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BROODSTOCK DEVELOPMENT, BREEDING & LARVAL REARING OF COBIA AND POMPANO

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In recent years, finfish mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. One of the major reasons for the growth of sea cage farming is the availability of breeding techniques that can produce sufficient quantity of seeds of different high value marine finfish. Many countries in the Asia-Pacific Region like Australia, China, Japan, Taiwan, Philippines, Indonesia, Thailand, Malaysia and Vietnam have made substantial progress in the development of commercial level seed production technologies of many high value finfish suitable for sea farming. But even in these countries, seed stock supply is one of the vital issues for further expansion of mariculture.

In India, much research attention was not given for developing seed production methods for high value finfishes suited for sea farming. At present we have commercial seed production of only one marine finfish – sea bass (*Lates calcarifer*). Here also private

entrepreneurship has not yet been developed. Unless an intensified research on the development of commercial level seed production technologies is taken up, sea farming cannot emerge as a significant seafood production sector in the country. In the recent past, the Central Marine Fisheries Research Institute (CMFRI) has been intensifying its research activities on the breeding and seed production of high value marine finfish and success was achieved in the breeding and seed production of cobia and silver pompano for the first time in the country at Mandapam Regional Centre of CMFRI.

Cobia (*Rachycentron canadum*) and silver pompano (*Trachinotus blochii*) are two marine finfish species with very high potential for aquaculture in India. Fast growth rate, adaptability for captive breeding, low cost of production, good meat quality and high market demand especially for *sashimi* industry are some of the attributes that make cobia an excellent species for aquaculture. In recent years the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Similarly, pompano is having fast growth rate, good meat quality and high market demand. Envisaging the prospects of cobia and pompano farming in India, broodstock development was initiated at the Mandapam Regional Centre of Central Marine Fisheries Research Institute in sea cages during 2008 and the first successful induced breeding and seed production was achieved for cobia in March – April 2010 and for pompano during July 2011.

Broodstock development Broodstock Collection and handling

Broodstock fish are generally collected from the wild and are conditioned and matured in captivity. The main selection criteria to identify suitable adult fish as broodstock fishes are size, age (for those collected from grow-out farms) and appearance. The following are the details of the selection criteria:-

- body shape, age and colour,
- absence of deformities,

- absence of wounds, haemorrhages, infections and parasites
- behaviour like quick response to feed and fast swimming
- It is advantageous to collect sub-adults for broodstock develo pment. Larger fishes would have crossed the reproductive age and very small fishes will take longer time to sexually mature.

Cobia weighing between 8 to 15 kg can be collected for broodstock development. Whereas,the pompano brooders could be procured in weight range of 750 gm to 1.5 kg.

Stress should always be minimised during capturing and handling of broodstock. It is best to collect broodstock fishes caught using trap nets, hook & line, etc., which cause minimum stress to the fishes.

Quarantine treatment

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish Anaesthetics like MS 222 (50-100 ppm), Aqui-S (4 ml / 100 L), 2-phenoxyethanol (200-300 ppm) and quinaldine dissolved in acetone (3-5 ppm) can be used for broodstock handling. The prophylactic treatment is given to limit the risk of introducing parasites or bacterial diseases into the hatchery facility. Short time exposure of brooders (5 – 15 minutes) in freshwater will help to remove the external parasites. The prophylactic treatment in hatcheries includes a sequence of medicated baths in formalin, malachite green and Oxytetracycline. Prophylactic treatment can be repeated three to four times within a week.

Broodstock holding and maturation

After quarantine, broodstock fishes are moved into 100 tonne capacity RCC tanks for maturation and long-term holding in the hatchery. During gonadal maturation, water salinity needs to be 31-35 ppt. Water quality parameters like salinity, temperature, dissolved oxygen, pH, ammonia, and fish stock condition viz., general behaviour, feeding activity, disease

symptoms, prophylactic treatments, etc. are monitored regularly. Normally sex ratio of 1 female: 2 males are maintained for cobia while it is 1: 3 for pompano.

Fig.1.Cobia broodstock fishes

Fig.2. Pompano brooder

Broodstock development in cages

For larger fishes like cobia, broodstock development in FRP tanks/ RCC tanks is possible only with recirculating aquaculture system due to its high metabolic rate. Alternatively, broodstock development can more effectively be practised in circular (6 meter diameter and 4.5 meter net cage length) or square (5 m X 5m) sea cages.

Broodstock Feeding

For quicker maturation, the broodstock fishes are to be fed with highly nutritive diet. Diet rich in vitamins, poly-unsaturated fatty acids (n- 3 PUFA) and other micro-nutrients is essential for obtaining viable eggs and larvae. The brood fishes can be fed *ad libitum* once a day with chopped oil-sardines, crabs, shrimps and squids stuffed with vitamin, micro- and macro- nutrient premixes.

Fig.3. Broodstock cages at Mandapam

Tagging of Fish

Tagging or physical marking of broodstock fishes through easily detectable methods is very much essential for selection of broodstock for identification, selective breeding and segregation. The most popular method is Passive Integrated Transponder (PIT) tagging. PIT tagging also known as 'microchips' is a radio frequency device to permanently mark fishes internally. The tag is designed to last the life of the fishes providing a reliable, long term identification method.

Maturation and spawning

The natural process of sexual maturation of the broodstock fishes can be accelerated by altering the photo-thermal period and it is also possible to obtain viable larvae almost throughout

the year. At the onset of the spawning season, it is necessary to move selected broodstock fishes from maturation tank to spawning tank after assessing the ovarian development through cannulation using flexible sterile catheters (1.2 mm internal diameter, Fig.4). Only females with oocytes in the late-vitellogenic stage, with a diameter round 700 µ in cobia and 500 µ in pompano, are selected.

Fig.4. Cannulation of Cobia

Induced spawning

Spawning can be obtained either by natural or inducing with hormonal treatment. Induced breeding is commonly practiced in most commercial hatcheries. The hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. The human chorionic

gonadotropin (HCG) is used at a dosage of 500 IU per kg of body weight in cobia females and 250 IU per kg body weight for males. Whereas, for pompano 350 IU per kg body weight is used for male and female. This dosage can be administered as a single dose on the dorsal muscles. The HCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethylamide, acetate salt]. It is a small molecule with 10 peptides and acts on the pituitary gland to induce the release of gonadotropins which, in turn, act on the gonads. Almost 100% of injected fish spawn eggs whose quality usually matches that of natural spawning. The LHRHa is used in very low dosages, usually around 20 µg / kg of body weight

Fig.5.Hormonal administration to cobia

Spawning tanks

The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners and possible risk of disease contamination. However, for economic reasons, it is usual to keep the brooders inside the hatchery in a specific dedicated area. Though we use only rectangular tanks based on availability, it is preferable to use

circular tanks with at least 1.20 m depth.Shape and depth counts for easy and free movement of brooders.

Normally the spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place normally between late night and early morning hours.The number of eggs spawned by cobia ranges from 0.4 to 2.5 million. Whereas, the pompano brooders spawn 0.5 to 1.5 lakh eggs.

Egg harvest

The fertilized eggs of cobia and pompano float and are scooped gently using 500 µm net. To minimise the presence of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs found at the water surface. The egg samples must be thoroughly examined to assess their quality, number and development stage using a microscope.

Incubation of eggs

Incubation of eggs can be carried out in incubation tanks of 3-5 tonne capacity. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. After hatching, only the hatched fish larvae have to be moved to the larval rearing tanks filled with filtered seawater. Prior to this, the aeration should be stopped briefly to enable the debris and exuviae to settle at the bottom which can be removed by siphoning. The development of embryo can be observed at frequent intervals under a stereo/compound bionocular microscope. The hatching of eggs takes place from 18 to 24 hours.

Larviculture

Newly hatched larvae have to be checked to assess their viability and condition prior to stocking in the larviculture tanks. At least 10 to 20 fish larvae have to be observed under the microscope for the following:

- shape and dimensions
- deformities, erosions and abnormalities

- appearance of internal organs
- pigmentation
- absence of external parasites

The larvae hatched in the incubation tanks or larval rearing tanks need to be distributed in larviculture tanks to have minimal stocking density of 10 to 20 larvae/ litre for cobia and 20-30 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage. Soon after hatching, the mouth remains closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 9-11 dph. Newly hatched cobia larvae generally start feeding at 3 dph and they can be fed with the enriched rotifer (*Brachionus rotundiformis*) at the rate of 10-12 nos / ml, four times a day till 10 dph. From 8 dph, the larvae can be fed with enriched Artemia nauplii at the rate of 1-3 nos / ml, 2-3 times per day. During the rotifer and Artemia feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsis occulata* at the cell density of $1x10^5$ cells / ml. The weaning to artificial larval diets has to be started from 15- 18 dph. While weaning, formulated feed should be given 30 minutes prior to feeding with live feed. Size of the artificial feed has to be smaller than the mouth size of the fish. Continuous water exchange is required during weaning stage. Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every four days interval. During this stage, the fry could be weaned totally to artificial diets. Larval rearing can be practised both intensively in tanks and extensively in ponds. The major factors affecting the growth and survival of larvae are nutrition, environmental conditions and handling stress. Since there is high demand for essential fatty acids (EFAs), enrichment protocols are needed for live-

feeds. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 12 dph. The environmental conditions required during the larviculture period are DO_2: > 5mg / l , NH3: < 0.1mg / l, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature : 27-33° C.

Green water has to be maintained in appropriate densities in the larval tanks. While weaning the fish larvae from rotifers to artemia nauplii, co-feeding with rotifers has to be continued due to the presence of different size groups of larvae. The detail of weaning protocol is as follows.

Table 1.Detail of weaning protocol

Stage of Larvae (dph)	Size of Larvae (cm)	Size of Feed (µ)
18 – 19	2.3 – 2.6	100-200
20 – 23	2.5 – 3.5	300-500
23 – 30	3.5 – 8.0	500-800
31 onwards	> 8.0	800-1200

The juveniles measuring 10 cm length were ready for stocking in happas/ nursery tanks.

Nursery and grow-out rearing of cobia

Nursery phase of cobia can be carried out in happas or sea cages or indoor FRP / cement tanks. During nursery rearing, it is advisable to feed the juveniles with formulated feed of 1200 µ size which can be increased to 1800 µ size from 55 dph onwards. Once the juveniles reach a size of 15 gm, they are ready to stock in sea cages or land based ponds for grow-out farming.

Larviculture of Pompano

The newly hatched larvae are stocked at a density of 10000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks are provided with mild aeration and green water at a cell density of 1 $x10^5$/ml. The mouth of the larvae opens on 3 dph and the mouth size was around 230 µ.

The larvae are fed from 3 dph to 10 dph with enriched rotifers at a density of 5-6 nos. per ml, wherever possible, wild collected copepods could also be added as supplements. Enriched Artemia nauplii are provided at a density of 1-2 nos. per ml from 8-19 dph. Weaning to larval inert feeds was started from 15 dph. From 25 dph onwards, feeding can be entirely on larval inert feeds. The metamorphosis of the larvae starts from 18 dph and all the larvae metamorphose into juveniles by 25 dph. Critical stage of mortality would occur during 3-5 dph and subsequent mortalities are negligible. The water exchange can be practically nil till 7dph and it can be gradually increased from 10-100 % from 8 to 14 dph.

Nursery Rearing of Pompano

Nursery rearing could be initiated from 25 to 30 dph. At this stage, artificial feed of 800 µ size could be provided. Thereafter, fingerlings were fed with progressively higher size range of floating extruded larval feeds. Daily water exchange of 100% is advisable. Water quality parameters like salinity, temperature, pH, oxygen level and ammonia are closely monitored during the entire larviculture period.

After 55dph, the fingerlings with size range from 1 to 1.5 inch size can be supplied to farmers for stocking in the happas/ tanks for further nursery rearing and grow-out farming thereafter.

Live feed culture

Micro-algal culture

Microalgae are the important live feeds required for larviculture of marine fin fishes. Algae like *Chlorella sp., Nannochloropsis sp., Tetraselmis sp., Dunaliella sp*., *Pavolova sp*., and *Isochrysis sp.* can be used as algal diet for growing the rotifers. The size, nutritive value, proliferation rate and digestibility of the algae are the critical factors for selecting the algae for the use in marine hatchery use.

Copepod culture

Copepods have almost become inevitable because they are the only acceptable sized prey for small larvae of many marine

fin fish species and the only type of live feed that will support the altritial type of larvae. Copepod nauplii offer a diverse size spectra and nutritious prey that can meet the specialized needs of small fast growing fish larvae. Over the past few years, several articles have been published and many conferences were dedicated to discussions of copepod culture and the important role that copepods can play as live feed for marine finfish larviculture.

Rotifer culture

Rotifers are the smaller size zooplanktons widely used in marine fin fish hatchery operations. The marine fin fish larvae initially feeds on the such smaller size zooplanktons and hence suitable size of rotifers need to be cultured in mass to feed the fish larvae. The important criteria for selecting the rotifer depends on the mouth size of the fish larvae, digestibility, nutritive value of the rotifer and easy for culture and proliferation. Marine and brackish water rotifer species can be artificially propagated in seawater and more popular rotifer species used for marine fin fish hatcheries are *Brachionus plicatilis* and *Brachionus rotundiformis.*

Based on the length of lorica, *Brachionus* is separated into 3 strains: *B. plicatilis* as L type (large) with long of lorica 200 – 360 µm; *B. rotundiformis* as S type (small) with long of lorica 150 – 220 µm; *B. rotundiformis* as SS type (super small) with long of lorica 70 – 160 µm.

Artemia nauplii

Having a larger size than rotifers, the nauplii of brine shrimp *Artemia* are used as the second live food to fed fish larvae. Commercially available *Artemia* cysts are purchased and hatched whenever required. The first *Artemia* larval form is the nauplii, which are smaller in size and richest in yolk, and followed by larger size metanauplii, whose nutritional value has to be boosted by feeding them with special enrichment diets 12 to 24 hours before feeding them to the fish larvae.

Prospects of cobia and pompano farming in India

Trials on sea cage farming carried out at Mandapam showed that the fishes attained an average weight of 2.5 kg in six months and 7.3 kg in twelve months. The species can be grown in salinity as low as 15 ppt and our experiments revealed that the growth and survival at 15 ppt is comparable to that in seawater. Further, a trial of earthen pond culture of cobia which is underway at Anthervedi in Andhara Pradesh shows very encouraging results. All these outcomes point out the possibility of developing a lucrative cobia aquaculture enterprise in the country. Similarly the silver pompano was able to acclimatize and grow well even at a lower salinity of about 10 ppt and hence is suitable for farming in the vast low saline waters of our country besides its potential for sea cage farming. Our farming trials at Anthervedi and Aakkivedu in Andhra Pradesh and at Turicorin and Vedalai in Tamil Nadu show encouraging results. This can be considered as a milestone towards the development of pompano aquaculture in the country.