

# **Broodstock development and breeding of marine finfishes**

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## Introduction

In recent years, mariculture has been growing rapidly on a global basis especially with the development and expansion of sea cage farming. On a global basis, a rapid growth in marine finfish culture is noted. It has increased at an annual average growth rate of 9.3% from 1990 to 2010. Salmonids, amberjacks, sea breams, sea basses, croakers, groupers, drums, mullets, turbot, other flatfishes, snappers, cobia, pompano, cods, puffers and tunas are the major groups which are maricultured. 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 high value finfish suitable for sea farming. In India, the broodstock development and seed production of sea bass, cobia and silver pompano were developed and standardized for commercial level production.

The major steps involved in marine finfish broodstock development and breeding are the following:

1. Broodstock collection
2. Transportation
3. Quarantine
4. Broodstock development
5. PIT tagging
6. Cannulation
7. Induction of spawning
8. Egg collection
9. Incubation

## Broodstock Collection and handling

Broodstock development is the vital and time

consuming procedure in marine finfish seed production. It is not easy to obtain broodstock fish directly from the wild and hence broodstock development is to be done in captivity. The main selection criteria to identify suitable adult fish as broodstock fishes are as follows:

- Body shape, age and colour,
- Absence of deformities,
- Absence of wounds, haemorrhages, infections and parasites,
- Behaviours like quick response to feed and fast swimming

It is advantageous to collect sub-adults for broodstock development. Larger fishes would have crossed the reproductive age and very small fishes will take longer time to sexually mature. In the case of cobia, fish weighing between 8 to 15 kg could be procured while silver pompano 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 from trap nets, hook & line, etc., as they cause minimum stress to the fishes. Adequate dissolved Oxygen (DO) should be ensured during transportation.

## Quarantine

Upon arrival at the hatchery, broodstock fishes are released into the quarantine tanks for prophylactic treatment. Fish anesthetics like MS 222 (50-100 ppm) and Aqui-S (4 ml / 100 L), 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 (maximum 5 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 (OTC). Prophylactic treatment can be repeated three to four times within a week. It is preferable to have a flow-through water circulation in quarantine tanks when treatments are not

underway. Smooth inner surface in tanks allow easy and complete cleaning. The following sequence of treatments can be followed:

Day 1: Fresh water bath for 10 minutes and then Oxytetracycline treatment (50 ppm) in seawater for 30 minutes.

Day 2 to Day 7: Treatment with a mix of 200 ppm formalin and 0.2 ppm malachite green for 1-2 minutes, followed by a freshwater dip for 5 minutes. Before returning the fishes to quarantine tanks with filtered seawater, they can be given an Oxytetracycline treatment at 50 ppm for 30 minutes. The fishes should be closely observed during treatments.

During the quarantine, fish should be closely monitored. If the fishes suddenly become immobile or are found with very less opercular movements or are turning upside down, they should be immediately transferred to filtered seawater. The fishes can be fed during the day time when it is not undergoing treatment. Over feeding should be avoided and the fishes can be transferred to maturation tanks after the treatments are over. Apart from quarantine treatment, the broodstock fishes should be given regular prophylactic treatment with freshwater with or without OTC at least once in a month.

### Broodstock development

After quarantine, broodstock fishes are moved into Recirculation Aquaculture Systems (RAS) or sea cages for broodstock development. Broodstock development in sea cages was successfully done for cobia at Mandapam Regional Centre of CMFRI. Circular cages of 6 m diameter and 3.5 m depth with HDPE frame were employed for the purpose. The major problem in the development and maintenance of the broodstock in sea cages is the risk of contracting diseases and subsequent loss of broodstock. The sudden loss of broodstock will affect the seed production, since loss of broodstock cannot be made good from the wild immediately. Hence, on shore facilities like RAS is advised for development and maintenance of biosecured broodstock.

The vital aspects which affect development of broodstock are the photoperiod, temperature and broodstock nutrition. In a shore based facility, the photo thermal conditioning can be practiced which will accelerate the gonadal maturation. In

addition, it is also possible to obtain year round spawning in such a controlled system.

### Broodstock nutrition

The viability of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet, the feed intake rate or the feeding period can all affect spawning, egg and larval quality. In the case of tropical fishes, ovarian development is often asynchronous – oocytes in all stages of development are present at the same time and sometimes independent of season. The ovarian development starts with the formation of primary oocytes. During the primary growth phase, the surrounding granulosa and theca cells envelop the oocyte to form the ovarian follicle. In the early stages of secondary growth, cortical alveoli appear and accumulate in the periphery of the oocyte. Even though the oocyte may increase in size several fold during primary and early secondary growth, the most conspicuous size increase occurs during the last part of secondary growth, vitellogenesis. Vitellogenesis is the process of yolk formation and incorporation in the growing oocytes. The yolk protein precursors, vitellogenins, are high molecular weight lipoproteins that are synthesized in the liver and secreted into the blood. The fatty acid composition of the vitellogenins can be affected by long term imbalances in the broodstock diet. It has been well established that feeding broodstock fish with squid, cuttlefish or meals made from cephalopods have beneficial effects. These feed ingredients make the diet more attractive and therefore increase feed intake. Squid and cuttlefish also contain high levels of essential fatty acids.

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. During gametogenesis, female fish require a food, richer than usual, in proteins and lipids to produce the vitellogenin. As the sole source of food for the developing embryo and the early larval stage until feeding on live preys starts, yolk quality and quantity are key factors for a successful reproduction. Both dry pellets and moist food are also employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 n-3) and

DHA (20:6  $\square$  3), which cannot be produced by fish metabolism. Broodstock fishes are fed ad libitum once a day with squids, cuttlefish, crabs, shrimps and chopped oil-sardines depending on the availability.

### Tagging

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 PIT Tagging. Passive Integrated Transponder (PIT) tag, also known as 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. The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term “passive”, so the microchip remains inactive until read with a reader. The reader sends a low frequency signal to the microchip of the tag providing the power needed to send its unique code back to the reader and therefore fish is positively identified.

The distance from which a tag can be read is the read range. Most read ranges using hand-held readers are 3 to 9 inches depending on the reader. There are currently three basic tag frequencies. The 400-kHz tag was one of the first developed but it has limited read range. As microchip technology evolved, the 125-kHz and 134.2-kHz tags became available. Compared to the older 400-kHz tags, they have a much better read range and reduced read time. The 134.2-kHz tag was developed to meet international standards for code format. It is very much important that the tag type and reader unit should be compatible. Most readers are capable of detecting both 125-kHz and 134.2-kHz frequencies.

Design engineers’ calculations suggest that PIT tags can last as long as 75 years or more. There is no battery to fail and the glass encapsulation is impervious to almost everything. PIT tags can be removed or recovered from a primary location and reused indefinitely. Reducing stress to the fish is the prime factor in ensuring the success of the tagging and safety of the fish. Therefore, the fish should be anesthetized during the implantation of PIT tags. Species, size and age should be considered when making a decision about anesthesia and restraint. Sterile implants are advised

but many field conditions do not allow for sterile implants. Equipment can be disinfected prior to use with alcohol and iodine-based solutions. The tag is encased in glass that protects the electronic components and prevents tissue irritation, thereby very much safe to the fish.



### Advantages of PIT tag over other tags

- Highly reliable individual identification
- Permanent identification marker
- Small size and no interference with the behaviour of fish
- No error in recording data
- Rapid data collection

### Disadvantages

- Initial cost is high
- Low detection distance

## Procedure of tagging

The implant site depends upon the species, size of the fish and the size of the tag. It is preferable to implant the tag on the dorsal musculature of the fish which will be convenient.

### Stepwise protocol

- Use sterile needle or implanter to tag the fish. In field condition, disinfect all the components prior to use with alcohol and iodine-based solutions.
- Read the tag before inserting into the fish and record the identification code or number.
- Catch the fish and anaesthetize it with suitable anaesthetic. In sea cages, it is easier to restrain the fish inside the catching net.
- Disinfect the site of implantation with alcohol or iodine-based solution.
- It is a better practice to keep a standard site of implantation so that the reading will be easier and quicker.
- The tag loaded inside the implanter has to be inserted into the muscle tissues. It is advisable to insert the tag parallel to the muscle fibres to avoid much damage to the tissues.
- The tag should be released slowly and steadily from the implanter while removing the implanter from the tissue in such a way that the tag fills the space created by the implanter needle.
- Once implanter needle is taken out, the site should be disinfected again with alcohol or iodine-based solutions to avoid secondary infection.
- Release the fish as soon as the tagging is over or once it has recovered from anaesthesia.

### Cannulation

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. Only females with oocytes in the late-vitellogenic stage, with a diameter around 700µm in cobia and 500 µm in pompano, are selected.

### Ovarian biopsy can be carried out as follows:

- Female brooders have to be transferred to a small tank containing anaesthesia in sufficient quantity.
- Flexible sterile catheters (1.2 mm internal diameter) can be used for cannulation biopsy.
- Introduce the sterile catheter into the oviduct, up to the ovary for a few cm; then suck carefully a small sample of oocytes up into the catheter and place the sample on a glass slide.
- After sampling, release the animal into the spawning tank, where recovery from sedation will take place.
- Put few drops of filtered sea water on the biopsy sample and examine under the microscope and measure the diameter of the oocytes and record the measurements.



### Induction of spawning

Spawning can be obtained either naturally or by inducing with hormones. 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 both male and female. This dosage can be administered as a single dose on the dorsal muscles. Use of hCG treatment sometimes

gives serious setbacks like not all females respond to it, egg quality may be below acceptable standards with hatching rate lower than 80%, being a large molecule it may provoke immunization reaction, and as a result, fish treated with hCG may not respond when treated repeatedly with this hormone. However, 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.



*Hormonal administration to cobia*

## Spawning

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. It is preferable to use circular tanks with at least 1.20 m depth. Shape and depth of tanks count 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  $\mu\text{m}$  net. To minimise the presence of poor-quality eggs, which

usually float deeper in the water, it is advisable to collect only the eggs which float at the water surface. Therefore, aeration can be switched off allowing the unfertilized / dead eggs to settle at the bottom of the tank. The floating layer of eggs thicker than one cm should be avoided. A thicker layer may reduce oxygen supply to the eggs, leading to possible anoxia after a short time. Then in the temporary container, eggs must be thoroughly examined to assess their quality, number and developmental stages. With a pipette eggs should be taken from the floating egg layer in the temporary container, and should be placed on a watch-glass or on a Petri dish for observation under microscope. Few dozens of eggs, which are placed under a microscope or a transmitted-light stereomicroscope have to be observed for the egg developmental stages.

As fertilised cobia/ pompano eggs float in the seawater, they can be collected using egg collectors. If well dimensioned and properly placed, these devices harvest only the floating eggs, while the dead or unfertilised ones sink to the bottom. The presence of eggs in the collectors should be checked rather frequently in the case of cobia, as its spawning releases a large amount of eggs in a very short time there is risk of clogging the collectors or of mechanical stress to the eggs.

## Check for the following egg characteristics:

- Presence of opaque, whitish eggs which are unfertilised. Similarly, eggs in the sample with transparent, but without evidence of cell divisions
- Regular rounded shape and size (diameter 900-1000  $\mu\text{m}$  in cobia; 800-900  $\mu\text{m}$  in pompano), regular cell division that can be observed only in the first blastomers; regular shape of yolk (it should occupy the egg volume entirely, without perivitelline space),
- Absence of parasites or associated micro-organisms on the chorion surface.

## Incubation of eggs

It is done in incubation tanks of 3-5 tonne capacity. 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. Aeration needs to be adjusted suitably, not too strong to avoid excessive physical collision among eggs, but not too weak either, to keep the eggs suspended in water column. The main purpose of aeration is to prevent clumping and settling down of eggs. Air bubbles should not be too small as seen while using air diffusers instead of stones, as it results in

clumped eggs and damage of the eggs. It is suggested to limit as much the number of air stones as possible. Stocking density can be maintained at a moderate level of 200 to 500 eggs per litre. The development of embryo can be observed at frequent intervals under a stereo / compound binocular microscope. The hatching of eggs takes place from 18 to 24 hours.