Hormonal Inducement of Marine Finfishes with Special Reference to Silver Pompano (*Trachinotus blochii*)

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Introduction

Induced breeding is a technique by which the economically valued ripe fish brooders are stimulated by pituitary extract or other synthetic hormones to breed in captive conditions. Most fishes won't breed in captivity due to insufficient release of pituitary hormones aroused by the disturbance in environmental conditions in controlled systems. The technique was first evolved in Argentina by Houssay, (1930). In 1934, Brazilian researchers could succeed in inducing ovulation by pituitary gland injection. Since then, the technique has been extensively used by various workers. In India, the first attempt on induced breeding was conducted by Khan (1938) on *Cirrhinas mrigala*. Later Choudhuri, (1955) tried it on minor carps (*Esomus danricus*, *Pseudotropius atherinoide*). Ramaswamy and Sundararaj (1956) had done the experiments on catfishes (*Clarias batrachus* and *Heteropneustes fossilis*). Induced breeding was successfully carried out in Indian carps such as *Labeo rohita*, *Cirrhinus mrigala*, *Cirrhinus reba*, *Labeo bata* by Chaudhuri and Alikunhii, (1957) by hormone injection. Since then, the application of this technique has spread widely and now, with modifications, forms a regular part of fish culture programs all over the country.

Breeding and seed production of marine fishes depends extensively on quality broodstocks, induced maturation, spawning, larval rearing, live feeds culture, and the availability of good quality water. A combination of water temperature control and photoperiod regulation in a recirculation system could further speed up the maturity. It may prove helpful in a commercial broodstock development system (Gopakumar, *et al*., 2012). Following the successful seed production of cobia, silver pompano, Indian pompano, vermiculated spine foot, pink ear emperor, orange spotted grouper and the farming demonstration of those species in marine cages and brackishwater ponds, poularized the technology among the farmers about its suitability for aquaculture.

This chapter mainly details the process of Tagging brood fishes, Cannulation techniques for sex identification, gonadal maturity assessment, and finally, the hormonal inducement of marine fishes with reference to Silver Pompano in RAS.

Silver Pompano

Pompanos are marine fishes in the genus *Trachinotus* belongs to the family Carangidae. The genus *Trachinotus*, occurs in all tropical oceans and comprises 19 species (Fricke *et al*., 2019). *Trachinotus blochii* is a deep-bodied carangid usually found around shallow coastal waters over rocky and coral reefs. Occasionally they are in small schools.

Diet consists mainly of bivalve molluscs and other hard-shelled invertebrates. Silver pompano, is one of the suitable species for brackish water and marine water aquaculture due to its fast growth, adaptability to different salinity regimes, good quality meat, and high market demand. Silver pompano has high Omega 3 fatty acids such as EPA and DHA in their meat (Jayakumar *et al*., 2019). The availability of silver pompano in commercial fishery is relatively scarce and its high market demand raises the culture potential all over the Indian coast. The aquaculture of pompano has been established successfully in many countries. Silver pompano aquaculture was done in Indo-Pacific countries, especially in China,Vietnam, Malaysia, India, and the Philippines. Total global aquaculture production of all species of pompano is more than 110,000 tonnes and appears to be growing. Silver pompano produced in Indonesia is being sold to restaurants and higher-end grocery stores in the USA. In addition to cage farming, the excellent growth rate in low saline ponds indicates potential commercial expansion. The farming of silver pompano has been carried out in ponds, tanks, and floating sea cages (R. Jayakumar, *et al*., 2014).

In India, the Central Marine Fisheries Research Institute (CMFRI) has initiated aquaculture research on pompano at its Mandapam Regional Centre in 2007, and the first successful broodstock development, induced breeding, and seed production was achieved in July 2011 (Gopakumar, *et al*., 2012). The national broodbank developed at Vizhinjam Regional Centre of CMFRI has initiated the collection of Silver pompano broodstocks and maintained in the RAS for breeding and seed production.

Techniques in induced breeding of marine finfish

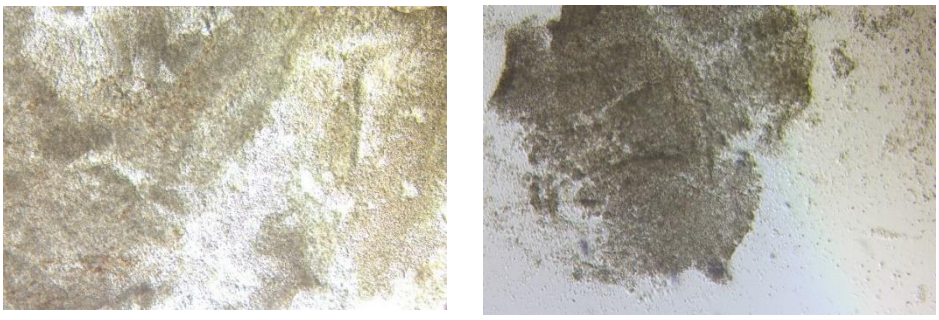
Tagging

Tagging of fish is one of the essential techniques for breeding and seed production. This allows the breeders to gather a wide variety of information about fishes during their rearing at tanks. A better understanding of the gonadal development of fishes, selection of fishes for brood pair formation, identification of fish during cannulation, and other species selected studies will be only possible through a safe and permanent tagging method. Tagging and marking are widely used in studying the migratory pattern in fishes; stock assessment provides population estimates, fish growth, and fishing and natural mortality (Sanford *et al.*, 2019). There are different types of tagging in fishes; biological, chemical, and physical. Biological tagging or natural tagging is by means of parasitic marks, morphometric marks, and genetic marks. Chemical marking can be carried out by using chemicals and dyes. This can be done by immersion, injection, or feeding of different chemicals (Oxytetracycline, Alizarin, Calcein). Physical tagging mainly includes internal and external tagging methods. Here we are using one of the internal tags, Passive integrated Transponder Tag (PIT tag), for marking the brooders.

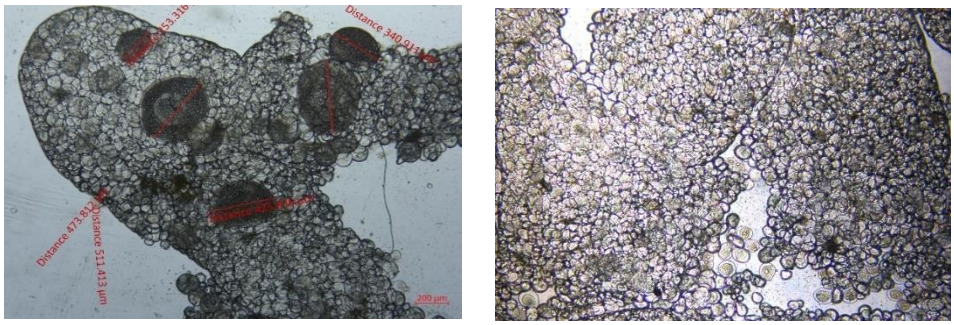
The sub-adults of Silver pompano were collected from different locations of Kerala and Tamilnadu for rearing. After proper quarantine for maintenance and further gonadal development, the wild-collected silver pompano broodstocks were transferred into the RAS.

The fishes were cannulated to identify the sex once it was acclimatized to the system. The microscopic view of male and female gonads of Silver pompano is depicted in figure 1. After sex identification, the fish was tagged and kept in the system as per the required sex ratio. Passive Integrated Transponder (PIT) tag, also known as radio-frequency device, is used to mark fishes internally permanently. The PIT tag contains a microprocessor chip and antenna. It has no internal battery, hence the term "passive," so the microchip remains inactive until reading with a reader. The reader sends a low-frequency signal to the tag's microchip 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. Tags can be read through materials such as soil, wood, and water. The durability of the PIT tag is 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. The tag is designed to last throughout the life of the fishes providing a reliable, long-term identification method. The procedure of tagging (Gopakumar, *et al* 2013) and reading is explained below and depicted in Figure 2.

a) Microscopic view of milt



b) Microscopic view of oocytes (Immature)

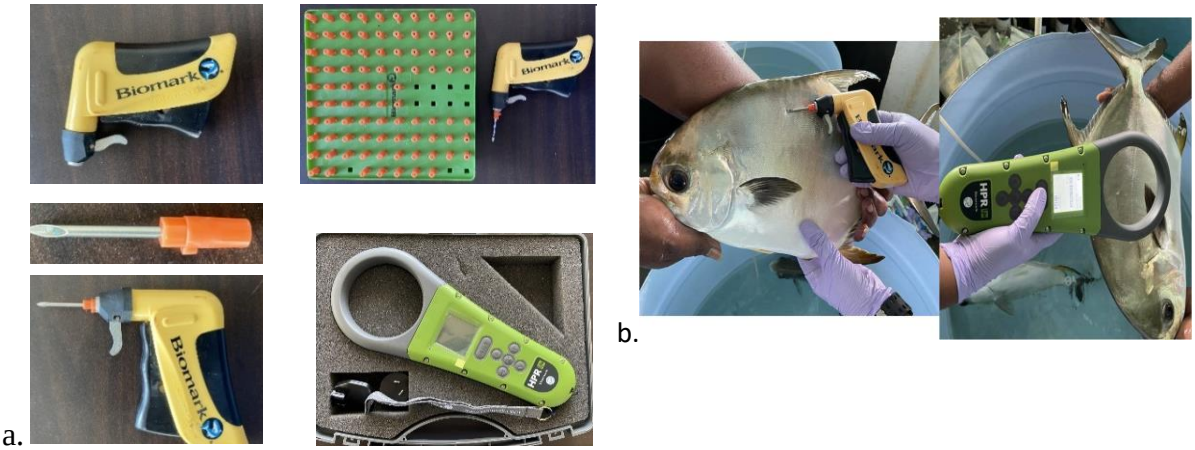


Microscopic view of male (a) and female gonads (b)

The preferred implantation site will be the dorsal musculature of the fish, and it depends mainly on the species, size of fish, and the tag size. Disinfect all the components of tagging with alcohol before use. Always use a sterile needle or implanter to tag the fish. Catch the fish and anesthetize it with a suitable anesthetic (2-phenoxy ethanol, 10 ml for 100 L seawater). Read the tag before inserting it into the fish and record the identification code or

number. Hold the fish on the palm and disinfect the site of implantation with alcohol or iodine-based solution before tagging. The tag loaded inside the implanter needle has to be inserted into the muscle tissues and inserted parallel to the muscle fibers. Once the needle has sufficiently inserted into the body, place the tag in the proper position by triggering the gun. After the tag placement, the inserted needle should be released slowly and steadily from the implanter site so that it doesn't hamper the fish. Always maintain a standard implantation site so that it will be convenient to read. Once the implanter needle is taken out, the area should be disinfected 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 anesthesia.

The advantages of PIT tags are; highly reliable permanent identification marker, create no stress to the behavior of fishes, small size, no error in recording data, and easy data retrieval. The high initial cost and low detection distance form the only negative part of PIT tags.



a. b.

a. Components of PIT tagging and reading b. Tagging and reading

Once the brood pairs were identified, it is better to keep them in a separate RAS for effective synchronization for breeding. The male fishes were kept undisturbed once they were determined, tagged, and placed.

Ovarian biopsy to assess gonadal maturity (Cannulation)

Monitoring of oocyte development in fishes was carried out at frequent intervals to determine its maturity. Oocytes collected via cannulation were observed under microscopes to analyze the diameter of the eggs. The procedure of Ovarian biopsy is given below and represented in figure 3.

1. Female brooders have to be transferred to a small tank containing anesthesia, the anesthetic agent used is 2-phenoxy ethanol (standardized to 10ml for 100 L seawater).
2. Flexible, sterile catheters, an infant feeding tube with a total length of 520 mm and size 2.00 mm, (FG -06) (Fig. 3) are introduced through the genital pore into the oviduct for a few cm up to the ovary. A mild pressure will be applied to suck out the

oocytes till a small sample of oocytes up into the catheter and placed sample on a glass slide.

3. After sampling, release the animal into the tank, where recovery from sedation will take place. The fish will be recovered from the sedation within 2-3 minutes. Take care to hold the fish in the tank till it recovered from sedation to avoid bleeding in fish due to slap at tank due to anesthesia
4. The biopsy sample (Fig. 3) is then examined under the microscope, measure the diameter of the oocytes, and analyze the different development stages of gonad development.

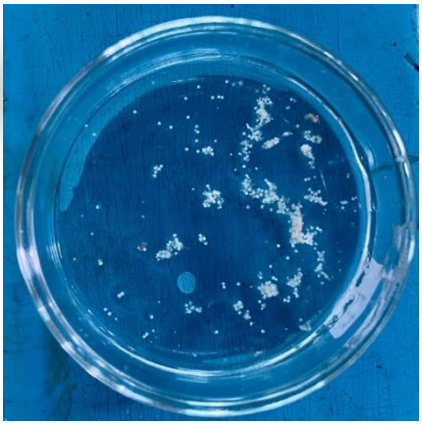
Different stages of maturity of Silver pompano were observed during the ovarian biopsy, and it is depicted in figure 4. The different maturity stages are;

a) Early developing stage- Immature ovaries are relatively small with size of 80-120 µ in diameter. Ovarian tissue was dominated by pre-vitellogenic oocytes and strands of stromal tissue. Ovary with different stages of previtellogenic oocytes visible in early developing stage ie; pre perinuclear oocyte, early perinuclear oocyte, and late perinuclear oocyte.

b) Maturing stage-Vitellogenesis begins with the appearance of oil droplets, yolk vesicles, and cortical alveoli vesicles surrounding the nucleus stage. Oocytes increase in size with a diameter of 350- 450 µ.

a

b



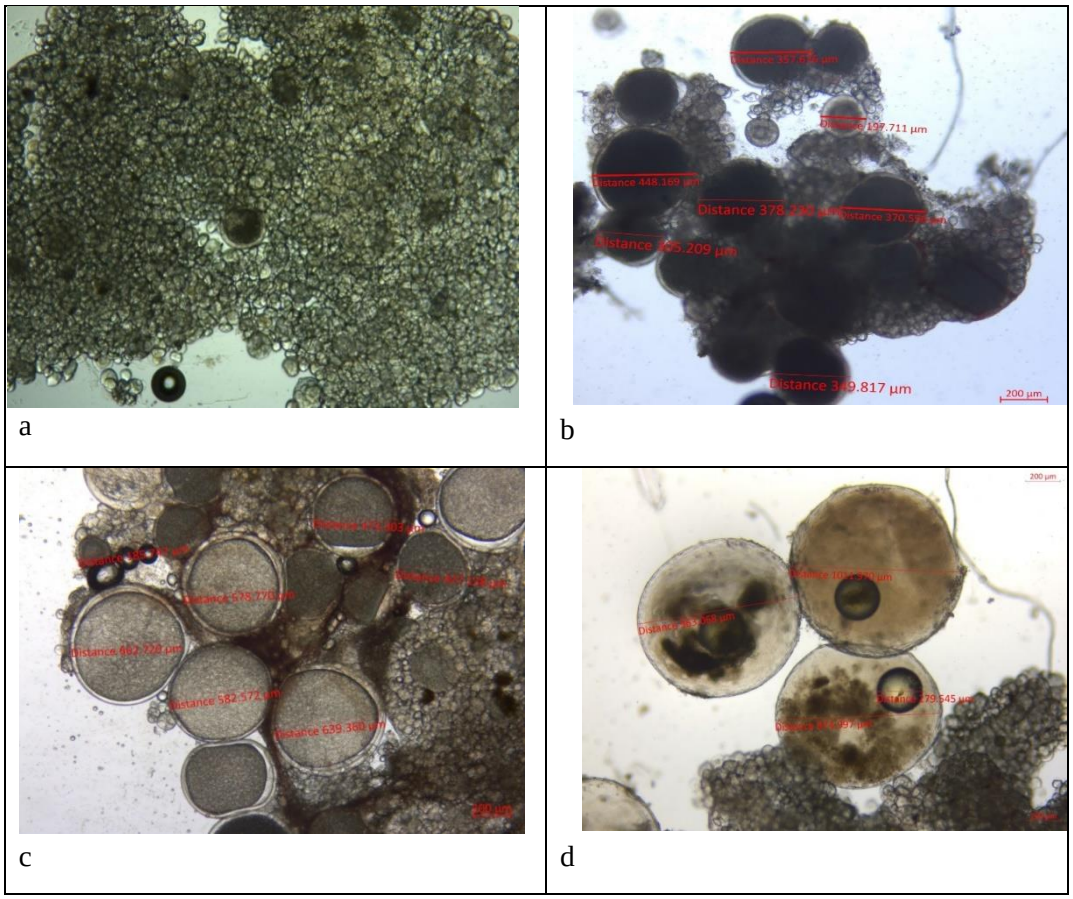
c

a) Procedure of cannulation b)Flexible, sterile catheter used for ovarian biopsy c) Biopsy sample (cannulated eggs)

c) Mature egg stage-Gonad wall becomes thin due to the expansion of the ovary as it ripened and oocytes matured. Induce breeding is carried out at this stage with an egg diameter of >500 µ.

d) Spent/artesia stage- Characterized by the presence of previtellogenic atretic oocytes without yolk granules and vitellogenic atretic oocytes with yolk granules.

Microscopes connected to a system with ZEN software, a modular image-processing and analysis software for image acquisition, processing, analysis and documentation for ova diameter studies.



Developmental stages of oocytes a; immature/ honeycomb stage, b; Maturing stage, c; Mature, d; Spent/Artesia

Inducement of fishes using hormones

Most of the broodstocks collected from the wild maintained in the RAS do not breed in captivity. But volitional spawning of *Lethrinus lentjan* in RAS were reported from the Vizhinjam centre by Anil *et al* 2019. In captive conditions, there is an extreme lack of certain environmental parameters like photoperiods, rain, temperature, water current to activate the pituitary and gonads to release hormones. However may be the conditions provided under captivity need not satisfy the fishes as in the wild and may cause insufficient release of gonadotropins in captivity. In such cases fishes were induced with hormones to activate

maturity and gonadal development. Hormonal inducement provides pure spawn of certain fishes under cultivation, assures timely supply of seeds and fulfiles any quantity of seed demand in any time.

Silver pompano fishes are induced to spawn; when the oocyte has attained the ova diameter of the range 520- 580 µm (usually >520 µm). A sex ratio of 1:3 (female: male) has been maintained in the spawning tank for induced breeding, but 1:1 and 1:2 also had registered spawning success. The brooders selected for induced spawning must be healthy and ripe, males should be more prominent in size and weight, 2 –3 years of age is generally selected, and 1.5 –5 kg body weight is preferable. A minimum of 2 kg for males and 1.5 kg for females are a must for inducement. Spawning can be obtained either naturally or by inducing with hormones. Induced breeding is commonly practiced in most of the commercial hatcheries for Silver pompano. The hormonal inducement is intended to trigger the final phase (vigorous egg hydration and its release) of oocyte maturation. Before hormonal inducement, make sure that the oocytes have attained the late-vitellogenic stage of development; otherwise, it doesn't work. The oocytes are matured enough to get it spawned while inducing with human chorionic gonadotropin hCG. Once the ovarian biopsy results have shown matured oocytes which completes the late-vitellogenic phase, the brood fishes will be induced within 2-3 days to get the proper spawning and high fecundity. The (hCG) is a hormone widely used to induce optimal spawning of fishes. After many experimental trials, a dosage of hCG was standardized for Silver pompano at the rate of 400 IU per kg body weight for both males and females and administered as a single dose on the dorsal muscles. One disadvantage regarding the hCG administration is the loss of immunity of brood fishes when it is not spawned. The fishes were likely to affect various infections and died within days. hCG can be successfully replaced by an analogue of luteinizing hormone-releasing hormone [LH-RHa des-Gly10 (D-Ala6) LH-RH ethyl amide, acetate salt]. The cost of research-grade LHRHa is too high as compared to hCG. But the low dosage of LHRHa works well for the inducement of fish. Here we are using an LHRHa, which is not research-grade but works well with the brooders of Silver pompano but to a bit higher dosage than the research grade. LHRHa has superactive biological properties to induce gamete maturation (ovulation and spermiation) and spawning in fishes; its low species specificity makes it worthiness in induced spawning success of a variety of fresh water and marine fishes.

Fishes won't get affected by any diseases due to LHRHA injection and its successive spawning failure; instead, it induces the gonadal maturation for the upcoming spawning. Initially, a dosage of 100 µl (non -research-grade LHRHa) per kg body weight for female silver pompano and 50 µl per kg body weight for males were administered into the dorsal body muscles of fishes as a single dose or split dose. Spawning success was not achieved at this rate of hormone dosage even though the fish was matured enough. So we slightly increased the dosage, and it was standardized at 150 µl per kg body for females and 75 µl per kg body for males. Injection given at split dose works well with our system, and a photograph showing the hormonal administration is shown in figure 5.

Brood fishes that responded to the above dosage continue to spawn throughout the year every 18 days. The procedure includes;

a) Selection of brood pair (1 female:2 male or 1 female:3 male). Here usually follows the sex ratio of 1:2.

b) Cannulate the selected female fish one or two days before inducement; male brooders need not be cannulated before inducement.

c) The cannulated oocyte diameter must be above 500- 520 µm. In the microscopic screen, a minimum of 10-15 eggs are of the above diameter to be observed to proceed with inducement

d) If the ova diameter readings are above 500-520 µm of the cannulated female fish, then induce the fish with hormone on the following day

e) Take the fish from the broodstock tank, anesthetize with 2-phenoxy ethanol and induce them with LHRHa. (either single dose or double dose). This dose will be administered on the dorsal muscles during the morning hours (9.00 am – 11.00 am)

f) After inducement, the brooders should be transferred to the spawning unit inside the hatchery in a specifically dedicated area to avoid disturbance to the brooders and the possible risk of contaminations.

g) If the hormone is administrated in split dosage, then the same procedure will be carried out on the next day at the same time to introduce the remaining dose of hormone.

h) It is preferable to use circular tanks (5-ton tanks) with at least 1.2 m depth to ease the movement of brooders inside the tank. Here we experimented with the spawning in brooder tank RAS and registered 100% success in spawning. If we are keeping the induced fishes in the same broodstock tanks with RAS, the system must be switched off at around 04.00 pm on the second day and attained spawning on the same day night between 09.00 pm to 02.00 am.

Usually, the spawning could be noted within 36 -48 hours after hormonal induction. The spawning in pompano usually takes place between late night and early morning hours. The number of eggs spawned by pompano brooders ranged from 1.5 to 3.0 lakh eggs (female brooder, bodyweight- 2-2.5kg). The fertilized eggs of pompano float and are scooped gently using a 500 µm net. The photograph of the collected pompano eggs is shown in Figure 6. To minimize the presence of poor-quality eggs, which usually float deeper in the water, it is advisable to collect only the eggs which float on the water surface. The post spawning mortality of fish was negligible when the fish was treated with LHRHa.

Hormonal inducement of Silver pompano

Collected Pompano eggs

Egg collection

Eggs are collected using a scoop net after switching off the aeration and allowing the unfertilized/ dead eggs to settle at the bottom of the tank. A thicker layer of eggs 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 placed on a watch-glass or a Petri dish for observation under the microscope. A few eggs, set under a microscope or a transmitted-light stereomicroscope, have to be observed for the egg developmental stages. As fertilized 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 frequently to avoid the risk of clogging the collectors leading to mechanical stress to the eggs.

Summary

The control of the reproductive function of marine fishes in captive conditions is essential for the sustainability of commercial mariculture and brackishwater culture production. The induced breeding of marine fishes can be eased by controlling the reproductive hormones of the brain, pituitary, and gonad. The Recirculatory Aquaculture System for keeping the brooders, the process of PIT tagging for easy identification of fishes for cannulation and ovarian biopsy studies, broodstock maintenance with adequate feed, and frequent monitoring of oocyte development eased the gonadal development in the case of Silver pompano. The female fish attained maturity at a minimum weight of 1.4 kg. We have never faced stress in fishes during anesthesia, cannulation, and hormone inducement. The hormone dosage standardization only took a bit more time in the entire process of Silver pompano breeding. Single-dose doesn't fit in the initial phase of the breeding programme, where we administered the hormone in split doses. Later in the same fish with lower dosage at a single dose acts perfectly and attains spawning success with less response time. One of the brood pairs starts laying eggs volitionally after three years of induced spawning. The continuous effort for standardizing the breeding of Silver pompano at Vizhinjam Regional

Centre of CMFRI under the financial assistance from NFDB aids to create a national broodbank for Silver pompano at the centre. Mass production of pompano seeds from the broodbank under controlled conditions be a key success to the industry.

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