MARINE FINFISH BREEDING AND SEED PRODUCTION WITH SPECIAL REFERENCE TO COBIA AND SILVER POMPANO

G. Tamilmani,A.K. Abdul Nazar, R. Jayakumar, M. Sakthivel, P. Rameshkumar, K.K. Anikuttan, and M. Sankar

Mariculture Division, MRC of CMFRI, Mandapam Camp, Tamil Nadu

Introduction

Since many centuries mankind grows almost all its food on cultivated farm land,as far as this food consists of terrestrial plants and animals.But, when it comes to sea water plants and animals, modern man still continues to collect them from natural sources like his forefathers. Marine animals have always been and still are an important source of protein and minerals in human food. With the rapid increase in human population the demand for sea food will certainly increase. However, production from capture fisheries has stagnated and fisheries cannot expand much further. Further increase in exploitation of the seas will lead to destruction of ecosystems and species extinction.

Mariculture - the farming and husbandry of marine plants and animals can augment the marine fish production and can supplement the capture fisheries. One of the major requirements for the establishment of a sustainable mariculture industry is the availability of quality seeds. Seed collection from the wild is unpredictable and cannot be relied upon. The ability to produce viable offspring from captive brood stock can ensure a steady supply of seeds. Countries like Australia, China, Indonesia, Japan, Malaysia, Philippines, Taiwan, Thailand 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 commercial mariculture industry for finfish is in its infancy and the primary technological bottleneck is the lack of commercial-scale hatchery technologies for targeted species.The ICAR –CMFRI is addressing the problem of seed availability through its research on breeding and seed production of several marine species. To date, technologies for breeding and seed production of cobia (*Rachycentroncanadum*), silver pompano (*Trachinotusblochii*), orange spotted grouper (*Epinepheluscoioides*), Indian pompano (*Trachinotusmookalee*) and pink ear emperor (*Lethrinuslentjan*) have been developed and are being refined continuously to make them commercially viable. With the expanding interest in aquaculture and the market demand for more diversified species hatchery technology is being developed for an increasing number of species.

This chapter aims at giving a general view of the major steps involved in marine finfish breeding and seed production with special emphasis on cobia and silver pompano.

Broodstock Collection and handling

It is not easy to obtain fully mature broodstock fish directly from the wild and hence broodstock development has to be done in captivity. Fish broodstock may be collected from the wild or captive stock.

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. Cobia and silver pompano does not have swim bladder as juveniles or adults, and there is no need to vent the fish after capture

Stress should be minimised during capture and handling of broodstock. It is best to collect broodstock fishes from hook & line and trap nets, as they cause minimum stress to the fishes. During transportation, dissolved Oxygen (DO) should be maintained at or above saturation. For handling and transfer, fish are anesthetized with 10-20 ppm clove oil (Aqui-S). Once anesthetized, the fish can be weighed, measured, tagged, sexed and sampled for assessment of sexual maturity.

On arrival at the hatchery prophylactic treatment is given to the fishes to reduce the risk of introducing ectoparasites or bacterial diseases into the hatchery facility. Formalin bath (100 ppm) for 2-5min followed by freshwater bath for 5-10 min is normally given. Silver pompano has a high tolerance for freshwater and freshwater bath can be given for more than 15 minutes also without any problem. During the treatment, fishes should be closely monitored. If the fishes suddenly become immobile or if their opercular movements become very slow or if the fishes are turning upside down, they should be immediately transferred to filtered seawater.

Broodstock Development

The basis for any hatchery operation is the maintenance of a healthy group of sexually mature fish (brooders) conditioned to spawn naturally or in response to hormonal induction. Broodstock development is the vital and time consuming procedure in marine finfish seed production.Good broodstock management involves providing close to natural, non-stressful environmental conditions as well as a nutritious and balanced diet.

Generally, broodstock development of marine finfish is being practiced either in sea cages or land-based cement concrete or fibre-reinforced plastic (FRP) tanks.Broodstock developed in sea cages are susceptible to changes in the water quality of the cage site, disease problems and impact of harmful algal blooms. Therefore, the broodstock developed in sea cages are not bio-secure. In land based broodstock development systems, continuous flow through is provided with 300-500 % of water exchange for maintaining the water quality. This involves huge expenditure. Further,this exposes the broodstock to varying water quality and parasites.

For practical and economic considerations, young adults are first reared in cages to reduce maintenance cost and later transferred to land-based facilities when the fish are ready for spawning.

Recirculation aquaculture systems (RAS) use land based units to pump water in a closed loop through fish rearing tanks and include a series of sub-systems for water treatment which include equipment for sterilization, heating or cooling, solid waste removal, water chemistry control, biological filtration and dissolved gas control. If the broodstock development is practiced in recirculation systems, it is possible to have control on the environment in which the broodstock are reared, the duration and intensity of light and water temperature can be manipulated to accelerate the gonadal maturation. Sustainable production of bio-secure marine finfish seed all through the year employing photo-thermal conditioning is possible only by recirculating systems and this can pave the way for the commercial level seed production.

FRP tanks of 10MT capacity are suitable for broodstock development of silver pompano. For cobia, tanks of 60 to 100 MT capacities with RAS were found suitable. The size of the tanks should be large enough for the species selected to reduce the stress of captivity and to enhance the chances of spawning. Circular tanks were found more suitable than rectangular or square tanks. The broodstock fishes should be given regular prophylactic treatments with freshwater with or without oxytetracycline (OTC) at least once in a month.

Broodstock Nutrition

The viability of the larvae is very much dependent on broodstock nutrition. The nutritional components in the diet can affect spawning, egg and larval quality. Yolk is the sole source of food for the developing embryo and the early larvae until feeding on live preys starts. Therefore, the yolk quality and quantity are key factors for a successful seed production. The process through which maturing oocytes in the ovary accumulate yolk is called as vitellogenesis. 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.

The broodstock fishes are to be fed with a 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.Both dry pellets and moist food can be employed during maturation. Dry pellets should include essential nutritional components like polyunsaturated fatty acids (n-3 PUFA), in particular EPA (20:5 ω 3) and DHA (20:6 ω 3), which cannot be produced by fish metabolism. Broodstock fishes are fed *ad libitum* once a day with squids, crabs, shrimps and chopped oil-sardines depending on the availability.The amount of the food given to the fish is about 3-5% of biomass day-1.

Induction of spawning

Spawning,the release of eggs and sperm can be obtained either naturally by placing the fish in an appropriate environment or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. Hormones injected cannot produce the gametes; they can only trigger the release of fully developed gametes. In females the hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. Only females with oocytes in the late-vitellogenic stage, with a diameter around 750 microns in cobia and 500 microns in pompano, are selected. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the hormone treatment does not work; hence ovarian biopsy is essential for assessing the ovarian development. Flexible sterile catheters (1.2 mm internal diameter) can be used for gonadal biopsy.

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. Being a large molecule, hCG 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.

Normally spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place usually between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, pompano spawn 0.5 to 1.5 lakh eggs. The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners.

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 which float at the water surface. Aeration can be switched off to allow 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, have to be observed for the egg developmental stages.

As fertilized 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 unfertilized 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.

Incubation of eggs

It is done in incubation tanks of 3-5 tonne capacity.As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank per female.

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. 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 microscope. The hatching of eggs takes place from 18 to 24 hours.

After hatching, only the hatched fish larvae/yolk sac fry 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.

Larviculture

Many of the marine fin fishes which are suitable for aquaculture are having altricial type of larvae. These larvae are having very less yolk reserves at hatching. Therefore, the larvae are are very small with a small mouth gape and are still in an under developed stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive and the perceptive powers for searching and taking external feed is also very less in this type of larvae.

The time when the yolk reserves are fully exhausted and the larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage. It is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

In addition, since most of the larvae are visual feeders providing the required light also affects the larval survival. During the critical period, the density of the live feed and its nutritional qualities determines the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence size grading from an early stage is also very much needed for increasing the survival. In addition, a variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., also affects the larval survival and growth. Larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very less.

The larvae hatched in the incubation tanks need to be distributed in larviculture tanks to have a stocking density of 5 to 10 larvae/ litre for cobia and 10-20 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage.

Larviculture of cobia

Newly hatched larvae of cobia normally measures 3.4 mm size. Soon after hatching, the mouth is closed and the digestive tract is not fully developed. During this period the larvae survive on its reserves in the yolk sac.

Larval mouth opens at 3-5 days post hatch (dph). Metamorphosis starts from 18-21 dph. Newly hatched cobia larvae generally starts feeding at 3 dph and they can be fed with the rotifer (*Brachionusrotundiformis*) at the rate of 10-12 nos / ml, two times a day till 10 dph.Before being fed to fry, rotifers require enrichment to enhance their nutritional value. Six-hour enrichment with an INVE product called Protein Selco Plus is usually carried out.

As fry get larger, they quickly outgrow the prey size represented by rotifers (200 to 400 microns), and a larger live feed is required. From 8 dph, the larvae can be fed with enriched *Artemia*nauplii (500 to 900 microns) at the rate of 2-3 nos / ml, 2 times a day. During the rotifer and *Artemia* feeding stage, green water technique can be used in the larviculture system with the microalgae *Nannocloropsisocculata*at the cell density of $1x10^{7}$ cells / ml. The addition of this algae to the tank water gives the water a green hue, and is thus commonly referred to as the “greenwater” technique.

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. 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.

Between 25-40 dph, the larvae are highly cannibalistic and hence size-grading has to be undertaken at every three days interval. During this stage, the fry could be weaned totally to formulated diets. Different sizes of formulated feeds need to be used as per the mouth size of the larvae. The fry are considered weaned once they are feeding solely on dry diets. At this point, they can be considered as fingerlings.

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 a 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. But, tank bottom siphoning should be carried out from day 1. The environmental conditions required during the larviculture period are DO_2: > 5mg / l, NH_3: < 0.1mg / l, pH: 7.8 – 8.4, Salinity: 25-35 ppt, water temperature: 27-33° C.

The juveniles measuring 10 cm length are 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 were stocked at a density of 15000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 $x10^7$/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 µ.

The larvae were fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks. Co-feeding of rotifers with enriched *Artemia*nauplii has to be done during 12-14 dph, and thereafter upto 19 dph with enriched *Artemia*nauplii alone by maintaining a density of 3-5 nos. per ml in the larviculture tanks. Weaning to larval inert feeds has to be started from 15 dph and co-feeding with *Artemia* needs to be continued until 19 dph. From 20 dph 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. Though cannibalism is less, grading has to be done during 20-25 dph to separate the shooters. 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.

Conclusion

Research in the areas of broodstock nutrition, hormonal manipulations, live feed and larviculture technologies are required for the development of reliable techniques for mass production of fingerlings of many marine finfish species. This is necessary for establishing a sustainable mariculture industry in the region.

Fish Reproduction, Reproductive dysfunctions in captivity and Hormonal Induction of Spawning

G.Tamilmani, A.K.Abdul Nazar, R.Jayakumar, M.Sakthivel, P.Rameshkumar, K.K Anikuttan and M.Sankar

ICAR-CMFRI, Mandapam Regional Centre

Introduction

Since many centuries mankind grows almost all its food on cultivated farm land, as far as this food consists of terrestrial plants and animals. But, when it comes to sea water plants and animals, modern man still continues to collect them from natural sources like his forefathers.Marine animalshave always been and still are an important source of protein and minerals in human food. With the rapid increase in human population the demand for sea food will certainly increase. However, production from capture fisheries has stagnatedand fisheries cannot expand much further. Further increase in exploitation of the seas will lead to destruction of ecosystems and species extinction. When we consider that the amount of wild-captured fish has not increased in recent years, only one alternative remains: fish farming, or aquaculture.

Mariculture- the farming and husbandry of marine plants and animals can augment the marine fish production and can supplement the capture fisheries. One of the major requirements for the establishment of a sustainable mariculture industry is the availability of quality seeds. Seed collection from the wild is unpredictable and cannot be relied upon. The ability to produce viable offspring from captive brood stock can ensure a steady supply of seeds. However, most of the fishes exhibit reproductive dysfunctions when reared in captivity. The dysfunctions may be due to a combination of captivity induced stress and lack of appropriate natural spawning environments. Hormone-induced spawning is the only reliable method to induce reproduction in these fishes.

This chapter aims at giving a general view of fish reproduction, the reproductive dysfunctions found in captive-reared fishes and the hormonal interventions for the control of fish reproduction.

Reproduction of fish in nature

Like many other animals, fish also have evolved to reproduce during environmental conditions that are favourable to the survival of the off spring. Long before spawning (the release of eggs and sperm), seasonal cues begin the process of gametogenesis (gamete growth and differentiation) which leads to the formation of the female oocyte (oogenesis) or the male spermatozoon (spermatogenesis). In many fish, this can take up to a year.

The reproductive cycle of fish is separated into two major phases. The first phase (spermatogenesis and vitellogenesis) involves the proliferation, growth and differentiation of the gametes. The second phase (spermiation and oocyte maturation) involves the maturation and preparation of the oocytes and spermatozoa for release and insemination.

Maturation of the egg is a long process that involves complex physiological and biochemical changes. One important step, vitellogenesis, is a process in which yolk proteins are produced in the liver, transported to the ovary, and stored in the egg, resulting in tremendous egg enlargement. The yolk is important as a source of nutrition for the developing embryo. Also

critical is germinal vesicle migration and germinal vesicle breakdown (GVBD). Before it migrates, the germinal vesicle, or nucleus, is located at the center of the egg in an arrested stage of development. At this stage, the egg is physiologically and genetically incapable of being fertilized, even though it has the outward appearance of a fully mature egg. When conditions are appropriate for final maturation, nuclear development resumes, and the germinal vesicle migrates to one side. Finally, the walls of the germinal vesicle break down, releasing the chromosomes into the cell. In mature eggs, the migration of the germinal vesicle to the side of the cell will be complete. After the egg has matured; a class of compounds called prostaglandins are synthesized. These stimulate ovulation, which is the rupture of the follicle cells that hold the egg. The egg is then released into the body cavity or ovarian lumen, from where it may subsequently be released to the outside environment.

When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Examples of environmental stimuli are changes in photoperiod, temperature, rainfall, and food availability. A variety of sensory receptors including the eye, olfactory organs, taste buds, and thermo receptors detect these cues.

Control of reproduction

Marine fishes produce and release sex cells based on maturity of the individuals, their nutrition and overall health, triggered by cues from the environment 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.

Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms. The external environmental factors that control reproduction (temperature, light/dark duration, tides, presence of conspecifics, mates, etc.), vary among species. However, the internal mechanisms (hormones) that regulate spawning are similar for most fishes. Therefore, more is known about the internal regulatory mechanism of fish reproduction than the specific environmental requirements for spawning each species.Knowledge of the normal sequence of endocrine changes taking place during oocyte maturation, ovulation, and egg release is necessary for understanding the endocrine framework that is manipulated by hormonal induction of spawning.

A cascade of hormones along the hypothalamus –pituitary–gonad (HPG) axis regulates the gametogenesis and final maturation.The hypothalamus, located at the base of the brain, is sensitive to signals from sensory receptors and releases hormones in response to environmental cues. Principal among these hormones is Gonadotrophin releasing hormone (GnRH), which travels from the hypothalamus to the pituitary gland. Certain cells of the pituitary receive GnRH and release gonadotrophic hormones, Follicle Stimuating Hormone (FSH) and Luteinizing Hormone (LH) into the bloodstream. The gonadotrophic hormones travel to the gonads, which synthesize steroids responsible for the final maturation of the gametes.Successful release of mature gametes could be achieved by providing proper environmental stimuli and /or administration of hormones acting at the level of the hypothalamus, pituitary, or gonads.

Reproductive dysfunctions in captive-reared fishes

Reproductive problems are usually more serious in female broodstocks, and can be classified into three types. The first and most severe reproductive dysfunction of captive fishes is the

complete absence of reproductive development observed in freshwater eel.The second type of reproductive dysfunction in cultured females is the absence of spawning at the end of the reproductive cycle. In species exhibiting this problem, the oocytes undergo normal vitellogenesis, FOM and ovulation in response to the appropriate physiological and environmental stimuli, but the ovulated eggs are not released into the water.This absence of only gamete release (i.e., spawning) is observed in cultured salmonids. In most other fishes, the reproductive dysfunction often observed in culture is that fish undergo vitellogenesis during the reproductive period, but fail to undergo FOM and, as a result, there is no ovulation and no spawning of eggs. The endocrine cause of the failure of female fish to undergo FOM has been identified to be a dysfunctional release of LH from the pituitary at the end of vitellogenesis. Comparison of plasma levels of reproductive hormones between cultured fish that fail to undergo FOM and wild fish captured on their spawning grounds showed that a plasma LH surge accompanied FOM and ovulation in wild females, but in females reared in captivity plasma LH levels remained low at the end of vitellogenesis.

The reproductive dysfunctions observed in male cultured broodstocks are limited to captivity-induced reductions in milt production or milt quality during the spermiation period. Reproductive problems often diminish over the years after domestication.

Assessing the ovarian development

Spawning, the release of eggs and sperm can be obtained either naturally by placing the fish in an appropriate environment or by inducing with hormones. Induced breeding is commonly practiced in most commercial hatcheries. Hormones injected cannot produce the gametes; they can only trigger the release of fully developed gametes. 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. Care must be exercised in assessing 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. Hence, ovarian biopsy is essential for assessing the ovarian development. Flexible sterile catheters (1.2 mm internal diameter) can be used for gonadal biopsy.

In females the hormonal treatment is intended to trigger the last phases in egg maturation, i.e. a strong egg hydration followed by their release. Only females with oocytes in the late-vitellogenic stage, with a diameter around 750 microns in cobia and 500 microns in pompano, are selected. However, if eggs have not reached the late-vitellogenic (or post-vitellogenic) stage, the hormone treatment does not work;

Hormonal therapies

Based on the evidence that the failure of cultured fishes to undergo full spermiation and FOM is the result of diminished LH release from the pituitary the hormonal therapies developed and applied for fish aquaculture can be grouped into two major types, “first generation” and “second generation” techniques. The first generation is the pituitary hormone based preparations, and includes the pituitary extracts and purified GTHs. The second generations are the brain hormone based treatments and includes the GnRH agonists (GnRHa) and dopamine (DA) antagonists. These two types of hormonal therapies act at different levels of the reproductive HPG axis. Exogenous LH preparations act directly at the level of the gonad.

GnRHa releases the endogenous LH stores from pituitary. Endogenous LH, in turn, acts at the level of the gonad to induce steroidogenesis and the process of FOM and spermiation.

Luteinizing hormone preparations include

(a) homogenates and purified extracts from the pituitary of mature fish during the reproductive season—most commonly of carp and salmonids—that contain high amounts of LH. Pituitary homogenates were the first type of exogenous hormonal treatments used by aquaculturists for the induction of maturation and spawning. Professor B.A.Houssay, an Argentinean, was the first to report (1930) on the ability of exogenous hormones to induce FOM and ovulation in fish. He injected female fish with ground pituitaries from another species and observed that the females underwent ovulation. However, use of ground pituitaries, is associated with various drawbacks, like the great variability in pituitary LH content, the administration of additional hormones present in the pituitary that may adversely affect the physiology of the treated fish, and the potential for transmission of diseases from donor fish to recipient broodstocks. Realizing the above drawbacks, efforts were later directed towards the acquisition of purified or partially purified preparations of LH.

(b) **GtH preparations of mammalian or human origin**
A wide range of GtH preparations of mammalian or human origin including mammalian FSH and LH, ovine LH, Pregnant Mare Serum Gonadotropin (PMSG) and human Chorionic Gonadotropin (hCG) have been tested in some fishes. However, only hCG – which is purified from the urine of pregnant women – has been used routinely in aquaculture. hCG has a very strong LH activity.

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. Being a large molecule, hCG 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. 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.

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

Normally spawning could be noted within 36 -48 hours after hormonal induction. The spawning in cobia and pompano takes place usually between late night and early morning hours. The number of eggs spawned by cobia ranges from 0.4 to 2.5 million, whereas, pompano spawn 0.5 to 1.5 lakh eggs. The spawning unit should preferably be kept separated from the main hatchery building to avoid disturbance to the spawners.

Larviculture of Cobia and Silver Pompano

Abdul Nazar, A.K*., Jayakumar, R., Tamilmani,G., Sakthivel, M., Rameshkumar, P., Anikuttan, K.K., Sankar,M., Hanumanta Rao, G and Krishnaveni, N.
Mandapam Regional Centre of CMFRI, Madapam Camp – 623520, Tamil Nadu, India
**Madras Research Centre, CMFRI, Chennai, Tamil Nadu, India*
aknazar77@gmail.com

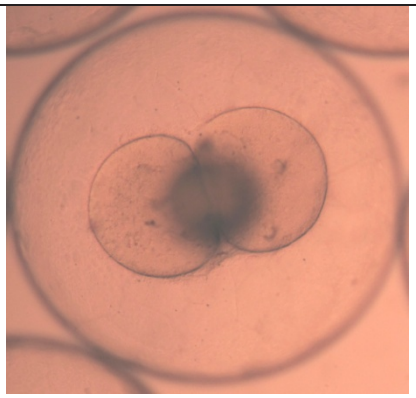
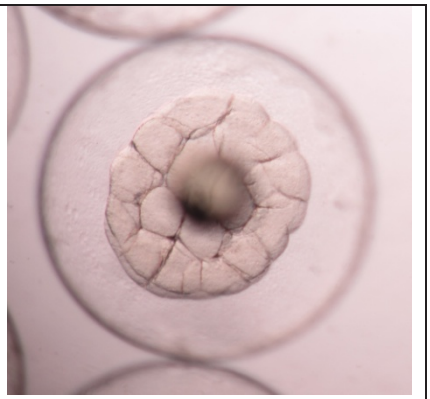
In India, technology for production of marine finfish seeds is in primitive stage except for sea bass. The Mandapam Regional Centre of the Central Marine Fisheries Research Institute (CMFRI) has developed hatchery technology for cobia, *Rachycentron canadum* during March 2010 for the first time in the country. Again the Centre has developed hatchery seed production technology for the silver pompano, *Trachinotus blochii* for the first time in the country. Both the technologies are standardised and hence the CMFRI has entered into agreements with interested entrepreneurs and farmers for dissemination of technologies for development of cobia and silver pompano aquaculture sector in our country.

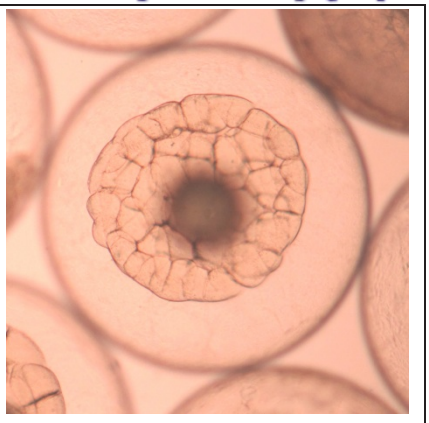
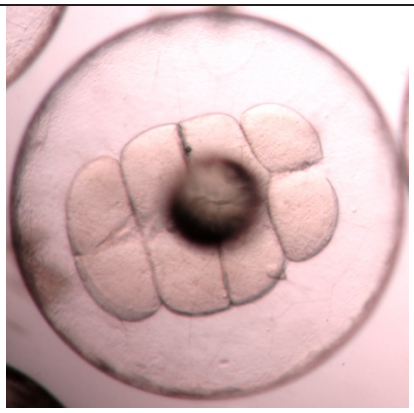
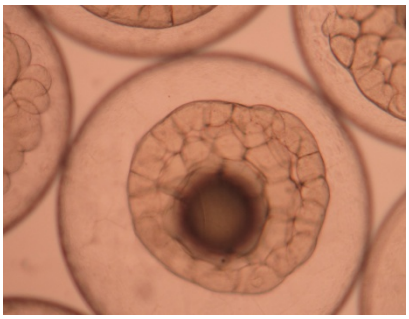
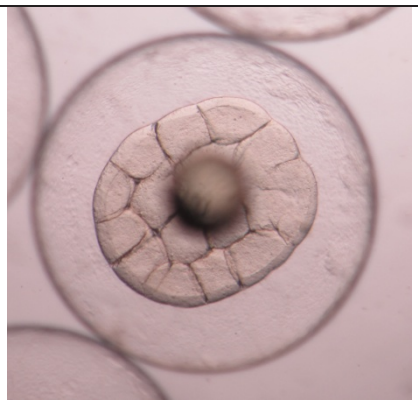
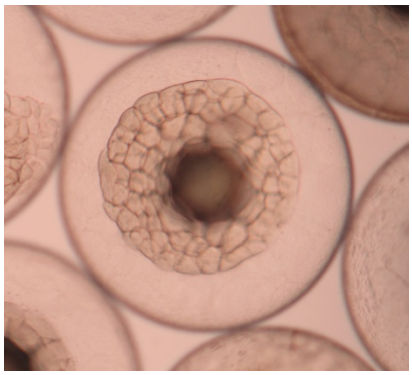
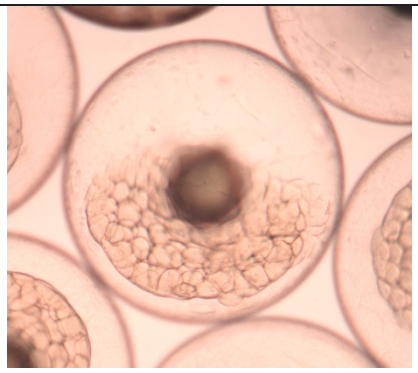
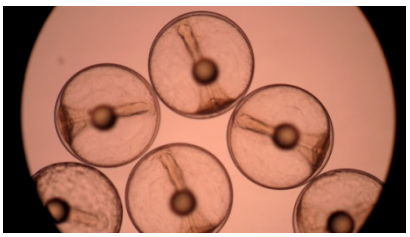
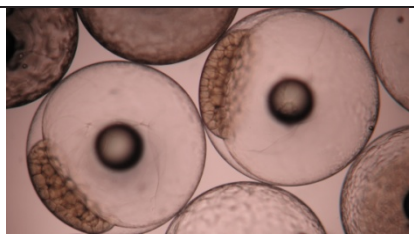
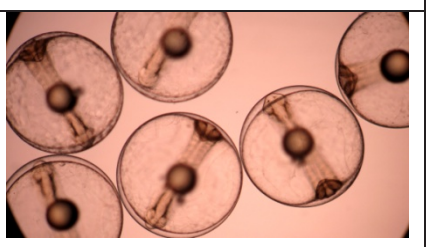
Cobia *Rachycentron canadum* is a marine finfish species with emerging global potential for mariculture and its positive culture attributes include capacity for natural and induced tank spawning, growth rates in excess of 7-9 kg/year. Further cobia has high disease resistance, survival rates (post larviculture stage) in tanks, pens and cages with adaptability to commercially available extruded diets. It also has high-quality meat for making fillets suitable for sashimi as well as white tablecloth restaurant markets. Similarly, among the many high value marine tropical finfish that could be farmed, the Silver pompano, *Trachinotus blochii* is one of the topmost, mainly owing to its fast growth rate, good meat quality and high market demand.

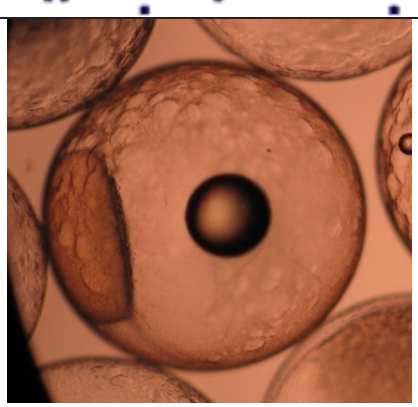
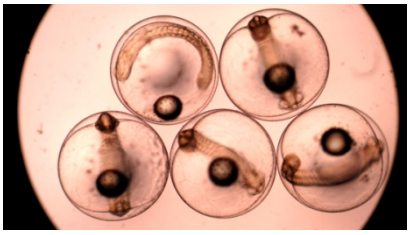
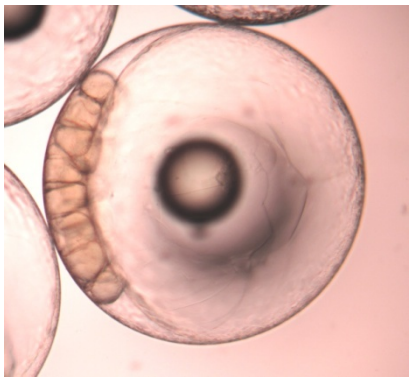
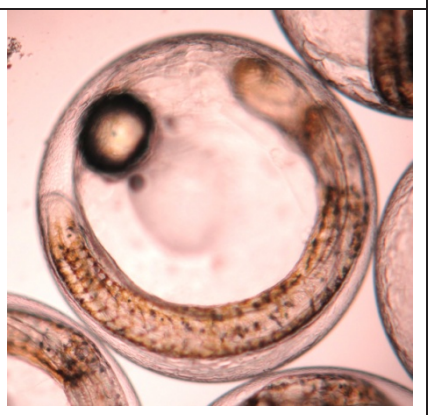
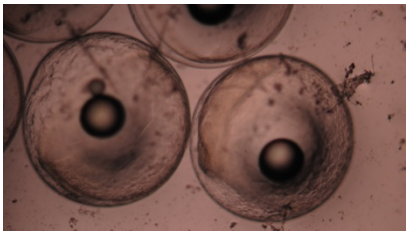
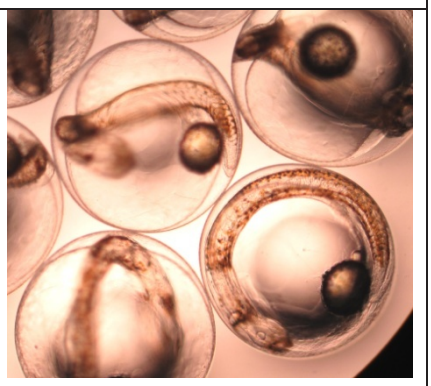
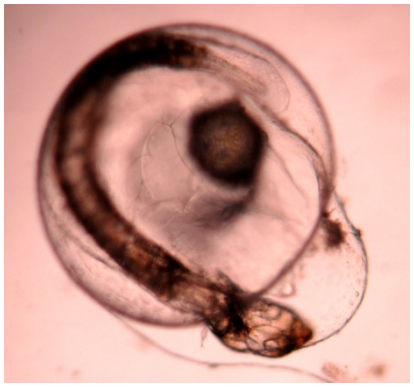
The larval rearing procedures of cobia and pompano are described below:-

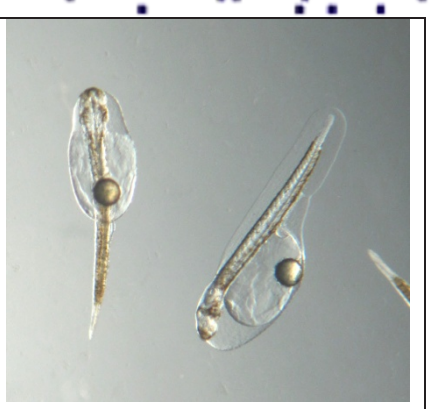
As the fecundity is normally high in cobia, we may require more incubation tanks, whereas the pompano requires only one tank /female.

The embryonic developmental stages of cobia and pompano normally look alike except for the duration of development and size of the larvae. The photos of embryonic development and newly hatched larvae are provided below;

Two-cell stage		32-cell stage	

Four-cell stage		64-cell stage	
Eight-cell stage		Early Morula	
16-cell stage		Late Morula	
Early Blastula		Early Gastrula	
High		Mid Gastrula	

Dome		Late Gastrula	
Oblong		Bud	
Epiboly		Segmentation	
High-pec		Hatching in progress	

Newly hatched larvae		Larvae-12 hour post hatch	
2dph			

Larviculture

The marine fish larvae are generally classified into altricial and precocial type. The altricial type of larvae is having very less yolk reserves at hatching and hence, the larvae are in an undeveloped stage when the yolk sac is completely resorbed. The development of digestive system is also very primitive in these types of larvae. Many of the marine fin fishes which are suitable for aquaculture are having the altricial type of larvae which poses challenges in their larviculture. When the yolk reserves are fully exhausted, the larval size and mouth gape are very small and the perceptive powers for searching and taking external feed is also very less. The period when the yolk reserves are fully exhausted and larvae need to resort to exogenous feeding is a critical period in the larviculture of most marine fin fishes. Unless proper live feeds of required size is provided in sufficient densities in larviculture media and its nutritional requirements especially in terms of PUFA are met, large scale mortality is bound to happen at this stage and hence it is evident that the larviculture of marine finfish having altricial larvae is really challenging and proper management of live feed is the most vital pre-requisite for the success in terms of survival and growth of the larvae.

In addition, since most of the larvae are visual feeders providing the required light also affect the larval survival. During the critical period, the density of the live feed and its nutritional qualities determines the percentage of the survival of the larvae. The density of the larvae of the concerned species should also be regulated in the larviculture tanks for getting good survival. The marine fish larvae exhibit highly differential growth even from very early stages (in the case of cobia, starting from the first week) and hence grading from an early stage is also very much needed for increasing the survival. In addition, variety of other factors such as tank colour, size of the tank, water temperature, water quality, etc., affect the larval survival and growth. From the foregoing, it is clear that the larviculture of marine finfish is highly complicated, unless each and every factor is taken care of, the survival and growth of the larvae will be very meagre.

The larvae hatched in the incubation tanks or larval rearing tanks need to be distributed in larviculture tanks to have minimal stocking density of 5 to 10 larvae/ litre for cobia and 10-20 larvae per litre for pompano. Care should be taken to avoid any mechanical stress or damage. Soon after hatching, the mouth is 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 18-21 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, two times a day till 10 dph. From 8 dph, the larvae can be fed with enriched *Artemia* nauplii at the rate of 2-3 nos / ml, 2 times a 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 $1x10^7$ 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 three 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. But, tank bottom siphoning should be carried out from day 1. The environmental conditions required during the larviculture period are DO_2: > 5mg / l , NH_3: < 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.

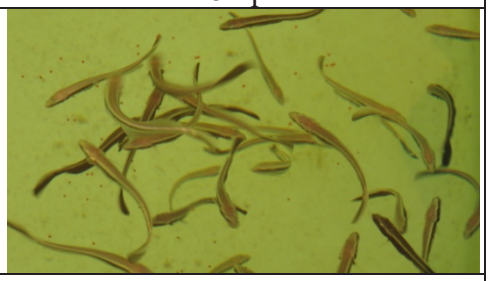
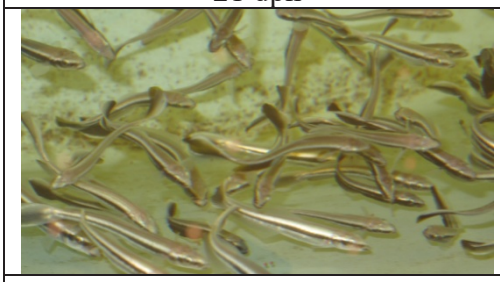
Stage of Larvae (dph)	**Size of Larvae (cm)**	**Size of Feed (µ)**
18 – 19	2.3 – 2.6	100-200
20 – 23	2.5 – 3.5	300-500
23 – 30	3.5 – 8.0	500-800
31 onwards	> 8.0	800-1200

The juveniles measuring 10 cm length are 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.

Few photos of larvae and fingerlings are provided below (*dph = day post hatch*)

	
2dph	4 dph
	
6 dph	10 dph
	
13 dph	15 dph
	
21 dph	40 dph
	
45 dph	62 ph

Larviculture of Silver Pompano

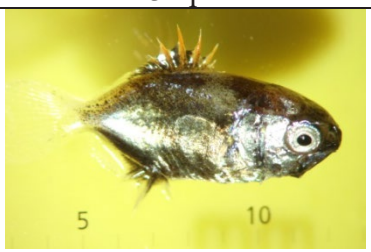
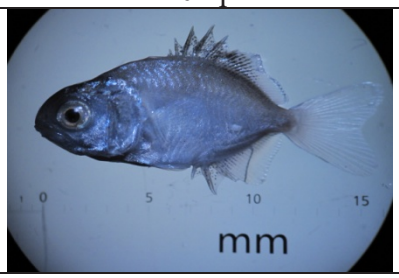
The newly hatched larvae were stocked at a density of 15000 larvae in FRP tanks of 2 m^3 capacity filled with 1.5 m^3 filtered seawater. The tanks were provided with mild aeration and green water at a cell density of 1 $x10^7$/ml. The mouth of the larvae opens on 3dph and the mouth size was around 230 µ. The larvae were fed from 3dph to 14 dph with enriched rotifers at a density of 6-8 nos. per ml in the larviculture tanks. Wherever possible, wild collected copepods could also be added as supplements. Co-feeding of rotifers with enriched *Artemia* nauplii has to be done during 12-14 dph, and thereafter upto 19 dph with enriched *Artemia* nauplii alone by maintaining a density of 3-5 nos. per ml in the larviculture tanks. Weaning to larval inert feeds has to be started from 15 dph and co-feeding with *Artemia* needs to be continued until 19 dph . From 20 dph 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. Though cannibalism is not witnessed, grading has to be done during 20-25 dph to separate the shooters. 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 Silver 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.

The pompano fingerlings can be reared at salinities as low as 5 ppt. At lower salinities i.e. from 10 to 15 ppt, they grow faster than in pure seawater.

Some photos of larval stages of pompano are given below

<table>
<tr><td> 
</td><td>  </td></tr>
<tr><td>3 dph</td><td>10 dph</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>15 dph</td><td>25 dph</td></tr>
</table>