

Hormonal induction of spawning in marine finfishes

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Introduction

In recent years, especially with the development and expansion of sea cage farming, mariculture is growing rapidly. 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. For most of the cultured species, supply of wild fry from natural sources is insufficient and fluctuates with environmental and climatic conditions. One of the major requirements 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. By manipulating environmental and hormonal factors seed can be produced year round rather than relying on wild-collection thereby reducing cost and disease.

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, lowest cost of production, good meat quality and high market demand especially for sashimi industry are some of the attributes that makes cobia an excellent species for aquaculture. In recent years, the seed production and farming of cobia is rapidly gaining momentum in many Asian countries. Envisaging the prospects of cobia 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 during March 2010. Subsequently, successful captive breeding and larviculture of silver pompano were achieved during July 2011.

Gametogenesis and reproductive behaviour

A cascade of events leads to release of mature

gametes from ovaries and testes. Marine fishes produce and release sex cells based on maturity of the individuals, their nutrition and overall health, triggered by cues from the environment (temperature, light/dark duration, tides, presence of conspecifics, mates, etc.) that in turn influence their hormonal/endocrine systems. Along with endocrine control there is also a steady, intimate, more sudden interplay of the fishes' nervous system.

Conditioning and triggering of actual spawning involves combining knowledge of modes of reproduction, social factors such as sex ratios, environmental manipulation and possibly direct/exogenous hormonal administration. Either proper environmental stimuli or administration of hormones acting at the level of the hypothalamus, pituitary, or gonads will affect successful release of mature gametes.

Hormonal manipulation

The endocrine system acts like a chemical link between an organism and its environment. Hormones are slow-acting chemical messengers. Along with the faster acting central nervous system they serve to moderate, direct and sustain the physiology of all animals.

Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod, water temperature or spawning substrate. However, the ecobiology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters (i.e., spawning migration, depth, riverine hydraulics, etc.) for natural reproductive performance. Almost all fishes reared in captivity exhibits some form of reproductive dysfunctions. The dysfunctions probably result from the combination of captivity induced stress and the lack of the appropriate natural spawning environments. In females there is a failure to undergo final oocyte maturation, ovulation and spawning while in males; there is a reduction of milt quantity and quality. In these instances, use of exogenous hor-

mones is an effective way to induce final oocyte maturation (FOM) and ovulation in females and spermiation in males and produce fertilized eggs. In some fishes, these hormonal manipulations are used only as a management tool to enhance the efficiency of egg production and facilitate hatchery operations, but in others exogenous hormones are the only way to produce fertilized eggs reliably.

Hormonal manipulations of reproductive function in cultured fishes have focused on the use of either exogenous luteinizing hormone (LH) preparations that act directly at the level of the gonad, or synthetic agonists of gonadotropin-releasing hormone (GnRH α) that act at the level of the pituitary to induce release of the endogenous LH stores, which, in turn act at the level of the gonad to induce steroidogenesis and the process of FOM and spermiation. After hormonal induction of maturation, broodstock should spawn spontaneously in their rearing enclosures.

Effectiveness of hypophysation (injection with pituitary hormones) is dependent on the stage of reproductive development of recipients. Injection of hormones in an unripe adult will not generally induce gametogenesis or ripening of eggs. Determination of spawning-readiness is sometimes associated with color or marking changes and distension of the body. There are chemical assays of body fluids which can also be used as guides of readiness, but these are not as commonly employed as much as simple hand-stripping of gametes, their mix and microscopic examination as a guide to broodstock fitness.

Care must be exercised in assaying sexual readiness in spawners. Sometimes generally adopted parameters have proven unreliable. An example of this is females with enlarged abdomens, reddish coloration and protrusion of the cloacal region may be due to engorgement of the intestine, or disease, even during the spawning season. It is often necessary to sacrifice some of the broodstock to assess their reproductive stage.

Two methods of injection are in wide practice (1) Intramuscular, in the flank just below the dorsal fin and behind the gill cover. This method is safer but slower working. (2) Intraperitoneal injections are faster acting but involve a greater chance of injury or death as the injections are made into the body cavity.

Maturation and spawning

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 700 μm 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.

Induced spawning

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 repeat-

edly with this hormone. However, hCG can be successfully replaced by an analogue of the luteinizing hormone-releasing hormone [LHRHa 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 cost of LHRHa is very high compared to that of hCG. But, LHRHa is used in very low dosages, usually around 20 μg / kg of body weight