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Breeding and spawning of fishes: Role of endocrine gland

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Abstract
The fish pituitary is an endocrine gland of dual origin found on the ventral side of mid brain attached to it by means of stalk. The pituitary gland produces and stores gonadotropin hormones (GtH), which play a decisive role in ovulation and spermiation. Hypophysation is a technique whereby ripe fish brooders are stimulated by pituitary hormone introduction to spawn in captive condition. Injected pituitary gland extract bypasses the brain-pituitary link, acting directly on the ovaries and testes, providing the surge in blood GtH levels that normally precede spawning. This technique has been developed for the production of good quality spawn of certain fish species in captivity by manipulating its reproduction with control timing and synchrony of egg production, while natural spawn collection yields some undesirable wild species and is not profitable for culture. This paper explains general organisation and morphology of pituitary gland and it's the role in induce breeding of fishes.

Keywords: Pituitary gland, gonadotropin, breeding, spawning, teleost

Introduction

Fish pituitary

The general organization and morphology of the pituitary gland has been described in a number of teleost fishes. The pituitary is a major endocrine gland, a pea-sized body attached to the base of the brain that is important in controlling growth, development and the functioning of the other endocrine glands. It is situated on the ventral side of the brain. It is a small, soft, whitish body whose size and shape vary with species. It is more or less round in carps; oval in catla and rohu and pear-shaped in mrigal. It is located in a concave cavity called sella turcica and enclosed by a membrane called durameter, which is attached to the brain by an infundibular stalk (Fig. 1) [1]. It produces gonadotropic hormones (GtH) that are essential for the maturation and spawning of fish. These gonadotropins include Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) which are secreted and correlated with the cycles of gonadal maturity.

As in higher vertebrates the pituitary or hypophysis of fishes consists of two distinct tissues capable of producing hormones-adenohypophysis (anterior pituitary) which is derived from the embryonic pouch, Rathke's pouch arising from the roof of the buccal cavity as an outward evagination and the neurohypophysis (posterior pituitary) which originates from the downward evagination from floor to third ventricle [2]. The adenohypophysis regulates gonadal functions in fishes and is the site of synthesis, storage and release into circulation of several peptide and hormones. The adenohypophysis is divided into the rostral (pro-adenohypophysis) and the proximal (meso-adenohypophysis) pars distalis, and the pars intermedia (meta-adenohypophysis) [3, 4].

The neurosecretory nuclei of the hypothalamus produce neurohormones control pituitary hormone secretion, thus constituting the hypothalamo-hypophysial axis. The neurohypophysial hormones are structurally and functionally related peptide hormone. Their main representatives are oxytocin and vasopressin. The neurohypophysis interdigitates into all areas of adenohypophysis but major interdigitations are found in pars intermedia. The neurosecretory materials from the neurons of preoptic nucleus are transported through the axons and stored in the axonal terminals ending at the junction between neurohypophysis and adenohypophysis which is called as neuroadeno interface. This interface is highly vascular and it is the centre for storage and release of hormones.

Gonadotropins

The gonadotropins are the secreting cells and are most often located in the proximal pars distalis. These are identified on the basis of tinctorial reactions with the periodic acid (Schiff reagent and Herlant'a tetrachrome stain) location. However, their numbers, granulation of the gonadotrophs and role in the reproductive cycle is researched in a few cultured species such viz., *Cyprinus carpio* (Leray 1965; Blanc-Livni and Abraham 1968), *Cirrhinus mrigala* (Moitra and Sarkar, 1976), *Clarias batrachus* (Lehri 1966, 1970), *Heteropneustes fossilis* (Sundararaj 1959 & 1960), and *Mugil cephalus* (Blanc-Livni and Abraham 1968). Generally, these are high in numbers and granulated during active breeding seasons compared to other non-breeding seasons. The fish pituitary gland produces and secretes two gonadotropins: 1) Follicle Stimulating Hormone (FSH) and 2) Luteinizing Hormone (LH).Both FSH and LH act on two different cells which in turn help in synthesising estrogen as explained below (Fig. 2).

In vertebrates, reproduction is controlled by two master gonadotrophic hormones from the pituitary gland, FSH and LH. Both gonadotropins belong to the glycoprotein hormone family, consisting of one common α-subunit and one hormone-specific β-subunit [13]. Numerous studies have been reported in a variety of fish species on the physiological roles of FSH and LH [14] and their regulation [15]; however, our knowledge of FSH and LH functions still remains fragmentary. It is now generally believed that similar to their mammalian counterparts, fish FSH is mostly involved in promoting early gonadal development and growth, whereas LH plays an important role in regulating the late stage of gametogenesis, including the final gamete maturation and release (ovulation and spermiation) [16, 17].

Some studies indicate that in teleost, just like the case in mammalian species, the functional changes of the pituitary and the ovary are directly and indirectly under the control of the hypothalamic releasing hormone. The medium lobe of the grass carp's pituitary has only one type of gonadotroph which contains two kinds of granules differing in size and staining reaction. The gonadotrophs show extensive activity of hormone synthesis and release following the LH-RH treatment. This is evidenced by the progressing decrease in number of the small granules, increase in number and size of the huge heterogeneous granules and the appearance of cytoplasmic vacuolation. The small granules are likely to be the LH secreting granules and the huge granules, the FSH secreting granules. Owing to the rapid elevation of pituitary LH, the ovary is activated.

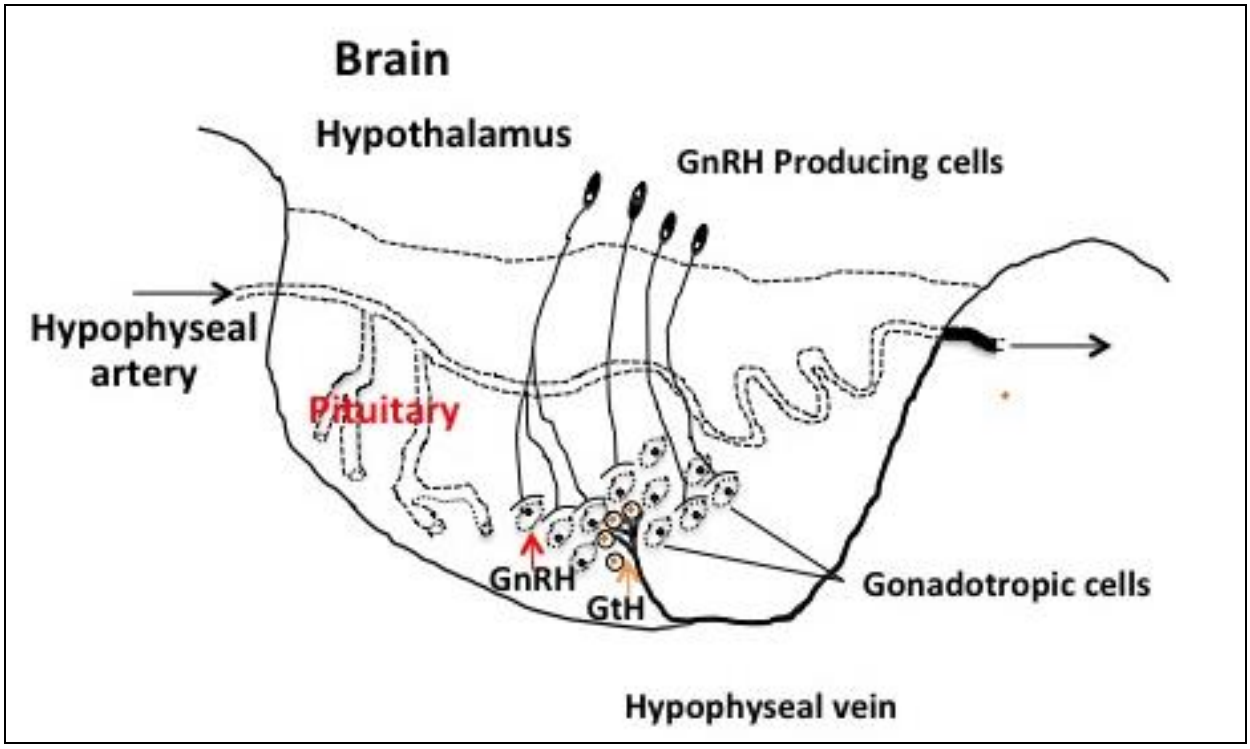


Fig 1: Fish pituitary gland and gonadotropins production. Note: GnRH-Gonadotropin Releasing Hormone, GtH-Gonadotropins. Blood enters from the hypophyseal artery and exits to through the hypophyseal vein. Gonadotropins produced by gonadotropic cells stimulated by GnRH from the hypothalamic nerve cells, is collected along the way.Figure adapted from Brian Harvey and Carolsfeld (and modified).

Biosynthesis of estrogen

In the estrogen biosynthesis pathway, a series of androgens (steroids) are formed in theca and leydig cells of females and males respectively with the help of a number of enzymes under the stimulation of LH. The androgens include testosterone and androstenedione formed are then get diffused into the granulosa cells in females and sertoli cells in males, after which the granulosa/sertoli cells convert the final steroids of the androgens (androstenedione or testosterone) to estrogens by an enzyme called P450 aromatase (P450 cytochrome Cyp19a1) under the influence of FSH (Fig. 2). The entire process takes place in two different cells hence referred as two cell hypothesis. The protein Cyp19a1 is the only enzyme which plays a key role in the sex differentiation [18, 19]. In addition, FSH is an important gonadotropin, since it stimulates the ovarian development and testicular spermatogenesis during gametogenesis; and LH is mainly constitutes for final gamete maturation (ovulation or spermiation). In spite of many studies on FSH and LH in teleosts, their functions in reproduction still remain poorly defined. This could be because of lack of studies on genetic approaches in adult teleosts [20].

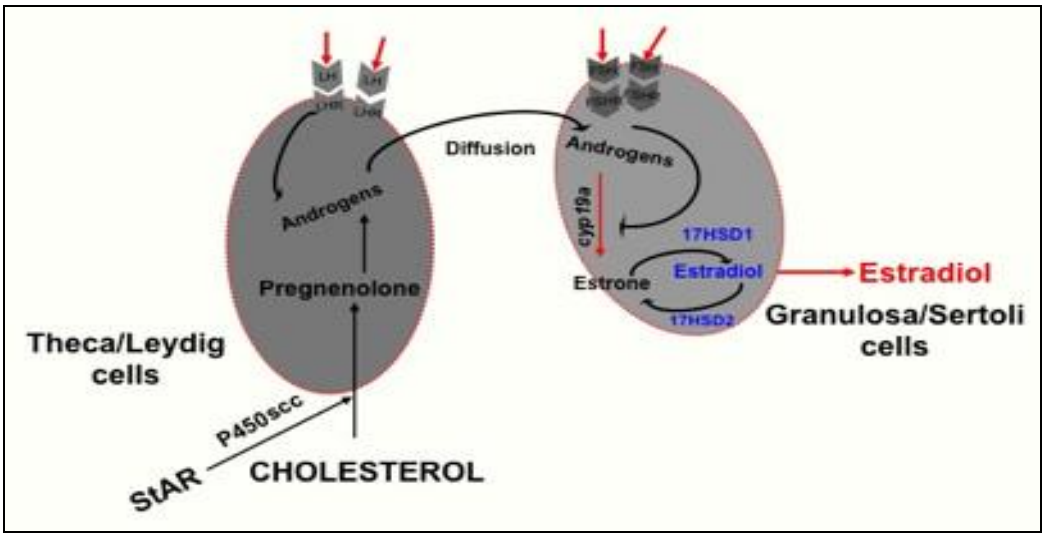


Fig 2: Schematic diagram showing two-cell hypothesis. Note: StAR-Steroid Acute Regulatory Protein, P450scc-cholesterol side-chain cleavage, LH-Luteinizing Hormone, LHR-Luteinizing Hormone Receptor, FSH-Follicle Stimulating Hormone, FSHR-Follicle Stimulating Hormone Receptor. Adapted from: Lubzens *et al*. 2010 [21]; Schulz *et al*. 2010 [22] (and modified).

The LH produced by the pituitary gland binds with the LHR (LH receptors) on theca and leydig cells and stimulate androgen synthesis by the cholesterol which has entered with the help of Star protein and in turn cause luteinisation in females and promotes the production of testosterone in males. Whereas, FSH stimulates granulosa and sertoli cells for estrogen synthesis by binding to FSHR (FSH receptors) causing growth and maturation of ovarian follicles in females and spermatogenesis in the testes of males. However, the process is not species specific; but, there is a great variability in its effectiveness in different species.

History of induce breeding in fishes

The first attempt of induced breeding by using pituitary extract was done by B.A. Houssay (1930) in Argentina on a viviparous fish [23]. He was successful in obtaining premature birth of young fish. Subsequently, based on the lines of Houssay, Von Ihering and his team of Brazil, in 1934, successfully induced bred a catfish with pituitary hormones and hence credit for the present day concept of induced breeding of fish goes to Brazilians [24]. In India, the first attempt on induced breeding was conducted by Hameed Khan in 1938 on mrigal, *Cirrhinus mrigala* by administration of mammalian pituitary gland but eggs were not fertilized [25]. Later in 1955, H.L. Chaudhari succeeded in induced spawning on small carp species, *Esomus danricus* by administering the intra-peritoneal injection of catla pituitary gland. The first success in induced breeding of Indian Major Carps (*L. rohita* and *C. mrigala*) was in the year 1957 by H.L Chaudhari and K. H. Alikunhi at Central Inland Fisheries Research Institute, substation, Cuttack (Orissa) [26, 27]. Since then, the technique has been standardized and refined for the large-scale production of fish seed.

Hypophysation in fishes

In order for the pituitary extract to be effective, donor fish should be sexually mature whether male or female. Also, the extraction of pituitary gland is preferred to be close to or within the spawning season. During breeding season (June-August) mature brooders are selected from the brood stock pond and injected with hormone injection for induced breeding/hypophysation. The following process consists of collection and identification of brooders, collection and preservation of pituitary glands, preparation of pituitary extract and site, dose, method and time of injection [28].

Identification of brooders for breeding purpose

1. Female brooder characters: Bulging belly, swollen pink vent and eggs oozes out on pressing the belly
2. Male brooder characters: White coloured liquid (Milt) oozes out when belly pressed, Pectoral fins rough to touch

Brooders ratio

For every one female two males (weight of female=weight of males) are preferred for a successful. The 1:2 ratio is followed since males in carps are generally smaller to females and to ensure complete fertilization of eggs produced by the female it is recommended to use two males per female and at the ideal temperature between 24-31 ºC for breeding.

Induced breeding using pituitary glands

Pituitary Gland Extract (PGE) of same or closely related species are normally injected. However**,** commercially available synthetic hormones e.g. Ovaprim and Ovatide are also used for breeding purposes, but many of the farmers in Punjab import the glands from West Bengal and the extract is being injected during breeding and farmers are gaining better results.

Selection of fish for pituitary gland

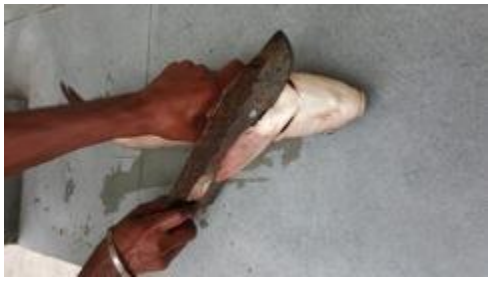
For the purpose of induced breeding using pituitary gland, mature fish (freshly killed or ice preserved) of either sex are selected of the same species (homoplastic) and or closely related but different species (heteroplastic). The fish which is used for collection of pituitary gland is referred as 'donor fish'. Among the carps in India, common carp is the most commonly preferred donor fish due to its availability of mature fish round the year.

Pituitary glands collection

Fish pituitary gland was collected by dissecting and removing a portion of the scalp as shown (Fig. 3) and the following steps were followed [29].

- The brain case (cranium) was removed obliquely using a butcher's knife and the skull was removed.

- The brain was exposed by removing blood, grey matter and fatty substance with foreceps and cotton.
- The anterior end was then detached, i.e. optic and olfactory nerves of the brain using foreceps and carefully lifted the entire brain up without disturbing the pituitary gland and laid it back to expose the pituitary under the membrane.
- By taking care, the membrane was removed which covers the gland and the fluid using cotton and then pituitary gland was completely exposed.
- Then carefully collected the pituitary gland by inserting the blunt end of the forceps and transferred to a vial containing preservative, i.e. either absolute alcohol or acetone.



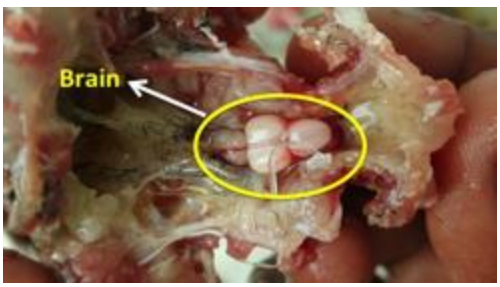
3A. Cut near pelvic fin

3B.Cut near pelvic fin

3C. Make incisions behind the skull

3D. Remove gills for convenience

3E. Sella turcica



3F. Fish brain

3G. Location of Pituitary gland

3H. Pituitary glands in preservatives

Fig 3: Steps showing collection and preservation of pituitary gland in fish

Preservation of pituitary glands

There are three methods for preservation of fish pituitary glands according the facilities available with the farmers, which are as described below.

A. Preservation in absolute alcohol

Immediately after collection, the glands are washed and transferred to fresh absolute alcohol in amber coloured glass vials. Every after 24 hrs, pituitary glands are washed with absolute alcohol and stored at room temperature in a dark place to avoid the sunlight. To increase the shelf life of the glands it is advised to refrigerate. During washing and storage, absolute alcohol dehydrates the glands, dissolves fats and preserves it for a longer time (up to 2 years). Vials are labelled for information includes date of collection, fish name, maturity stage and other details if any.

B. Preservation in acetone

Upon collection, glands are transferred to ice-chilled acetone and stored in a refrigerator for 2-3 days. During refrigeration,

acetone is changed 2-3 times and stored in a refrigerator. Like absolute alcohol, acetone also has dehydrating and defattening effect and vials are labelled as explained above.

C. Immediate freezing

Immediately upon collection the glands are kept in freezer.

Preparation of pituitary gland extract

Most often, pituitary gland extract (PGE) is prepared just before the injection. Based on the weight of the brooders to be injected, total quantity of glands required are calculated and used for extract preparation. About 10% extra glands are taken to compensate the wastage during the extraction procedure. The preserved glands are taken out and dried using blotting paper. If acetone-dried glands are used, they can directly be taken for maceration. Use one-third of the medium for homogenization and the remaining two-thirds for rinsing the homogenizer and the glass rod. In order to prepare the extract, initially glands are weighed and homogenized in distilled water or 0.3% physiological saline and then the suspension is centrifuged at 5000rpm for 5-10 minutes. Maintain a dilution rate of 20-30 mg of pituitary in 1.0 mL of the medium. After transferring the contents from the tissue homogenizer to centrifuge tubes, centrifuge the extract at 5000 rpm for 5-10 minutes (at the farm site hand centrifuge could be used (Fig. 4D). Following which, supernatant is collected using syringes for induce breeding.

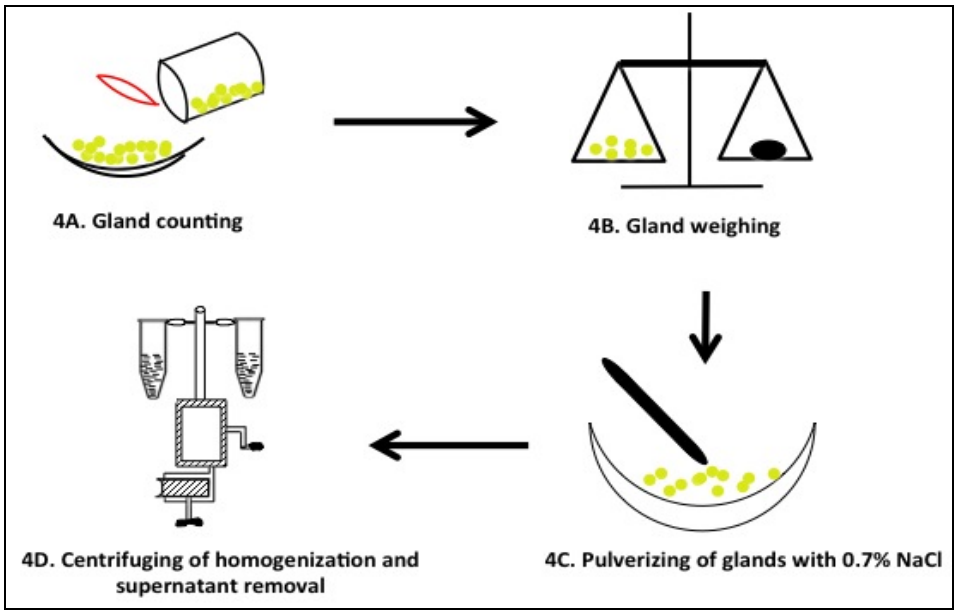


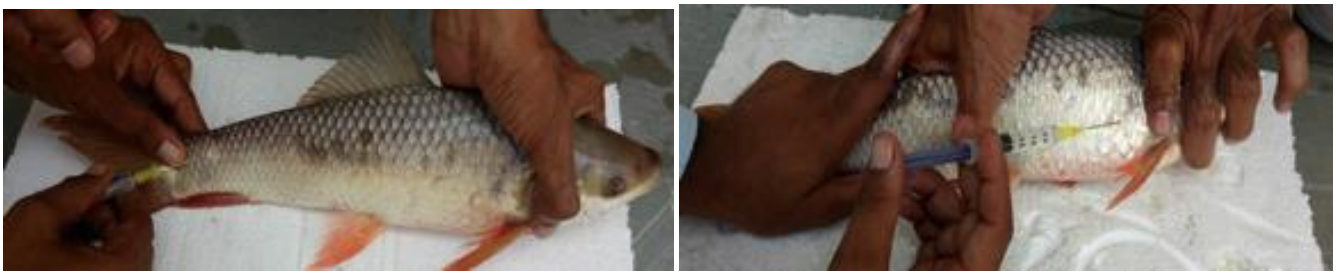
Fig 4: Schematic diagram showing preparation of pituitary gland extract.

Site of injection

Pituitary gland extract injection is performed either intramuscularly or intraperitonially. Intramuscular injection is performed at different locations of the fish include (i) near the ventral fin base, (ii) Below the dorsal fin base and above the lateral line, (iii) On the caudal peduncle just above the lateral line and (iv) Below the pectoral fin. While injecting care must be taken not to prick through the scale (Fig. 5).

5A. Near the ventral fin base

5B. Below the dorsal fin base and above the lateral line



5C. On the caudal peduncle and above the lateral line

5D. Below the pectoral fin

Fig 5: Sites of injection on brooder fish for efficient breeding

Breeding hapa

A pair of brooders is released into the breeding hapa for spawning after injection of pituitary extract. The breeding hapa is a rectangular case of fine netting. For larger fishes its size is 8′ x 3′ x 3′, but for the smaller fishes it is 5'x 3'x 3′. It is held on four bamboo poles, one at each corner of the rectangular case. The roof of the hapa may be open or closed. The hapa is made of mosquito net cloth through which laid eggs and milt cannot escape out. Three-fourth part of hapa is summarized in water whereas upper one-fourth part remains in air. After 3 to 6 hours of injection of pituitary extract spawning takes place. The fertilized eggs are white and opaque whereas unfertilized eggs are transparent and bead-like. A hatching hapa is also rectangular and made of muslin cloth and is open from above. The mosquito net hapa is present inside the hatching hapa.

Dose of pituitary gland extract

Female fishes are given two injections with a difference of 4-6 hrs, the first dose given is called initial dose or preparatory dose or priming dose and the later dose is called as final or decisive or resolving dose, whereas male is given a single dose at the time when female is given the second dose. Not surprisingly, the dosage varies within and between the species and also depends on the size of the fish. The dosage also depends on the readiness and sensitivity of the fish which reflects the health status. In addition, the interval between the first and second dose varies from species to species and or from place to place depending the agroclimatic conditions region.

Method of injection

One millilitre volume disposable syringe should be used for injecting PG hormone to the recipient fish. The appropriate amount of diluted hormone stock solution is to be taken in the syringe. Then the fishes are caught from the spawning tank by net. A piece of clean, soft and wet cloth was used to wrap up the fish and kept lying on a table. Following which, the accurate dose of PG extract is administered (Fig. 5). For an efficient discharge of the PG extract, the needle is inserted at an angle of 45° with the body [30].

Time of injection

Most commonly injections are given in the evening hours considering the water temperature is ideal during the night hours and expected successful breeding.

Conclusion

Quality seed is the prime requirement for the fish breeding of any species. A few years back, the only source for procurement of fish seed was from its natural spawning ground. By this method pure stock of seed of selected species in adequate quantity could not be made available for farming. Last three decades have witnessed a phenomenal growth in the technology of seed production of different species of fishes as a result of which, at present, seed of many species of cultural value are available on a commercial scale. More emphasis should be given to refine this technique species specifically to breed indigenous species of market importance and to preserve the biodiversity.

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