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REVIEW ARTICLE

A review on induced breeding in fishes

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Abstract: Endocrine system acts as a regulatory link between the environmental events and maturation and release of gametes in vertebrates. appearance of secondary sexual characteristics during the breeding season, breeding behavior during courtship and timing of reproduction are some of the activities controlled by pituitary hormones. The important pituitary hormones in this regard are LH and FSH.

Key words: Hypophysation; Pituitary; Pisciculture; Stocking; Induced breeding; hormone; Fish seed; Fry.

Introduction

Cro-magnon men were cave dwellers. They lived by the riverside. They used fish as food. From the ancient period fish is used to be considered as a source of nutritious food. It is rich in protein. Fish also provides proteins, fats, vitamins, essential amino acids and fatty acids. Above all, fish is rich in linolenic acid (omega-3) that helps in prevention of coronary heart diseases and other cardiovascular diseases. For this reason, Greenland Eskimos and Japanese fisherman do not suffer from heart attacks as they consume fish daily (250g to 400g). The omega-3 fatty acid also prevent blood clotting and artherosclerosis. So cardiologists recommend that if fish is taken twice a week, it will prevent heart diseases. Now-a-days about 30-40% of the world population is suffering from protein deficiency. So, fish has special importance as a supplement to ill balanced cereal diets.

It is estimated that about 8.5 million tons of fish is required annually to meet the present day demand of fish protein in the country. But it is seen that the annual production of fish is only 1-7 million tons. That means the naturally breeding fish cannot meet the demand of humans. So induced breeding is essential. The natural breeding grounds for fishes are rivers, ponds and bundhs. Fish seed collections from the natural site of spawning possess problems of being mixed with spawns of predaceous fishes. Even though much care is taken in identifying the fish seed by adopting various methods, their separation sometime becomes difficult. To overcome these difficulties, induced breeding has been developed.

The technique of induced breeding is simple and can be easily learnt without much training. The technique assures a timely available supply of seed spawn for fish culture. But availability of spawn from natural sources is uncertain, as it depends on monsoon. By applying this technique fish demand has already been achieved.

Induced breeding is a technique by which ripe fishes are stimulated by pituitary hormone introduction to breed in captivity. The stimulation promotes a timely release of eggs and sperms from ripe gonads. The active factors like LH and FSH are present in fish pituitary.

History of pituitary treatment in pisciculture

The technique of induced breeding was first evolved in Argentina. The pituitary extract was produced following the method of Houssay, (1931). When viviparous fishes were injected with fresh fish pituitary gland extracts, premature birth occurred. In 1934, Brazilian researchers could succeed in inducing ovulation by pituitary gland injection. Since then the technique is widely used by various workers. This technique was also followed in America and in Russia Gerebilisky, (1938). It has been observed that the Brazilian pisciculturists were the first to use fish pituitary gland for induced breeding in their indigenous fishes Houssay, (1931); Iherring, (1937); Fontenele, (1955).

In India the first attempt on induced breeding was conducted by Khan (1938) on *Cirrhina mrigala*. Russians had also developed the induced breeding technique Gerbil' skii, (1938); Kazanskii, (1939).

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 by hormone injection such as in *Labeo rohita, Cirrhina mrigala, Cirrhinus reba, Labeo bata* Chaudhuri and Alikunhii, (1957) and Alikunhi, (1959). There was a preliminary observation on hybridization of the common carps (*Cyprinus carpio*) with Indian carps. It was observed in the Indian carp spawns induced by injection of pituitary hormones Alikunhi and Ibrahim, (1960). The exotic chinese carps (*Hypophthalmichthys molitrix* and *Ctenopharyngodon idella)* were induced bred in India by Alikunhi and Vijayalakshmanan, (1960). Certain drugs had been tested for induced spawning in fishes with variation in the percentage of success Harvey and Hoar, (1979). It has been observed that the

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difference in dosage among different species is due to the varied level of dopamine activity Billard *et al.,* (1983); Peter *et al.,* (1986). Peter (1986) had described the self-potentiating action of the releasing hormone to some drugs when given in two doses.

Human chorionic gonadotropin (HCG) was then used as a substitute for pituitary gland but the result was not as good as pituitary hormones Chonder, (1985). Ovaprim was the widely used substitute for pituitary gland extract which was synthetically manufactured by Syndel laboratories inc, Vancouver, British, Columbia, Canada. All the fish breeders readily showed preference for this drug Nandeeshsa *et al.,* (1990).

It is well known that the pituitary gland extract from same species of fish gives the best result however extract from amphibians are also quiet effective in fishes Padhi *et al.,* (2015).

Culturable fresh water fishes

***Catla catla* (Catla):** This is the fastest growing carp in India. The adult fish and advanced fingerlings are distinguished by deep body with a conspicuous head, large upturned mouth, non-fringed lips, devoid of barbells and a broad dorsal fin with 14 to 16 branched rays. The body is ordinarily dull, silver white, but tends to be rather darkish in weedy weathers.

Catla is reported to grow very quick, even 7.5 to 10 cm per month. *Catla* grows to over 1- 5 meter in length. The fish in the second year attain sexual maturity and are ready to breed in the third season.

***Labeo rohita* (Rohu):** Rohu is considered the tastiest of Indian carps. It is easily distinguished by its relatively small or pointed head, almost terminal mouth with fringed lower lip, dull reddish scale on the sides and pink reddish fins. The dorsal fin has 12-13 branched rays. The body is more linear than that of *Catla.*

Rohu grows quickly. A growth of 35-50 cm can be expected in the first year in a well-stocked pond. Sexual maturity is attained towards the end of the second year. Rohu grows to over one meter in length.

***Cirrhina mrigala* (Mrigal):** Next to *Catla* and rohu for culture purpose is the mrigal. It is easily distinguished by the relatively linear body, small head with blunt snout, terminal mouth with thin non-fringed lips, bright silvery body and reddish fins. The dorsal fin has 12-13 branched rays.

Mrigal grows slower than *Catla* or rohu. The species attains a maximum length of over 0.75 meter. It becomes sexually matured in the second year.

***Labeo calbasu* (Calbasu):**

The species is suitable for cultivation in confined waters. Its body is oblong, moderately compressed, mouth narrow, lips thick and fringed, each with a distinct inner fold. Two pair of barbell arose from dorsal fin midway between the snout and base of the caudal fin which is deeply forked. Color of the body is slate black, scales sometimes with a scarlet centre and eyes red in color. Maximum length is about 90 cm.

***Cyprinus carpio* (common carp)**:

The common carp (Bankok strain) has been found to adapt to these warm waters and grow satisfactorily. It reaches sexual maturity on attaining 35 to 40 cm length and 1-1.5kg weight in the first year of its life. The fish unlike Indian major carps breed in ponds almost throughout the year with a peak period from January to April.

Identification of eggs, spawn, fry and fingerlings of culturable fishes of India

Identification and segregation of different species from a mixed collection of fish seed are of vital importance for selective stocking of ponds in order to avoid wasteful culture of economic species.

Table: Identification of Eggs of Some Cultured Fishes Based On Their Characteristics

Nature	Diameter of eggs (mm)	Shape	Colour	Species
		1-Non-Floating		
	i) 5.3 to 6.5	Round	yellow	*Catla catla*
	ii) 5.5	Round	Brownish	*Cirrhinus mrigala*
	iii) 5.0	Round	Reddish	*Labeo rohita*
	iv) 3.4	Round	Bluish	*Labeo calbasu*
(a) Non-adhesive	v) 4.5	Round	Bluish	*Labeo gonius*
	vi) 3.8	Round	Faint Pale Bluish	*Labeo kontius*
	vii) 3.5	Round	Bluish tinge	*Labeo funbriatus*
	viii) 2.1-2.3	Round Segmented with numerous Oil Globules	Light red	*Hilsa ilisha*
b) Adhesive				
	i) 1.5 x 1.3	Oval	Greenish	*Clarias batrachus*
	ii) 1.5	Round	Greenish	*Heteopneustes fossilis*
i) Non filamentous	iii) 1.2-1.5	Round	Yellowish	*Wallago attu*
	iv) 3.5	Round	Yellowish	*Notopterus notopterus*
	v) 6.5	Round	Yellowish	*Notopterus chitala*
	vi) 2.0	Round	Light brown	*Mastocembelus*
	i) 0.9 -1.0	Round	Pale-transparent	*Rhinomugil corsula*
	ii) 1.0	Round	Gloden amber	*Channa punctatus.*
2. Floating	iii) 1.25 – 1.35	Round	Amber	*Channa striatus*
	iv) 1.5- 1.7	Oval	Amber	*Channa marulius*
	v) 0.7	Round	Transparent	*Anabas testudinus*

Fish seed requirements:

The present demand for fish seed has been assessed by different states in different ways and does not seem to be based on scientific considerations. In view of the widely divergent stocking rates, the fish seed committee felt that it is necessary to fix certain factors, like stocking rates and mortality rates, for estimating the optimum seed requirements. It is as follows:

1. Mortality rate from spawn to fry stage – 70%.
2. Mortality rate from fry to fingerling stage – 50%.
3. Stocking of fries to fingerling in various bodies of water.

Fish pituitary gland: The pituitary gland of fish is a small body situated as the ventral aspect of the brain in a concavity called as sella turcica and is connected to the brain by means of a stalk. Like higher vertebrates, fish pituitary gland also controls a wide variety of physiological processes by secreting a number of hormones. The most important are gonad stimulating hormones (FSH and LH) which take part in stimulating the development and maturity of the sexual organs and induce spawning in fishes.

Hypophysation technique:
Since the first success in induced breeding of Indian major carps by injections of fish pituitary hormones in the year 1957, systematic attempts were made to standardize the technique for commercial production of carp seed and improve breeding. Uniform techniques are followed throughout India and Burma, with slight modifications suited for particular environment.

Mechanism of induced breeding:
Hormone injection is the most common method of induced breeding in which the pituitary extract injected into the ripe breeders (both male and female) which force them to spawn. Induced spawning of this type depends upon the dosage of injection, the state of maturity of the breeders and environmental factors like temperature, water currents and rain etc. The mechanism can be completed in following steps.

8.1. Selection of breeders.
8.2. Segregation of breeders.
8.3. Stocking of breeders.
8.4. Maintenance of breeders.
8.5. Extraction of pituitary gland.
8.6. Storage of pituitary gland.
8.7. Preparation of pituitary extract and preservation.
8.8. Injection or administration of pituitary extract.
8.9. Collection of fertilized eggs and transfer to hatching hapa.

8.1. **Selection of breeders:**
Proper selection of breeders is the key to success in induced breeding. The breeding fishes should be healthy, fully ripe and of medium size. These are collected from their natural habitat quite in advance of their breeding season and raised in fertilization pond of the fish farm. They are stocked at a rate of 1000-2000 per hectare area (e.g. carps). The breeders should preferably be in the age group ranging from two to four years and have a weight averaging 1-5 kg. Large size breeders are avoided for difficulty in handling. A fish farm may have its own stocking ponds from which the breeders of suitable age are selected and transferred to fertilization pond. Fully ripe male and female carps are easily distinguishable. The male shows roughness on pectoral fin and when its belly is pressured, milt freely oozes out. The ripe female shows a relatively soft, round and bulging belly and its vent is swollen, protruding and pinkish in color. It is wiser practice to keep ready adequate stock of potential breeders. For this, a few months before breeding season potential breeders are kept separately under care and fed on supplementary food.

8.2. **Segregation of breeders:** To ensure a higher percentage of fertilization during induced spawning, it is necessary that there is synchronization between ovulation and milt shedding, i.e., release of sperm and egg takes place at the same time.

To raise the breeders at the farm, suitable male and female fishes are nested and stocked in the pond. Individuals of opposite sexes are kept separately in the ponds. In order to avoid

Suitable measures are taken to keep the breeders healthy and free from infection. If during transmission, the fishes are injured, then 20% of $KMNO_4$ solution is applied on wounds, keeping in view that $KMN0_4$ is toxic at high concentration and the gills never come in contact with solution.

To check the bacterial growth, protozoan parasites and the fungi, the breeders are treated with 10 ppm of $KMNO_4$ solution for an hour and 1 ppm acriflavin for another 5-12 hours, in separate earthwaves or pools. These solutions kill bacteria, protozoan, fungi and another parasite.

The physio - chemical and biological conditions of water are regularly checked and kept in accordance to the fish species selected for the purpose.

Breeders are weighed, in order to ascertain the dose of pituitary extract to be given later, prior to spawning.

8.3. **Stocking of breeders:** The stocking of breeders is done in various locations as the fish culture operation comprises the eggs, spawn, fry and fingerlings of Indian major carps. The stock is procured from natural sources by

i) Collecting eggs from breeding grounds.
ii) Collecting of spawn, fry and fingerlings from rivers.

8.3.1. Fish seed collection from river:
The seed of major carps namely *Catla,* rohu and mrigal and some medium carps are collected from rivers during the monsoon months.

8.3.1.1. Collection of eggs: From the actual breeding grounds the eggs are scooped out from shallower grounds by means of rectangular pieces of mosquito netting of varying sizes. However,

they are collected by benchi jal (conical bag net) from fast flowing waters.

8.3.1.2. Collection of spawn: Carp spawn which emerge out of the eggs in 18 to 24 hours and measures 5-7 mm in length are collected by means of a specially designed gear – the benchi Jal (shooting net).

The shooting net differs with regard to its size, shape, material and construction in different states. The larger ones are made out of mosquito netting cloth, conical in shape and open at both ends, measuring about 24 feet in length and having a mouth diameter of about 18 ft. The mouth is provided with two lateral wings to widen the respective area of the mouth. The cod end, measuring about 9"-12" in diameter is provided with a cane or bamboo ring which gives it stable shape during operation. A detachable piece of the size 2ft x 1ft, a gamcha made out of fine muslin cloth, is attached to the cod end during the operation to serve as a receptacle (Figure-1).

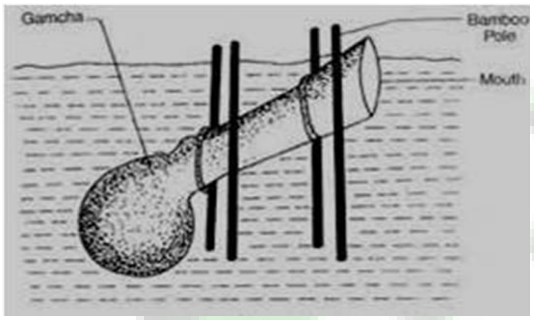

Figure 1: Gamcha net

8.3.1.3. Collection of fry and fingerlings: Apart from eggs and spawn, the fry and fingerlings are also collected from rivers. They are collected by fry collection nets which are fine meshed drag nets. The fingerlings are collected by cast nets, traps or fine muslin cloth while they tend jump over to cross the irrigation barriers. Basket traps are used for collecting fry and fingerlings in Godavari, Krishna and Cauvery rivers.

8.3.2. Terminology of the developmental stages commonly applied in fishery:

i. Larva: Any stage from hatching to metamorphosis, bearing yolk.
ii. Hatchling or Sac fry: A stage just hatched, bearing large amount of yolk but mouth not still open.
iii. Fry: A stage when mouth is open for feeding.
iv. Fingerling: A stage beyond advanced fry to a size of 5 inches in length.
v. Post larva: A stage with no yolk, but structurally similar to young.
vi. Alevin or Advanced fry: A stage from complete absorption of yolk to a size of 1 inch in length and resembling the young in structure.
vii. Yearling: A stage at age group 1year i.e. in the second year of life.
viii. Juvenile: A stage of fish between young and adult.
ix. Year-class: A group of fish that spawned and hatched in same calendar year.
x. Recruit: A stage of younger fish which can be easily handled and contributes to adult population size.

8.3.3. Breeding fish in bundhs:

Bundhs are nothing but special type of ponds where riverine conditions are simulated. They are constructed in the middle of a vast low lying area, with proper embankments and receive large quantities of rain water after a heavy shower. Bundhs are provided with an outlet for the overflow of excess water, and shallow areas which serve as spawning grounds for the fish. A large number of bundh type of tanks are found in west Bengal and Bihar. Bundh breeding has been conducted on a large scale in Madhya Pradesh too. Bundhs are generally two types.

8.3.3.1. Perennial bundh also called wet bundhs.
8.3.3.2. Seasonal bundh or the dry bundhs.

8.3.3.1. Wet bundh

It is a perennial pond situated in the slope of a vast catchment area, with embankment on three sides. The main pond retains water throughout the year, but its shallow marginal areas dry up during the summer months. The bundh has an inlet towards the high catchment area and an outlet at the opposite lower side. The bundh usually gets flooded with water from the upland area after heavy showers. The shallow area of the bundh called 'moan' gets inundated and excess of water flows out. The outlet is protected by a bamboo fencing (Figure 2). The breeders which are either grown in the perennial pond or released from other ponds, get stimulated by the flow of silt laden and oxygenated rain water. They spawn in the shallow areas.

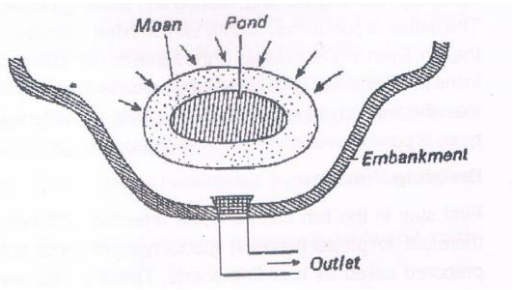

Figure 2: Wet bundh

8.3.3.2. Dry bundh:

A dry bundh is a seasonal shallow pond enclosed by an earthen wall (embankment) on three sides.

During the monsoon season, rain water rushes from the vast catchment area and accumulates in the pond. Breeders from nearby ponds are introduced in the shallow ponds. Breeding takes place after a heavy shower when the bundh is flooded with fresh water. It has been observed that the carps migrate to shallow water after a little sexual play and eventually they spawn.

8.4. Maintenance of breeders: Indian major carp breeders are usually raised in fish farms. Ordinarily 2-4 years old carps are collected and stocked in brood fish ponds at the rate of 1000-2000 kg. per hectare, a few months prior to fish breeding season. They are fed with equal quantities of rice bran and oil cakes at the rate of 1% of the body weight per day. Occasional checks are made to examine the general condition and stage of maturity of the breeders. Male and female brood stocks are kept in separate ponds.

8.5. Extraction of pituitary gland:
For success in induced spawning pituitary should be obtained from fully mature, healthy donor fish. Generally pituitary from the fish of the same species or from a phylogenetically related one is preferred. But hormones from an unrelated fish can also be used. Glands from either male or female fish can be used and are equally effective.

The pituitary gland can be collected by any one of the following two methods.

a) Through the foramen magnum.
b) By dissecting and cutting through the roof of skull.

a) Collection of gland through the foramen magnum is easier and economical too, because the fish head can be sold after taking out the pituitary. A large number of heads of fresh fishes are available in the market. The foramen magnum is first exposed by removing muscles and the parts of vertebrae left with the cut head. The fatty substance covering the brain from the above is removed by means of forceps taking adequate care not to damage the brain. The anterior part of the brain is now detached by means of a fine forceps and the brain is carefully taken out. The pituitary gland is exposed, cleared of the membrane, and picked by means of tweezers and kept in a Petri-dish.

b) For collecting the gland by dissecting the head, the roof of the brain case is cut by means of a sharp butcher's knife. The dorsal side of the brain is thus exposed. The olfactory and optic nerves are cut to free the brain. Then the brain is carefully lifted up and placed in a Petri-dish. The pituitary situated behind the optic chiasma is then exposed and removed. The gland is carefully picked up taking care that it is not damaged.

8.6. Storage of pituitary gland:
Pituitary gland can be used immediately after collection in fresh condition. However, it is usually preserved and stored for future use. The glands if properly preserved, is known to retain its potency for several months. It can be preserved by any of the following methods.

8.6.1. By freezing: Fresh gland is immediately frozen after collection and kept in a freezer in frozen condition.

8.6.2. In absolute alcohol:
Each gland is kept in absolute alcohol in a marked phial. After 24 hours, the alcohol is changed and the phials are kept at room temperature or in a refrigerator. The phials must be air tight to prevent moisture from getting in or the phials are kept in a descicator containing anhydrous $Cacl_2$. Absolute alcohol de fattens and dehydrates the gland, and should be changed occasionally to maintain the gland in good condition for a long time. Each gland is weighed accurately, for calculating the dose to be given to the fish. For this, the gland is taken out, kept on a filter paper for 2 minutes to dry, and weighed. It is then preserved in absolute alcohol in air tight phial.

8.6.3. Preservation in acetone:
In USA and Russia, the gland is preserved in acetone, and is considered to give better results. The gland is put in fresh acetone or ice-chilled acetone in a phial immediately after its collection. In this procedure it is kept in a refrigerator at 50^0F (10^0c) for 36 hrs during which acetone is changed after every 12 hrs. The acetone de fattens and dehydrates the gland, which is then taken out on a filter paper and dried for one hour at room temperature. The dried gland is then accurately weighed, and stored in a phial in a refrigerator for future use.

8.7. Preparation of pituitary extract and preservation:
The pituitaries obtained from donor fishes are macerated to form an extract which is injected into the ripe breeders and compel them to spawn. The method of preservation and the dosage required are described below.

The dose of pituitary extract depends upon the size and the state of maturity of recepient fishes. Normally in carps, 2-5mg of dried pituitary gland is required per kg body weight of the fish. After selecting the required amount, they are dried in filter paper and weighed accurately in a chemical balance. Weighed pituitaries are then kept in mortar and grinded with pestle or in a tissue homogenizer with a little amount of distilled water or 0.3% of sodium chloride solution. Grinded pituitaries are thus reduced to a pulpy mass which

is diluted by the medium used before and centrifuged at about 1000 rpm for 5 minutes. The supernatant fluid is used for injection. However, it is better to prepare the extract in advance and preserve it for injection when needed.

The extract is preserved in glycerin in air tight phials. The ratio of distilled water and glycerin should be 1:2 and a concentration of 20-40 mg of gland in 1 ml of water and glycerin is considered to be most convenient. After 3-4 days sediments settle down in the bottom, it is filtered and stored in sealed glass ampoules. Pituitary extract can be preserved in propane and kept in refrigerator up to 30 days for further use.

8.8. Injection or administration of pituitary extract:

Determination of correct dosage of pituitary extract to be given to the breeders depends upon the size and state of maturity of the recepient as well as upon the state of maturity of the donor fish. It has been found that the potency of the extract is influenced by the size, age and the sex of the donor fish. One set of breeders usually consists of one female and two males.

Method of injection: For fish pituitary extract, intramuscular injection (Figure-4) is given usually in the region of the caudal peduncle, a little below the lateral line. The needle is inserted under a scale parallel to the body of the recipient fish and then the muscle is pierced at 45^0. Clinical needles no 24, 22 and 19 are used for breeders of about 1kg, 1.3 kg and 3 kg body weight respectively. For administering the injection, the breeder is wrapped in a hand net and is placed on a cushion. At least two persons are needed for giving the injection, while one holds the head of the breeder firmly against the cushion, the other presses the tail with one hand and injects the extract.

Generally, the female is given an initial dose of 2-3 mg of dried pituitary per kg body weight and another dose of 5-8 mg per kg body weight after an interval of 6 hours. Males are given only a single dose of 2-3 mg per kg of their body weight. After the injection, the breeders are released immediately in the breeding hapa or a cistern.

Breeding hapa (Figure-3) is a box-shaped container, fixed with the help of four bamboo poles in a pond. The hapa is made of fine-meshed markin cloth or close – meshed mosquito net cloth. A thick cloth is not recommended as it would restrict proper circulation of water causing suffocation. The meshes of the cloth should not be large to allow the eggs and milt to pass out through them. The hapa is usually of the size 3-5 m x 1-5 m x 1.0 m, and is closed on all sides, except a part of the roof which can be opened or closed when required, using loops or buttons. The hapa is fixed to the bamboo poles in such a way that about 1/3 of it remains above the water surface, and its lower surface should be above the muddy pond bottom. Cemented cisterns of the required size having arrangement for circulation of water are also used in place of cloth hapa.

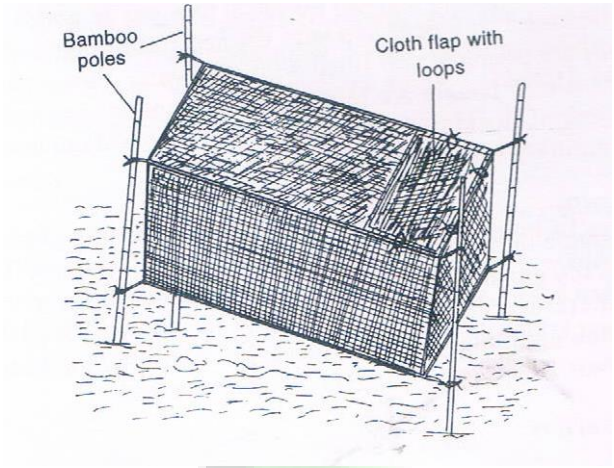

Figure 3: Breeding hapa

After 2-3 hrs of the second injection, the breeders start swimming actively, become excited and restless. Males start chasing the female, pushing her with the snout. Spawning usually occurs within 6 hrs of the second injection. Although injection may be given at any time in the day, it is better to select a cool, cloudy day and injection is given in the evening when the temperature is low. Spawning occurs at midnight or a little later, but the hapa should not be disturbed till the next morning for observation of eggs. A few eggs are examined, and if unfertilized they appear opaque and whitish. The fertilized eggs are crystalline, transparent and look like a pearl. They come up to the surface on a slight movement of water and are transferred to hatching hapas for hatching.

Figure 4: Method of Intramuscular injection

8.9. Collection of fertilized eggs and their transfer to hatching hapa:

- The fertilized eggs are collected by means of a plastic bucket or mug.
- Then transferred to another bucket.
- The collected eggs are transferred to a hatching hapa (inner). Hatching hapa consists of two separate halves i.e. inner and outer.

- Hatching hapas (Figure-5) have average measurements of 2 x1 x 1 m for the outer hapa and 1.75 x 0.75 x 0.5 m. for the inner one.

After 24 hours, each embryo hatches into a hatchling. Then the hatchlings pass out through the mosquito netting cloth and are placed at outer hatching hapa. The inner hapa contains only the shells of the embryos and outer hapa contains hatchlings.

Figure 5: A hatching hapa

9. Use of natural and synthetic hormones in induced breeding of fishes:

After introduction of hypophysation, several methods of artificial inducement by several hormone extracts were practiced for breeding the fish up to 100% perfection. They are as mentioned below:

9.1. Human Chorionic Gonadotropin (HCG):

HCG is a glycoprotein hormone which is produced by the placenta in the pregnant woman. During early pregnancy, the hormone appears in the urine in large quantities. When it is injected to mature fish, the hormone is known to cause maturation and release of gametes. The action of inducing sperm release and ovulation is a joint action, synergistically with the circulating pituitary hormones. When HCG is injected singly, it is not so effective as when it is injected together with pituitary gland extract.

9.2. Sumaach and Synahorin:

INFAR (India) Ltd. has brought out a product which is cheaper as compared to the pituitary gland extract and has a long shelf life. The product is grinded in distilled water (2mg in 0.2ml) and the mixture is centrifuged. The supernatant is then used for injection.

Dose: First injection is given to the female which is followed by the second injection given simultaneously to both the female and male. The first dose is smaller. The second dose is given together with the pituitary gland extract. The dose is weight dependent. For best results, fully ripe breeder and favorable weather condition is required.

9.3. Synahorin:

It is another preparation of chorionic gonadotropin and mammalian hypophysial extract.

9.4. Ovaprim (salmon gonadotropin RH):

"Ovaprim" is a preparation of salmon gonadotropin RH and Dopamine antagonist in a stable solution. It is prepared in glycerin and alcohol at particular proportion. This pioneering work was first done by Dr. Richard Peter of the University of Alberta, Canada. He found that dopamine, a neuromodulator of the hypothalamus acts as an inhibitor in the synthesis and release of gonadotropins from the pituitary in the fish. In the hypothalamus dopamine neurons have synaptic connections with that of gonadotrophic releasing hormone (GnRH) neurons. Thus, the inhibitory signal from dopamine neurons can be transmitted to the GnRH neurons through the synaptic connections. This hormone is available commercially since 1988 and is extensively used. These are introduced into the fish with a dose of 0.3 to 0.5 mg /kg body weight of female and .01 to 0.3 mg / kg body weight of male. In India, good results have been observed in the use of it in Uttar Pradesh, West Bengal, Bihar, Assam, Madhya Pradesh, Andhra Pradesh, Karnataka, Kerala, Odisha and Maharashtra. It has been observed that it is better than pituitary extract. Production of eggs from "rohu" achieved by use of pituitary extract is 1.15 lakh whereas with the use of ovaprim it has increased to 1.41 lakh. Ovaprim can be stored at ambient temperature even in the tropics for more than a year.

9.5. Pimozide and LH RH-A

Pimozide is a dopamine antagonist having ovulatory role of LHRH-A. It is quite effective on Indian major carps. The LHRH (Luteininsing hormone releasing Hormone) and its analogue (LHRH –A) are very effective on brakish water fishes (*Mugil* and *Lates)*. They are cheap but, at present preparations are short-lived. Their application shall await production of long lasting preparations.

9.6. DOCA (II-Desoxycorticosterone – acetate):

DOCA is another effective drug which has been tried on catfish, *Clarias* and *Heteropneustes*. They are a bit different in the sense that they not only cause ovulation but may also bring about maturation of eggs.

Antiestrogen Tamoxifen: (I-CP-(Beta-dimethyl aminoethoxy) phenyl)-1, 2 – transdiphenyl but-1-ene) has given good results on coho salmon especially when administered in conjuction with a primer (pituitary extract).

Factors influencing induced breeding:
Favorable climatic and hydrological conditions increase the chances of successful breeding. Failures are mostly due to incorrect choice of breeders, wrong doses of pituitary extracts and unfavorable climatic conditions. Generally hot, salty or sunny days are not suitable for undertaking induced breeding. Environmental factors such as light, temperature, water condition etc. are known to play important roles in stimulating the release of pituitary gonadotropins within the organism and thereby controlling reproduction of fish.

10.1. Light:
Light is an important factor in controlling the reproduction of fishes. Early maturation and spawning of fish takes place as a result of enhanced photoperiodic conditions.

For example: *Salvelinus fontinalis* attain early maturity under environmental conditions of short light periods and delayed maturation under long light periods. In India, *Cirrhinus reba* is observed to attain early maturity in day time.

10.2. Temperature:
The role of environmental temperature on the sexual maturation and breeding of fish has been studied by several investigators. All observations show that there are optimum temperature ranges for induced breeding of cultivated fishes and critical temperature limits, above and below which fish will not reproduce. Warm temperature plays a primary role in stimulating the maturation of gonads in a number of fishes and also accelerates spermiation. Thus it seems that temperature has a direct effect on gonads, an indirect effect on the gonads regulating their ability to respond to pituitary stimulation and effects the pituitary synthesis and release of gonadotropins.

The Indian major carps have been observed to breed within a range of temperature varying from 24^0 C-37^0C and optimum temperature is 27^0C Chaudhary, (1968). It is found that breeding was very poor above 30^0C. It seems probable that pituitary injection at higher temperature, in addition to ripening the sexual products, may imparts the necessary nervous stimuli for spawning and the subsequent lower temperature may provide a favorable external environment for ensuring maximum fertilization and embryonic development.

10.3. Dissolved oxygen (DO_2):
High dissolved oxygen is most important for hatching as they require more oxygen. Many fishes do not breed in water which is poor in oxygen contents. Renewal of water induces them to breed.

10.4. Water current and rain:
Rheotactic response to water current is well established for fishes. Rain becomes a pre-requisite to spawning of major carps, even when they are injected with pituitary extract.

Rainy Season:
It is seen that more the monsoon→ more rain→ more water current→ more stimulation→ more maturation→ more gonadal activity.

10.5. Cloudy weather:
Successful spawning in majority of fishes has been induced on cloudy and rainy days especially after heavy shower. This factor is highly essential because the weather remains cool and cloudy weather attracts fishes.

10.6. pH:
The carps are found to breed at a fairly wide range of pH. For successful breeding alkaline pH is necessary.

Advantages of induced breeding:
Several advantages are there in induced breeding. These are

i. A high quality fish seed of a particular species can be produced.
ii. The fish with maximum growth rate can be produced by genetic manipulations.
iii. Huge number of seeds can be produced i.e. 5,00,000 to 1 million at a time.
iv. At a time, several breeders can be bred at a single location with different species types.
v. Hybrids with high growth rate can be produced by artificial inducement and by applying several genetic techniques like gynaegenesis, androgenesis, sex reversal etc.

Example: Jayanti (carp): It is a carp produced at Central Institute of Freshwater Aquaculture (CIFA) following Scandinavian technique of induced breeding. The seed can be produced based on the time and the site of demand.

Conclusion

Success in inducing major Indian carps to breed in confined waters by injection of fish pituitary gland hormones is an important landmark in the history of fish culture in India. It has revolutionized the age-old practices of fish rearing and has provided vast scope for the development of pond fish culture, not only in India but also in other Asiatic countries where fish culture is practiced more or less on the same lines as in India.

With further improvement of the breeding and hatching techniques and standardization of doses, it would be possible to meet a larger requirement of quality fish seed in the country. The method is a simple one and can be learnt by private fish

culturists with conscientious effort. For popularization of the technique, sufficient numbers of demonstration centers have to be engaged all over the country with the help of the Fishery experts.

The need of artificial production of fish seed is expected to grow tremendously in near future as there is every possibility of scarcity of fish seed in the natural habitats. With the rapid industrialization in India, large numbers of factories are being established and consequently large quantities of factory wastes are also discharged into the rivers throughout the country. These factory effluents pollute the waters and adversely affect the riverine fisheries.

Besides, construction of dams across rivers have restricted to a large extent the migration of fishes and has brought about considerable changes in the environment and natural spawning grounds of many fishes, thus depleting the fishery in those waters.

Lastly, the frequent natural calamities brought about by flood in most of our rivers during monsoon months are affecting spawn collection from rivers considerably. Embankments constructed across many rivers to prevent flood have also destroyed many natural spawning grounds of carps.

Considering the above adverse factors, it is apprehended that in future the riverine fisheries will be impoverished more and more and the chance of procuring sufficient quantity of fish seed from those areas will be remote. This dependence on the artificial breeding of fishes will be felt all the more.

In conclusion it may be suggested that more emphasis should be given to develop the technique of induced breeding of fish and popularize it all over the country. It is a fact that the first and foremost prerequisite for successful intensive fish cultivation and development of Inland Fisheries is an assured supply of pure quality of fish seed. This method should profitably be utilized in making the important economic estuarine varieties of fishes to breed and obtain seed for brackish water fish farming.

The experiments so far conducted have been highly encouraging and have shown great promise for further research. Concerted efforts are being made to perfect the techniques, and it is believed that earnest endeavors and co-operation from all quarters would lead to the production of fish seed of desirable varieties on a commercial scale to meet the demand for fish seed in India.

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