# STUDIES ON INDUCED BREEDING OF SOME CARPS FOR SEED PRODUCTION IN FISH SEED HATCHERY OF BOUDH

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## CHAPTER 1: INTRODUCTION

### 1.1: INDUCED BREEDING:

When the breeding is not allowed to occur naturally but it is induced artificially in fishes it is called induced breeding. Availability of required quantity of fish seed of desired species is one of the most important requisite for successful fish farming. The widely cultured Indian major carps in inland waters like Catla,Rohu ,Mrigal and Chinese carps like silver carp, grass carp, mud carp, black carp etc. normally do not breed in confined waters. They do mature there , but only breed in the flooded shallow areas along the course of rivers during monsoon months which are their natural habitat. The Indian major carp do spawn in the specialized 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 fish seed 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 gonadotropic 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 induced breeding in fish in India was made by Hamid Khan in 1937 when khan tried to induce spawn *C. mrigal* by the injection of mammalian pituitary gland. Later H.L Chaudhuri succeeded in induced spawning of small carp species *Esomus danricus* by administering the intra-peritoneal injection of Catla pituitary gland. The first success to inducing the Indian major carps was achieved in 1957 ( Chaudhuri and Alikunhi,1957) and Silver and Grass carp introduced in India in 1959, in the year 1962( Alikunhi et al.,1963).Since then several experiments on induced breeding of fish have been carried out.

Induced Breeding (IB) is the most significant advancement in the field of aquaculture to induce reproduction in fish. It is a technique to stimulate ripe fish breeders by pituitary hormone or any other synthetic hormone to breed in captive condition by promotion of timely release of sperms and eggs. Farmed carp seed production has increased from 6,321 million fry in 1985-86 to over 45,000 million fry in recent year.

India is the third largest farmed fish producer ranking second globally. The current fish production in India has reached 8.3 million tons, which expected to reach 12.5 million tons by 2025. Aquaculture is the fastest growing food producing sector and by 2025, one out of two fish eaten may come from aquaculture. Quality fish seed is a key factor for increase of aquaculture production. Fish seed from natural sources of spawning process uncertainty in availability, quality, mixing of predatory and weed fish seeds. The technique of induced breeding helped in mass production of quality carp seed under controlled condition and assured timely supply of India is the third largest farmed fish producer ranking second globally. The current fish production in India has reached 8.3 million tons, which expected to reach 12.5 million tons by 2025. Aquaculture is the fastest growing food producing sector and by 2025, one out of two fish eaten may come from aquaculture. Quality fish seed is a key factor for increase of aquaculture production. Fish seed from natural sources of spawning process uncertainty in availability, quality, mixing of predatory and weed fish seeds. The technique of induced breeding helped in mass production of quality carp seed under controlled condition and assured timely supply of stocking material for culture farms. Induced breeding is based on the principles of manipulating hormonal

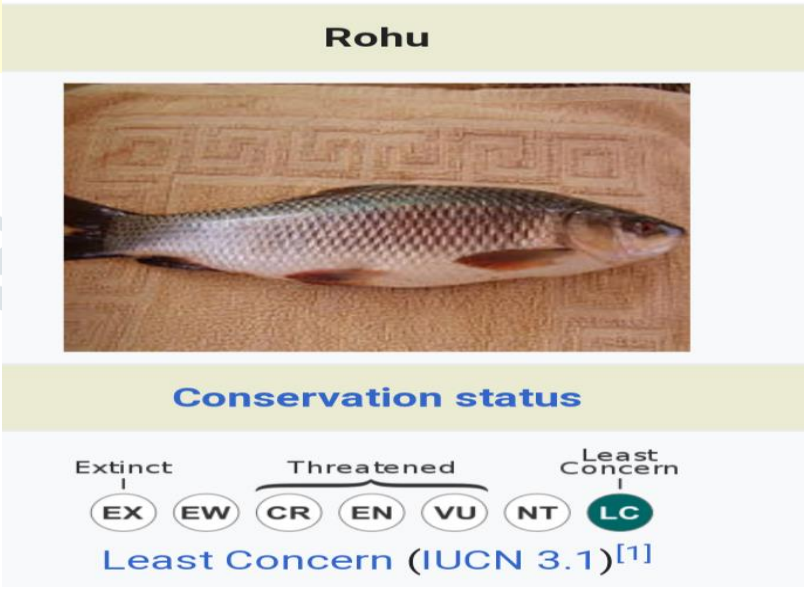
or environmental factors for stimulation of reproduction in fishes. The need for the production of quality fish seed to stock artificial ponds and natural water bodies through artificial propagation has steadily been encouraged , as it is the only practicable means of producing enough quality fish seeds.

Scarcity of carp spawn is a major problem for fish farmers in village to stock their ponds with seed collected from rivers and due to lack of technical knowledge and basic infrastructure facility, such as hatchery system ; induced breeding of carps was rarely adopted by farmers. The contribution of several researchers starting from hapa breeding to cemented eco-hatchery, and then to portable FRP hatchery have made easy in availability of fish seed for aqua-farming. For timely production of quality seed , "ICAR-AICRP on plasticulture engineering and technology" center at ICAR-CIFA, Bhubaneswar has designed and developed portable fibre glass reinforced plastic ( FRP) carp hatchery[9-14] and it has been installed and successfully operated in 26 states of India. The system is designed to create an environment friendly atmosphere for fish breeding in the farmers 'field. It is capable to breed 10to 12 kg of female carps and equal quantity of male in one operation in one run, 1.0-1.2 million spawn can be produced from the hatchery which is sufficient to stock pond area of about 30 hectares with stocking density 5000 fingerlings/ha. There are several benefits of hatchery viz., it is portable, easy to install and operate, requires less quantity of water during fish breeding and spawn production, needs less space for installation and the durable for about fifteen years. In lean lesson, the hatchery can be used for ornamental fish rearing in which village women may take part in leisure time. One complete operation of the hatchery for 1.0 million spawn production requires $90m^3$ water. This hatchery can be a tool for biodiversity conservation through production of seed of endangered and threatened fish species

The present work was carried out in Fishery Office of Boudh through induced breeding of carps in Fish seed Hatchery of Boudh. I have taken two fishes *Labeo rohita* (Rohu) and *Cyprinus carpio* ( Chinese carp).

1. Fish 1( Rohu):

| Scientific classification | |
|---|---|
| Kingdom | Animalia |
| Phylum | Chordata |
| Class | Actinopterygii |
| Order | Cypriniformes |
| Family | Cyprinidae |
| Subfamily: | Labeoninae |
| Genus | Labeo |
| Species | L. rohita |
| Binomial name | |
| *Labeo rohita* | |
| F.Hamiltion, 1822 | |




IDENTIFICATION:

Body moderately elongate (Figures) Mouth inferior and lips thick and fringed (Figure), with distinct inner fold. Fin ray counts: dorsal (3/12-13), pectoral (17), pelvic (9), anal (7) and caudal (19). Dorsal fin inserts anterior to pelvic fins and ends in line with or slightly anterior to anal fin. Scales cycloid and moderate in size. Lateral line with 40-44 scales. Body colour blue to brownish along the back, silvery on the sides and belly. There is a red mark on each scale during the breeding season and the fins become grayish or black. up to the juvenile stage, where after it changes rapidly because of the change in feeding habit (i.e. from zooplankton to plant feeding). For example, the RGL for rohu fingerling is about 4, juvenile is 6, small mature adult 10 and in large mature adults the gut may be 12 times the body length(DasandSrivastava,1979).

Male and female rohu attain first sexual maturity at two years of age and 50 percent maturity is attained at three years. Complete maturity of male and female is observed at age four and five years respectively (Khan and Jhingran, 1975). The minimum size at first maturity of male is about 46 cm and in the case of female it is about 51 cm. One hundred percent maturity has been found in 65 cm length group in the case of male while in the case of female hundred cent percent maturity has been found in 70 cm group (Chondar, 1999). During the breeding season the pectoral fins of mature males are rough on the dorsal surface, while those of females are smooth. Rohu is highly fecund (109 000 to 535 000 eggs per kg) (Chondar, 1999). The species breeds naturally in rivers, reservoirs, and in large lakes in which fluvial conditions are simulated. The breeding season generally coincides with the southwest monsoon. The fish can be spawned through hypophysation with 2-3 mg pituitary/kg body weight followed by a second dose of 5-8 mg/kg after an interval of 4-6 hours. Males are given only a single dose of 2-3 mg/kg at the time of the second injection given to females. Fish generally spawn within six hours of the second injection.

The alimentary canal of rohu consists of the mouth, buccal cavity, pharynx, oesophagus, intestinal bulb, intestine and rectum. The free edges of the lips are broad and have four to five rows of conical papillae. The buccal cavity is dorso-ventrally compressed. The mucous membrane lining the buccal cavity contains minute papillae. The pharynx is well demarcated into an anterior respiratory part and a posterior masticatory section, that has well developed molariform pharyngeal teeth and the teeth are all alike (homodont) and arranged in three rows on the inferior pharyngeal bones.

The oesophagus is short and a true stomach is absent. The anterior part of the intestine, behind the oesophagus, is modified into a sac like structure (intestinal bulb) in which food is stored. There are no gastric glands in the intestinal bulb. The intestine is extremely long (Figures 3a and b). The length of the intestine relative to body length (relative length of gut - RLG) increases slowly from fry stage rohu have low the intestinal bulb. The intestinal fluid has a pH ranging from 6.8-7.1 in the intestinal bulb, but decreases to a range of 6.2 to 6.5 RLG i.e. (0.7-1.0) and the value varies between 1.1–2.5 for fingerlings and the adults have value ranging from 2.6 – 19 (Sinha and Moitra, 1975). The intestine is more or less uniform in diameter and forms a number of coils (Figures 3a and b). Elongation of intestine and its extensive coiling are related to its herbivorous feeding habit and the intestinal coil may provide a large surface for absorption of food. The terminal part of the intestine is slightly dilated and forms the rectum. Pyloric caecae are absent.The absence of stomach is compensated by the production of pancreatic trypsin and erepsin as well as enterokinase from intestinal mucosa and the digestive function is performed in the anterior part of in the hind gut. The intestinal bulb serves the role of a store house for the acceptable food materials. Protein digestion occurs under pH 7.0 to 7.1.

2-FISH 2( Chinese Carp)

| Scientific classification | |
|---|---|
| Kingdom | Animalia |
| Phylum | Chordata |
| Class | Actinopterygii |
| Order | Cypriniformes |
| Family | Cyprinidae |
| Subfamily: | Labeoninae |
| Genus | Cyprinus |
| Species | C.carpio |
| Binomial name | |
| ***Cyprinus carpio*** | |
| Linnaeus, 1758 | |

**Conservation status**


Vulnerable (IUCN 3.1)[1]

IDENTIFICATION:

Body elongated and somewhat compressed. Lips thick. Two pairs of barbels at angle of mouth, shorter ones on the upper lip. Dorsal fin base long with 17-22 branched rays and a strong, toothed spine in front; dorsal fin outline concave anteriorly. Anal fin with 6-7 soft rays; posterior edge of 3rd dorsal and anal fin spines with sharp spinules. Lateral line with 32 to 38 scales. Pharyngeal teeth 5:5, teeth with flattened crowns. Colour variable, wild carp are brownish-green on the back and upper sides, shading to golden yellow ventrally. The fins are dusky, ventrally with a reddish tinge. Golden carp are bred for ornamental purposes. Carp generally spawn in the spring and early summer depending upon the climate. They segregate into groups in the shallows to spawn. Carp prefer shallow waters with dense macrophyte cover. Males externally fertilize eggs, which the females scatter over macrophytes in a very active manner. The eggs stick to the substrate upon which they are scattered. A typical female (about 45 cm) may produce 300,000 eggs, with some estimates as high as one million over the breeding season. Incubation is related to water temperature and has been documented at three days at temperatures of 25 to 32C. Fry average 5 to 5.5 mm in total length. Temperature, stocking density, and availability of food influence individual growth. By the time the fish reach 8 mm the yolk has disappeared and they begin to actively feed. Males typically become sexually mature at 3 to 5 years and females at 4 to 5 years. (Froese and Pauly, 2002; McCrimmon, 1968)

## 1.2: Necessity of Induced Breeding:

The demand for fish for food, recreation and ornamental aquariums is steadily increasing natural fish populations have declined during the cast several decades because of environmental degradation like photoperiod rain, temperature ,currents of water and over fishing etc. this has resulted in an increased effort in the development of increased effort in the in the development of techniques for hatchery production of fish traditional aquaculture species such as trout, cat fish ,common carp, golden shiner and goldfish reach sexual maturity and spawn in hatcheries or ponds, when conditions are appropriate.

However ,a number of fish species that have not or potentially have great economic significance for aquaculture do not reproduce spontaneously in captivity many of these fish spawn in environments that are nearly impossible to stimulate in hatchery hormone induced spewing is the only reliable method to induce reproduction in these fishes

Induced breeding is a technique where organism is stimulated by particular hormone or other synthetic hormone or by providing condition .introduced to breed in captive condition .the stimulation promotes timely release of sperms and ova from ripe gonads .

Induced breeding is necessary for fish culture because it gives pure spawn of certain species of fishes under cultivation.

## 1.3 History of induced breeding in Boudh.

The Boudh district came into exist stance after the district reorganization notification published during 1994-95 it has 3 blocks namely -Boudh sadar, Harabhanga & kantamal.There are four rivers passing in the district i.e, Mahanadi,Telnadi,Baghnadi,Salunki.

The following are the brief information about the dist and its pisciculture resources.

a) Geographical area of the Dist.3440.70 Sq.km
b) Total population of the Dist. 373088
c) Total no. of village-1152
d) Total no of fisherman village -158
e) Total no of fisherman-4939

Male-2466, Female 2473

The District establishment of fisheries is situated in Boudh Govt. fish seed farm campus at Murusundhi which is 4km away from the Boudh town area. It is running laboratory cum farm building. The District establishment of fisheries has been running of 22 staffs comprising to its headquarters as well as functionaries at block level.

First aim of the District Fisheries Office is to produce and Provide disease free, pure quality fish and fresh water seed to the fish farmers for their Pisciculture tanks Secondly, to develop the unutilized water lodging, derelict water bodies or low productive, low lying land( Govt. & Private owned tanks) into a culturable status by excavation or renovation providing financial assistance through bankable schemes with subsidy of FFDA for promoting the production of pisciculture in the district.

The fisherman community residing on the riverbanks or reservoir, who are completely depend to maintain their livelihood on capture fisheries, the dept. of fisheries through its district networks providing technical schemes for their fishing equipment with bank finance on Govt. subsidy( SGSY,SCST FDC) etc. The District Fishery office through FFDA has been providing training to the fisherman for implementing intensive pisciculture scheme i.e, fish seed production, hatchery management, fishing in MIP and reservoir etc.

The District fisheries install new fish rearing projects in order to meet the fish seed demand of the District. By using new technologies like induced breeding it boost the fish production and to meet the per capita fish consumption of the District.

## 1.4: Hormones used in Induced Breeding:

In fish, hormones play a critical role in the reproductive process. Hormones are the chemical messengers released into blood stream by specific tissues, such as pituitary gland.The hormones travel through the bloodstream to other tissues, which respond in a variety of ways. One response is to release another hormone which elicit a response in yet another tissue. The primary tissues involved in this hormonal cascade are hypothalamus, pituitary gland and gonads.

Numerous hormones have been used to induce reproduction in fishes, Mainly the injection of a GnRH analog with dopamine antagonist and injection of Gonadotropin.

### =>GnRH analog with Dopamine antagonist:

Leutinizing Hormone Releasing Hormone (LHRH) is the name of mammalian hormone that has been employed successfully to induce the reproductive hormonal cascade. In recent years, synthetic analogues of LHRH, referred to as LHRHa, have been developed that are far more effective, because they are purer and are not rapidly metabolized by fish, they remain active for longer periods.

### =>Gonadotropin:

Two types of Gonadotropin extracts are used to induce ovulation in fish. Human Chorionic Gonadotropin(HCG) and fish pituitary extract. Pituitary extracts are made by removing the pituitary from a fish and extracting the hormones,whichmay then be injected into another fish. HCG offers three major advantages over the pituitary extract:

1) It is much less expensive

2) It is more stable and thus has longer shelf life

3) It comes in a purified form

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.

=>Ovaprim:

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 act as an inhibitor in the synthesis & release of gonadotropin from the pituitary gland 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.

Ovaprim is a mixture of the analogue of salmon gonadotropin releasing hormone (SGnRHa) and a dopamine antagonist domperidone (Leelapatra, 1988). The administration of ovaprim was based on Linpe method, which was developed after extensive research on the combined effects of the LHRH analogue, sGnRH in combination with a dopamine antagonist such as domperidone or pimozide on the Chinese bach (Lin et al., 1987a 1988) common carp (Lin et al., 1987b, 1988) and Chinese carps (Peter et al., 1987). Ovaprim, which is marketed by Syndel laboratories Ltd of Canada has been successfully tested on Thai carp Puntius gonionotus in Thailand (Leelapatra, 1988), the Indian major carps (Nandeesha et al., 1990), Channel catfish Ictalurus punctatus in the USA (Goudie et al., 1992), walking catfish C. batrachus in Malaysia (Cheah and Yeo, 1994) and Sand whiting Sillago ciliata in Australia (Battaglene, 1996). SGnRH analogue (Glu-His-Trp-Ser-Tyr-DArg6-TrpLeu-Pro-NH-CH2-CH3) has amino acid change in the sixth and tenth position of the native form (Glu-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly NH2). D-Amino acids, which are mirror image form of the naturally occurring L-forms, have been substituted at position b making the molecule much more resistant to degradation in the body and therefore it is long lasting. Ovaprim acts at pituitary level leading to the secretion of endogenous gonadotropin whereas in the case of hypophysation technique, exogenous gonadotropins have been introduced into the body (Habibi et al., 1989). 52 Endogenous gonadototropins appear to significantly enhance the secretion of the right type of steroids in abundant quality enabling complete maturity of ova for spawning. Peter et al., (1988) reported that ovaprim is 17 times more potent than the mammalian LHRHa. This increased effectiveness of SGnRHa predicted to have higher affinity for the binding site on pituitary (Habibi et al., 1989). An additional advantage of domperidon is that it does not cross blood brain barrier in teleosts (Omeijaniuk et at., 1987) there by reducing the probability of detrimental side effect in the injected fish. Trails conducted with ovaprim on number of species of fishes administering in a single dose to both male and female brooders simultaneously yielded encouraging results in inducing complete spawning (Nandeesha et at., 1990).

=>OVATIDE

Ovatide is a newly launched ovulating agent developed by an Indian company (Hemmo pharma, Mumbai) which was successfully tested by Central Institute of Fisheries Education (ICAR) Mumbai in several places of India during 1997. Ovatide is a synthetic preparation containing salmon GnRH analogue and dopamine antagonist. It is composed of 20 pg of salmon GnRH and 10 mg of domperidone/mi. It has been successfully tested in C. catla, L. rohita, C. mrigala, C. idella, L. calbasu, P. javanicus, T. putitora, T. musullah, T. khudree by Thakur and Reddy (1997); 0. pabo by Mukherjee and Das (2001); C. carpio by Sarnia et at., (2000) and C. catla by Pandey et at., (2000).

Wova-FH


**WOVA-FH™**

It is an alternative to traditional practice. Traditionally, the farmer has been using pituitary gland for controlled spawning in fishes. However, usage of pituitary gland has its own disadvantages. The probability of success is low because it is practically not possible to maintain uniformity in the product. Besides, it causes handling and post-breeding stress to the brooders, which may lead to death. It is also difficult to handle, store and transport, above all, there is uncertainty about the availability. Problems like those as mentioned above have always forced the farmer to search for alternative products. Wockhardt, one of the largest pharmaceutical companies in India with state of the art Research and Development Centre, has come up with **WOVA-FH™**- Synthetic Gonadotropin releasing hormone analogue (SGnRH), a product developed through years of research.

**WOVA-FH™** can be used for induced breeding in carp and catfish. Performance of **WOVA-FH™** has been assessed in some of the government and private hatcheries in Andhra Pradesh, West Bengal, Orissa and other places. Results proved that **WOVA-FH™** is a very effective synthetic fish hormone for induced breeding.

**Composition:**

Synthetic Gonadotropin Releasing Hormone Analogue (SGnRH).

**Advantages:**

Observed advantages of environ-ac over other probiotics

- High fertilization & hatching rate.
- Single dose administration.
- No stress to the broodstock.
- Easy injectability.
- Cost effective.
- Stable at room temperature and long shelf life.
- Easily available and ready to use.

**Dosage and Application:**

| Species | Sex | ml/kg body weight |
|---|---|---|
| Rohu | Male | 0.10 - 0.30 |
| | Female | 0.30 - 0.50 |
| Catla | Male | 0.10 - 0.30 |
| | Female | 0.30 - 0.50 |
| Mrigal | Male | 0.10 - 0.30 |
| | Female | 0.30 - 0.50 |
| Chinese carp | Male | 0.10 - 0.30 |
| | Female | 0.40 - 0.80 |
| Silver carp | Male | 0.10 - 0.30 |
| | Female | 0.40 - 0.80 |
| Cat Fishes | Male | 0.20 - 0.40 |
| | Female | 0.60 - 0.90 |

**Storage and Caution:**

**Storage**

Store below or at 25oC.

# CHPTER 2

## 2.1:MILESTONES IN THE FIELD OF INDUCED BREEDING

Induced breeding is a technique by which the economically important fish generally which do not breed in captive condition, are bred through artificial stimulation. Hormone induced technique for fish spawning has been used for almost 60 years. Surprisingly, with the same procedure only minor modifications have been used to spawn. A primary requisite for adopting advanced techniques in fish culture is to ensure adequate production of fish seeds under controlled conditions. Procurement of quality fish seeds in large scale is crucial input in aquaculture enterprises and their timely availability is an essential prelude to any ambitious plan of completing the aquaculture development project. The commonly adopted technique for induced spawning of carp is hypophysation.

Induced spawning 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 Brazillian researches could succed in inducing ovulation by pituitary gland injection. Induced breeding in fish developed by Von Ihering of Brazil in(1937). This technique was followed by

America and Russia Gerebilisky,(1938). This technique is widely used by various worker. It observed that the Brazilian pisciculturists like Houssay(1931), Iherring(1937), Frontencle(1955), were the first to use fish pituitary gland for induced breeding in their indigenous fishes.

Traditional methods of induced spawing for culture fish are based on the injection of GTH-II from different sources including extract of carp pituitary gland , partially purified fish GTH-II and mammalia GTH especially Human Chorionic Gonodotropin (HCG) [ (Lam,1982); Donaslon &Hunter 1983; peter et al 1988 ]. Among these hormones,fish GTH-II is generally of high species specificity. Thus, for example, carp or salmon GTH-II are infective in others, such as grass carp and black carp (Lin & Peter in 1996).

Several researches and seed producers in the private sectors soon adopted the technology during last decades. A recent development in the technology of induced breeding is the stimulation of endogenous gonadotropin release from the pituitary of the treated fish using of synthetic analogue of gonadotropin releasing hormone, GnRH. (Anonymous,1977)

A major breakthrough in fish breeding research was the advent of Dopamine, which act as an inhibitory factor for the synthetic of Gonadotropins (Peter et al 1986). Several hormones and chemical agents intervene at different levels of hypothalamus-pituitary-ovarian-axis in maturation & breeding. Gonadotropin releasing hormones, a peptide in turn triggers the hormonal cascade of the brain-pituitary gonadal maturation ultimately depends on the endocrine system. Hormonal manipulation appears as the most direct approach to brood stock development.

Most species of teleost fishes are seasonal breeders and only a few breeds continuously. Among the seasonal breeders there is variation in the time of the year. Fish integrate their reproductive activities with seasonal environmental cycles and certain environmental factor viz. Temperature, Photoperiod & Rainfall act as cues for the approaching season which is favorable for the reproduction signals from environmental cues and endogenous physiological cycles accelerate the neuroendocrine system, which in turn regulates pituitary gonadal functions (Line & Peter 1996).

Snakeheads breed naturally during southwest monsoon and northeast monsoon in flooded rivers & ponds ( Haniffa et al; 1996,2000).But monsoon failure often limits the seed production. Hence ,to facilitates a steady supply of seeds , oocyte maturation & ovulation need to be induced.

In using Hormones for artificial reproduction it should be considered that several items such as side effects of mixture of hormones , the cost of some hormones , also at the time of treatment , the fish should be at an advanced .stage of gonadal development. Otherwise hormone injection of improper of time or insufficient of the dosage may damage or even will kill the fish. Almost in all cultured fishes , hormonal manipulations may be used as management tools to enhance the efficiency of egg production , increase spermination & facilitate hatchery operations. Also in artificial fertilization for genetic selection programs .(Mylonas et al 2010).

The first successful induced breeding in Indian major carps was reported in India in 1957( Chaudhuri & Alikunhi (1957). Chaudhuri & Alikunhi induced spawning in five carps at Cuttack station of Central Inland Fisheries Research Institute through intramuscular injection of carp pituitary extract. Chaudhuri(1960) standardized hypophysation technique for induced spawning of Indian major carps. Alikunhi et al (1963) succeeded the induced breeding of Chinese siver carp & grass carp in the ponds of Cuttack. Parameswaran & Murugesan (1976) used carp pituitary glands for induced breeding in Murrels , but due to inadequate supply of two cumbersome methods of collection and preservation of pituitary gland, their attempts were not cent percent successful.

The bulk of Indian fish seed supply is derived from riverine collection, which almost inevitably comprise a mixed lot and is uneconomic. As such seeds are available at some specific centers located on river only.

Procurement of pure seed of cultivable fishes from a dependable source posed a problem in India until the technique of hypophysation was successfully applied in 1957-59 at Cuttack.

## Chapter 2.2 Objective:

To have a review of hormonal manipulation in fishes, we may first have an investigation of the general hormonal function in reproduction and then we use different hormones in different fish species. The objective of the present study is a brief study of different hormones used in fish reproduction and listing that, the hypophysation technique and provide information on the induced breeding technique of some carps in fish seed hatchery of Boudh.

## 2.3: Aim of Future Research:

The mission of future research should be to solve the following problems encountered in hypophysation of fishes.

1) The foremost need is of isolation and determination of active ingredient of pituitary hormones of chemical purity responsible for breeding in different species of fishes. Such a development may lead to the synthesis of the fish gonadotropic hormone. To synthesize chemically pure gonadotropic hormones of fish pituitary in terms of measureable potency will indeed be a scientific feat for great destination.
2) There is a need to acquire through understanding of the physiological conditions of natural fish pituitary gonadotropin in relation to season, sex, age development of donor species.
3) It is necessary to understand cytogenetically fish maturity and ovulation brought about by the administration of pituitary hormone as well as the process of resorption which may lead to a more satisfactory solution to the problem of ovarian resorption.
4) The role of food on the development of pituitary itself needs through investigation
5) It is necessary to hybridization so as to evolve pure breeding stains and have predictable results.

## CHAPTER 3: MATERIAL & METHOD:

Indian Major carps like *Labeo rohita* and Chinese carp *Cirrhinus mrigala* were taken for the induced breeding.

The propagation of carps through induced breeding involves a chain of activities as follows:-

**1.SELECTION OF FISH**:-

ROHU:

A fully matured male rohu is about 65 cm length and female rohu is about 70 cm. During breeding season the pectoral fins of mature males are rough on the dorsal surface,while those of females are smooth. The male release a few drops of thick milt when its abdomen is pressed slightly. The females can be recognized by comparatively larger buldging abdomen. Those having a satisfactory buldging rounded and soft abdomen with slightly swollen and reddish vent can be cathetered to determine the condition of eggs before selection.

CHINESE CARP:

Shortly before and during spawning, the carp develops breeding tubercles appearing as small granules. In males, the tubercles appear primarily on the opercle, pre-opercle & under the eyes, although may present on the head, caudal peduncle, fin rays & the scales below the lateral line. In females, the breeding tubercle occurs only on the head.

**2.COLLECTION OF BROOD FISH:-**

Sexually ripe and healthy fishes are prerequisite for successful induced breeding. They can be collected from natural water prior to spawning season or farm stocking ponds. Generally potential breeders having 2-4 kg weight are stocked in brood fish pond at the rate of 100-200 kg per hectare, a few months before the commencement of spawning season. They are fed daily with rice bran and oil cake (1:1) at the rate of 1:1 of total body weight. The brood fish were fed one time a day in the morning or in the evening. Occasionally fishes are examined to determine their stage of maturity.

The brood fishes were collected and male and female were separated. Then they were injected with synthetic hormone Wova-FH by a hypodermic syringe for injection.

When the receipient nearer to the spawning stage, a very low dose may be sufficient to induce spawning, but when they are reserving a higher dose is require.

**3.INDUCING METHOD (DOSAGE OF INJECTION):-**

Female require more hormone than the males. A dose of 0.4 ml per kg body weight is administrated to the female Rohu and the male were given 0.2 ml per Kg.. A dose of 5.0 ml per kg body weight is injected to the females Chinese carp and the male carp were given 3.5 ml per Kg body weight. Care should be taken to handle the fish gently at the time of spawn.

**4. TIMING OF INJECTION**:

The brooders were collected at 5 pm and then male and female were injected hormone at 7 pm.

**5: BREEDING ENVIRONMENT**:

Immediately after injection ,the brooders were released into a breeding hapa for spawning. Usually one set comprising of two females and three males are introduced in a breeding hapa. The breeding hapa is a rectangular cloth container measuring usually 3.5*1.5*1.0 m stiched out of close meshed mosquito net or finely meshed muslin cloth having an opening on the top through which the brooders can be introduced and taken out and can be securely tied after introduction of fishes so as to prevent their escape. Breeding hapas are fixed to bamboo pole in the marginal waters of the pond and spawning takes place inside these cloth container

**6. HATCHERY OPERATION**:

One unit of FRP hatchery consisting of four parts *i.e.*, one breeding pool ,two hatching pools and one egg/spawn collection tank was installed at District Fishery Office, Boudh. The water supply was made to the hatchery from one cemented overhead tank of capacity 35$m^3$. The system operates with the principle of eco-hatchery. Breeding pool is 2.15m diameter,0.9m height,1:22 bottom slope and 3409 litre total capacity with operation capacity 2950 litre ; Hatching/incubation pool is 1.4 m diameter, 0.98m height,1400 litre total volume and 1200 litre net egg incubation volume with a FRP inner chamber of 0.4m diameter and 90 cm height covered with nylon blotting cloth to filter the excess water and Egg/Spawn collection chamber is 1.0m length,0.5m breadth,0.5 m height and 250 litre water holding capacity.

Carp eggs are non-adhesive, demersal and swells up generally to 3.5 to 5.5 mm in diameter. These eggs are removed from the breeding hapa after being properly hardened. Usually after 6-8 hours of fertilization the eggs are collected in baskets from breeding hapas. The total quantity of eggs is estimated by measuring the

total volume. The viable eggs appear transparent and dead eggs opaque. The eggs are then transferred to hatching cloth hapa or eco-carp hatchery.

A hatching hapa consists of two pieces of separate hapas, one fitted inside the other. The outer one is made of thick close meshed cloth netting while the inner is of mosquito netting. About 75,000 to 1,00,000 eggs are uniformly spread on the stretched bottom of inner hapa. The eggs hatch out in 14 to 18 hours after fertilization of a temperature of $27^0$ C to $31^0$ C. After hatching the larvae escape to the outer hapa through the meshes of inner hapa. These are removed when all the eggs hatched out. The hatchlings are left undisturbed till third day after hatching. By the time, the yolk gets almost absorbed and the young fry start feeding. They are collected and stocked in nursery tanks.

The carp fishes used for breeding trials reared in the same farm. Synthetic hormone Wova FH was used as the inducing agent for carp breeding. The dose of hormone was 0.4 ml/kg body weight of female and 0.2 ml/kg body weight of male. Latency period for egg release, effective spawning period, percentage of fertilization of eggs, hatching time, percentage of spawn recovery and spawn production per kg body weight of carps were calculated.

Latency period= Time between hormone administration and initiation of spawning in carps.

Effective spawning period=Time between initiation and stoppage of spawning.

Spawn production per kg body weight= Total spawn harvested / total weight of female.

Water parameters like pH, alkalinity and hardness were analyzed by APHA 2005 method and water temperature was measured by temperature probe.

## CHAPTER 4: RESULT AND DISCUSSIONS

Induced breeding of Indian major carp Rohu (*Labeo rohita*) and Chinese carp (*Cyprinus carpio*) was conducted from January to March 2019 for 21 times at District Fishery Office Boudh. Odisha. Total 198 lakh spawn was harvested *i.e.,* Rohu 125 lakh and Chinese carp 73 lakh from the breeding operation. Induced breeding was conducted for 11 times for *L.rohita* and 10 times for *C.carpio*. The results are shown in the Table-1 and 2. In this experiment of Rohu and Chinese carp 1.25 to 1.58 lakh egg/ kg bodyweight of female fish respectively. Effective spawning period for Rohu was 45 to 110 minutes and Chinese carp was of 50 to 95 minutes.

**Table 1: Induced breeding of Rohu ( *Labeo rohita*) at Fishery Office, Boudh, Odisha**

| Parameters | Number of male breeders | Number of female breeders | Total weight of male breeder (kg) | Total weight of male breeder (kg) | Time of first egg released after hormone injection(minute) period (A) | Completion time of egg release from time of injection given(minute)(B) | Effective spawning period (B-A)(minute) | Egg released(Lakh) |
|---|---|---|---|---|---|---|---|---|
| I | 6 | 6 | 7.6 | 8.4 | 390 | 460 | 70 | 13 |
| II | 6 | 5 | 7 | 7.3 | 390 | 470 | 80 | 9.5 |
| III | 6 | 6 | 8.1 | 8.7 | 375 | 450 | 75 | 12.5 |
| IV | 5 | 5 | 6.9 | 7.2 | 360 | 410 | 50 | 10 |
| V | 5 | 5 | 6.5 | 7 | 340 | 420 | 80 | 11 |
| VI | 8 | 8 | 10 | 12 | 370 | 480 | 110 | 20 |
| VII | 7 | 6 | 8.4 | 9.3 | 330 | 430 | 100 | 14 |
| VIII | 4 | 4 | 4.7 | 4.9 | 375 | 420 | 45 | 7.5 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| IX | 10 | 9 | 12 | 11 | 345 | 440 | 95 | 20 |
| X | 5 | 5 | 6.5 | 6.7 | 350 | 400 | 50 | 10 |
| XI | 8 | 7 | 8.4 | 9.1 | 380 | 450 | 70 | 14 |

**Table 2: Induced breeding of Chinese carp (*Cyprinus carpio*) at Fishery Office, Boudh, Odisha**

| **Parameters** | **Number of male breeders** | **Number of female breeders** | **Total weight of male breeder (kg)** | **Total weight of male breeder (kg)** | **Time of first egg released after hormone injection(minute)period (A)** | **Completion time of egg release from time of injection given(minute)(B)** | **Effective spawning period (B-A)(minute)** | **Egg released( Lakh)** |
|---|---|---|---|---|---|---|---|---|
| I | 4 | 4 | 5.8 | 7.0 | 375 | 440 | 65 | 10 |
| II | 4 | 4 | 6.2 | 6.5 | 360 | 420 | 60 | 8.5 |
| III | 8 | 7 | 9.0 | 9.6 | 350 | 430 | 80 | 14 |
| IV | 6 | 6 | 6.9 | 6.8 | 380 | 450 | 70 | 9 |
| V | 6 | 6 | 7.6 | 8.2 | 375 | 430 | 55 | 13 |
| VI | 7 | 7 | 8.5 | 8.7 | 345 | 440 | 95 | 12 |
| VII | 7 | 6 | 7.3 | 7.1 | 360 | 430 | 50 | 10 |
| VIII | 4 | 4 | 6.0 | 6.4 | 375 | 420 | 45 | 8 |
| IX | 10 | 9 | 12 | 11 | 345 | 440 | 95 | 20 |
| X | 5 | 5 | 6.5 | 6.7 | 350 | 400 | 50 | 10 |

Water parameters were found within suitable range for induced breeding of carps ( Table-3). Water temperature during breeding operations ranged 26.3 to 33.8. Total alkalinity and total hardness of hatchery water were found in ideal for carp breeding *i.e.*, 60 – 70 mg/ l and 50 – 60 mg/l respectively.

**Table 3: Physico-chemical parameters of intake water for hatchery operation at District Fishery Office Boudh**

| Sl. No | Species | Water Temperature$^0$C | pH | Total alkalinity( mg/l) | Total hardness(mg/l) |
|---|---|---|---|---|---|
| I | *L. rohita* | 28.4-33.6 | 7.3-7.7 | 70 | 60 |
| II | *L. rohita* | 27.9-33.8 | 7.4-7.9 | 70 | 60 |
| III | *L. rohita* | 28.6-33.4 | 7.4-8.0 | 70 | 60 |
| IV | *L. rohita* | 28.1-33.4 | 7.3-7.8 | 70 | 60 |
| V | *L. rohita* | 26.4-32.6 | 7.2-7.9 | 70 | 50 |
| VI | *L. rohita* | 27.3-33.4 | 7.1-7.6 | 60 | 60 |
| VII | *L. rohita* | 26.5-32.1 | 7.2-7.8 | 60 | 60 |
| VIII | *L. rohita* | 28.4-32.1 | 7.2-7.7 | 60 | 50 |
| IX | *L. rohita* | 27.1-33.9 | 7.3-7.4 | 60 | 50 |
| X | *L. rohita* | 26.8-33.2 | 7.1-7.5 | 60 | 50 |
| XI | *L.rohita* | 26.3-32.7 | 7.1-7.4 | 70 | 50 |
| I | *C.carpio* | 26.5-32.3 | 7.3-8.0 | 70 | 60 |
| II | *C.carpio* | 26.7-32.5 | 7.4-7.8 | 70 | 60 |
| III | *C. carpio* | 27.8-34.1 | 7.5-7.8 | 70 | 60 |
| IV | *C. carpio* | 28.2-33.6 | 7.2-7.5 | 70 | 60 |
| V | *C. carpio* | 27.7-33.5 | 7.3-7.8 | 60 | 50 |
| VI | *C. carpio* | 27.9-32.8 | 7.1-7.7 | 60 | 50 |
| VII | *C. carpio* | 26.8-32.6 | 7.0-7.6 | 70 | 60 |
| VIII | *C. carpio* | 26.6-33.2 | 7.2-7.5 | 70 | 60 |
| IX | *C. carpio* | 26.3-32.9 | 7.4-7.9 | 60 | 50 |
| X | *C. carpio* | 27.1-33.7 | 7.3-7.7 | 60 | 50 |

Odisha Watershed Development Mission(OWDM) conducted fish breeding operations in FRP carp hatcheries under the project " Western Odisha Rural Livelihood Project (WORLP)"

at Nuapada and Bargarh Districts (Sudhin,2007; Alan Casebow,2008). In its first year of operation in 2005, the hatchery supported to nurse 5.5 million fish seeds ,which in turn led to take up grow out culture fish in 530 ha of pond area in Western Odisha. The water quality and temperature regime were within the limits of hatchery operation in field condition. At Nuagaon, Nayagarh District of Odisha , the rohu breeder was transport from a reservoir 22 km away from the hatchery, reared in a less deep pond prior to breeding produced 0.8-0.925 lakh eggs/ kg of female (Mahapatra et al., 2011). In the present experiment the breeders of Rohu and Chinese carp were maintained in the same farm one month before the breeding. Percentage of fertilized eggs during spawning was found to be 90-95%. Spawn production per kg female body weight was found to be 1.07-1.36 lakh/ kg of rohu and 1.17-1.36 lakh/kg of Chinese carp.

## CHAPTER 5: CONCLUSION:-

The number of fish species currently under domestication efforts is rising up, due to the development of commercial aquaculture. To establish a sustainable aquaculture, it is necessary to stipulate the fish and control reproduction process of fish in captivity to acquire high quality seed. Different species of fish have their own physiological and characteristic in reproduction therefore by using separate protocol of hormones and to illustrate the exact dosage and hormonal combination.

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