

CMFRI

Course Manual

*Winter School on
Recent Advances in Breeding and Larviculture
of Marine Finfish and Shellfish*

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ASSESSMENT OF MATURATION AND INDUCTION OF SPAWNING IN MARINE FINFISH

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Introduction

The cultivation of many economically important species has been helped greatly by the growing use of the technique of artificial fertilization and incubation. The discovery of artificial fertilization is supposed to be very old and this technique is said to have been practiced in the middle ages by the monk Don Pinchon in trout. The rediscovery of wet artificial fertilization in salmonids in 1842 by two anglers Gehin and Remy and the discovery of dry fertilization by the Russian Vrassky and its application between 1856 and 1870 led to great technical progress. Artificial spawning was first achieved in striped mullet in 1930 in Italy. In general the technique of hypophysation has triggered rapid progress in the induced breeding of cultured species, in the controlled rearing of fish larvae and in selective breeding.

Maturity determination

The effectiveness of hypophysation technique for artificial spawning depends mainly on the selection of suitable recipient fish at the proper stage of ovarian development. For finfish species which undergo normal gonad development but fail to spawn in captivity, identification of the correct stage of development is important and critical. There are various methods for maturity determination. External anatomical characteristics have been described and used ex., depth and fullness of belly, colour and state of swelling of the cloaca, softness and resilience of the belly, roughness of pectoral fins or presence of head tubercles.. Other methods include microscopic appearance of the oocytes, histological structures of eggs, etc.

Physiological parameters associated with sexual maturity such as elevated plasma proteins and calcium concentrations are of little practical use. Shehadeh, Kuo & Milison, 1973) described a method for assessment of ovarian biopsy *in vivo* which is accurate and reliable. In this method intra-ovarian oocytes are removed *in vivo* from an unanaesthetised female through a polyethylene canula of known orifice diameter. The canula is inserted into the oviduct for a distance of 6-7 cm from the cloaca, and the oocytes sucked orally into the tube by the operator as the canula is withdrawn. Oocytes from the mid portion of the ovary are the most representative and sampling error is minimized by avoiding extremities.

The oocytes are removed from the canula, washed and preserved in a solution of 1% formalin in 0.6% Sodium chloride solution. They are then placed in a plexiglass plate and measured with an ocular micrometer. Fine grooves cut in the plate align the oocytes and facilitate measurement. Egg diameters are measured along the horizontal axis and the measurements grouped into 50 μ m class intervals. The sexual maturity of the fish is expressed in terms of mean egg diameter, calculated from the egg diameter frequency distribution. In many fishes where the oocytes develop in synchrony, this method helps in determining the ovarian development accurately without sacrificing the female fish. This method also provides a means to observe and record oocyte development in individual fish and thus precludes variations between females in the brood stock. Apart from the ova diameter this method also helps in assessing/observing the shape, quality and stage of maturity of the ova. In mullets ova in the tertiary oil globule stage are selected for experimentation on induced breeding. Ova which are dark, opaque and perfectly round are found to be ideal to initiate experimentation. Fishes wherein ova are in the process of disintegration are to be avoided.

Apart from the knowledge of ova diameter, the knowledge of the exact stage of maturity of ova thus sampled is a pre-requisite. Most of the researchers have accepted the ova maturity classification described by Yamamoto (1956) and Yamamoto *et al.*, (1965) Kuo *et al.* (1974) have classified the oogenesis in oocytes of *Mugil cephalus* along the



pattern of Yamamoto and compared them microscopically and histologically. As per the classification there are five stages which are primary oocyte stage (12-170 μ m in dia.), yolk vesicle stage (170-210 μ m) yolk globule stage (200-700 μ m), ripe stage and atresia. . Kuo *et.al.* (1974) advocated initiation of hypophysation in *M.cephalus* when the ova are in the tertiary yolk globule stage in which the intraovarian eggs are fully yolk laden. . In milk fish an ova dia. of 850 μ m is ideal to start with. In mullets, fishes with ova dia. of above 600 μ m alone are taken for inducement. The actual relationship between initial mean ova diameter of recipient females and the amount of gonadotropin required to induce spawning in *M.cephalus* has been worked out.

Source of breeders used

A review of earlier works on induced spawning of cultivable fishes will show that the experiments on breeding were performed in wild ripe fish caught just before natural spawning or enroute their spawning migration. Later workers relied on brood stock reared in captivity. This has the advantage of easier availability and the breeders are less active compared to wild caught breeders. Pond reared fish are easier to spawn since they are docile and free from injuries. Wild caught brood stock is restless and easily prone to handling stress. This may sometimes lead to degeneration of ova.

Hormones and their use

The influence of environmental factors on gametogenesis of fishes is mediated through the hypothalamo-pituitary-gonad axis. The hormones involved in the process of maturation are gonadotropin releasing hormones, gonadotropins and steroids. It is this basic knowledge which enables us to manipulate gametogenesis by exogenous hormones. The role of pituitary glands in reproduction of vertebrates was understood quite early. Many workers give elaborate accounts of the techniques of collection, processing and storage of pituitary and their methods of application with reference to cultured fishes. Usage of pituitary glands in hypophysation suffers in lacking standardisation especially on the doses. So also are the inherent problems connected with the variations in fish pituitary gonadotropin activity which in turn is related to the phylogeny, sex and season. It is generally believed that pituitary from either sexes are equally potent and the difference if any can be minimized by the preparation of mixed pituitary extracts from both the sexes. . The common practice is to collect pituitary from mature fishes during spawning season. This leads to sacrifice of valuable breeders. The use of salmon pituitaries revolutionized the further use of pituitary gonadotropins. A partially purified form of salmon gonadotropin has also been described. (PPSG). This led to the preparation of purified salmon gonadotropin. Different preparations of pituitary such as in the form of powders LPE (lyophilized salmon pituitary extract) , ADP (acetone dried salmon pituitary powder) , acetone dried pituitary powder of carp (CPH) are available. Information on spawning of fishes using pituitary glands alone or in combination with other hormones is in plenty. Whatever hormones are used in spawning a fish, a priming dose with pituitary extract (30 –50mg. CPH/Kg.) helps in achieving good spawning. The dosages used by workers on different species vary.

The use of mammalian hormones for inducement of cultured fishes has many advantages such as reliable sources, readily available always, easily stored, no sacrifice of valuable breeders etc. . Their use revolutionized the breeding of many cultivable fishes. Beginning with Human pregnancy urine and pregnant mare's serum, the available mammalian hormones are in plenty such as H.C.G., F.S.H., L.H, puberogen and Synahorin (a mixture of chorionic hormone and mammalian pituitary extracts). HCG is widely used in many food fishes in concentrations beginning from 20 –30 IU/ gm body wt. Many workers use HCG in combination with CPH to get spawning. HCG can also be used to induce spermiation in males as well as seminal thinning. Mammalian hormones have been used in getting successful spawning of mullets, rabbit fishes, milk fish, sea bass , many species of catfishes, striped bass, groupers, etc.

The use of synthetic hormones came into existence in recent years. The most important of these is the synthetic form Leutinizing hormone Releasing Hormone analogue (LHRH-a). The effective doses begin from 200 to 300 mgm/ Kg. wt. of fish. Another advancement in hormone therapy is the use of steroids such as D.O.C. (Deoxycorticosterone). The steroids are cheap and are available as pure preparations. The use of hormones such as Methyl testosterone in prolonging spermatogenesis in males is quite common. Similarly the use of MT in sex reversal of protogynous species such as groupers to obtain functional males is well established.

Administration of hormones

Administration of hormones is mainly intramuscular and sometimes intraperitoneal. But other methods of hormone administration such as fixation of silastic tubing, coelomic administration via a catheter, intraperitoneal implantation of osmotic pumps or intramuscular implantation of cholesterol hormone pellets are all in use in modern laboratories. These new avenues of hormone administration reduce the stress due to repetitive injections. . The use of plastic hoods in covering the head portion of the breeder while injecting is helpful. The number and frequency of hormone injections vary and are related to the response of the breeder and in other words the maturity stage of the breeder, the environmental conditions and the type of hormone used. Live ovarian biopsy gives a clear understanding of the response of the ova to the hormone used. Visible reactions such as the enlargement of the belly or production calcium salt depositions as seen in mullets and milkfish are the direct results of the hydration of ova. The release of a few hydrated eggs by the female shows the onset of spawning. The males are generally released along with the females at the time of giving the resolving dose. Male female ratio is also important and varies from species to species. Natural spawning is always preferred compared to stripping since it gives better quality eggs which in turn result in better survival of larvae. Stripping can be done either through the dry or wet method. Stripping has to be performed at the right time mainly through observation of the breeder through visible symptoms of courtship or release of hydrated eggs by the female.

Egg collection and incubation

The collection of spawned eggs in hatchery bred fishes is done with the help of appropriate scoop nets. But collection is difficult in caged brood stock in open enclosures or in ponds. The quality of the incubating systems, the control of environmental conditions especially water flow, aeration etc help in better hatching.

Preservation of sex products

Methods for storage of fish sperm and ova have several advantages mainly in selective breeding and hybridization. Short term storage of sperm by chilling at 5 °c up to 23 days helps in extending their fertile period in tropical and subtropical species. Many descriptions of cryogenic preservation of sex products in liquid nitrogen are available. Cryoprotective agents such as glycerine, DMSO are used. Shiro Hara *et al.* (1982) studied various extenders for preservation of milk fish sperm. The work by Withler and Crim (1982) throw much light on the storage of grouper sperm.

