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Azadi Ka Amrit Mahotsav

GOOD AQUACULTURE PRACTICES FOR MARINE FINFISH HATCHERY

INDIAN COUNCIL OF AGRICULTURAL RESEARCH
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE

Good Aquaculture Practices
for
MARINE FINFISH HATCHERY

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Indian Council of Agricultural Research
Central Marine Fisheries Research Institute

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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 "Good Aquaculture Practices for Marine Finfish Hatchery" 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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1. Introduction

The domestic demand for fish in India is growing and is expected to touch 15.61 million metric tonnes by 2030. With the present annual fish production of 12.39 million metric tonnes, a gap of 3.22 million metric tonnes will need to be bridged to meet the projected domestic fish demand. This can be achieved by diversification of the culture system, cultivable fish species and by increase in the cultivable area. India, with 8119 km length of coastline, encompassing nine coastal states and possessing an Exclusive Economic Zone area of 2.172 million square km offers immense potential for mariculture. However the mariculture has failed to achieve its true potential because of several issues, most important of which is non-availability of adequate number of quality seeds for stocking. However, this potential is contingent on (1) building and equipping facilities to be able to produce marine finfish seed/fingerlings and (2) farmers being able to access them in various regions throughout the country. Until recently, India had hardly any marine finfish hatchery to produce fingerlings for coastal aquaculture. However, recent interventions by ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI) have led to the development of technology for marine finfish breeding and seed production of atleast 8 commercially important species and further, it has paved a way for establishing marine finfish hatcheries in private sector for producing fingerlings and supplying to the farmers of the country. These private sector infrastructure establishments will contribute to the growth of the marine finfish culture in the country. It is needless to say, that it is a very important sector and needs special attention to ensure the supply of good quality seed-stock and inturn, ensure the sustainability of marine finfish farming in India. This can be achieved following the proper management guidelines by developing good aquaculture practices (GAPs). GAPs are a set of management guidelines and are not standards, which should ensure that the adoption of the guidelines is relatively easy to achieve without increased costs. GAPs are an ever evolving process, open to improvement and indeed, needs

perfection as the practices progress. GAPs are necessary for broodstock management, egg and larval rearing, fry nursing and fingerlings grading, and water quality management. Whenever GAPs are not followed, losses in the hatcheries reach as high as 100%. Hence, the present manual provides a preliminary attempt to document the set of management guidelines for marine finfish hatchery with special reference to orange spotted grouper and Indian pompano. The objective of this document is to provide information on GAPs for preventing common errors during commercial production. This will minimize losses, especially at the larval, fry and fingerlings stages of the production cycle.

The quality of seed stock depends on a number of factors. These include physical status of the broodstock such as size, age, level of maturity, number of spawns per year, appearance and health condition; and these are also largely dependent on broodstock conditioning practices. In addition, genetic quality of the broodstock also impacts significantly on the quality of the seed-stock.

From an aquaculture perspective, hatcheries in the long-term should aim at consistently providing good quality seed to the nursery or grow out sectors;

- Through proper husbandry practices; and
- Ensuring long-term genetic quality of the broodstock with a sound genetic management plan.

2. Marine finfish hatchery

Generally, a controlled environment is required for the marine finfish breeding and seed production, and the best way to do it is by using an indoor hatchery.

2.1 Site Selection

The site selection for establishing a hatchery is the first important step, which should be done with careful examination of the following points.

- Good quality sea water, with salinity of more than 30 gL^{-1} (preferably 30-34 gL^{-1}) in sufficient quantities
- Proper connectivity for transporting various requirements of the hatchery and seeds from the hatchery to other places
- Constant power supply
- Source of freshwater in sufficient quantities
- Sufficient area/land for accommodating different components of the hatchery
- Should be away from cyclone prone areas/ frequent flooding areas
- Avoiding areas with longer winter

2.2 Hatchery components:

A typical marine finfish hatchery will have the following components

1. Seawater intake facility
2. Broodstock area
3. Larval rearing area
4. Live feed production area
5. Nursery rearing area

2.2.1. Sea water intake facility

A permanent sea water intake facility (preferably a bore well) is required for drawing quality sea water of salinity > 30 gL^{-1}. The drawn seawater is passed through reverse-slow sand filter for pre-treatment, prior to ozonisation and storage. All incoming water should be ozonized at a level of 0.1 ppm, and de-ozonised using charcoal bed, before use in any sections of the hatchery.

2.2.2. Broodstock area

It is better to use Re-circulatory Aquaculture System (RAS) for broodstock development of marine finfishes. Sufficient broodstock

area is required for accommodating various components of RAS; fish tank, filtration system, protein skimmer, biological filter, pumps, and egg collection chamber. This section can be situated in outdoor; however it should be in shade to avoid direct penetration of sunlight. A minimum of 12 x 12 m area is required for one broodstock tank of 125 t capacity attached with different component of RAS.

2.2.3. Larval rearing area

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

2.2.4. Live feed production area

A dedicated area is required for culturing the phytoplankton, mainly *Nannochloropsis* sp. and *Isochrysis* sp. and zooplankton, mostly rotifers, copepods and *Artemia*. The phytoplankton and zooplankton culture area should be separate to ensure that the zooplankton does not contaminate the phytoplankton. It is better to place the copepods and rotifers apart, so that the rotifers do not contaminate the copepods. The space requirement for the phytoplankton will be more than the zooplankton, and generally, three times more volume is required for phytoplankton than for zooplankton production.

2.2.5. Nursery rearing area

A separate dedicated area is required for nursery rearing of the fry produced in larval rearing section. It is better to establish RAS for nursery rearing, because more requirement for area and water during nursery rearing if carried out in traditional ways like flow-through or water exchanges.

3. Broodstock and spawning

3.1. Acquisition of brooders

- Adult fish of more than 3 kg (Indian pompano, *Trachinotus mookalee*) or 2 kg (Orange spotted grouper, *Epinephelus coioides*) should be collected, either from the wild or from the pond or cage (farmed), and are further raised for broodstock development.
- Adult fishes selected for broodstock development should be of normal body shape and colour; should have absence of skeletal deformities, large wounds, haemorrhages, infections and parasites; and should exhibit normal behaviour, such as good response to feed distribution, controlled buoyancy to maintain position in the water column and best growth and feed conversions within its age group, when selected from farmed fish.

3.2. Transportation

Orange spotted grouper collected from wild showing barotrauma

- The collected fish should be transported in covered tanks, containing aerated or oxygenated water mixed with approved fish sedatives, such as 2 phenoxyethanol @ 50 ppm, for reducing stress and for easier handling of the fish.
- After arrival into the hatchery, orange spotted grouper should be degassed by puncturing the air bladder to relieve the barotraumatic effect.

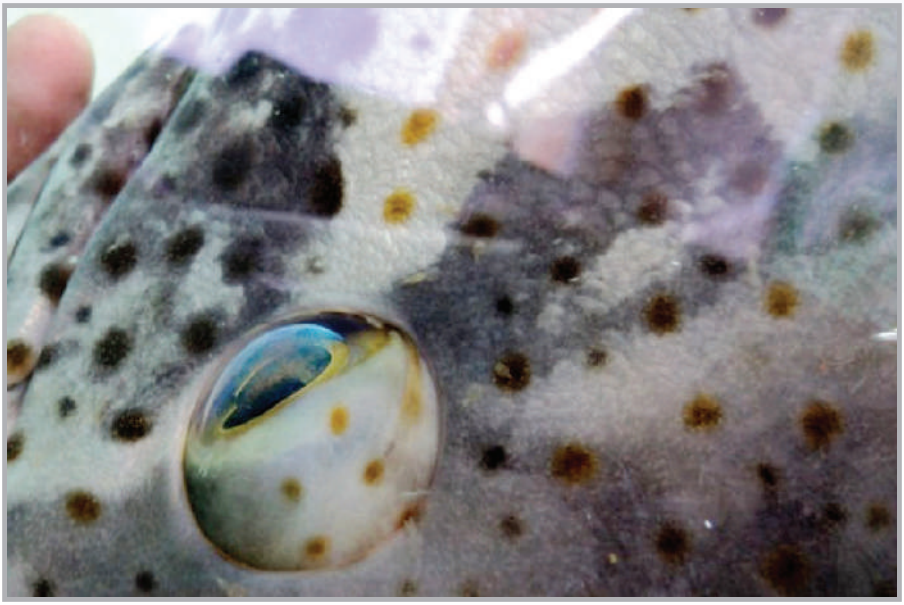
Fish showing balanced swimming behavior after relieving the barotrauma stress

3.3. Quarantine

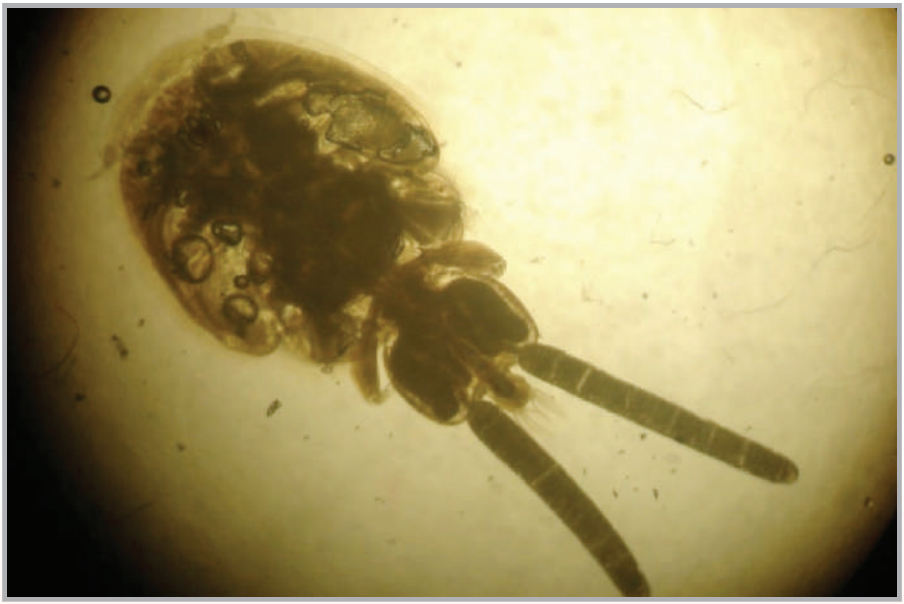
- Collected Indian pompano should be given a bath treatment of formalin in freshwater at the rate of 30 mg L^{-1} for 15 min, once in four days for a period of 3 to 4 weeks.
- Collected orange spotted grouper should be given a bath treatment of formalin in seawater at the rate of 200 mg L^{-1} for 30 min, followed by 5 minutes dip in freshwater, once in three days for a period of 1 to 2 weeks.

External parasite, *Benedenia* infestation in Indian pompano

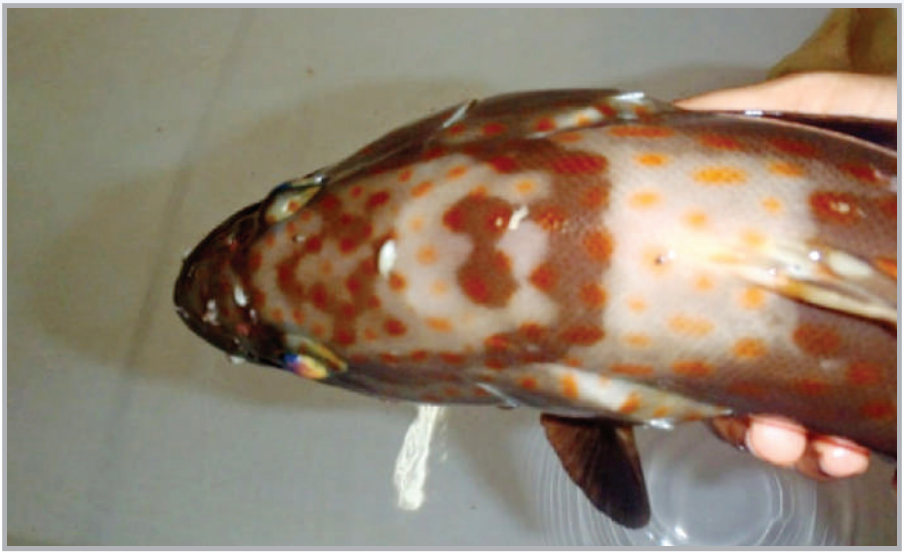
External parasite, *Benedenia*



External parasite, *Lepeophtheirus kabatai* infestation in orange spotted grouper



External parasite, *Lepeophtheirus kabatai*



External parasite, *Isopod* attached to orange spotted grouper

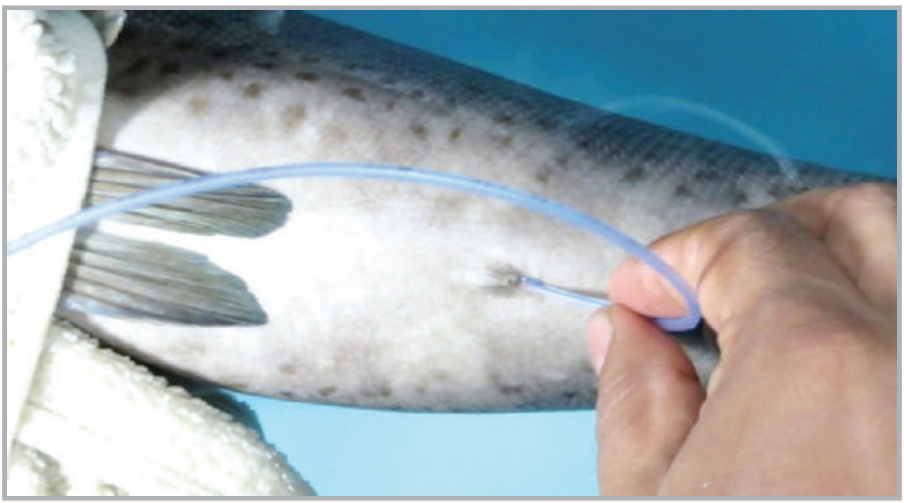
External parasite, Isopod

3.4. Gender identification

- Sexing should be carried out by anaesthetizing the fish in 200 ppm 2-phenoxy ethanol and by covering the eye with wet cloth.

Live ovarian biopsy of Indian pompano using baby feeding tube

- Fish cannula or baby feeding tube CH 6 should be inserted gently inside the urinogenital orifice of males and the oviduct of females for a distance of upto 6-7 cm.
- The fish should be tagged for keeping the record of the individual fish.



Live ovarian biopsy of orange spotted grouper using baby feeding tube

Passive Integrated Transponder used for tagging fish

Tagging of Indian pompano

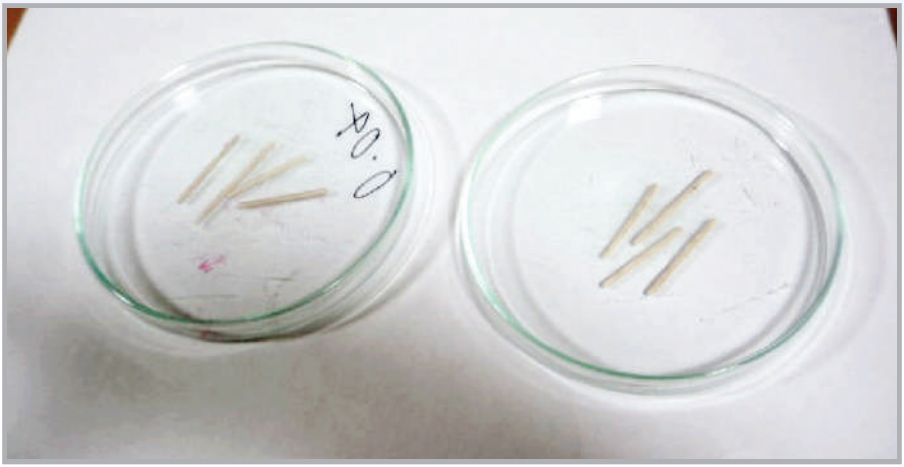
3.5. Broodstock development

- Broodstock tanks of more than 30 t capacities, round in shape, and preferably of grey colour, connected with RAS possessing a minimum of 300% water circulation, should be used for broodstock development - cum spawning tank.

Broodstock tank connected with re-circulating aquaculture system

- The brrodstock tank used for orange spotted grouper should have a minimum depth of 2 m.

Orange spotted grouper brooders stocked in Re-circulating aquaculture system



Hormonal pellet used for sex reversal of orange spotted grouper

- Broodstock density should not be more than 1 kg/m^3 and the sex ratio should preferably be 1:2 (female:male).
- Adult orange spotted grouper should be implanted with cholesterol based pellet containing 17α methyl testosterone and Letrazole at the rate of 5 and 0.2 mg kg^{-1} body weight for converting female to male.

Hormone pellet implantation to orange spotted grouper for sex reversal

3.6. Feeding

- Broodstock should be fed at least once in a day till satiation.
- Indian pompano should be fed with squid and clam meat, and the feed should be supplemented with Vitamin A, C, E and mineral-mix atleast once in a week.

Squid used for feeding brooders

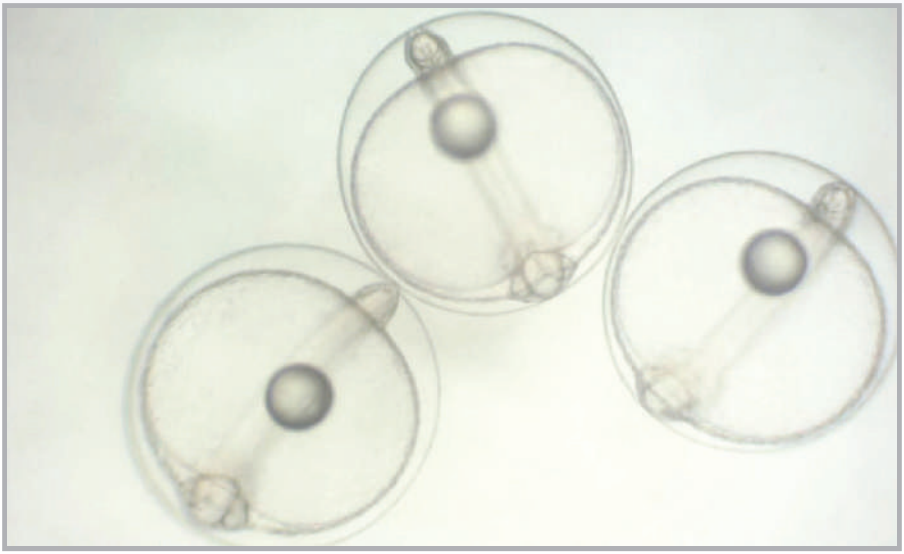
Clam meat for feeding Indian pompano brooders

- Orange spotted grouper should be fed with squid, and supplemented with squid oil and Vitamin A, C, E and mineral-mix atleast once a week.

3.7. Spawning and egg collection

- Orange spotted grouper brooders don't need induction for spawning, and the RAS should be stopped from 17.00 hrs in the evening to next day morning, until egg collection has commenced. The fishes spawn before sunset round the year. The eggs should be collected in the morning, once the eggs reach the eyed stage.

Hormonal induction of Indian pompano



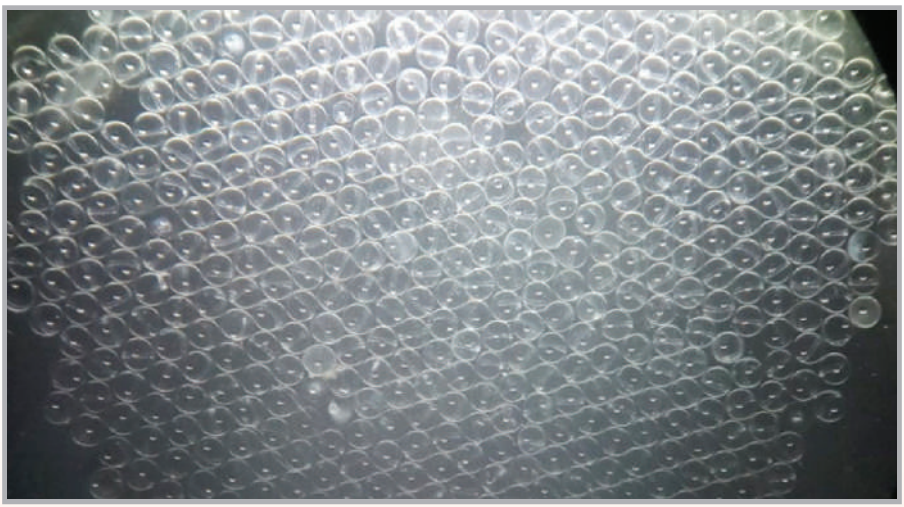
Eyed stage embryo of orange spotted grouper

- Indian pompano females, with ova sizes >500µm and oozing males, with release of milt on slight pressure of the abdomen, in the sex ratio of 1:2 (female:male) should be induced with inducing hormone hCG @350 IU per kg body weight. Fishes respond after 36-42 hrs of injection at 28-30 °C. The eggs are collected at the eyed stage.

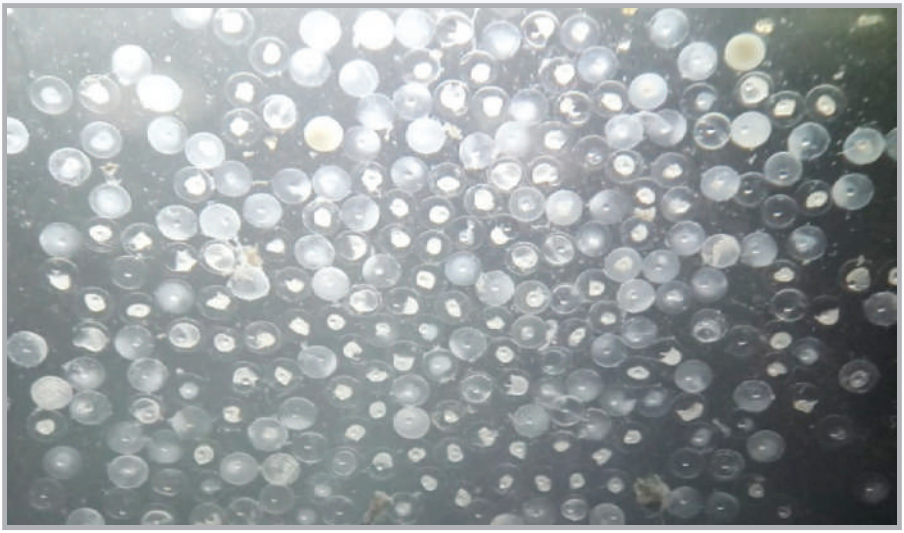
Eyed stage embryo of Indian pompano

- The fertilized eggs are collected in an egg collection chamber, where a hapa made up of 500 μm mesh is fixed and through which, surface water of the tank is directed and is allowed to flow from the tank.

Egg collection after spawning

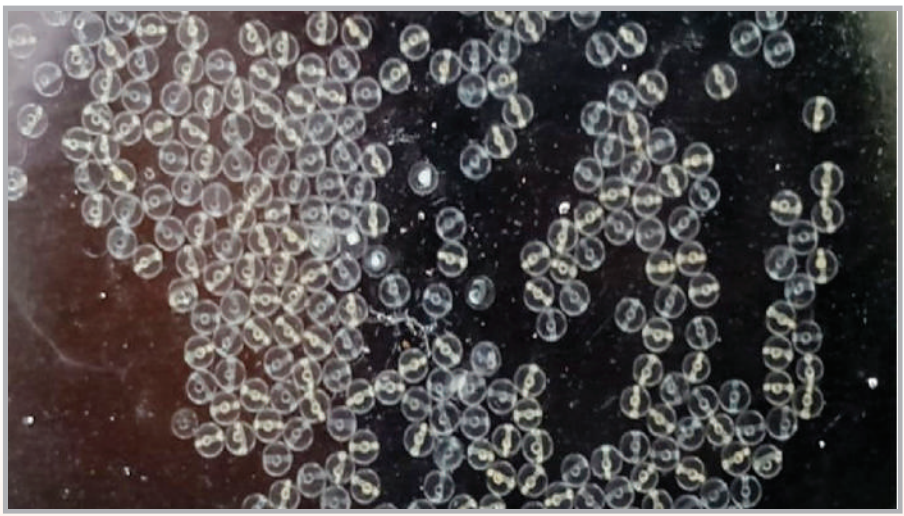


Fertilized eggs of orange spotted grouper

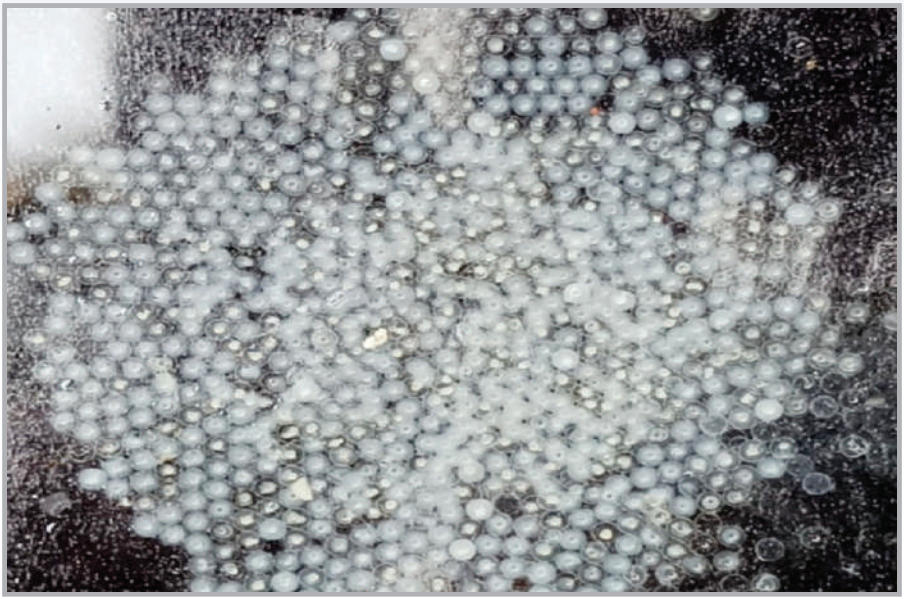


Unfertilized eggs of orange spotted grouper

- The eggs collected in egg collecting chamber should be sieved through a hand net, and treated with 20 ppm iodine for 10 minutes with aeration, after which, the eggs should be washed in de-ozonised sea water and kept in an aquarium at a stocking density of 200 eggs/L.



Fertilized eggs of Indian Pompano



Unfertilized eggs of Indian Pompano

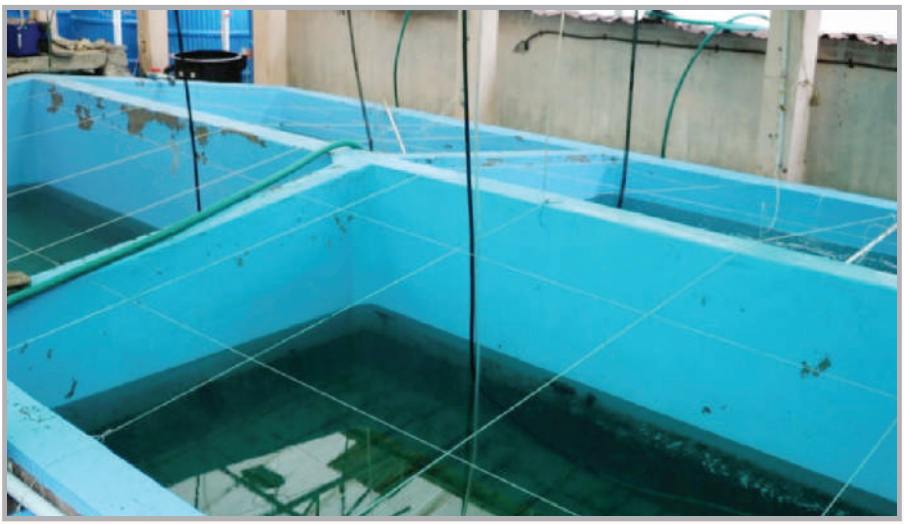
4. Larval rearing

4.1. Larval rearing system

- Generally, round and rectangular tanks of 2 to 10 t capacity, made up-of fiber re-enforced plastic (FRP) or RCC are used for larval rearing.
- Preferable to use RCC tanks for avoiding temperature fluctuations during larval rearing.
- Inside wall of the tank should be dark blue or yellow in colour.
- Water depth of the tank should be more than 80 cm.
- Water used for larval rearing should have salinity more than 30 g L^{-1}, and should be ozonised and then, de-ozonised.
- Aeration should be mild during the early stages (at least upto 10 days) of larval rearing, to avoid physical damage of the larvae. With progression of the larval-rearing cycle, as the larvae become more robust, aeration is gradually increased.

Circular tanks used for larval rearing of orange spotted grouper and Indian pompano

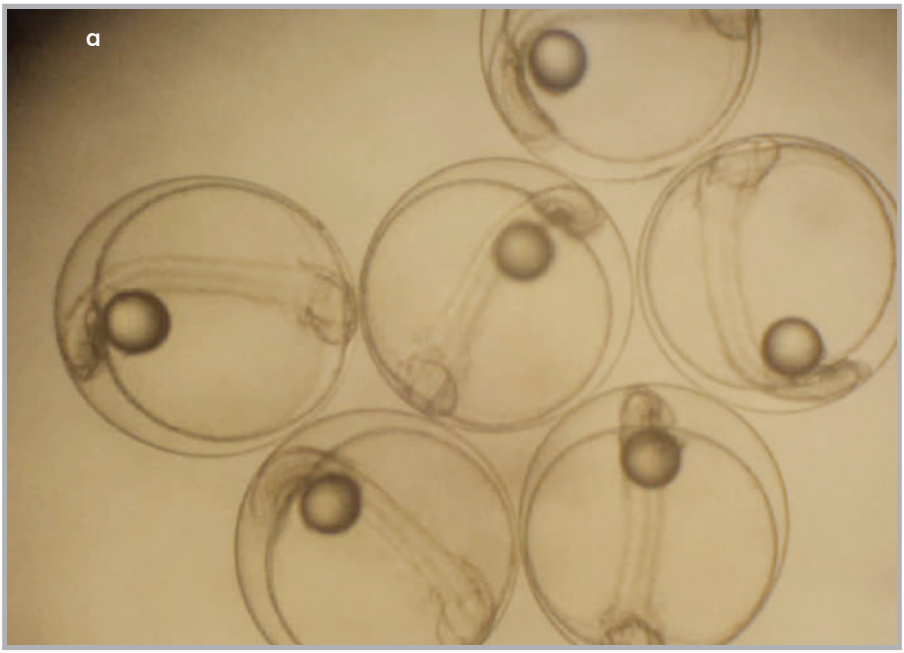
- The tank should be cleaned, either with liquid bleach or washed with mild acid, and air dried atleast for two days prior to stocking.



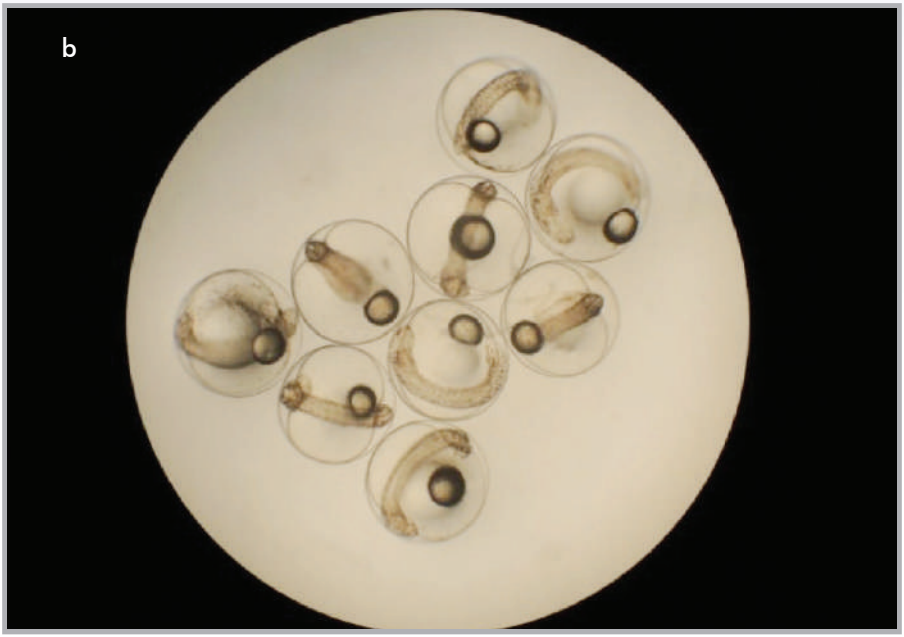
Rectangular tanks used for larval rearing of orange spotted grouper and Indian pompano

4.2. Stocking

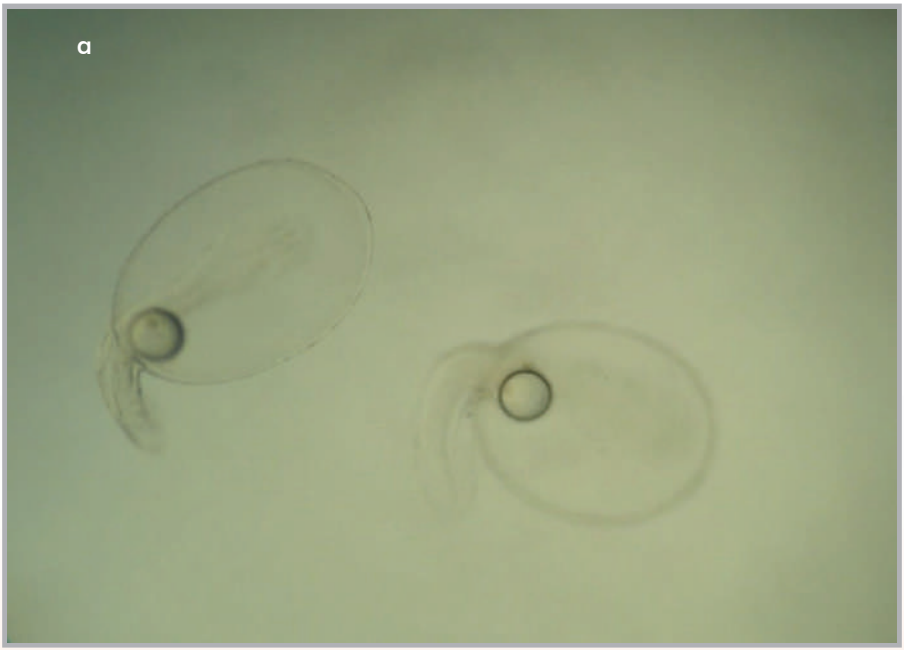
- It is preferable to stock the larval rearing tanks with fertilized eggs in the eyed stage of embryonic development, just before hatching.
- Only surface floating eggs should be stocked, and the stocking density should not be more than 10 eggs L^{-1}.
- On termination of hatching of the eggs to larvae, the tank bottom should be siphoned out immediately.
- For orange spotted grouper, if the egg hatching rate is less than 80%, it is better to discard that batch for fresh stocking.
- After hatching, it is preferable to cover the tank with black cloth till the commencement of first feeding, for avoiding any light penetration to the tank.



Eyed stage embryo of orange spotted grouper (a) and Indian pompano



(b) for stocking in larval rearing tanks



Newly hatched larvae of orange spotted grouper (a)

and Indian pompano (b)

4.3. Feeding

- Larval rearing should be carried out using green water technology, where *Nannochloropsis* sp. and *Isochrysis* sp. are used as micro-algal source at the rate of 10^5 cells mL^{-1} in the ratio of 2:1. The micro-algae should be added on the day of feeding i.e. 2 DPH (day post hatch)

4.3.1. Indian pompano

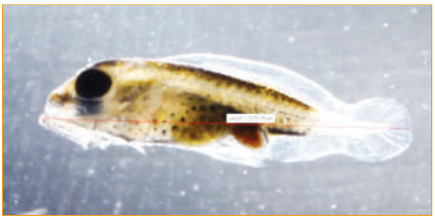
- Initial feeding should commence after 45-48 hrs of hatching at a temperature of 28-30 °C.
- Larvae should be fed with copepod nauplii (*Parvoclanus* sp.) and screened rotifers (*Brachionus rotundiformis*) of < 100 µm at the rate of 2 and 10 individual mL^{-1}, respectively during the initial 5 DPH.

- After 5th DPH, rotifer densities should be increased and maintained at 20 individual mL^{-1}, which is again, gradually increased to 30 individual mL^{-1}, from 8th to 10th DPH.
- Freshly hatched out *Artemia* nauplii should be fed at a density of 0.5 individual mL^{-1} from 8th DPH, and further increasing size should be fed with advancement in rearing period.
- Weaning of pompano larvae to artificial diets should start from 11th DPH. Initially, artificial diet with a particle size of 200-300 μm is used at the rate of 2 g/m^3/day.
- Formulated feed should be 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, and excess feed do not accumulate on the bottom of the tank where it gets decomposed and degrade the water quality. The size of the particulate feed is increased to 400–800 μm from 22nd DPH.
- Bottom of the larval-rearing tanks should be siphoned on the day of hatching to remove unhatched eggs, as well as hatched out eggs shells; afterwhich water is maintained static upto 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 5th DPH, and is carried out once in every 3 days. From 12th DPH; faeces, dead larvae and uneaten food accumulated on the tank bottom are siphoned out at least once daily for maintaining water quality.
- Water exchange should be increased to 20%/day, when both rotifers and *Artemia* are fed together (8th DPH). Water exchange is gradually increased to 50%/day from 11th DPH, and is 100%/day from 16th DPH.

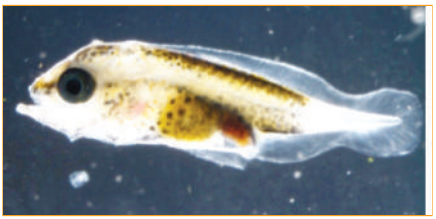
1st DPH

2nd DPH

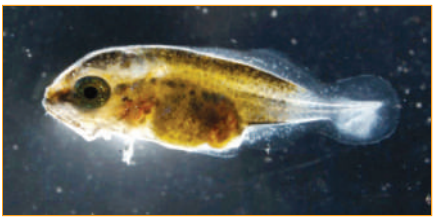
3rd DPH



4th DPH

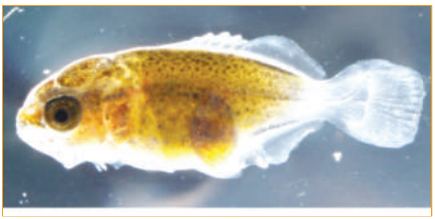


5th DPH



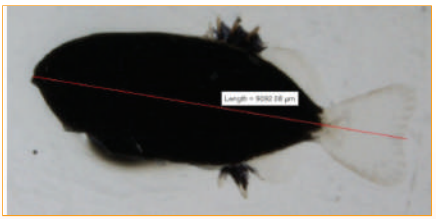
6th DPH

7th DPH

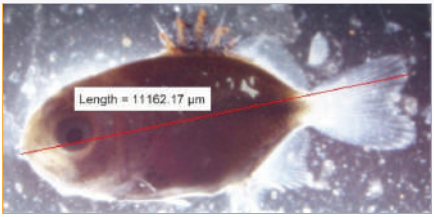


8th DPH

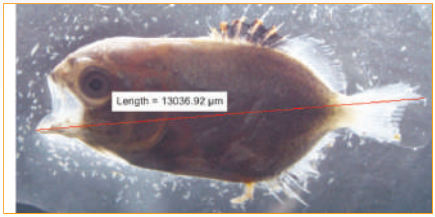
9th DPH



10th DPH



11th DPH



12th DPH

13th DPH

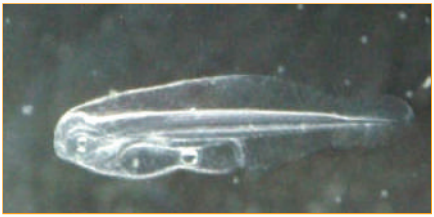
14th DPH

Larval developmental stages of Indian pompano

Metamorphosed fry of Indian pompano

4.3.2. Orange spotted grouper

- Oil (preferably squid oil) should be added to form a thin film on the water surface (around 0.2 ml/m^2) during 1st-4th DPH, for preventing surface aggregation related mortality in early-stage grouper larvae.
- Initial feeding should commence 56-60 hrs after hatching at a temperature of 28-30 °C. The larvae should be fed with copepod nauplii (*Parvoclanus* sp.) and screened rotifers (*Brachionus rotundiformis*) of < 100 µm at the rate of 2 and 5 individual mL^{-1}, respectively till 5 DPH.
- After 5th DPH, small rotifers (filtered with 150 µm mesh) should be added and maintained at a density of 10-15 individuals/ml, which is gradually increased to 20 individual mL^{-1}, from 11th to 18th DPH.
- Freshly hatched-out *Artemia* nauplii should be fed at a density of 0.5 individual mL^{-1} from 17th DPH, and their size increasing with advancement in rearing period.
- Adult copepods should be fed, atleast at the rate of 0.75 individual mL^{-1} during 16th-20th DPH.
- Weaning of grouper larvae to artificial diets should start from 20th DPH. Artificial diet with a particle size of 200-300 µm is initially used. 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 gets decomposed and degrade the water quality.
- The size of the particulate feed should be increased to 400–800 µm from 30th-45th DPH.



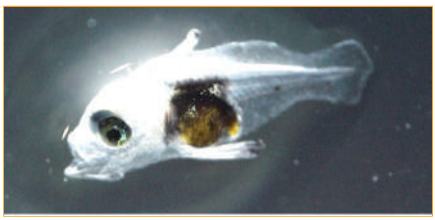
1st DPH

2nd DPH

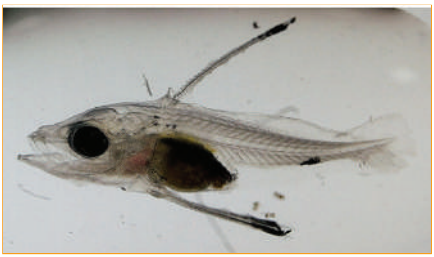
3rd DPH

4th DPH

6th DPH



8th DPH

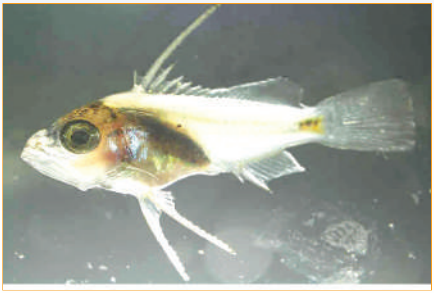


11th DPH

13th DPH

15th DPH

19th DPH



25th DPH

29th DPH

Larval developmental stages of orange spotted grouper

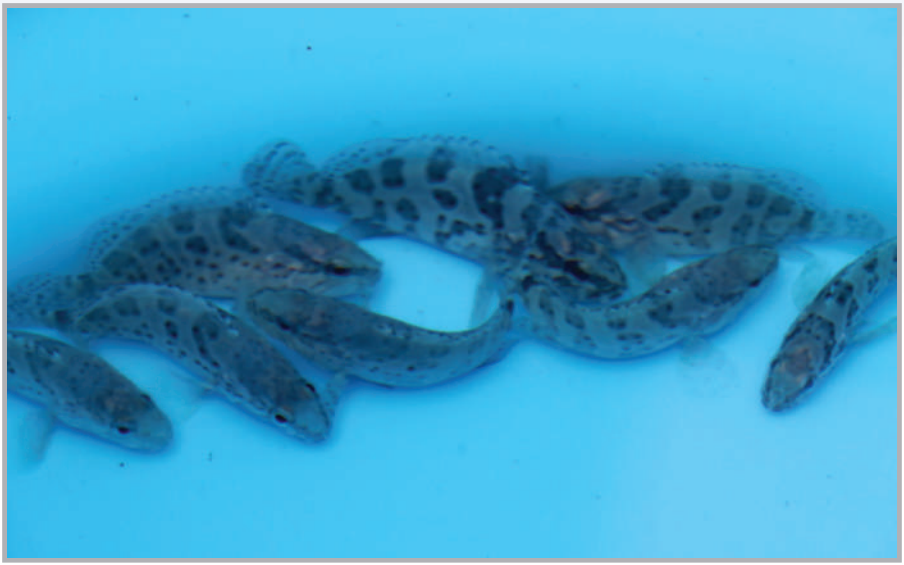
Metamorphosed larvae of orange spotted grouper *(Epinephelus coioides)*

- Larval-rearing tanks should be 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 start on 7th DPH. From 12th DPH; faeces, dead larvae and uneaten food accumulating 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 fed together (15th-20th DPH). Water exchange gradually increases to 50%/day from 25th DPH, and is 100%/day from 35th DPH.

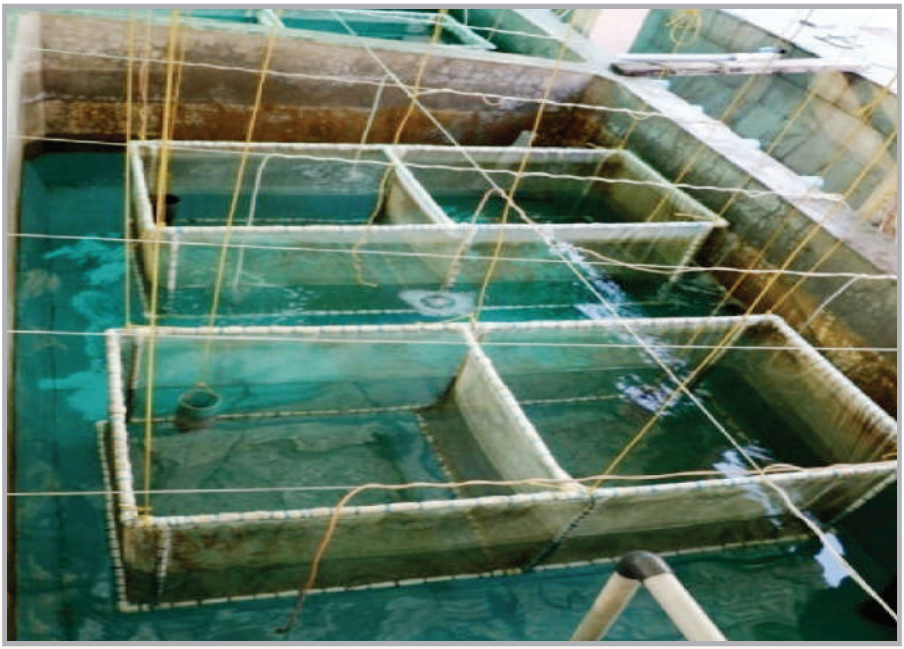
5. Nursery rearing

5.1. Orange spotted grouper

- The nursery rearing of orange spotted grouper has been 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 completely, by 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 onto artificial feed. Artificial feed containing 45% protein and 10% fat of 0.8 mm and 1.2 mm pellet sizes are used. Feed is 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 in the 1st month. The water quality should be managed at optimum level either by water exchange or by flow through.
- Second phase of nursery rearing consists of growing 5-6 cm size to 10-15 cm size 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), or in flow-through cement tank, or RAS.
- Pelleted floating feed with 45% protein and 10% fat of 1.2 mm and 1.8 mm sizes are used @ 10% of body weight, 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 limiting actions related to water exchange, bottom



Fingerlings of orange spotted grouper



Hapa-based nursery rearing system in cement tank

Hapa-based nursery rearing system in pond

Orange spotted grouper nursed in pond based hapa

siphoning, tank cleaning; which are essential for the maintenance of water quality. Therefore, large number of grouper fingerlings is nursed without the requirement of additional manpower, and with a high growth rate as compared to other systems.

5.2. Indian pompano

- Nursery rearing of Indian pompano has been standardized with different feed and culture conditions, such as hapa in pond, and FRP or cement tank. Even the cages installed in sea, is used for nursery rearing with 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 reach 28.08 g with a weight gain percentage of 610.
- Fry are fed artificial pelleted feed containing 45 % protein and 10 % fat @ 10% of biomass, four times a day during this period.
- 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 attain 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 in 8 mm mesh size inner net at 35 nos/m^3. Fish fry are fed at 10% of body weight with commercial floating diet containing 45% crude protein and 10% fat, twice a day.

Nursery rearing of Indian pompano in FRP tanks (a) and pond (b)

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