

## INDUCED BREEDING/ SEED PRODUCTION AND HATCHERY MANAGEMENT

### INTRODUCTION

When the breeding is not allowed to occur naturally but is induced artificially in fishes such a process is called induced breeding. Availability of required quantity of fish seed of desired species is one of the most important pre-requisite for successful fish farming. The widely cultured Indian major carps in inland waters like catla (*Catla catla*), rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and calbasu (*Labeo calbasu*) and Chinese carps, silver carp (*Hypophthalmichthys molitrix*), big head (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idella*), black carp (*Mylopharyngodon piceus*) and mud carp (*Cirrhina molitirella*), normally do not breed in confined waters. They do mature there, but only breed in the flooded shallow areas along the course of the rivers during monsoon months which are their natural habitat. The Indian major carps do spawn in the specialised environments of bundhs, both wet and dry where lot of rain water is accumulated during monsoon period. Under such circumstances the fish culturists had to depend for the fish seed on collection from river systems and the collected fish seed consisted of not only of desired species, but also of uneconomic species including predators. The inability of Asiatic carps to breed in confined stagnant waters is owing to the lack of needed ecological stimuli to effect secretion of required quantity of gonadotrophic hormones and so extraneous hormones such as pituitary extract or synthetic hormones are injected to brood fish to induce them to breed. The first success in inducing the Indian major carps was achieved in 1957 (Chaudhuri and Alikunhi, 1957) and in silver carp and grass carp introduced in India in 1959, in the year 1962 (Alikunhi *et al.*, 1963).

### Collection and Preservation of Pituitary Gland

In India, generally, pituitary glands are used as an inducing agent to breed Indian major carps and Chinese carps. The glands are collected from fresh gravid fishes as well as fishes well preserved for a period of a week or so. Using a sharp butcher's knife the scalp is removed exposing the brain. The entire brain is lifted carefully, disconnecting the olfactory and optic nerves. On removal of the brain, the pituitary gland can be seen covered with a membrane, thin or thick and removed with a pair of forceps. Sufficient care has to be taken while removing the gland, so as to avoid any damage to the gland. Glands are also collected from the fish heads cut off from the body of the fish. While cutting the head, posterior part of the

cranium also is cut, leaving sufficient space to remove the brain and collect the gland. After removal, the glands are immediately kept in absolute alcohol in phials or bottles for preservation. After 24 hours the glands are kept in fresh absolute alcohol. The glands of females and males can be preserved together and also the glands of Indian major carps and Chinese carps. The glands are kept under refrigeration for long duration but for short period may be kept in room temperature. Absolute alcohol dehydrates and defattens the glands. The glands may be weighed individually or in small lots before hand for use and their record kept. Common carp is a good donor of glands. Since in tropical countries they mature throughout the year, year round collection is possible. Glands from induced bred fishes, soon after spawning are also collected, as they are found potent, but glands from immature fishes are normally not used.

### **BROOD STOCK REARING**

An adequate stock of brood fishes has to be built up and reared into prime mature condition for the success in hypophysation or induced breeding. The prospective spawners have to be reared with proper care 4–5 months ahead of the breeding season. The brood stock ponds may be 0.2 to 0.5 ha in size, capable of retaining 1.5 m water in peak summer. The ponds must be cleared of weeds and other fishes by pumping out water or by poisoning and stocked with healthy spawners of 2–4 kg weight at the rate of 1000 to 2000 kg/ha. The age of the fishes must be above one year, but preferably 2–3 years old, since in India, the Indian major carps and Chinese carps are generally found to mature when they are about 2 years old.

The ponds are suitably treated initially with lime, organic manures and inorganic fertilizers periodically depending upon the nutrient level of the ponds. The fishes may be provided with supplementary feed consisting of groundnut oil cake and rice polish (1:1 by weight) and the quantity of feed given daily may be 1–3% of the body weight of the fishes. In case algal bloom develops, supplementary feeding and manuring may be suspended till normal water conditions are restored. Pumping out the water and then adding fresh water into the ponds are desirable since this is conducive for breeding.

Indian major carps and Chinese carps can be reared together, but it is better, not to stock catla and silver carp in the same pond. In case they are stocked together, their condition has to be regularly monitored. The males and females also can be stocked together but if sufficient ponds are available they can be stocked separately and so also, Chinese carps and Indian major carps. The grass carp has to be provided with suitable aquatic weeds like Hydrilla, Najas or duck weeds at the rate of 20 to 25% of their body weight daily. Feeding with napier

grass or hybrid napier grass or other tender terrestrial grass appears to help in proper maturation of grass carp. The fishes are examined monthly to find out the stage of maturity and also to see whether they are healthy and free from diseases and remedial measures are adopted if found not satisfactory.

Instead of feeding with traditional oil cake and rice polish (20 to 22% protein) formulated feed prepared with the above in combination with fish meal, dibasic calcium phosphate, multivitamins, vitamin C and E and trace elements with a protein level of 30% carbohydrate 35% and fat 11% at the rate of 3% of the body weight of stocked fishes gives better results.

### **BREEDING TECHNIQUE**

#### **Indian Major Carps**

##### **Selection of breeders**

Induced breeding is generally taken up at the onset of monsoon, with the accumulation of fresh rain water, when the temperature goes down. The spawners are netted out and selected carefully and are weighed individually. The males are distinguished by the roughness of the pectoral fin, whereas in the female, it is smooth. By gently pressing the belly near the vent, if the milt oozes out freely or easily, the males can be selected for injection. The females can be recognized, because of their more bulging abdomen. If they possess a fully bulging abdomen which is soft to touch but not loose and having slightly swollen pinkish vent, they may be chosen for injection under normal conditions. By using a catheter, a sample of ovarian eggs (oocytes) may be collected and examined to find out the ripeness of oocytes.

##### **Dosage of pituitary injection**

Depending upon the ripeness of gonad and prevailing climatic conditions, the dosage of pituitary extract to be administered is decided. When the recipient is in prime condition, a very low dose is sufficient to precipitate spawning. Normally, a slightly higher dose is tried during the beginning of the season, which may be reduced subsequently and by the fag end of the season the dose is again increased. The females are given the pituitary extract in increasing quantities in split doses of generally two, whereas the males receive only one injection. The two split doses in the case of female may vary from 2–4 mg/kg body for the first injection and 5–10 mg/kg body weight for the second injection. The interval between two injections generally is 6 hours and males get the one injection along with the final injection to the female and the dose may range from 2–4 mg/kg body weight. If the females are in prime condition even with the low dose of first injection they may breed. The dosage also will depend upon the potency of the glands used.

**Preparation of pituitary extract and injection**

After deciding the dosage, the quantity of glands required for injecting the spawners is calculated. The required quantity of glands is taken out. The excess alcohol allowed to evaporate for a minute or so and weighed if not already weighed before and macerated in a homogenizer in a known volume of distilled water or 0.3% saline solution. The homogenized gland is then diluted to the desired volume. The dilution is made at the rate of 0.2 ml/kg body weight of recipient fish. The pituitary extract is then centrifuged and only the supernatant solution is used for injection.

The pituitary extract may also be prepared before-hand in bulk and stored and ampouled and kept in refrigerator. In this case the extract is prepared in distilled water and preserved in glycerine in the ratio of distilled water and glycerine 1:2 (Ibrahim & Chaudhuri, 1966). If the pituitary gland extract is prepared in bulk, it can be utilized in a large number of sets and the results will be comparable as the potency of the extract is uniform, unlike in the extracts prepared at each time.

**Breeding**

After the first injection, a set consisting of one female and two males is released in the breeding hapa. Breeding hapa is a rectangular cloth container, stitched with close meshed cloth, having an opening on one side (breadth side) through which breeders are introduced and taken out and can be securely tied. Breeding hapas are fixed in the marginal waters of ponds, canals, lakes and reservoirs. While fixing the hapas, water bodies having common carp and tilapia are avoided and so also ponds recently-manured and having algal blooms.

Breeding generally takes place within 3–6 hours after the final injection to females. Stripping method is not followed, since the Indian major carps breed fully after injection with very high percentage of fertilization.

**Silver Carp and Grass Carp****Selection of breeders**

Healthy males which readily ooze milt by a gentle pressure are generally preferred. In males, the pectoral fins are rough to touch whereas in females, smooth as in Indian major carps. In females the general criteria followed for selection is soft, round and bulging abdomen with slightly swollen and pinkish genital opening. A catheter is helpful in selecting the females of silver carp and grass carp. Inserting the catheter some oocytes are taken out and examined in the field by keeping them in petridish. Silver carp female with uniform size of eggs very pale blue in colour, but slightly pinkish in smaller ones are usually selected for breeding. The use

of catheter is more helpful in selecting grass carp females to ensure that they are gravid since well-fed females may appear mature. Females having brownish or copper coloured eggs of uniform size are preferred as they will be in a better stage of maturity.

### **Breeding technique**

The breeding technique followed in silver carp and grass carp is almost the same as in Indian major carps (Alikunhi *et al.*, 1960 and Chaudhuri, 1960).

The first success in induced breeding of both the species was achieved in July, 1962 (Alikunhi *et al.*, 1963), and subsequently in 1963, silver carp which were one year old, were successfully induced bred (Alikunhi *et al.*, 1965) and their offspring again matured in one year (Sukumaran, 1969).

The females are given pituitary gland extract at the rate of 10–14 mg/kg body weight and males, 3–4 mg/kg body weight; two injections to females in increasing doses and one to males as in the case of Indian major carps. The injected fishes, one set consisting of one female and two males are introduced in the breeding hapas.

The females are examined 3–4 hours after the final injection to find out whether they are ready for stripping. Keeping the ventral side up by a slight pressure at the genital opening, if the eggs are soon oozing out, the fish is ready for stripping. At times there is vigorous movement of fishes and then some eggs are released. Otherwise the fish are released back and examined again after half an hour or one hour depending upon the condition. Dry method of stripping is followed, when the fishes are wiped with a towel to remove the excess of water and the eggs are stripped into well cleaned dry enamel basin and immediately fertilized with milt. With the help of clean feathers, eggs and milt are mixed thoroughly for one or two minutes and some water is added and subsequently washed 3–4 times to remove the excess of milt or any other foreign material. The eggs are then kept in breeding hapas for proper swelling and water hardening.

When grass carps are allowed to spawn themselves after receiving pituitary injections, high percentage of success is achieved, whereas in silver carp, rarely fertilized eggs are obtained and if fertilized, the percentage is low (Chaudhuri *et al.*, 1967).

In silver carp males, quantity of milt obtained often is insufficient and so extra males injected and kept separately are used when necessary.

On cool rainy days, free release of eggs have been observed in grass carp and silver carp even with a single low dose of 3–5 mg/kg body weight of pituitary extract and the eggs could be fertilized with uninjected males.

### **Multiple breeding**

Under natural condition major Indian carps and Chinese carps breed once in a year during monsoon. Till a few years back, it was the impression that the induced breeding of the females can be carried out only once in a year. However, this concept has changed, since most of these carps were made to breed a second time in the same season from the second half of seventies. Bhowmick *et al.* (1977) could breed catla and rohu at Cuttack and Rao and Mahanta (1978) could succeed in rohu and mrigal at Assam and at Bhavanisagar (South India) in mrigal by another team of workers, a second time in the same season in an interval of 30–60 days. In the case of *Labeo fimbriatus*, a medium size carp, two breedings in the same female during the season was achieved in Kerala, South India in the year 1980 and 1981 (Sukumaran *et al.*, MS). This success is normally achieved in fishes breeding early in the season, when they are properly looked after.

Sen Gupta *et al.*, (1984) has stated that silver carp and grass carp which have bred in February/March, become mature a second time and they could be hypophysed a second time during May-July and the spawners lay more eggs during the second breeding.

Chen (personal communication) says that in Malaysia in silver carp and big head two or three spawnings could be obtained in one year, whereas in grass carp two spawnings are recorded.

In natural waters in China, Chinese carps spawn once in a year. The carps under cultivation can spawn several times in a year. This change in reproduction cycle is induced by the change in environmental conditions. Intensified culture of post spawner is carried out when temperature is above 20°C. The brood stock will reach maturity again and thus grass carp could be bred three times in a year from April to August (Anonymous, 1980).

### **Breeding period**

Under natural conditions major Indian carps breed in flooded rivers during monsoon season. Normally, this period ranges from April to August/September in different parts of India. Extensive and intensive induced breeding of these fishes and also Chinese carps in India coincide with the same period.

However, when examining the induced breeding experiments conducted in Indian major carps and Chinese carps from late fifties to this time, it was found that there are occasions when fishes could be successfully bred for a longer period than generally thought possible. At Cuttack Station of CIFRI, rohu, *Labeo rohita* could be bred till the end of September in certain years - Alikunhi *et al.* (1965), could breed silver carp from June to September in 1963. Breeding of silver carp upto November in 1978 was reported by Singh *et al.* (1979).

Grass carp could be induced to breed in the month of September in 1980, though the general successful period is till August. Most probably continued monsoon for a longer duration would have been responsible for this, providing favourable weather conditions.

Sukumaran *et al.* (MS) could breed *Labeo fimbriatus* from April to August and then in November in 1980 and 1981 in Kerala and the same species is successfully induced bred in Tamil Nadu in October, apart from breeding them in July-August.

Silver carp and mrigal were induced to breed in the months of March and April at Rahara Centre of Central Inland Fisheries Research Institute (CIFRI). At Jaunpur (Uttar Pradesh) and Karnal (Haryana) Centres of All India Coordinated Research Project on Composite Fish Culture, rohu, mrigal and silver carp could be induced to breed 40–50 days ahead of monsoon at Jaunpur under water temperature of 38°C.

Sen Gupta (1984) could breed successfully silver carp, grass carp and Indian major carps from last week of March to September. Sukumarn and Rahman (1984) could breed mrigal by hypophysation in the month of December 1983, bringing the fish into proper maturity by injecting pituitary extracts in the month of November.

### **BREEDING ENVIRONMENT AND CONDUSIVE ECOLOGICAL FACTORS FOR INDUCED BREEDING**

In India injected breeders are normally released in hapas fixed in marginal waters of ponds, lakes, reservoirs and in canal for breeding. The water will be stagnant except in canal systems and water temperature can shoot up to 35°C and exceptionally to 38°C in certain parts of the country, where it is not raining for 2 or 3 days. However, with the advent of hatchery system in several parts of the country, though still limited, better facilities are available in some stations, where running water or circulation of water with provision to control water temperature to some extent are possible.

During breeding of Indian major carps in ponds by hypophysation, the weather and water conditions studied at Cuttack Station of CIFRI indicated that spawning takes in clear and turbid water at temperatures ranging from 24–36°C. Chaudhuri (1968) is of the opinion, cool raining days with the water temperature of 27°C as optimum, whereas Ibrahim *et al.*(1969) found best results at 30 to 33°C. Alikunhi *et al.* (1964) obtained better spawning at controlled temperature of 28°C compared to field conditions where temperature was 3.1 to 5.6°C higher. Sukumaran *et al.*, (MS) could breed *L. fimbriatus* as early as 10th April in Kerala under controlled temperature of 29–30°C. Though Indian major carps breed in wide range of

temperature under hypophysation, high percentage of fertilization and better hatching are observed when water temperature is 30°C or below.

In silver carp and grass carp rain, sufficient accumulation of rain water, sudden rise in water level and water temperature below 30°C are found conducive to spawning in hapas fixed in stagnant pond water (Chaudhuri *et al.*, 1966), water temperature above 33°C usually affected spawning and hatching of eggs and brood fish kept in hapas (Singh *et al.*, 1970).

While conducting experiments in a pond with only accumulated rain water of the season and with continuous slow rise in water level due to the flow of rain water into pond, almost cent percent breeding of silver carp and grass carp was achieved on three consecutive days by Sukumaran *et al.*, (MS) in water temperature 24–28°C.

At Rahara Centre of CIFRI, silver carp and mrigal were successfully induced to breed in March and April under controlled temperature of 25–26°C. Sen Gupta *et al.*, (1984) used deep tube well water of constant temperature of 28–29°C after mechanically oxygenating it to breed Indian major carps and Chinese carps in modified version of Chinese hatchery system successfully from March to September. Wu and Chung (1964) while describing the technique of Chinese carps stated that running water (flow of 0.2–0.4 m/second) proves effective in stimulating the fish to spawn and water temperature between 20–30°C (preferably 23–29°C) is considered as important. Sinha *et al.*, (1974) reviewed the factors responsible for carp breeding in nature and by hypophysation and indicated the role of water/flood in lowering down the electrolytic level of water causing gonadal hydration for successful spawning. They put forward a hypothesis regarding the spawning of carps, wherein they suggested that during induced breeding, gonadal hydration was achieved by additional amount of exogenous pituitary extract, whereas in natural spawning, a sudden drop in electrolytic level in water by flood or heavy monsoon rain induced hydration of the fish as a whole and consequently the gonad and those fish which are ripe in condition started spawning in nature.

It is well-known that when fish move from water of higher electrolytic level, i.e. from sea water to fresh water, they gain water and thus gain weight and reverse is true when they come from lower electrolytic level to higher level. In rivers and reservoirs, there is marked increase in electrolytic level during summer months and this level decreases suddenly after monsoon rains. Further, the total water content of the ovary, the appearance of fluid in space between the follicular cells, loosening of adhesion - between the cells and eggs and increase in diameter of eggs suggest that these morphological changes accompanying ovulation are preceded by a remarkable absorption of water into ovary and ovarian eggs. Whether the condition

prevailing in ponds during the spawning season favour such absorption of water or not needs investigation, especially because these carps do mature in ponds, but do not spawn there. The water in these ponds is perhaps not that much diluted with rain or flood water as that of river where they normally breed. In this context, it is important to note that males spermiate in ponds but females do not ovulate there. This may be because the sperms have no mechanical barrier to water movement unlike the chorion which may mechanically oppose or support the entry of ions and water to egg for its final maturation (Sinha, 1980).

### **FISH PITUITARY GLANDS AND THEIR SUBSTITUTE**

In India, the glands of major Indian carps, Chinese carps and common carp are commonly utilized for hypophysation technique. Glands from other fresh water, brackish water and marine fishes were tried in Indian major carps but not in Chinese carps.

The glands of fresh water catfishes like *Pangasius pangasius*, *Silonia siondia*, *Bagarius bagarius*, *Mystus seenghala* and *Wallago attu* when injected @ 10–16 mg/kg body weight induced successful spawning. At the same time the pituitary glands of *Channa* sp., *Tilapia mossambica* and *Netopterus chitala* when administered @ 8–20 mg/kg were ineffective in females.

The pituitary glands of marine cat fishes like *Arius* sp., *M. gulis* and *Tachysurus* sp. @ 12–20 mg/kg body weight failed to induce spawning; whereas *Tachysurus* sp. gland extract administered @ 20–30 mg/kg body weight has been reported to effect spawning in Indian major carps.

HCG (Organon) when injected to *L. rohita* @ 460–2010 IU/kg body weight did not precipitate spawning. However it has been reported that *L. rohita* could be bred by injecting HCG @ 600 IU/kg body weight in a few cases.

In the case of silver carp, successful spawning could be achieved by injecting HCG (Organon) alone @ 630–660 IU and also with HCG 240 IU + 12 mg carp pituitary per kg body weight.

Synahorin along with carp pituitary gland was successful in breeding rohu and silver carp, but failed to induce spawning when tried alone in rohu.

Limited experiments conducted on rohu and silver carp with Hce 766 Vet + Progesterone have yielded positive results.

### **HATCHERY SYSTEMS**

At present an integrated system is developed wherein breeding and hatching eggs and rearing of hatchlings for a few days are taken up as a part of single system.

### **Modern Indian Hatchery**

The system consists of a unit of breeding tanks of metal, cement or plastic pools with cooling tower or air conditioners to control the water temperature and a slow continuous flow of water may or may not be maintained. The water may be filtered by means of an in situ filter and a pump is used to pump in water to the storage tank.

The carp hatchery unit consists of source of water where *in situ* filter can be kept, pump, storage tank, aeration facilities with an air blower or compressor, vertical hatchery jars made of glass, galvanized iron sheets or polythelene, water supply system from the storage tank to the hatching jars and spawnery or hatchling storage tanks (water temperature control is optional). The pilot hatchery at CIFRI, Cuttack and the one used at Malampuzha, Kerala and hatchery model CIFE-D-81 come under this category with some modifications.

### **Chinese Hatchery**

As per this system, breeding and hatching are taken up in cisterns which may be circular and with running water. There are separate sections for breeding and hatching eggs and large volume of water is necessary. It is an open system wherein temperature is not controlled, a combination of ground water and canal water or pond water are used. Gravitational force is normally used for the eggs to get into the hatching cistern where in continuous circulation of water is maintained.

### **Hatching**

The hatching techniques of carps eggs have undergone a lot of changes to get better hatching and survival from the earthen hatchery pits and cement hatchery pits at spawn collection centres. Use of double hatching hapas is being replaced by running water systems, with or without temperature control. The water hardened eggs are measured, their number calculated and a sample is examined to find out percentage of fertilization. Under field conditions, the eggs are kept in double-walled hapas, outer made of this close-meshed cotton or nylon and inner made of thin round-meshed mosquito net. The inner hapa is smaller, leaving gaps of 15 cm on all sides and also at bottom between the inner and outer hapa. In an outer hapa of the size 2 m × 1 m × 1 m and a suitable size inner hapa a total number of 50,000 to 100,000 eggs can be kept for hatching depending upon fertilization. The eggs are distributed in the inner hatching hapa uniformly. The hatching time is 16–18 hours in the case of Indian major carps and nearly two hours more in silver carp and grass carp at optimum water temperature range of 26–31°C. The hatchlings pass through the inner hapa to outer hapa and inner hapa is

removed. Inner hapa with wooden frame is found quite convenient for uniform distribution of developing eggs. Floating hapas are also used for hatching purpose.

In a vertical jar hatchery, hatching jars with a capacity of 6.0 to 6.35 litres, 50,000 eggs can be kept and a rate of flow of water is maintained at 600–800 ml per minute for Indian major carps and 800–1000 ml for silver carp and grass carp. In hatching jars of higher capacity more eggs can be introduced but the flow of water has to be suitably increased. The hatching time inside the hatchery is nearly 2–3 hours less than in the field.

Chinese hatchery:

The Chinese hatchery system is meant for incubating very high density of eggs i.e. 7,00,000–12,00,000 eggs per cubic meter and provided with running water. This consists of hatching trough/cistern with filtering screen, nozzles, water inlet and outlet valve and fry collecting compartment.

### **CONCLUSION**

In recent years it was possible to breed the carps by hypophysation technique in India almost throughout the year, except in the month of January. However, the breeding success in the month of February and December is extremely limited. The prolific season continues to be South-West monsoon months, i.e. from May-August/September in different parts of the country. Successful breeding of Indian carps in October to December is confined to South India where winter season is mild and consequently water temperature does not fall very low and day is not much reduced.

Multiple breeding of Indian major carps at different parts in Indian and in Chinese carps to some extent also has been achieved. In China, grass carp is bred thrice in a year from April-August with appropriate care when the water temperature is above 20°C. In Malaysia also Chinese carps are seen to mature throughout the year and more than one spawning during the year is reported.

Proper hatchery facilities are needed to breed the fishes. Under natural condition, a certain percentage of carps are seen to mature ahead of the season and with proper management of brood stock still higher percentage can be obtained. In the regions where the influence of South-West monsoon and North-East monsoon is felt, there is every possibility of breeding all the cultured important species, during both the monsoons, though presently during north-east monsoon breeding is limited and confined to some species in South India.

Separately rearing the offsprings obtained from early breeding and late breeding and monitoring their growth and maturation cycle may culminate in evolving strains which can

breed early and late, whereby desired fish seed in adequate quantities are available throughout the year which may serve to tide over the paucity of the fish seed which hampers fish culture often.

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