanks containing aerated or oxygenated water to reduce stress. Mild sedation, using approved sedatives for fish, such as 2 phenoxyethanol @ 50 ppm can be used to reduce stress and make handling the fish easier and safer. If the fish is collected from the culture system, they should not be fed for the previous 24 h to avoid deterioration of water quality during transportation. Generally, fish regurgitate the feed if they are handled after feeding, thus it will spoil the water quality as well as induce stress to the collected fish.

Treatment after collection

Once the fish are transported to the hatchery, the fish need to be shifted in quarantine area. It is advisable to quarantine them to reduce the parasitic or bacterial infection. Generally the quarantine period varies from 3 to 4 weeks and can be carried out in small tanks of 1 m^3 to facilitate easy handling. Groupers are demersal fish and hence after catching, the fish from wild shows barotrauma (Fig. 5). Barotrauma occurs due to the differences in the pressure from where they are caught and the sea surface. Due to differences in pressure, air bladder fills with air and bulges and gives stress to the internal organs. This needs to be relieved by inserting a needle in gas bladder through anus. Once the fish are swimming normally with controlled buoyancy to maintain

Fig. 5. Orange spotted grouper collected from wild show barotrauma

Fig. 6. Wild collected Indian pompano treated with formalin in fresh water

position in the water column, they need to be shifted in appropriate tank. The wild collected orange spotted grouper should be given bath treatment in formalin at the rate of 200 mg L^{-1} for 30 min followed by 5 min, dip in freshwater. Fish should be shifted to another tank after the treatment with fresh seawater. This treatment should be repeated every 4 to 5 days for atleast 6 times. In case of Indian pompano, the fish should be treated by giving bath treatment of formalin in freshwater at the rate of 30 mg L^{-1} for 15 min (Fig. 6). Fish should be shifted to another tank after the treatment with fresh seawater. This treatment need to be repeated every 4 to 5 days for atleast 8 times consequently. Once the fishes are free from the parasite, the fish can be shifted from quarantine area to broodstock tank.

Broodstock tanks

Generally broodstock tanks are used for culture and maturation of broodstock as well as for spawning. Due to the size of the broodstock and the natural spawning behavior of the fish, larger tank of 50 -100 m^3 are preferred for broodstock tanks. At Visakhapatnam Regional Centre of ICAR-CMFRI, 125 m^3 concrete reinforced tanks with water volume of 100 m^3 are used for broodstock development cum spawning (Fig. 7). Generally tanks should be round, or square or rectangular with rounded corners, however round tank is preferable. Medium-range blue, green or grey colour is preferable for the broodstock tank. Tanks should be at least 2.0 m deep and preferably 2.5 m to allow sufficient room for spawning behavior of orange spotted grouper, which involves pairs or group of fish swimming upward from the tank bottom while releasing ova and sperm (Ranjan *et al*., 2017). However, in case of Indian pompano, tank of smaller size of 20-30 m^3 can also be used for broodstock development. Generally the broodstock tanks are used as flow-through system, however re-circulating aquaculture system are better for broodstock development and spawning. An overflow pipe is fixed 15 cm down from the surface of the broodstock tank to the egg collection tank. The egg collection chamber is installed with 500 µm mesh for sieving the eggs (Fig. 20). The egg collecting chambers are

Fig. 7. Broodstock tank with re-circulating facilities

Fig. 8. Orange spotted grouper brooders in re-circulating aquaculture system

Fig. 9. Indian pompano brooders stocked in RAS

connected with the re-circulating loop so that the water is pumped back to the broodstock tank. It is advisable that broodstock area should be roofed in order to reduce the growth of algae on the tank wall and bottom, which makes egg collection difficult and increases the risk of failure of re-circulating system. Moreover, dirty tanks need to be cleaned frequently which may stress the broodstock and cause spawning failure or lower the quality of eggs. Broodstock tanks should have re-circulating facilities with 300% water re-circulation. Sea water used for broodstock development should be filtered and clear with stable salinity of 30-35 ppt and water temperature of 27-32°C.

BROODSTOCK MANAGEMENT

Gender identification

The male and female of orange spotted grouper and Indian pompano cannot be identified morphologically. The sex of individual fish can be confirmed only by physical examination i.e live ovarian biopsy (LOB). Fishes need to be anaesthetized by using 2 phenoxy ethanol at

Fig. 10. Cannulation of orange spotted grouper using baby feeding tube

Fig. 11. Cannulation of Indian pompano using baby feeding tube

Fig. 12. PIT tag used for tagging and identification of a particular brooder

Fig. 13. PIT tagging of a brooder

the rate of 200 ppm for 2-3 minutes. The anaesthetized fish is then cannulated using the fish cannula or baby feeding tube CH 6 having an inner diameter of 1 mm and an outer diameter of 2 mm. The cannula is inserted into the urinogenital orifice of males and the oviduct of females (Figs. 10 & 11). Fish to be cannulated are anaesthetised and a wet cloth or towel is placed over the eyes to assist in calming the fish. The cannula is guided into the fish for a distance of 6–7 cm and suction is applied to the other end of the cannula as it is withdrawn. After withdrawal, the sample within the cannula is expelled onto a microscope slide for immediate examination or into a vial containing 1% neutral buffered formalin for later measurement of egg diameter. Generally, females of orange spotted grouper and Indian pompano in spawning condition will have oocytes in range of 400–500 and 500-600 µm diameter respectively. To confirm matured male, the abdomen of an anaesthetised fish is gently massaged in a head-to-tail direction. A sexually ripe male spawner will extrude copious milt from its urinogenital pore. If no milt is expressed, the fish is immature, a male not in spawning condition. Once the sexes of the fishes are confirmed, they are tagged with passive integrated transponder (PIT) and the tag number need to be maintained for future use (Figs. 12 & 13).

Sex-reversal

As noted earlier, orange spotted grouper are diandric protogynous, and getting male from wild is very difficult; thus a hatchery manager needs to manipulate the sex by using hormones and enzyme. The fifty percent of the stocked and acclimatized fishes should be implanted with pellet containing 17 α methyl testosterone and letrazole at the rate of 5 and 0.2 mg kg^{-1} body weight respectively (Ranjan *et al., 2015)*. The pellet can be prepared using gum acacia, cholesterol and 17 α methyl testosterone in the ratio of 1:2:1 (Fig. 14). These chemicals need to be weighted accordingly, and mixed with few drops of water, which then form dough and is prepared in required size as per the implanter. This prepared pellet needs to be implanted on dorsal side of the brooders below to the dorsal fin in musculature (Fig. 15). Before

implantation, fish should be anaesthetized to avoid handling stress. This hormonal dose will convert the female to male within 2 months.

Fig. 14. Pellet prepared using gum acacia, cholesterol and 17 α methyl testosterone for implanting brooder

Fig. 15. Implantation of hormonal pellet to grouper brooders

Feeding

At Visakhapatnam RC of CMFRI, broodstocks are fed to satiation at least once daily in the morning with fresh or frozen squid (Fig. 16) for orange spotted grouper and squid along with clam meat (Fig. 17) for Indian pompano. Various vitamins namely, 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) are supplemented twice a week along with the feed to avoid any possible nutritional deficiencies. These vitamin pre-mix tablet are inserted inside the squid and fed one by one till the satiation of the fish.

Tank cleaning

If the broodstock tank is connected with re-circulating system, faeces and other waste material such as dead eggs and feed remains will not

Fig. 16. Squid used for feeding brooders

Fig. 17. Clam meat fed to the Indian pompano brooders

accumulate in the tank. The broodstock tank doesn't require any cleaning; however it is advisable to clean the tank at least once in a year by removing all fish from broodstock tank.

Spawning/ Induced spawning

Orange spotted grouper broodstock are allowed to spawn naturally in the tanks. If the tank is stocked with matured female (2.0 kg) and hormone pellet implanted male, spawning will start from 3rd month onwards. Spawning generally occurs at dusk (3 pm – 6 pm) for ten to fifteen times in each month. At Visakhapatnam RC of CMFRI, grouper broodstock spawn throughout the year (Ritesh *et al*., 2017). During the spawning period, orange spotted grouper may spawn between 0.8 and 6.0 million eggs each time. The re-circulating system needs to be stopped by 15.00 h during winter and 17.00 h in summer to avoid washing out of eggs. If the spawning happened then, the re-circulatory system should be in OFF till the next morning when the eggs are being collected. Otherwise, if the spawning did not happen by 18.00 h, the re-circulating system should be ON.

Fig. 18. Administering the hCG hormone to the brooder for inducing spawning

In the case of Indian pompano, natural spawning is rare and in most situations, needs to be stimulated with the use of hCG or LhRH hormones for the spawning. Once the female ova size exceeds 500 µm, and the males are oozing, then the both sexes needs to be injected hCG at the rate of 350 IU/kg body weight, or LhRH at the rate of 100 and 50 µg/kg body weight of female and male respectively (Fig. 18). The fishes for cannulation should be anesthetized to avoid stress during the cannulation and injection. As far as possible, avoid the checking of male before inducing for spawning. The fishes are then shifted back to the same tank for spawning. The fishes respond after 36 h of injection at a temperature range of 28-32 °C. The re-circulating system needs to be stopped after 35 h of induction to avoid washing out of eggs. Generally the Indian pompano will respond in night between 23.00 to 24.00 h

EGG-HANDLING

Collection

The fertilized eggs of orange spotted grouper and Indian pompano are floating in nature at a salinity of more than 30 ppt. This floating nature of eggs is helpful in collection by overflowing the egg in collecting chamber. The fertilized eggs of orange spotted grouper and Indian pompano are non-adhesive and pelagic, and range from 0.8 to 0.9 mm and 0.9 to 1.0 mm in diameter respectively. A 500 µm mesh size bag is tied in egg collecting chamber, which is connected to broodstock tank via a 3 inch PVC pipe (Fig. 20). The water level in the broodstock tanks should be raised and the re-circulating system should be started in the morning at 6.00 h by switching ON the water flow from the egg collecting chamber to the re-circulating system. The eggs collected in egg collecting chamber are scooped with the help of 500 µm mesh size sieve and transported to the larval rearing section of

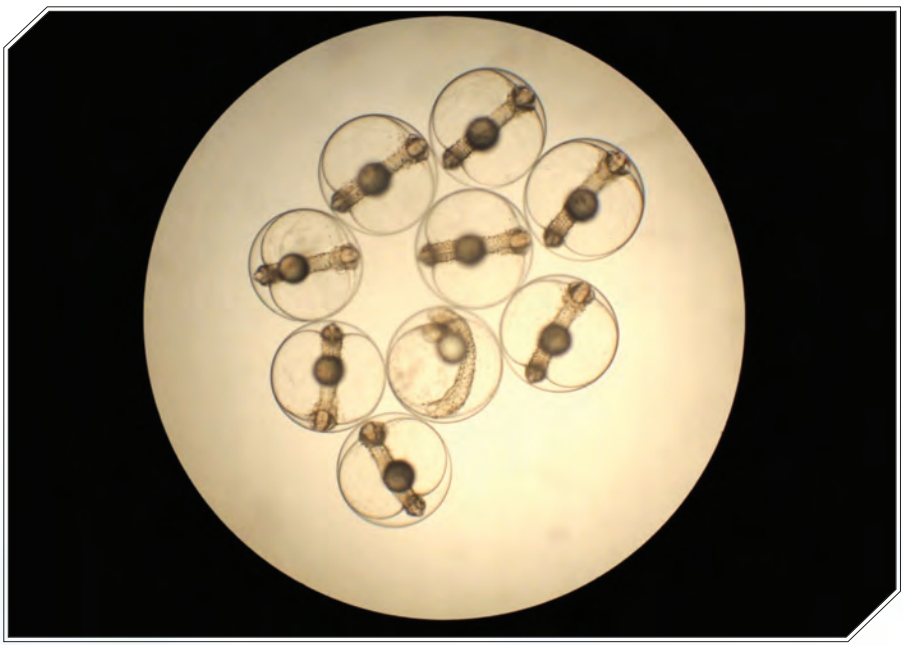


Fig. 19. Indian pompano embryo has developed optic vesicles, i.e. at the eyed stage, the eggs will be collected at this stage

Fig. 20. Water in the broodstock tank flows through the egg collecting chamber and the eggs are collected in the fine mesh net hapa

hatchery. The eggs are sensitive to handling stress during the early embryonic developmental stages and should only be collected after the embryo has developed optic vesicles, i.e. eyed stage (Fig. 19). Handling or iodine treatment of eggs before this stage will lead to increased mortality and higher incidence of failure of hatching.

Disinfection

The collected eggs should be disinfected with 20 ppm active iodine for 10 minutes in strong aeration (Fig. 21). The treated eggs are then washed with deionized sea water and stocked in glass aquarium with water salinity of 30-32 ppt (Fig. 22). As noted earlier, fertilized eggs will float on the water surface whereas unfertilized or dead eggs will settle in the bottom, , which needs to be removed by siphoning the bottom of the aquarium (Fig. 23). Only floating eggs on surface are used for larval rearing because these are more likely to be of good quality than sinking eggs, which are unfertilized or dead or inferior of quality.

Fig. 21 Egg treatment with iodine solution

Fig. 22. Eggs stocking in aquarium after treatment

Fig. 23. Removing dead and unfertilized eggs from the aquarium through siphoning

EGG QUALITY

Egg quality in marine finfish is generally evaluated using both qualitative and quantitative methods.

Qualitative evaluation of the eggs

Fertilized eggs are examined under a microscope (4x or 10x) for qualitative evaluation of embryos. The good fertilized eggs will have following characters:

- Eggs should be regular in shape
- During the early stages of embryonic development, the individual cells should be regular in size
- Eggs and embryos should be transparent, with no dark areas
- Chorions (eggs hells) should be free of any parasites or fouling organisms.

In case of orange spotted grouper, if there is only a small proportion of eggs with irregular shape, dark or with aberrant embryonic development, then the eggs can be used in the hatchery. However, if the proportion of eggs exhibiting abnormal characteristics (>10%) is high, the entire batch should be discarded, as there is very less chance of larval survival during the larval rearing period (Sugama *et al*., 2012). The eggs should be discarded when they have parasites or fouling organisms attached to them for the probability of transferring pathogens to the hatchery.

Quantitative evaluation of eggs

Fertilization and hatching rates are also used as indicators of egg quality. These rates for groupers are higher than 50%, and preferably more than 80%. Grouper larvae from batches of eggs with low fertilization and hatching rates (<30%) are regarded to be of poor quality (Sugama *et al*., 2012). Poor quality larvae exhibit low survival with a high incidence of deformities and other health problems and are usually discarded.

Fertilization rate

Estimation of fertilization rate is undertaken several hours after fertilization has taken place, but well before hatching, as because embryonic development makes it easier to discriminate the fertilized eggs (Fig. 24) from the unfertilized eggs (Fig. 25). For estimating fertilization rates, at least 10 egg samples are taken to have an accurate estimate. Before taking the sample, the eggs should be mixed properly so that the fertilized and unfertilized eggs have proper representation. The number of fertilized eggs and unfertilized eggs present in the samples are counted. Total number of eggs present in the sample is calculated by adding the numbers of fertilized eggs and unfertilized eggs of the sample. The numbers of fertilized eggs are divided by total numbers of eggs and multiplied by 100 to obtain fertilization rate (%). The fertilization rates of orange spotted grouper and Indian pompano is generally more than the 80% and 70% respectively at Visakhapatnam RC of CMFRI.

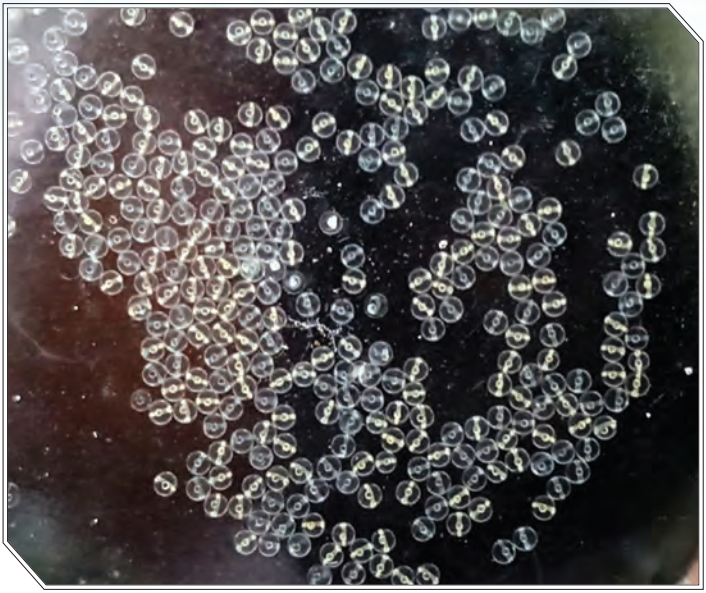


Fig. 24. Fertilized eggs of Indian pompano

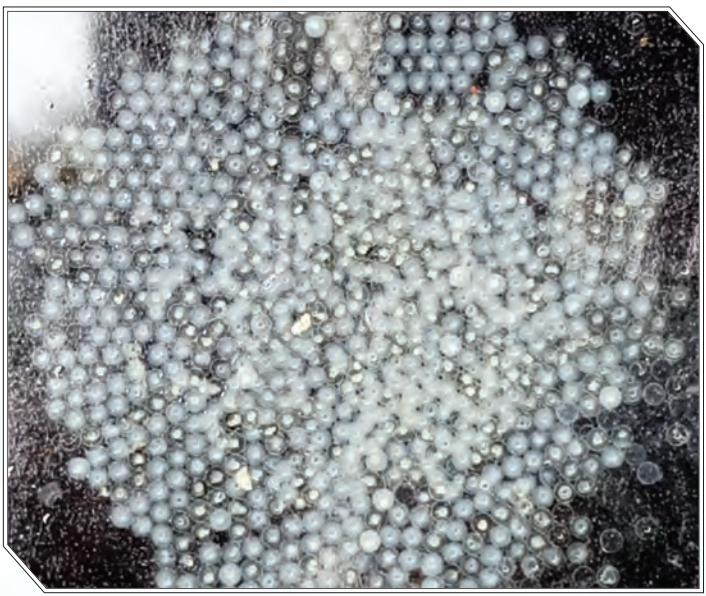


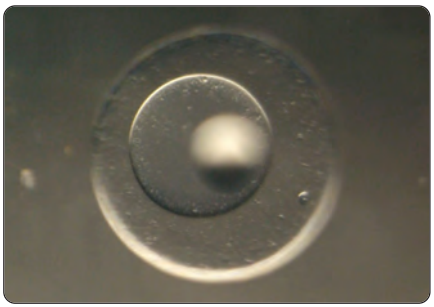
Fig. 25. Unfertilized eggs of Indian pompano make them opaque

Hatching rate

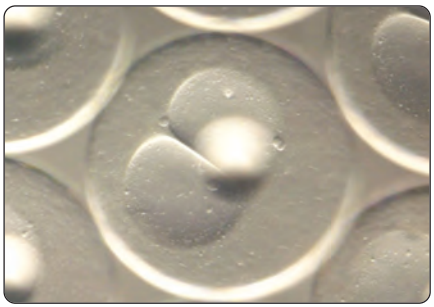
The stocked fertilized eggs usually hatch out 18-20 hrs after fertilization at a water temperature range of 28-30°C. Estimation of the hatching rate is undertaken when hatching is completed. The hatched out larvae drift on the water surface. The estimation of hatching rate is performed from random vertical water samples of hatching tank using PVC pipe from 10 different places. The volume of sampled water and number of larvae in it is estimated. The total number of larvae present in the hatching tank is calculated by extrapolating the sampled estimate for the whole tank. The total number of hatched out larvae are divided by the total number of fertilized eggs stocked and multiplied by 100 for calculating hatching rate (%). The hatching rate of orange spotted grouper and Indian pompano at Visakhapatnam RC of CMFRI is more than 85% and 80% respectively.

EGG EMBRYONIC DEVELOPMENT

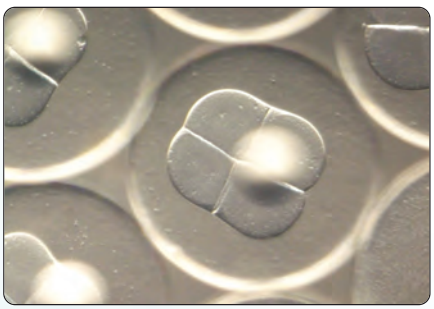
The embryonic development start after the fertilization of eggs and proceeds with different stages as shown in Fig. 26 and 27.



Fertilized egg

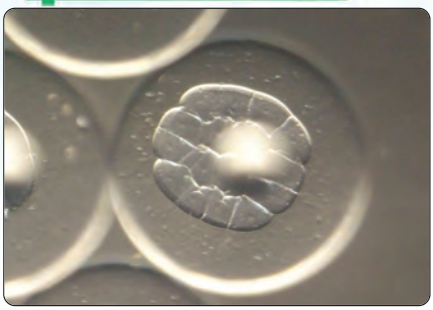


Two cell stage

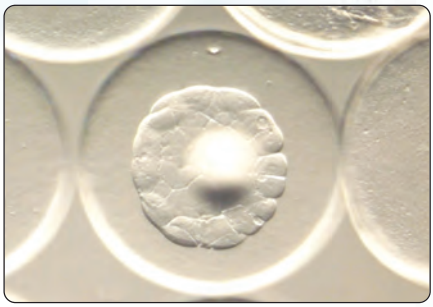


Four cell stage

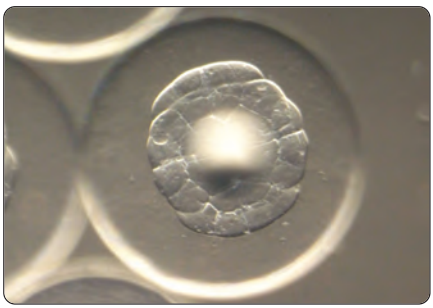
Eight cell stage



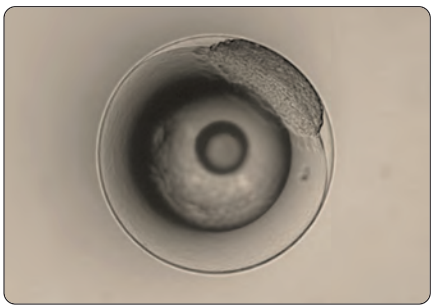
Sixteen cell stage



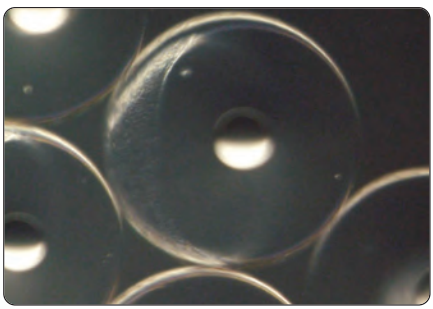
Morula stage



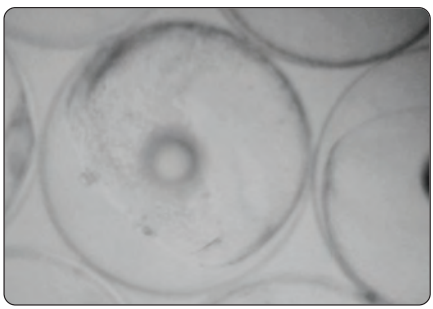
Blastula stage



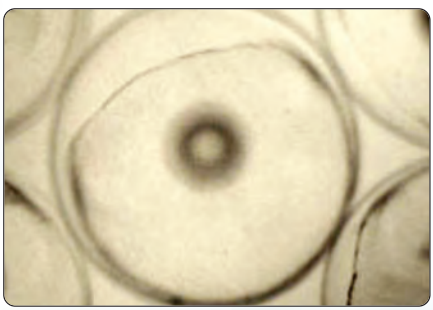
Early gastrula stage



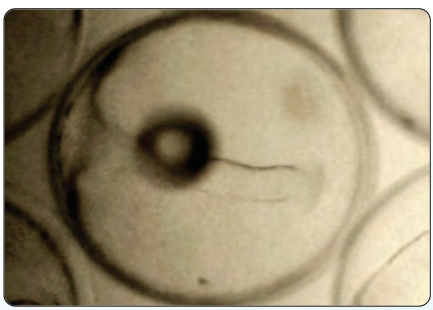
Gastrula (1/4 Epiboly)



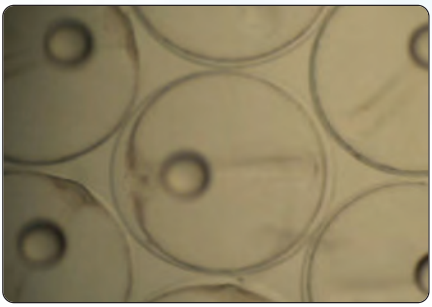
Epiboly ½.



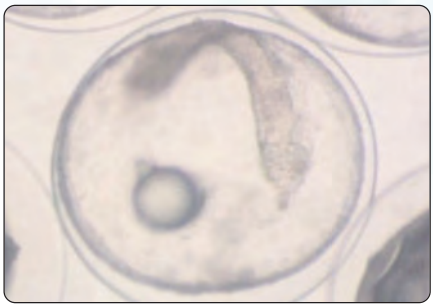
Epiboly ¾.



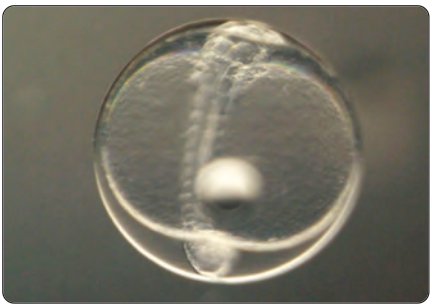
Late Gastrula (Primordial axis).



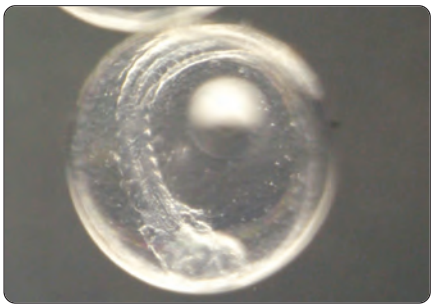
Early Neurula (Prominent Primordial axis).



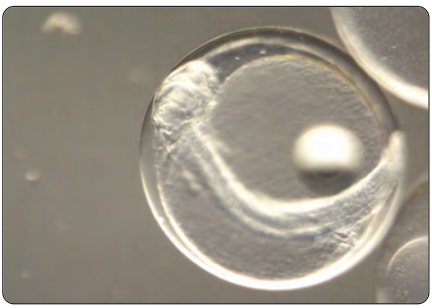
Late Neurula.



Embryo



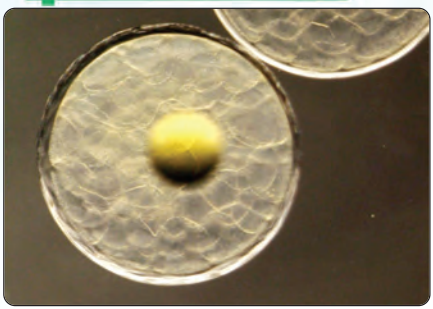
Embryo: High-pec.



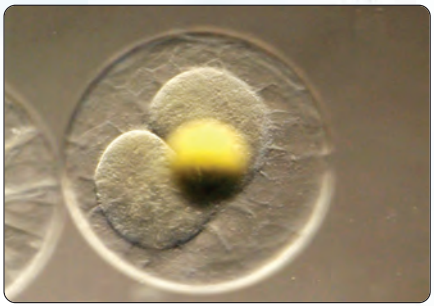
Embryo: Active embryo

Hatched out larva

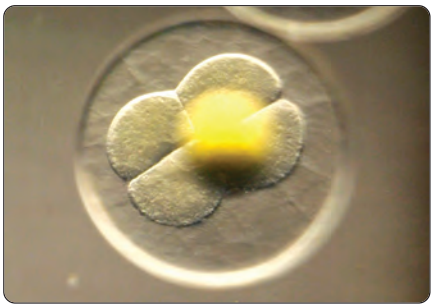
Fig. 26. Orange spotted grouper embryo developmental stages



Fertilized egg

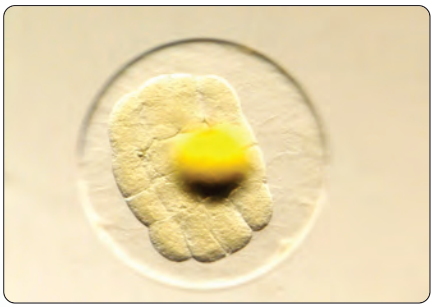


Two cell stage

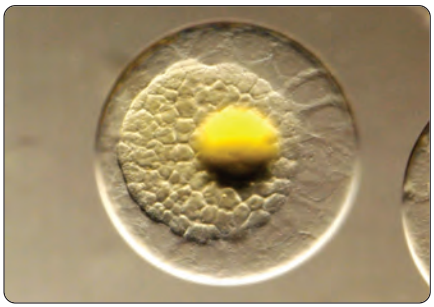


Four cell stage

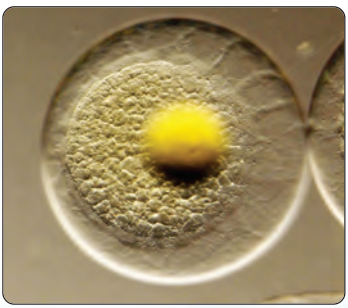
Eight cell stage



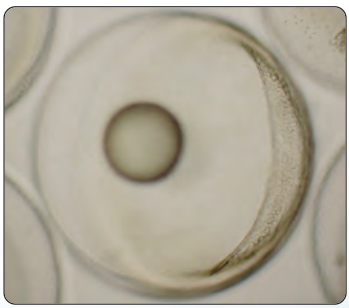
Sixteen cell stage



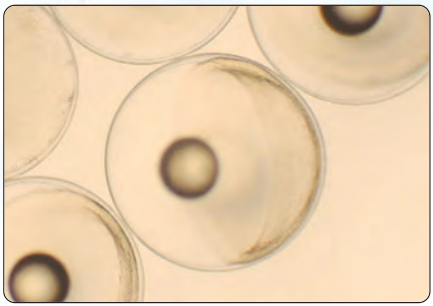
Morula stage



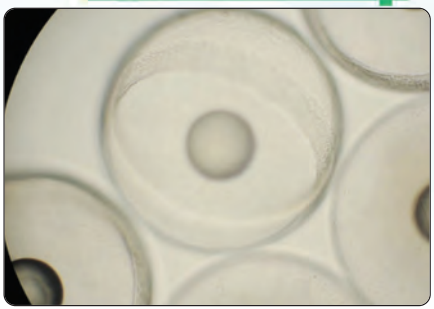
Blastula stage



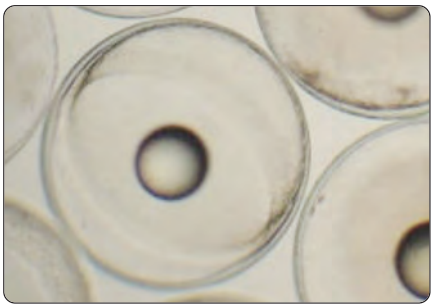
Early gastrula stage



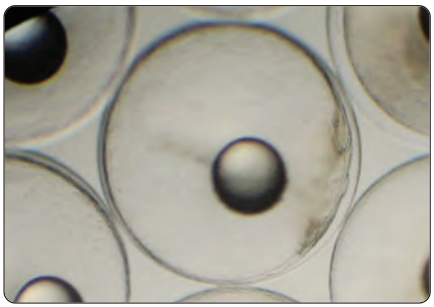
Gastrula (1/4 Epiboly)



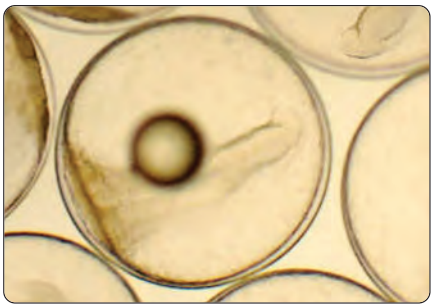
Epiboly ½.



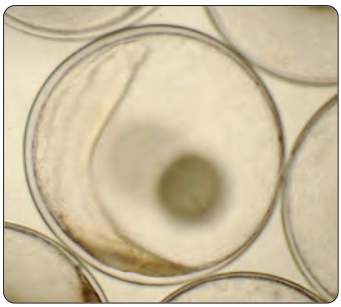
Epiboly ¾.



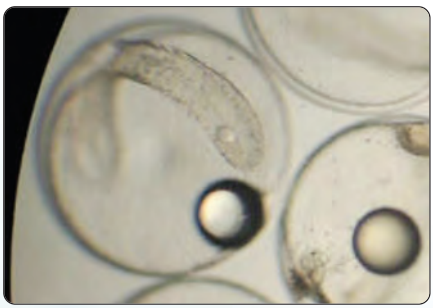
Late Gastrula (Primordial axis).



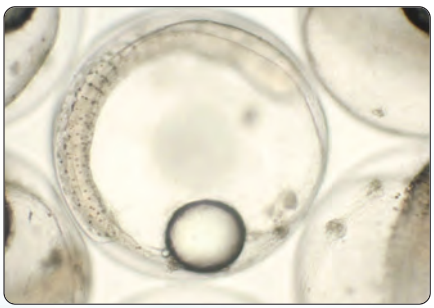
Early Neurula (Prominent Primordial axis).



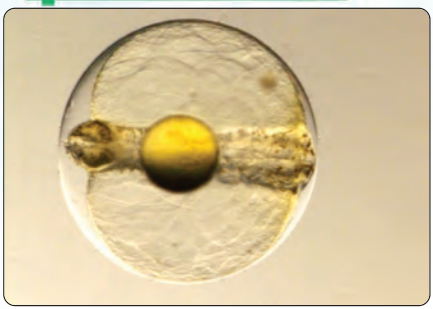
Late Neurula.



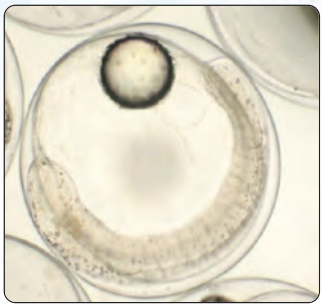
Embryo: Optical vesicle



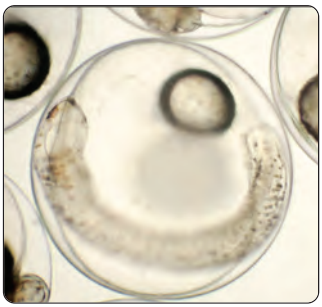
Embryo: Segmentation



Embryo



Embryo: High-pec.



Embryo: Active embryo

Hatched out larva

Fig. 28. Indian pompano embryo developmental stages

Stocking of larval rearing tanks

Generally, fertilized eggs are stocked at the later of eyed stage (Fig. 28 & 29) because they are more robust than the newly hatched larvae. Newly hatched larvae (Fig. 30) are very sensitive to physical shock or changes in water quality, and moving them to the larval-rearing tanks may result in high levels of mortality. Because the hatching rate is not known before the eggs are stocked in the larval-rearing tanks, the number of eggs to be stocked needs to be estimated using historical hatching rates for that hatchery. Accurate estimates of the number on larvae stocked can be back-calculated using data from the actual batch stocked, as described above. If hatching rates are low, the larvae in the larval-rearing tanks should be discarded, and the tanks cleaned and disinfected.

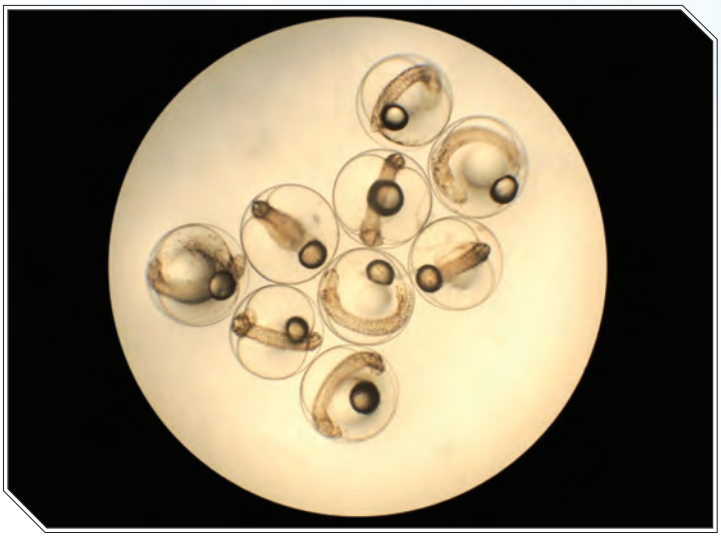


Fig. 28. Eyed stage Indian pompano eggs immediately before hatching. At this stage the larvae will be visibly 'twitching' within the chorion.

Fig. 29. Stocking of eggs in larval rearing tank

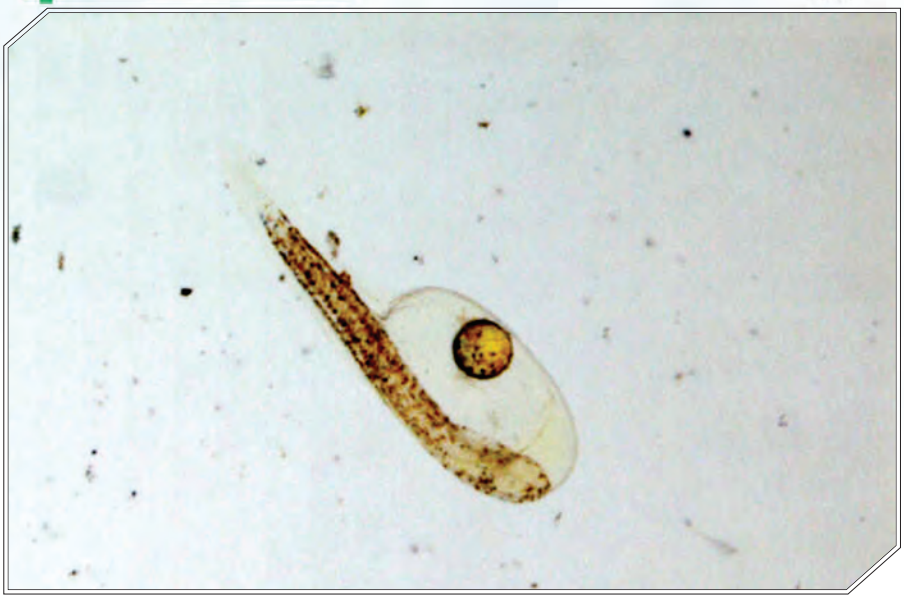


Fig. 30. Newly hatched Indian pompano larva

LARVAL-REARING PROCEDURES

Larval rearing tanks

Generally, round and rectangular tanks are used for larval rearing. At Visakhapatnam RC of CMFRI, round tanks of 2 and 5 m^3 and rectangular tank of 10 m^3 capacity with 1.2 m depth are used for larval rearing (Fig. 31). A single aeration point in the middle of the tank is provided, which is connected with either oxygen cylinder or PSA oxygen concentrator. The aeration should be mild during the early stages (at least upto 10 days) of larval rearing to avoid physical damage of the larvae. However, it is increased gradually with progression of the larval-rearing cycle, as the larvae becomes more robust. The tank should be cleaned with liquid bleach or acid washed and dried atleast for two days before stocking.

Sea water should be filtered with sand bed filter and treated with ozone at the rate of 0.1 ppm for 5 minutes. The ozonised water should be passed through carbon filter before filling in the larval rearing tank.

Fig. 31. Larval rearing tanks used for larval rearing

Larval-rearing tanks should be placed under roof to avoid direct sunlight and rain; It is preferable to have larval rearing tanks inside concrete building. Light is necessary for the larval rearing and it should be either diffused light or artificially provided with the help of tube lights. These tanks should be maintained as a separate quarantined area within the hatchery, with restricted entry only to authorized few persons. Their hands and feet should be washed on every entry and exit, and disinfection of all equipments should be performed before and after use.

LARVAL DEVELOPMENT

Orange spotted grouper

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 hrs) depending upon temperature and the yolk is completely absorbed by 3rd-4th DPH (Days post hatching). At the time of mouth

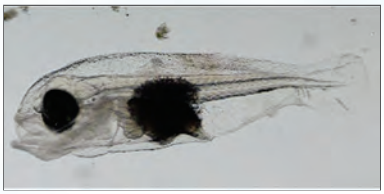
opening, mouth gape is 120 µm which increases to 180 µm after 10-12 hrs. The stomach and eyes becomes pigmented on 3rd DPH. From 4th to 6th DPH, there are no major morphological changes, but pigmentation around the stomach increases. At 6-8th DPH, the buds of the dorsal and pectoral spines appear and by 10th-25th DPH, most 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 often 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 other serranid larvae. The larvae before metamorphosis to the juvenile stage are highly sensitive to environmental conditions and substantial mortality occurs due to minor stresses. Orange spotted grouper larvae metamorphose to juveniles at about 30th-35th DPH, however this can be delayed because of low water temperatures and poor nutrition. As the orange spotted grouper larvae are highly sensitive, careful management is required throughout the larval-rearing phase.

1st DPH

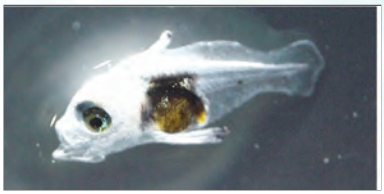
2nd DPH

3rd DPH

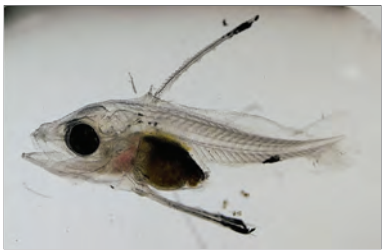
4th DPH



6th DPH



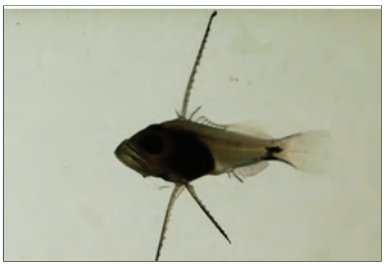
8th DPH



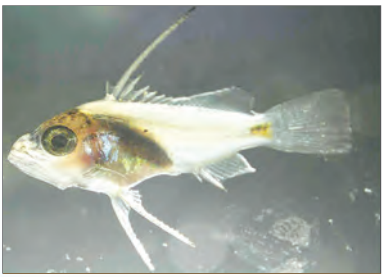
11th DPH

13th DPH

15th DPH



19th DPH



25th DPH

29th DPH

Fig. 32. Larval development stages of orange spotted grouper

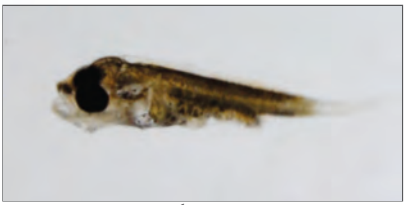
Fig. 33. Metamorphosed larvae of orange spotted grouper

Indian pompano

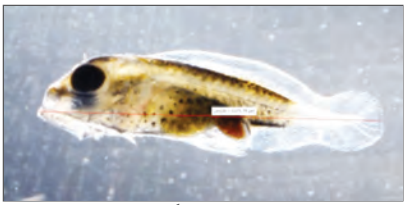
The newly hatched larvae of Indian pompano are 2.12 ± 0.02 mm in total length with an oval shaped yolk sac of 0.55 mm^2 and an oil droplet of 0.06 mm^2 in area. The body length increases to 2.58 mm on 1st DPH while the yolk sac decreases to 0.06 mm^2. By 46 h post hatch, the yolk sac is almost absorbed, the eyes starts to show visible pigmentation and mouth opens with a mouth gape of 228.10 ± 1.31 µm. Enriched rotifers screened with 100 µm and copepod nauplii are utilized as the initial feed during this stage. The yolk sac is completely absorbed by 3rd DPH, when the larval body length is 2.66 ± 0.03 mm. Larval body length reaches about 4.64 ± 0.3 mm by 6th DPH, by this time, amount as well as size of rotifers given is increased to satisfy the consumption demand. Larval body length reaches 6.35 ± 0.02 mm by 8th DPH; at this stage, appearance of the dorsal, caudal and pelvic fins begin. Enriched

1st DPH

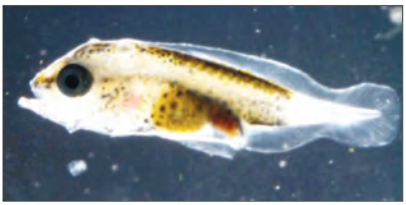
2nd DPH



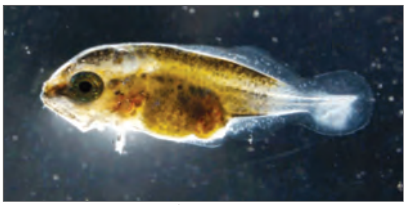
3rd DPH



4th DPH



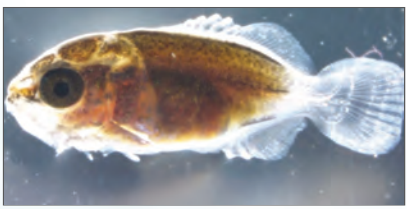
5th DPH



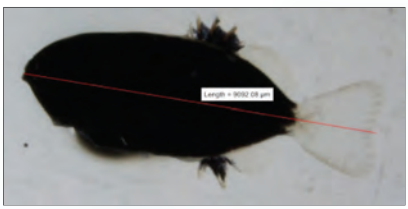
6th DPH

7th DPH

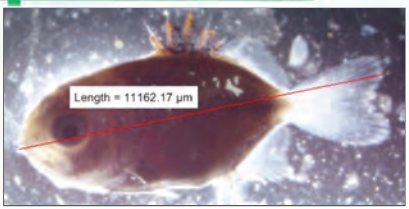
8th DPH



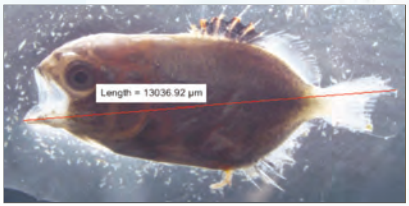
9th DPH



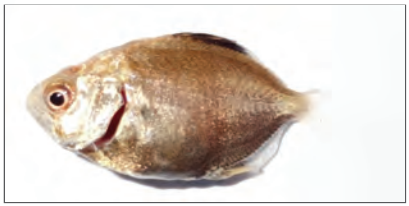
10th DPH



11th DPH



12th DPH



13th DPH

14th DPH

Fig. 34. Larval development stages of Indian pompano

Fig. 35. Metamorphosed larvae of Indian pompano

Artemia nauplii are fed to the larvae for faster growth. The larvae grows to 9.04 ± 0.06 mm by 10th DPH, by which time all fin types are well demarcated. Melanin pigmentation starts from embryo development onwards and becomes intense as the larvae grow. Therefore, larvae body colour is dark till 10th DPH. Artificial formulated feed was fed to the larvae from 12th DPH, when larval body length reaches 11.91 ± 0.07 mm. Larvae starts metamorphosis by 17th DPH onwards, when larval body length reaches 20.55 ± 0.08 mm, and metamorphosis is completed by 21st DPH, when larvae has grown to 27.33 ± 0.10 mm. The larval body colour changes from dark to silvery on completion of metamorphosis into juveniles. The juveniles have developed the entire components of all fins and starts feeding on artificial pellets of 0.8 mm.

LARVAL REARING

Orange spotted grouper

The sea water used in larval-rearing tanks is pre-treated through sand filter to remove particulate matter and is then ozone sterilized to eliminate pathogens. The recommended initial stocking density for orange spotted grouper is 10 larvae/L. Oil is generally added to form a thin film on the water surface (around 0.2 ml/m^2) during 1st - 4th DPH for preventing surface aggregation mortality in early-stage grouper larvae. Live feeds used for larval rearing comprises microalgae (*Nannochloropsis* sp. and *Isochrysis* sp.), copepod nauplii and adult, small rotifers (*Brachionus rotundiformis*), large rotifers (*Brachionous plicatilis*) and brine shrimp (*Artemia*) nauplii.

The yolk sac (endogenous source) continues as the sole source of nutrients for the developing embryos immediately after hatching. The endogenous source provide nutrient for 2-3 days in grouper larvae. Then, the exogenous feeding starts when the mouth opens after 2nd day. Their initial mouth gape is very less, so they have to be provided with appropriate size of feed *i.e.* copepod nauplii and screened rotifers. *Nannochloropsis sp* is introduced into the larval-rearing tanks on 2nd DPH at algal cell density of 1× 10^5 cells/ml (Fig. 36). Rotifers and

copepod nauplii filtered with 100 µm mesh are introduced into the larval rearing tanks on 2nd DPH, after the larval mouth opening has been formed. The rotifer and copepod nauplii density in the larval-rearing tanks is maintained at 5-7 and 2-3 individuals/ml respectively during 2nd -5th DPH. After 5th DPH, small rotifers (filtered with 150 µm mesh) are introduced at densities of 10-15 individuals/ml, which is gradually increased to 20 individuals/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 0.5 individual/ml from 17th 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 20th DPH. Artificial diet with a particle size of 200-300 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

Fig. 36. Dripping live phytoplankton in larval rearing tank

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. High-quality micro diets, specifically formulated for marine finfish, should be used and these should be stored in a refrigerator or freezer to maintain their quality. In addition, minced fresh fish meat is fed from 30th DPH onwards.

Fig. 37. Bottom siphoning of the larval rearing tank

Larval-rearing tanks are maintained static until 7th DPH, and then from 10th DPH, 5-10% of water exchange per day is required to maintain the rearing water quality. Bottom siphoning of the tank should be started on 7th DPH (Fig. 37). From 12th DPH, faeces, dead larvae and uneaten food accumulate on the tank bottom, and should be siphoned out at least once daily for maintaining water quality. Water exchange should be increased to 20%/day, when both rotifers and *Artemia* are being fed together (15th-20th DPH). Water exchange gradually increases to 50%/day from 25th DPH, and is 100%/day from 35th DPH.

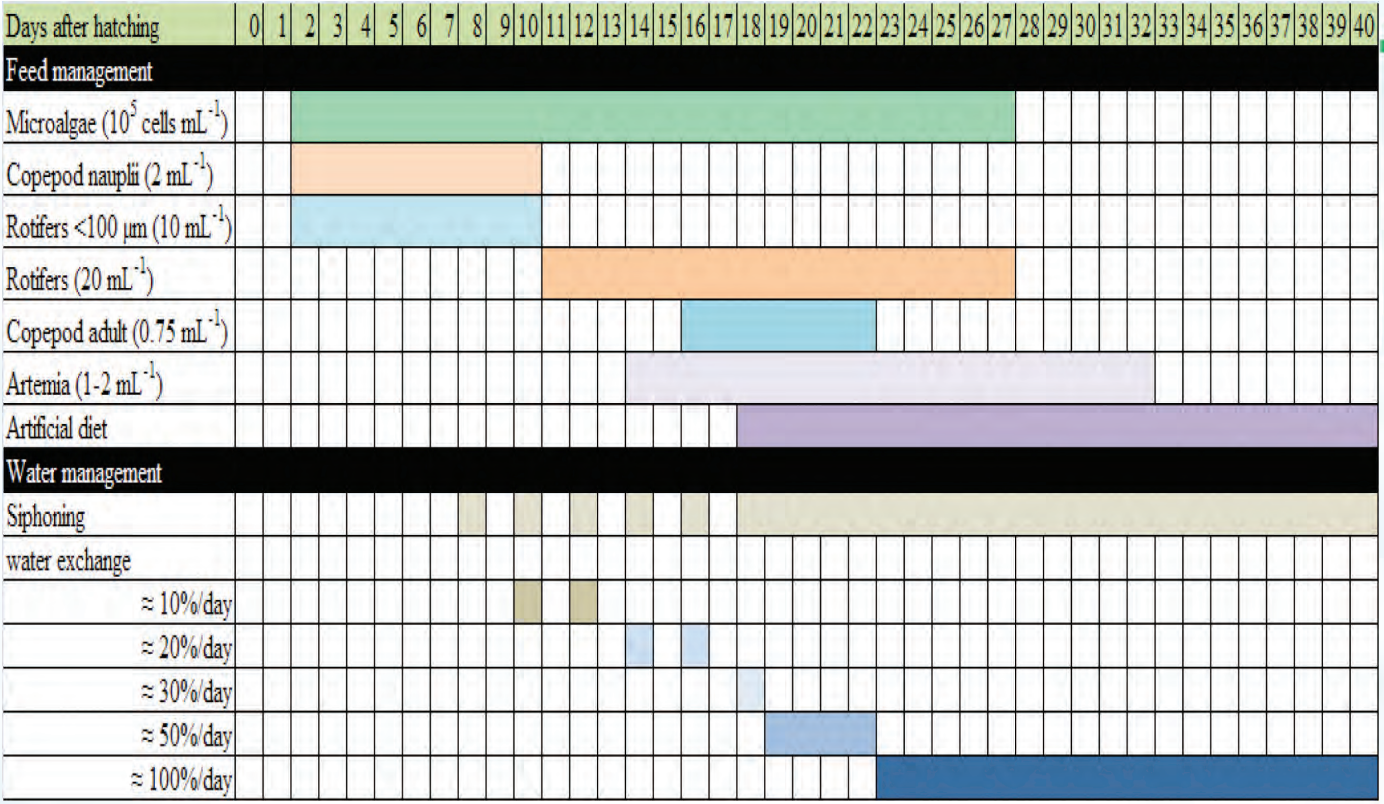


Fig.38. Standardized feeding and water management protocol developed for larval rearing of orange spotted grouper

It is of atmost importance to measure water quality regularly in the larval-rearing tanks (Table 3). If water quality degrades, it should be necessary to exchange the water at rates higher than the rates recommended above. However, replaced water should be of similar temperature and salinity to the water in the rearing tanks to avoid stress to the larvae.

Table 3. The optimum physico-chemical parameters for larval rearing of orange spotted grouper during early larval rearing.

Sl. No.	Parameters	Value	Reference
1.	Temperature*	28-30°C	Sugama *et al.*, 2012
2.	Salinity	16-24 ppt	Toledo *et al.*, 2004
3.	Light	500 to 700 lx	Toledo *et al.*, 2004
4.	Photoperiod*	Natural	Sugama *et al.*, 2012
5.	Aeration	0.62-1.25ml/min/L	Toledo *et al.*, 2004
6.	Dissolved oxygen*	Near saturation	Sugama *et al.*, 2012
7.	Ammonia*	<0.1 ppm	Sugama *et al.*, 2012
8.	Nitrite*	<1.0 ppm	Sugama *et al.*, 2012

*Reported for other species of grouper

Indian pompano

The sea water used in larval-rearing tanks is pre-treated through sand filter to remove particulate matter and is then ozone sterilized to eliminate pathogens. The recommended initial stocking density for Indian pompano is 10 larvae/L. Live feeds used for larval rearing comprises microalgae (*Nannochloropsis* sp. and *Isochrysis* sp.), copepod nauplii and adult, small rotifers (*Brachionus rotundiformis*), large rotifers (*Brachionous plicatilis*) and brine shrimp (*Artemia*) nauplii.

The yolk sac (endogenous source) continues as the sole source of nutrients for the developing embryos immediately after hatching. The

Fig. 39. A snap of larvae from larval rearing tank at 5 DPH

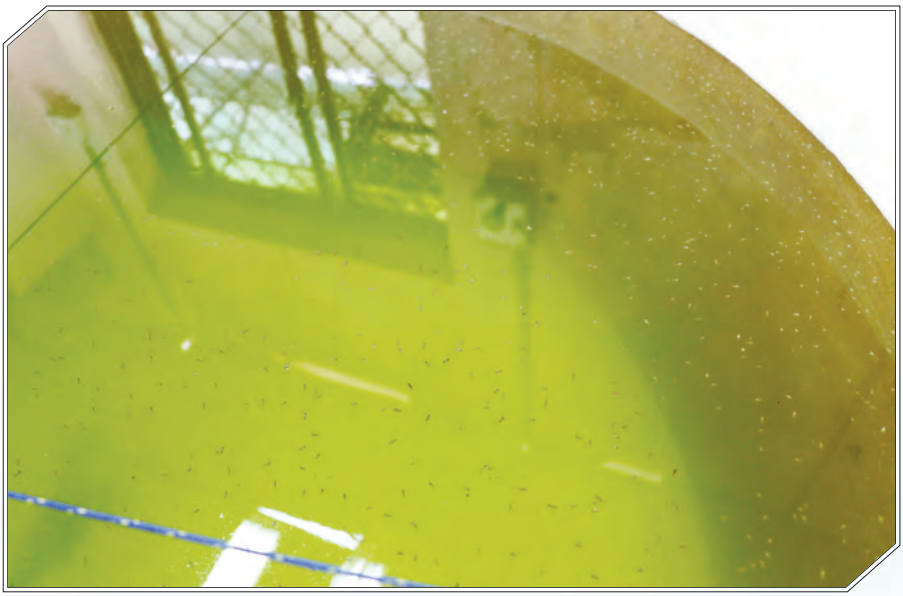


Fig. 40. A snap of larvae from larval rearing tank at 7 DPH

endogenous source provide nutrients for 2 days in Indian pompano larvae. Then, the exogenous feeding starts when the mouth opens after 2nd day. Their initial mouth gape is around 230µm, and hence appropriate size of feed *i.e.* copepod nauplii and screened rotifers needs to be provided. *Nannochloropsis sp* and *Isochrysis* sp in ratio of 2:1 is introduced into the larval-rearing tanks on 2nd DPH at algal cell density of 1× 10^5 cells/ml (Fig. 36). Rotifers and copepod nauplii filtered with 100 µm mesh are introduced into the larval rearing tanks on 2nd DPH, after the larval mouth opening has been formed. The rotifer and copepod nauplii density in the larval-rearing tanks is maintained at 10-15 and 2-3 individuals/ml respectively during 2nd -5th DPH. After 5th DPH, rotifers are introduced at densities of 20 individuals/ml, which is gradually increased to 30 individuals/ml from 8th to 10th DPH onwards. Rotifer density gradually decreases with increase in the rate of rotifer consumption by the larvae and eventually by 13th DPH, the rotifers disappear. Freshly hatched out *Artemia* nauplii are fed at density of 0.5 individual/ml from 8th DPH and their size is increasing with

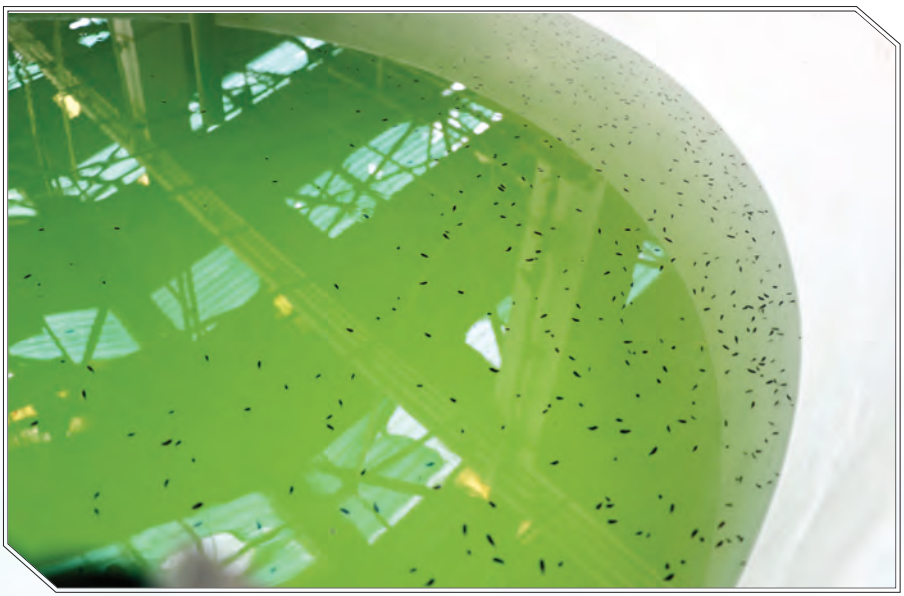


Fig. 41. A snap of larvae from larval rearing tank at 11 DPH

advancement in rearing period. Weaning of pompano larvae with artificial diets starts from 11th DPH. Artificial diet with a particle size of 200-300 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 (Fig. 42). The size of particulate feed is increased to 400–800 µm from 22nd DPH. High-quality micro diets, specifically formulated for marine finfish, should be used and these should be stored in a refrigerator or freezer to maintain their quality.

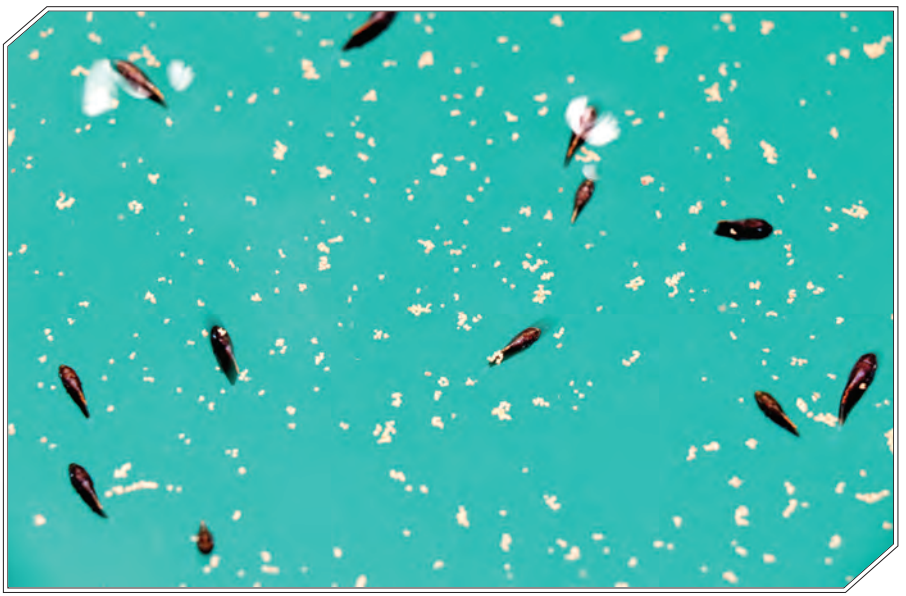


Fig. 42. Weaning of the larvae on inert diet

Larval-rearing tanks are bottom siphoned on the day of hatching to remove unhatched eggs as well as hatched out eggs shells, and are further maintained static until 4th DPH, and then from 5th DPH, 5-10% of water exchange per day is required to maintain the rearing water quality. Bottom siphoning of the tank should be started on 4th DPH and are carried out once every 3 days. From 12th DPH onwards, faeces,

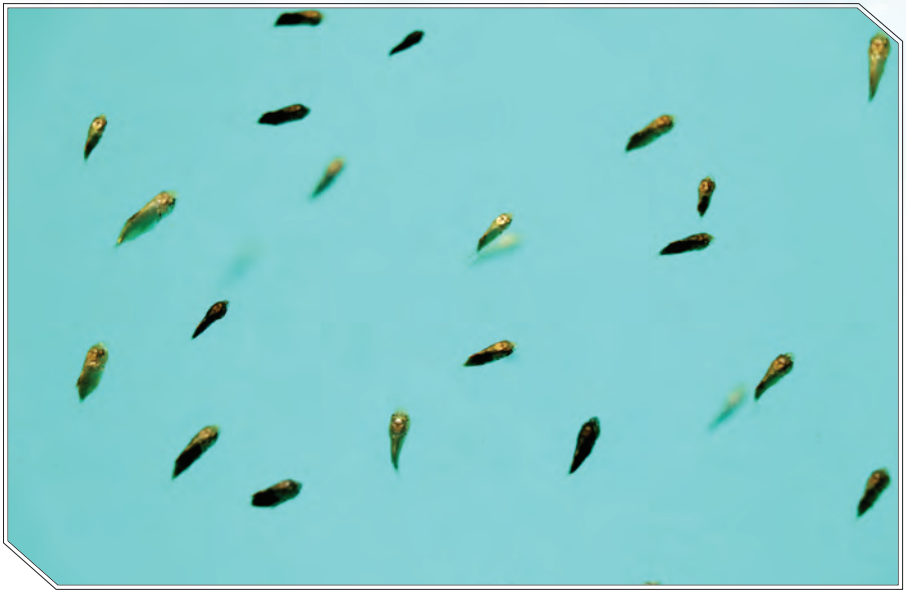


Fig. 43. A snap of larval tank; few metamorphosed larvae (silver colour) and yet to metamorphosed (black colour)

Fig. 44. Metamorphosed larvae of Indian pompano

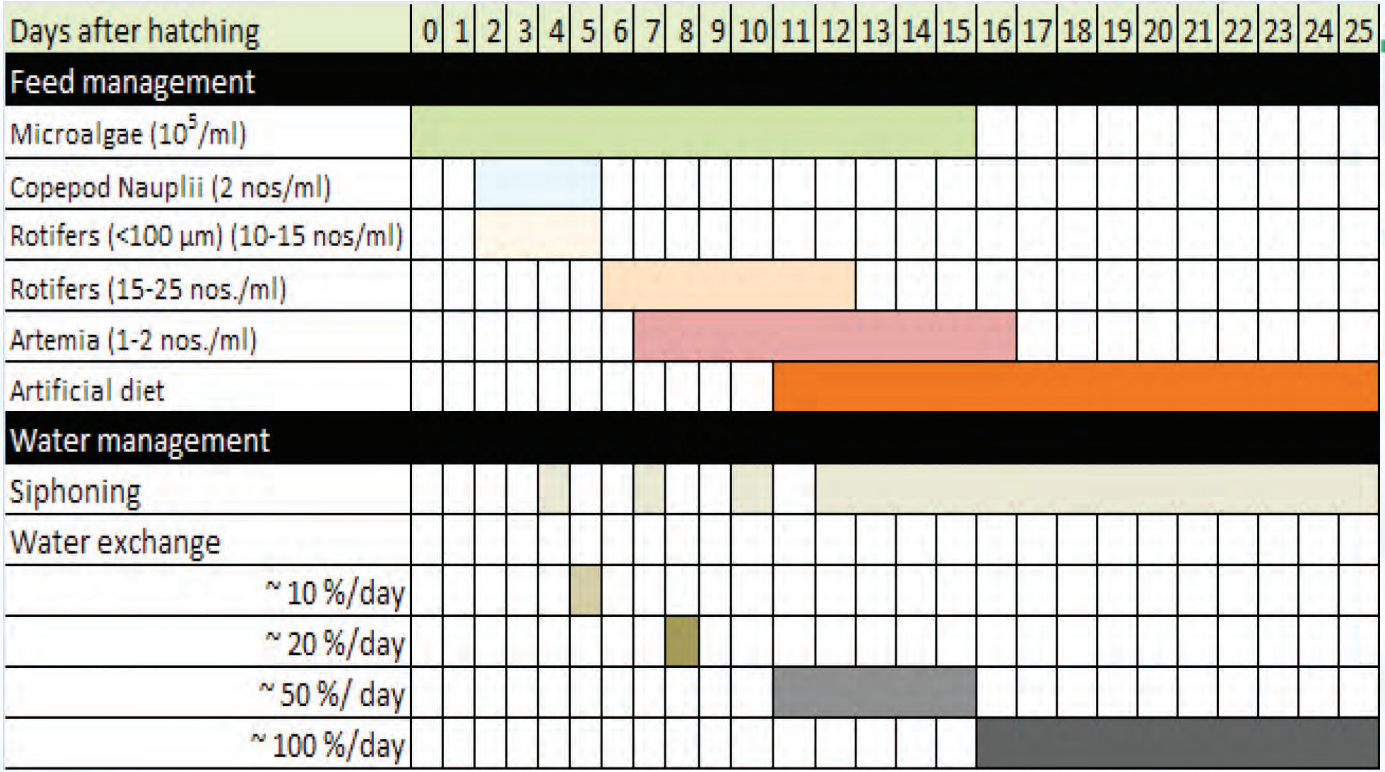


Fig. 45. Standardized feeding and water management protocol developed for larval rearing of Indian pompano

dead larvae and uneaten food accumulated on the tank bottom should be siphoned out at least once daily for maintaining water quality. Water exchange should be increased to 20%/day, when both rotifers and *Artemia* are being fed together (8th DPH). Water exchange is gradually increased to 50%/day from 11th DPH, and is 100%/day from 16th DPH.

It is of atmost importance to measure water quality regularly in the larval-rearing tanks (Table 3). If water quality degrades, it should be necessary to exchange the water at rates higher than the rates recommended above. However, replaced water should be of similar temperature and salinity to the water in the rearing tanks to avoid stress to the larvae.

Nutritional enhancement of live foods

Larvae of marine finfish require high levels of the unsaturated fatty acids; eicosapentaenoic acid (EPA, or 20:5n-3), arachidonic acid (ARA, or 20:4n-6) and docosahexaenoic acid (DHA, or 22:6n-3) for proper development, and provision of these fatty acids in the diet, via incorporation in the live feeds used for larval rearing; improves survival, growth and pigmentation of the larvae and fingerlings. However, rotifers are poor in terms of high level of unsaturated fatty acid such as DHA and EPA. *Artemia* nauplii though rich in EPA lack in DHA. Due to this, these live feed need to be enriched either with natural phytoplankton or artificial enrichment product to increase the levels of these essential fatty acids.

Various commercial preparations have been developed for nutritional enhancement of rotifers and brine shrimp (Alava *et al.*, 2004) such as, Ori 1 from Skretting, Easy DHA from Inve Aquaculture and Algamac from Bio-Marine. These enrichment products are packaged as liquid or spray-dried products. Generally, preparation involves measuring the required quantity, blending to hydrate (for spray-dried products) or emulsify (for liquid products) the material, then add to the live-feed culture tanks. The manufacturers, provide technical information on the application of their products. Of particular importance is the need to maintain high dissolved oxygen levels in the

culture tanks during the application period (usually <12 hours). This may require the use of pure oxygen, or oxygen-supplemented air, particularly if the live-feed organisms are at high density.

Problems in larval rearing

There are several commonly encountered difficulties in the larval rearing of groupers (Sugama *et al*., 2001), including that of orange spotted grouper.

1. Floating death

During DPH 1 to DPH 4, weak larvae are easily trapped on the water surface by surface tension. Once the larva is trapped on the water surface, they are unable to escape and eventually die. The trapped larvae at the water surface secrete a sticky mucous due to stress, which in turn accelerates the trapping of other larvae, causing the mortality of a significant number of larvae in a short period of time. This has been observed during the initial periods of larval rearing. The following measures are taken to avoid mortality from trapping at the surface.

- Addition of oil (squid or fish oil) to the tank (0.1ml/m^2 surface area) twice daily to form a thin film during 1^{st} - 4^{th} DPH.
- Maintaining green water for larval rearing is effective in lowering larval floatation on the water surface and subsequent aggregation.
- Air stones should be fitted away from the tank wall.

2. Larval mortality at first feeding

Usually, there is relativly high mortality among first-feeding larvae. Samples of first feeding larvae (3^{rd} DPH) are examined under a microscope for ensuring that they are feeding effectively on the copepod nauplii or rotifers present in the tank. If the larvae gut is found empty, the size and density of the live-feed organisms are checked to ensure that there are enough food items of appropriate size available for the larvae to feed.

3. Surface aggregation mortality

During DPH 10 to DPH 25, mass mortality of larvae is observed in the larval rearing tank due to entangling of their spines. Larvae develop elongated single dorsal and two pelvic spines after DPH 10. The grouper larvae gets attracted to patches of light, either natural or artificial in the tank surface, which results in intense crowding often leading to entanglement of spines. The situation worsens when, stressed larvae secretes mucous and mass entanglement and mass mortality is observed. The following measures can be taken to avoid mortality from surface aggregation mortality.

- Adjust light intensity as evenly as possible on larval rearing tank.
- Increase the number of air stones to provide aeration.
- Air stones should be fitted close to the tank wall.
- Maintaining green water for larval rearing is effective in controlling larval aggregation.

4. Shock syndrome

Larval mortality is observed from about 20th DPH and prevalence of high mortality occurs around 25th DPH. This is due to nutritional deficiency, particularly that of essential fatty acids. Improving the nutritional composition of live feed fed to orange spotted grouper (*E. coioides*) larvae, using larval nutritional supplements dramatically reduces the incidence of 'shock syndrome' in cultured larvae. The use of copepod, high in essential fatty acids, particularly DHA, will reduce the incidence and severity of 'shock syndrome' in grouper larvae.

5. Cannibalism

Cannibalism begins during the later stages of larval rearing, forming an obstacle in the larval-rearing cycle. The following measures are taken to avoid cannibalism in larval rearing tank.

- Ensure adequate feed availability to the larvae every morning at first sight.

- Feeding with particulate artificial pellets frequently, i.e. every 1–2 hours.
- For advanced stages of larval rearing, maintain light levels around 500-600 lux.
- Grading the larvae frequently after metamorphosis.

There are few commonly encountered difficulties in the larval rearing of Indian pompano, which are similar to the orange spotted grouper such as i. Larval mortality at first feeding and ii. Cannibalism.

NURSERY REARING

Orange spotted grouper

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 (0.4 g) fry are cultured in tank for 2 weeks till they accept artificial feeds fully, by

Fig. 46. Fingerlings of orange spotted grouper

Fig. 47. Hapa based nursery rearing system in pond

Fig. 48. Hapa based nursery rearing system in cement tank

which time they reach upto 5-6 cm. The fry during this period is reared in 1 t capacity tank @ 1no/L. They are fed on *Artemia* biomass and artificial diet for completely weaning the larvae to artificial feed. Artificial feed containing 45% protein and 10% fat of 0.8 and 1.2 mm pellet size are used in this period. Feeds are added frequently (at least once in 2-3 h interval) in the tank. Dissolved oxygen should not be less than 4 mg L^{-1} at any time. Grading is performed every 5 days to grade the larvae according to size during the initial phase of nursery rearing for 1 month. The water quality should be managed at optimum level either by water exchange or flow through.

Fig. 49. Grouper grown to 10-15 cm for stocking in cage as well as in pond.

Second phase of nursery rearing 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 (4 mm mesh size) (Fig. 47), flow through cement tank (Fig. 48) or in re-circulatory aquaculture system (RAS). Pellet feed with 45% protein and 10% fat of 1.2 mm and 1.8 mm floating pellets are used as feed @ 10% body 3-4

times in a day. The rearing system is also found to influence the growth rate, where highest average daily weight gain of 0.59g/day is observed in RAS, followed by 0.4g/day in pond and 0.26g /day in cement tanks after one month of rearing. The nursery rearing of grouper in RAS eases the operational activity by stopping the activity related to water exchange, bottom siphoning, tank cleaning that is required for the maintenance of water quality. Thus the large number of grouper fingerlings can be nursed without the requirement of more number of manpower and with high growth rate compared to other system.

Indian pompano

Nursery rearing of Indian pompano is standardized with different feed and culture conditions such as hapa either in cement tank or in pond (Fig. 50). Even the cage installed in sea can be used for nursery rearing of Indian pompano by using smaller mesh size nets. In tanks, when nursed for a period of two months at a density of 150 nos/m^3, fry weighing on an average 3.95 g reaches 28.08 g. Fry are fed artificial

Fig. 50. Hapa based nursery rearing system in pond.

pelleted feed containing 45 % protein and 10 % fat during this period @ 10% of biomass with feeding frequency of four times a day.

Fig. 51. Fingerlings of Indian pompano reared in RAS

In ponds, Indian pompano fry weighing on an average 2 g are nursed in hapas at a density of 150 nos/m^3 and the fry attains a weight of 20 g after 60 days of rearing. The feeding regime followed is similar. For stocking in ponds, advanced fingerlings of approximately 20 g size is ideal.

Nursery rearing and grow-out is carried out in marine cages (HDPE of 6 m in diameter) using hatchery produced seeds. Seeds weighing 2.5 g and measuring 5.25 cm are stocked at 35 nos/m^3 in 6 m dia HDPE cages with 8 mm mesh size inner net. Initially fish fingerlings are fed at 10% of body weight with commercial floating diet containing 45% crude protein and 10% fat twice a day.

APPENDIX

Tentative estimates for setting up of a hatchery for production of Grouper fingerlings (Proposed production capacity: 1 million / year)

A) Capital Expenditure (infrastructure facilities without land cost)

Sl. No.	Particulars	Quantity	Cost in INR (in Lakhs)
CAPITAL INVESTMENT			
1.	Building with light-roofing for office, laboratory, store facility, algal stock culture, pump house, power house, blower room, packing area, staff dormitory, kitchen and dining hall, and security cabins)	-	100.00
2.	Sheds for broodstock facility, larval, intermediary algal and rotifer sections	-	100.00
3.	Plumbing and electrical fittings	-	10.00
4.	Water intake and filtration system		10.00
5.	Sump (100 ton capacity)	3 nos	21.00
6.	Over Head Tank (100 ton capacity)	1 nos.	20.00
7.	Racks for algal stock culture with lights	10 nos.	3.00
8.	Intermediary stock culture tanks (3 ton capacity)	6 nos.	1.80
9.	Out-door algal culture tanks (5 ton capacity)	15 nos.	7.50
10.	Rotifer culture tanks (5 ton capacity)	6 nos.	3.00
11.	Effluent treatment system (CC tanks)	3 chambers	10.00
12.	Power supply		
13.	Transformer (250 KVA) with installation and commissioning	1 no.	3.00
14.	Generator (250 KVA)	1 no.	15 .00
15.	Laboratory equipment (water quality probe, PIT tag reader, microscope,		5.00

	camera, balances, laminar airflow chamber, hot air oven, autoclave, water distillation unit, bacterial incubator, etc.)		
16.	Hatchery machineries (water pumps, blowers, submersible pumps, UV sterilizers, ozonisers, rapid sand filters and cartridge filters)		15.00
17.	Vehicle (Multi Purpose Vehicle)	1 no.	7.50
18.	Broodstock tanks (100 ton capacity) with re-circulating facility	1 nos.	16.00
19.	Larviculture tanks (5 ton capacity each)	50 nos.	25.00
20.	Nursery tanks (20 ton capacity each) with 5 re-circulating facilities in grouping of 5 tanks	25 nos	65.00
21.	Miscellaneous items (furniture, air conditioners, fans, etc.)		5.00
	TOTAL		**442.8**

B) Operational cost

Sl. No.	Particulars	Quantity	Cost in INR (in Lakhs)
1.	Consumables (Chemicals, glassware, feeds, enrichment media, feed additives, hormones, disinfectants, plastic ware, nettings, PIT tags, tag implanters, broodstock, Artemia, etc.)		20.00
2.	Fuel and electricity	Lumpsum	10.00
3.	Manpower		
	Hatchery manager @ INR 25000/m	1 no.	3.00
	Section Supervisors @ INR 15000/m	3nos.	5.40
	Hatchery workers @ INR 10000/m	12 nos.	14.40
	Office cum Account Assistant @		
	INR 10000/month	1 no.	1.20
	Drivers @ INR 15000/m	1 no.	1.80
	Cook @ INR 10000/m	1 no.	1.20

	Cooking Assistant @ INR 8000/m	1 no	0.96
	Office and dormitory cleaner @ INR 8000/m	1 no.	0.96
	Security (1 nos. per shift) @ INR 8000/m/person	6 nos.	5.76
4.	Miscellaneous expenditure		5.00
	Total		**69.68**

ECONOMICS OF GROUPER HATCHERY

Sl. No	Production Estimates
	Total Expenditure (Capital cost + Operational cost) = 442.80 lakh + 69.68 lakh = 512.48 lakh
1.	Anticipated Production = 1.0 million fingerlings / year
2.	Average sale price = INR 25.00 / fingerling
3.	**Gross income** from sale of fingerlings **= INR 2.5 Crore**
4.	**Operational expenditure = INR 0.70 Crore**
5.	**Gross Profit =** Gross income - Operational expenditure **= INR 1.80 Crore**
6.	Partial repayment of the capital expenditure @ 14.29 % / year = INR 0.63 Crore Repayment of capital @ INR 0.63 crore/year x 7 years
7.	Interest on the total project cost @ 11% = INR 0.56 Crore
8.	**Partial repayment of Capital + interest = INR 0.63+ INR 0.56 = INR 1.19 Crore**
9.	**Net Profit = Gross Profit - (8) = INR 1.80 Crore - INR 1.19 Crore = INR 0.61 crore**
	✓ Net profit (after repayment of working capital, interest & part of capital expenditure) is 0.61 crore per annum for the first 7 years
	✓ Net Profit from 8th Year onwards [Gross Income - Operational Expenditure] = INR 1.80 Crore

Tentative estimates for setting up of a hatchery for production of Indian Pompano fingerlings (Proposed production capacity: 2 million / year)

A) Capital Expenditure (infrastructure facilities without land cost)

Sl. No.	Particulars	Quantity	Cost in INR (in Lakhs)
CAPITAL INVESTMENT			
22.	Building with light-roofing for office, laboratory, store facility, algal stock culture, pump house, power house, blower room, packing area, staff dormitory, kitchen and dining hall, and security cabins)	-	100.00
23.	Sheds for broodstock facility, larval, intermediary algal and rotifer sections	-	100.00
24.	Plumbing and electrical fittings	-	10.00
25.	Water intake and filtration system		10.00
26.	Sump (100 ton capacity)	3 nos	21.00
27.	Over Head Tank (100 ton capacity)	1 nos.	20.00
28.	Racks for algal stock culture with lights	10 nos.	3.00
29.	Intermediary stock culture tanks (3 ton capacity)	6 nos.	1.80
30.	Out-door algal culture tanks (5 ton capacity)	15 nos.	7.50
31.	Rotifer culture tanks (5 ton capacity)	6 nos.	3.00
32.	Effluent treatment system (CC tanks)	3 chambers	10.00
33.	Power supply		
34.	Transformer (250 KVA) with installation and commissioning	1 no.	3.00
35.	Generator (250 KVA)	1 no.	15 .00
36.	Laboratory equipment (water quality probe, PIT tag reader, microscope, camera, balances, laminar airflow chamber, hot air oven, autoclave,		5.00

	water distillation unit, bacterial incubator, etc.)		
37.	Hatchery machineries (water pumps, blowers, submersible pumps, UV sterilizers, ozonisers, rapid sand filters and cartridge filters)		15.00
38.	Vehicle (Multi Purpose Vehicle)	1 no.	7.50
39.	Broodstock tanks (80 ton capacity) with re-circulating facility	5 nos.	50.00
40.	Larviculture tanks (5 ton capacity each)	40 nos.	20.00
41.	Nursery tanks (20 ton capacity each) with 5 re-circulating facilities in grouping of 5 tanks	25 nos	65.00
42.	Miscellaneous items (furniture, air conditioners, fans, etc.)		5.00
	TOTAL		**471.8**

B) Operational cost

Sl. No.	Particulars	Quantity	Cost in INR (in Lakhs)
5.	Consumables (Chemicals, glassware, feeds, enrichment media, feed additives, hormones, disinfectants, plastic ware, nettings, PIT tags, tag implanters, broodstock, Artemia, etc.)		20.00
6.	Fuel and electricity	Lumpsum	10.00
7.	Manpower		
	Hatchery manager @ INR 25000/m	1 no.	3.00
	Section Supervisors @ INR 15000/m	3nos.	5.40
	Hatchery workers @ INR 10000/m	12 nos.	14.40
	Office cum Account Assistant@ INR 10000/month	1 no.	1.20
	Drivers @ INR 15000/month	1 no.	1.80
	Cook @ INR 10000/month	1 no.	1.20
	Cooking Assistant @ INR 8000/m	1 no	0.96

	Office and dormitory cleaner @ INR 8000/month	1 no.	0.96
	Security (1 nos. per shift) @ INR 8000/m/person	6 nos.	5.76
8.	Miscellaneous expenditure		5.00
	Total		**69.68**

ECONOMICS OF INDIAN POMPANO HATCHERY

Sl. No	Production Estimates
	Total Expenditure (Capital cost + Operational cost) = 471.8 lakh + 69.68 lakh = 541.48 lakh
10.	Anticipated Production = 2.0 million fingerlings / year
11.	Average sale price = INR 12.00 / fingerling
12.	**Gross income** from sale of fingerlings = **INR 2.4 Crore**
13.	**Operational expenditure = INR 0.70 Crore**
14.	**Gross Profit =** Gross income - Operational expenditure **= INR 1.70 Crore**
15.	Partial repayment of the capital expenditure @ 14.29 % / year = INR 0.67 Crore Repayment of capital @ INR 0.67 crore/year x 7 years
7.	Interest on the total project cost @ 11% = INR 0.60 Crore
8.	**Partial repayment of Capital + interest = INR 0.67+ INR 0.60 = INR 1.27 Crore**
9.	**Net Profit = Gross Profit - (8) = INR 1.70 Crore - INR 1.27 Crore = INR 0.43 crore**
	✓ Net profit (after repayment of working capital, interest & part of capital expenditure) is 0.43 crore per annum for the first 7 years
	✓ Net Profit from 8th Year onwards [Gross Income - Operational Expenditure] = INR 1.70 Crore

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Indian Council of Agricultural Research
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
सी एम एफ आर आइ
CMFRI

Post Box No. 1603; Ernakulam North P.O.
Kochi - 682018; Kerala, India
Phone: +91 484 2394357, 2394867,
Fax: +91 484 2394909 | E-mail: contact@cmfri.org.in
www.cmfri.org.in