COMPARATIVE EFFICIENCY OF CARP PITUITARY EXTRACT AND OVAPRIM IN BREEDING OF CATFISH, PABDA (*Ompok pabda*)

A Thesis
Submitted to the
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in partial fulfillment of the requirements for the award of the
degree of Master of Fishery Science
in

AQUACULTURE

By

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Dedicated to...

My Beloved Parents

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CERTIFICATE

This is to certify that the work embodied in the thesis entitled **"Comparative efficiency of carp pituitary extract and ovaprim in breeding of catfish, pabda (*Ompok pabda*)"** *submitted by* ***Mr. Avinash Kumar*** *in partial fulfillment of the requirements for the degree of* ***Master of Fishery Science (Aquaculture)*** *in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafied research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.*

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(Aquaculture)

We, the undersigned, have been satisfied with the performance of Mr. Avinash kumar, in the Viva-Voce Examination, conducted today, the 20th November., 2012, recommended that the thesis be accepted for the award of the Degree of Master of Fishery Science in Aquaculture.

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List of abbreviations

CPE	**:Carp pituitary extract**
PG	**: Pituitary gland**
GnRH	**: Gonadotropic releasing hormone**
LHRH	**: Leutenising hormone releasing hormone**
hr	**: Hour**
Max	**: Maximum**
Min	**:Minimum**
Ha	**: Hectare**
Beli	**: Spawn measuring cup**
ANOVA	**: Analysis of variance**
Cao	**:Calcium oxide**
M:F	**: Male :Female**
$KMnO_4$	**: Potassium permanganate**
GTH	**: Gonadotropic hormone**
Mg	**: Milligram**
MT	**: Metric ton**
Fig	**: Figure**
N/A	**: Not available**
HCG	**: Human chorionic gonadotropin**

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Ompok pabda, popularly known as "*Butter cat fish*", is a freshwater species native to India, Bangladesh, Pakistan, and Myanmar. This species is an indigenous catfish belongs to the family Siluridae of the order Siluriformes. It is listed as endangered fish species (CAMP, 1998). The fish has a wide geographical distribution covering Afghanistan, Bangladesh, Myanmar, the Indus plain and adjoining hill area of Pakistan, the North East States of India particularly in Bihar and West Bengal during the early 1970s. Due to rich lipo-protein content and soft bony structure this fish species is considered delicious and nutritious to the people of East India, North East India and Bangladesh as well (Fagede *et al.*, 1984; Demska-Zakes & Dlugosz, 1995; Pillay, 2000 and Jhingran, 2004).

This species occurs in the lotic and fresh water ecosystem (Banik *et al.*, 2011 and 2012). Also available in beels/mauns connected with rivers. In nature the fish breeds in rivers, reservoirs and water sheds, while in flooded condition during south west monsoon. However, in the early 1980s sharp decline in occurrence and abundance were observed (Bhowmick *et al.*, 2000 and Banik *et al.*, 2002). Despite its greater economic value this species did not receive sufficient attention in aquaculture. Meagere occurrence of live samples in nature and poor survival of the larvae are major constrains of the observations (Banik and Malla, 2011). As a result of indiscriminate fishing during monsoon, application of pesticide in agriculture, soil erosion and siltation formation in river, contamination of habit due to sewage and industrial pollution etc. The population of this species is sharply reduced (Chakrabarty *et al.*, 2009 and Banik & Bhattacharya, 2012).

The systematic position of ***Ompok pabda***, is ---

Phylum- *Chordata*

Class- *Actinopterygii,*

Order- *Siluriformes,*

Super family- *Siluridea,*

Family- *Siluridae (Sheat fish),*

Genus- *Ompok,*

Species- *O. pabda.*

The catfish belongs to the genus *Ompok* (La Cepede, 1803), mainly consists of medium-sized silurid fishes found in inland waters throughout South and Southeast Asia. The genus is traditionally diagnosed by the presence of a short dorsal fin with 4 rays, strongly forked caudal fin, subcutaneous eye, set immediately posterior to the mouth rectus and two patches of palatal teeth (Weber & de Beaufort, 1913). Bornbusch (1995) showed that *Ompok*, as currently understood, is probably paraphyletic. Distinguished by relatively short maxillary barbells (not extending beyond tip of pectoral fin) and 48 – 54 branched anal-fin rays. Males are more slender and have strong serrations on the posterior edge of the pectoral spine (females lack these serrations). *Ompok pabda* is highly priced, delicious, good taste, fine flesh with soft meat texture and high nutritional value and well preferred fish as possess relatively few bones. This species are silvery in colour with two one at distinguished spots, shoulder and another close to the base of tail; longitudinal stripes of dark colour often present on the sides of the body. This species which attains a length of 24 cm is caught in fairly large numbers and as a nourishing food fish especially for invalids; it fetches a very high price. The price is above Rs. 300/-per kg (US$1 = Rs. 52/-).

The species is recently prioritized as a new candidate species for freshwater aquaculture (Ponniah and Sarkar 2000; Ayyappan *et al.*, .2001). It is piscivorous as well as carnivorous and prefers to inhabit lakes, ponds and rivers. In India, It is widely distributed in the plains and sub mountainous regions. over the last decade, its wild population has undergone a steady decline (>50%), mainly due to overexploitation, loss of habitat, disease, pollution, siltation, poisoning, dynamiting, indiscriminate fishing during the breeding season, wide use of pesticide and insecticides from agriculture fields and other destructive fishing operations and changes in hydrology of the rivers due to human intervention.

Thus, *Ompok pabda* has been categorized as an endangered (EN) fish species according to IUCN based CAMP report (1998). It is necessary to develop captive breeding programme and culture techniques for these endangered species, in order to save them from extinction. It has not been received much attention in aquaculture mainly

due to non-availability of information regarding its breeding and culture technique. However, successes have been achieved in breeding and seed rearing of *Pabda* in India.

The Regional Research Centre of Central Institute of Freshwater Aquaculture, Kalyani, West Bengal has been working on the subject for the past five years to develop and standardize the breeding and seed production techniques of this fish. Recently this Center has successfully achieved mass breeding and seed production of *pabda*, after several trials.

In natural habitat, *pabda* achieves maturity at the end of first year of growth. The fecundity is as high as 8,400 to 13,800 nos./100g.of gravid fish weight. Since the availability of *pabda* seed in nature is poor and the procurement of seed is difficult, induced spawning has been adopted for its widespread propagation as well as large scale production of seed for extensive farming practices. Proper care and maintenance of broodfish is the primary step for successful breeding. Generally *pabda* weighing 40 g. and above (male & female) are considered as suitable for breeding. The sexes can be easily identified by examining the secondary sexual characters they develop during the breeding season. The abdomen of the gravid female is soft and bulged and genital papilla is fleshy and large in size. While in mature males the genital papilla is conical in shape. Mature fishes are generally stocked and raised up to the gravid stage in earthen ponds (0.01 – 0.04 ha). Lime applied at the @ of 500 kg/ha and the pond is manured with cattle dung @ 10 t/ha. In the broodstock pond, water *hyacinth* and *hydrilla* are grown to simulate the natural condition of their habitat. These plants provide as ideal shelter for the fishes and also serve as a source of periphytic food organisms. The depth of the pond was maintained between 1.0 – 1.5 m. The brood fishes are fed daily with a compounded feed of rice bran, oil cake and fish meal (1:1:0.5) @ 5% of average body weight of fishes under farming.

Artificial breeding:

The breeding season of *O. pabda* extends from May-August. The broodfishes of 35-40 g are usually selected for inducing them to breed successfully. Fishes of both sexes were induced by using either carp pituitary extract or the branded commercial product

"Ovaprim" The doses of Ovaprim varied at 1-1.5 ml/kg of female and male 0.5 ml/kg of male. The injected fishes were stripped after 10-15 hrs by gently squeezing the abdomen towards the tail. The eggs were collected in clean dry plastic /enamel/steel tray. A good female may yield ripe eggs to the extent of about 15-20 % of its body weight. The abdomens of males were cut open, testes were removed carefully. They were finely chopped and macerated to prepare sperm suspension. The collected milt was then added to eggs and thoroughly mixed using a feather. The eggs were cleaned by washing in freshwater and transferred to a flow- through system or a fiberglass tank with aeration facilities for incubation. The fertilized eggs were spread uniformly in a plastic tub or fiberglass tank with a water depth of 5 cm. A feeble current of water was created, having the support of an aeration facility. In about 30 minutes of fertilization of eggs, the blastodisc was formed. The yolk plug stage was attained in 5 hours. There was enclosed. A slight thickening of one end of the embryo indicated cephalic region. The notochord was formed rudimentarily in 8 hrs and the embryo acquired a kidney shape. In 18 hrs the caudal region appeared distinctly. The gut was faintly indicated, in a position posterior to the yolk sac. The egg hatched out in 24 hrs. The newly hatched larvae were cylindrical in shape. They were transparent with a pale greenish yolk sac, visible prominently. The yolk sac got absorbed in 3 days. After hatching, the larvae were collected by siphoning them out from the bottom of the hatching tank. The *O. pabda* larva with yolk measured about 4-5 mm in length. 4-7 days old spawn were fed with finely sieved zooplankton in alternate days and later with chopped tubifex worms. The fry attained 5-6 cm./3-4.5 g in a rearing period of 40-50 days with a survival of 80%. Fingerlings of this size are considered fit for stocking in grow-out farming ponds.

Breeding of *Pabda* is important; so as to stock them in natural habitats for restoring the gene bank of this endangered fish. Seed production and culture technologies also need to be developed for such non- conventional fish as Snakehead (Shoal and taki), climbing perch (Koi) catfish (Boal, Air, Rita) and Eel (Baim).

Objective:-

Ompok pabda fetch a good price in the market due to its palatability and nutritive value. Considering its economic importance and specially in the perspective of its rapidly declining population, efforts has been initiated to standardize the breeding and larval rearing of this unique species. Trial has been conducted by both the State Government as well as Kolkata centre of CIFA. All the trials were conducted using ovaprim as inducing agent, with not so satisfactory results. In this experiment we have used both Carp pituitary Extract and Ovaprim, on a comparative basis with the following objectives ----

1. Comparative evaluation of Carp Pituitary Extract and Ovaprim towards Spawning of Pabda (*Ompok pabda*) .
2. Standardization of the hatchings and larval rearing by placing the fertilized eggs in specially designe rectangular pool.
3. Standardization of larval feeding.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Back ground of fish breeding and hatchery operation

According to Andersen, O., and Hayes, B. (2005), 'The aim of fish breeding is not to change individual fish, but rather the fish population.' With the introduction of induce breeding technique for fish breeding, the technology has revolutionized the entire fishery industry. Since its introduction the technology for fish seed production in captivity was infused to innumerable hatcheries. Fish farming started 4000 to 5000 years ago in China. Artificial hatching of fish was already practiced in China around year 2000 B.C. (Lin, 1940).

In fact the technique of induce breeding proposed nearly 60 years ago by Prof. B.A.Housay of the University of Buenos Aires, Argentina and Dr. R. Von Ihering of the Department of fisheries, Brazil. In India, Khan (1938) was first to successfully induced *Cirrhinus mrigala* to spawn by injecting mammalian pituitary hormone. Dr. H.L.Choudhury (1956) succeeded in inducing *Esomus danricus* to spawn by intra-peritoneal injection of pituitary gland from *Catla catla*. Ramaswamy and Sundararaj (1956) succeeded in inducing spawning of *Heteropneustes fossilis* and *Clarius batrachus*. Later, several carps *Labeo rohita*, *Labeo bata*, *Cirrhinus mrigala*, *Cirrhinus reba*, etc. were successfully bred by injecting pituitary extract (Choudhury and Alikunhi, 1957). Ever since 1957, carp breeding by hypophysation is an established technology in India. An epoch discovery for carp seed production in confined waters revolutionized the fish seed production scenario in India.

Chakraborty *et al.* (2007a) reported that in nature the fish breeds in rivers, reservoirs and water sheds, while in flooded condition during south west monsoon. *Pabda* now shows a declining catch trend from the capture fishing ground of India. For this reason, it is enlisted as threatened species. The mandate of The Regional Research Centre of Central Institute of Freshwater Aquaculture, Kalyani, and West Bengal was to work on the subject for the past five years to developed mass breeding and seed production techniques of pabda fish. In the last year several trials had been conducted to standardise the breeding of pabda. In natural habitats, *Pabda* achieves maturity at the end of first year of the growth. The fecundity is as high as 8,400-13,800 nos./ 100g of fish weight. The species supported a strong fishery in North

Bihar and West Bengal during the early 1970s, but in the early 1980s sharp falls in catches were noticed, indicating swift declines in those areas. Consequently, *O.pabda* has been listed as an endangered fish species in India due to its decrease in abundance and restricted distribution. Causes of decline are likely to be indiscriminate fishing during the breeding season, wide use of pesticide and insecticides from agricultural fields causing pollution and siltation in habitat.

Khanam *et al.* (2005) mentioned fin fish hatchery was first established in Jessore by Mohoshin Master in 1967. Since then the number of fish hatchery increased uninterruptedly reaching over a thousand in 2010 to fulfil the ever increasing demand of the fin fish seeds for aquaculture industry of Bangladesh. This includes 126 govt. Hatcheries and a number of private hatcheries, most of which are located in Jessore, Comilla and Mymen district. Brahmaputra fish seed complex is a renowned hatchery in Mymensingh district (Bangladesh). This hatchery first produced huge amount of fry commercially from magur, shinghi, pabda, carps, koi and other species. In induced breeding, hormone doses differ according to different species. Some fish require single dose and some fish require double dose. Singhi (*Heteropneuestes fossilis*), pabda (*Ompok pabda*) gives egg naturally into the hapa and eggs are collected by siphoning and are kept into cistern for hatching. But in case of magur (*Clarias batrachus*) three different hormones (PG, HCG mix with PG, ovaprim mix with PG) were used to determine the effectiveness of hormones on ovulation, fertilization and hatching of magur. Here HCG mixed with PG was found to be effective hormone to maximize the ovulation, fertilization and hatching rate of magur. In Bangladesh, both public and private hatchery produced around 423986 kg hatching (DoF, 2008).

Sarkar *et al.* (2005) reported that the catfish, *Ompok pabda*, was successfully bred during 2002-2003 by inducing with ovaprim at two different doses (0.5 ml and 0.3 ml kg^{-1} body weight for female and 0.6 ml and 0.4 ml for male). The fish spawned 8-12 hours after single dose of injection. The spawn in time, fertilization rate, hatching rate, and survival rate were quantified in each set of breeding experiment. The latency period was less (8-10 hours) with 0.5 ml, where it was more (10-12 hours) with the dose of 0.3 ml kg^{-1} of body weight. All the female spawned successfully and the average rate of the fertilization (91.05%) and hatching rate (65.55%) was higher with dose @ 0.5 ml, where as lower fertilization (84.85%) and hatching rate (66.35%) was observed @ 0.3 ml kg^{-1} of body weight. The fecundity

ranged from 84-138 eggs gm^{-1} body weight. Eggs hatched between 14-15 hours after spawning at water temperature 29-30 ^{0}C. A total of 1, 64,185 hatchling were produced with a survival rate of 65.37% ± 2.25.

According to Mukherjee *et al.* (2002) for conservation of endangered fish stocks, some freshwater fish species like *Ompok pabda, Ompok pabo, Clarias batrachus,* and *Mystus gulio* etc. should be reared in captivity and on field trials should be conducted to artificially induce reproduction including larval rearing. Generally breeding is carried out in a bund where broodstocks are kept in hapa and conditioned for 24 hours. Two techniques are used for induce breeding, one with carp pituitary extract and second with a synthetic hormone 'ovatide' (Hemo Pharma, Mumbai, India). The dose of hormone used, as pituitary, was 16 mg/kg body weight and as ovatide 3.0mg/kg body weight. 'Ovaprim' is used in magur (sex ratio 1:1, doses 2.5 ml in female and 1.0 ml in male). The Synthetic hormone (ovatide) yielded best results, with spawning rate 80% higher than that of pituitary extract. Hatchery percentage was higher with ovatide and 28% higher than pituitary extract. The technique for induced breeding of *Pabda* and *Tangra* is comparable with the induced breeding of carp but special attention is needed during larval rearing as large mortalities occur mostly after 24 hours from hatching. Care should be taken for immediate removal of egg shell, which otherwise may induce higher mortality by deteriorating water quality.

Sharma, *et al.* (2012) reported that induced breeding and larval rearing of an endangered fish species like *Ompok pabda* should be carried out in captive condition using low cost technology. About 90% spawning was recorded and 76% fertilized eggs were collected through hapa breeding using ovatide (dose for male 0.5 ml/kg body weight and for female 0.6 ml/kg body weight) as inducing agent. Hatching of embryo are conducted in a series of earthen tubs fitted with continuous shower and renewal and removal of water. Hatching was occurred 22 hours after spawning. A total of 7,972 numbers hatchlings are obtained for further rearing.

Purkayastha, *et al.* (2012) reported that the catfish, *Ompok pabda* was successfully bred during 2008 by inducing with ovatide using two different doses (0.4 and 0.6 ml/kg of body weight of female) in the live gene bank facility (LGBF), Guwahati, Assam. The fish spawned 8-10 hours after single dose of injection. The spawning time, fertilization rate, hatching rate and survival rate were quantified in each set of breeding experiment. The latency period was 6-8 hours with 0.5 ml, where

it was 8-10 hours with the dose of 0.4 ml/kg body weight. All the female spawned successfully and the average rate of fertilization (75.5%) and hatching (60.5%) was higher with dose @ 0.6 ml/kg. While lower fertilization (65.1%) and hatching rate (45%) were observed @ 0.3 ml/kg of body weight. The fecundity ranged from 80 - 130 eggs gm^{-1}/body weight. Egg hatched 12-14 hours after spawning at water temperature of 28-32^{0}C.

Goswami, *et al.* (2007) reported about induced spawning of *Pangasius pangasius* in Circular Eco Hatchery (CEH) and studied the embryonic and larval development. Two breeding trials were conducted separately in altogether 18 fishes by administering synthetic ovatide hormone. Spawning response observed to a dose of 0.3-0.4 ml hormone per kg body weight (BW) in male and 0.6-0.7 ml per kg body weight in female. Hatching took place within 24-26 hours at 28-31^{0}C water temperature. Only 22-28% hatching success was recorded due to outbreak of fungal infection. Early embryonic development up to blastula stage was not distinct due to opaqueness of the egg. Formation of yolk plug was noticed at 8.2 hours. The hatchlings were light yellow in colour with a large yolk sac after 72 hours of fertilization. After 21 days of hatching the larvae developed structural configuration of adult. The larvae survived till 21 days in flow-through system in plastic and earthen tubs.

2.2. Status of hatchery operation in West Bengal

Species considered for induced spawning are mainly Indian major carps, Bighead carp (*Aristichthys nobilis*), Silver carp (*Hypophthalmichthys molitrix*), and grass carp, *Ctenopharyngodon idella.* Recently an enthusiasm has been observed for breeding of some exotic carps as well as indigenous catfishes.

2.3.Brood stock management

2.3.1. Age and size of brooders

Chakrabarty, *et al.* (2009) reported that the fish attained maturity at the end of the first year. Male matured earlier than females, which become mature at 30-40 g in weight.

Banik, *et al.* (2011) reported that maturity cycle of the *Ompok bimaculatus* species in first year. Mature females were induced bred and fertilized with spermatozoa suspension from the adult males. Ovaprim was used as at different dosages. 27-32^{0}C. Hatching time has been recorded @ 23-24 hrs after fertilization at about average fertilization rate is being72%.

Chakrabarty, *et al.* (2007b) reported that *Mystus vittatus* mature in the first year. This species matures during April-May in Bengal and Assam and have a fairly extended breeding season till the end of July.

Sarkar, *et al.* (2010) reported that three years old Pangus is better to ready for breeding.

2.3.2. Sex ratio

Mukherjee, *et al.* (2002) reported that for successful induced breeding of Pabda, free oozing males and ripe females in the ratio of 2:1 will be best.

Goswami, *et al.* (2007) reported that induced spawning of *Pangasius pangasius* in Circular Eco Hatchery (CEH). Two induced breeding trials were conducted. Male and female ratio used in the 1st trial was 2:1 and in the 2nd trial 1:1. With lower latency period and higher fertilization rate better result was obtained in the 2nd trial only. In the 1st trial surplus of milt in males were evident after the breeding.

Chakraborty, *et al.* (2007a) selected a ratio of 3:2 (male:female) for *Ompok pabda*. The brooders were injected with ovaprim, at a dose of 0.5 ml/kg body weight for male and 1.0 -1.5 ml/kg body weight for female fish.

Sarkar, *et al.* (2010) reported that for breeding the ratio of male and female at 1:1 is better. However, it can be 1:2. The breeding process takes 1-2 days.

Bondari (1983) demonstrated that cat fish farmers who use the open pond spawning technique have a skewed sex ratio of 4 females: 1 male but not affect fry production. The practice may be beneficial for the economics of the fingerling production but the genetic consequences cannot be ignored. Farmers are altered to use skewed sex ratios because they optimize fingerling production out of small number of brood fish needed to achieve the required production quotas.

Khanam (2005) reported that Singhi and Pabda become sexually mature at the age of 10-11 months. For breeding normally a ratio of 1:1 is maintained however spawning rate will be more if the ratio is 1.5:1.

Vijayakumar (2010) reported that an for *Ompok malabaricus* when a ratio of 2:1 (male:female) were selected with ovaprim (Syndel Labouratory, Canada) dose of 0.5 ml/kg body weight spawning occurred 12-15 hrs after injection. After spawning, fertilized eggs were collected, counted and the percentage of fertilization was determined. Fertilization rate was 78% with the survival rate varied from 54-63%.

Purkayastha *et al.* (2012) reported that the catfish *Ompok pabda*, was successfully bred by inducing with ovatide at two different doses (0.4 and 0.6 ml/kg of body weight of female) maintaining a ratio of 3:2. The latency period was 6-8 hours with 0.5 ml, where it was 8-10 hours with the dose of 0.4 ml/kg of body weight. All the female spawned successfully and average rate of fertilization (75.5%) and the hatching (60.5%) was higher with dose @ 0.6 ml/kgbody weight, while lower fertilization (65.1%) and hatching rate (45%) was observed @ 0.3 ml/kg body weight. The fecundity ranged from 80-130 eggs gm^{-1}body weight of the fish species. Eggs hatched 12-14 hours after spawning at water temperature of 28-32° C.

2.4.Inducing agent (hormone)

2.4.1. Pituitary Gland Extract

Haniffa *et al.* (2000) reported that hypophysation is a simple practical technique but suffers from the disadvantage regarding potency of which is unknown and difficult to standardize. Breeding experiment conducted by Haniffa et *al.* (2000), on striated murrel,*Channa striatus* and were injected both natural hormones (pituitary extract and human chorionic gonadotropin) and synthetic hormones (luteinizing hormone releasing hormone analogue and ovaprim). When compared to the LHRH and ovaprim, the latency period was long with pituitary (24 hr) and HCG injected (26 hr) fish. In the pituitary injected fish, the percentage of fertilization was the lowest (60-68%) and the duration of hatching was longest (39-43 hr) followed by HCG – (36-38 hr), LHRH (34-36 hr) and ovaprim injected (21-23 hr) individuals. In terms of fertilization (95-98%) and hatching, ovaprim yielded better results. Ova reached the

highest diameter (1.34–1.45 mm) in ovaprim injected fish, followed by HCG (1.22–1.27 mm). The lowest ova diameter (1.07-1.09 mm) was observed with LHRH.

Chondar (1994) made an assessment and opined that proper dosages of pituitary gland for induce breeding of fish is most important. Low dose may not be at all effective, while overdose inhibits spawning or initiates premature release of ova which either do not fertilize or at best lead to deformed progeny susceptible to mortality. The greatest difficulty in standardization of fish gonadotropin is encountered on account of various factors of which the size and sexual stage of maturity of the breeders are the most important. It is very difficult to know the exact stages of maturity in female breeders from external characters. Breeders belonging to particular species and having the same size may differ in the stages of maturity of their gonads. The potency of glands also varies according to sizes and sexual development of the donor as well as the species of the donor fish, time of collection of glands and their proper preservation.

Chondar (1994) studied the dose of PG in relation to the weight of the breeder to be injected. It was noticed that the identical dose to the breeders of similar weights (and similar sex) may give contradictory results owing to difference in maturity of gonads. Even heavy doses of hormones (12-17mg) may not be effective if the gonads are in recession stage. It is, thus, by individual experience that one can determine the proper dose needed to induce spawning in a particular fish. By careful selection of breeders and administering a known weight of pituitary gland per kg body weight of the breeder, successful breeding can be obtained in about 60-70% events when the weather conditions is favourable.

Chondar (1994) reported that in similar environmental conditions, catla usually requires a little higher dose than Rohu and Mrigal. However, for successful breeding of fully mature female Indian and Chinese major carps in confined waters, the average total dose of pituitary gland for Catla is 9-12 mg/kg (2-4mg and 7-8mg in 1^{st} and 2^{nd} injections, respectively); for Rohu and Mrigal is 7-9 mg/kg (2-3 mg and 5-6 mg in 1^{st} and 2^{nd} injections, respectively); for Silver carp 10-14 mg/kg (2-4 mg and 8-10 mg in 1^{st} and 2^{nd} injections); and for Grass carp is 9-13 mg/kg (2-4 mg and 7-9 mg in 1^{st} and 2^{nd} injections).

Ayyappan (2006) recommended one priming dose of 4-6 mg/kg body weight and a decisive dose of 10-12 mg/kg body weight of carp pituitary extract to female for induce breeding of IMC. The effective spawning time reported with pituitary gland extract is 3-5 hrs.

Thomas *et al.* (2003) stated that the initial dose for female is 2-3 mg/kg body weight and after 5-6 hours males are given PGE injection of 4-6 mg/kg and females 10-12 mg/kg. On an average female Grass carp and Silver carp require a dose of 10-14 mg/ kg and male require 2-5 mg/kg body weight of CPE. In case of Grass carp and Silver carp artificial insemination by stripping was also carried out at about 6-8 hours after the resolving dose.

Mukherjee, *et al.* (2002) reported that for experiment, 6 males and 3 females were kept in a separate breeding hapa. Single dose of hormone was administered to females only. The dose of hormone used, as pituitary, was 16 mg/kg body weight and the main water quality parameters of tanks (Brooders tank, breeding tank and larval rearing tank) were recorded regularly by APHA. The number of eggs released 4,200 and percentage hatching 50%, which was less than used of ovatide (70%) and ovaprim (80%).

Mukherjee and Das (2001) used ovaprim, ovatide and pituitary extract for breeding of *Pabda* and the range of hatchlings obtained was 14,350-21,000 nos. Chakrabarty *et al.*,(2006) reported that the production about 2.25 lakh nos. of *Pabda* seed by induced breeding.

Chakrabarty *et al.* (2007a) reported that the broodfish of 35-80 g are usually selected for artificial breeding. Fishes of both the sexes were induced by hormone therapy using either carp pituitary extract or the branded commercial product ovaprim.

Khanam (2005) reported that after selection of matured male and female, PG extracts are used to induce them to breed. Single dose is used for both male and female. Female is treated with 30 mg PG/kg body weight of fish and male is treated with 5-10 mg PG/kg body of fish. Male and Female ratio is 1:1. However maximum eggs will be found if the male and female ratio is 1.5:1, fertilization rate is higher.

2.4.2. Ovaprim

Thomas *et al.* (2003) reported that the ovaprim is highly successful and advantageous when compared to the traditional method of CPE administration. Use of ovaprim increases spawning, fecundity, fertilization and hatching rate.

Nandeesha *et al.* (1990) observed that studies based on 3 year field trial with Indian Major Carps from 1988-90s have indicated that average number of eggs obtained per kg body weight of brood fish was 1,14,000 with ovaprim as compared to 85,000 with CPGE. Average number of fry obtained per kg body weight of brooder was 72,000 with ovaprim as against 43,000 with CPGE. Thus 40% increase was recorded using ovaprim than CPGE.

Alok *et al.* (1993) and Nandeesha *et al.* (1990, 1993) achieved complete spawning for medium and high dose of ovaprim injected fish where as low dose injected fish did not respond. In terms of fertilization and hatching, ovaprim yielded better results. The highest percentage of fertilization (95-98%) was observed in ovaprim injected *Channa striatus.*

Sarkar *et al.* (2005) obtained breeding success in *Ompok pabda* by inducing with at two different doses (0.5 ml and 0.3 ml kg^{-1} body weight for female and 0.6 ml and 0.4 ml for male). The fish spawned 8-12 hours after single dose of injection. The latency period was less (8-10 hours) with 0.5 ml, where it was more (10-12 hours) with the dose of 0.3 ml kg^{-1} of body weight. All the female spawned successfully and the average rate of the fertilization (91.05%) and hatching rate (65.55%) was higher with dose @ 0.5 ml, where as lower (84.85%) and (66.35%) with a dose of 0.3 ml/ kg body weight.

Khanam *et al.* (2005.) reported that singhi (*Heteropneustes fossilis*), pabda (*Ompok pabda*) spawned naturally into the hapa, and eggs are collected by siphoning and are kept into cistern for hatching. But in case of magur (*Clarias batrachus*) three different hormones (PG, HCG mixed with PG, ovaprim mixed with PG) were used to determine the effectiveness of hormones on ovulation, fertilization and hatching. The dose of hormones were 5mgPG/kg female (1^{st} dose) and 15mg PG/kg female (2^{nd} dose), (5000IU HCG+5mg PG) /1.5kg female, (200 micro g ovaprim+5mg PG)/2kg

female. In case of pabda double dose (HCG hormone) treatment were used. HCG mixed with PG was found to be effective hormone to maximize the ovulation, fertilization and hatching rate of magur.

Chakraborty *et al.* (2006) reported that when single dose of ovaprim was applied in *Pabda* at 1-1.5 ml/kg body weight for female and 0.5-1.0 ml/kg body weight for males, ensures successful result compared to natural breeding. Generally *Pabda* weighing 35-40 g. and above (male and female) attain maturity and can be employed for breeding and seed production. The survivality and growth performance of *pabda* in the farmers' pond has been observed to be highly satisfactory and encouraging.

Chakrabarty *et al.* (2007c) reported that artificial spawning of *Ompok pabda* using ovaprim as inducing agent and fertilization of eggs with milt collected from males were attempted. The embryo hatched out in 23 hours of fertilization, the hatchling reared in fibre glass tank with the supply of natural food organisms, attained the average size of 30-35 mm/ 207-245 mg within fortnight with a survival of 61.0%.

Mijkherjee *et al.* (2002) reported the use of 'ovaprim' were to freshwater fish *Clarias batrachus* (sex ratio 1:1) and brackishwater fish *Mystus gulio* (sex ratio 2:1), dose selected were for *Clarias batrachus* 2.5 ml/kg body weight in female, 1.0 ml/kg body weight in male and for *Mystus gulio* 2.5 ml/kg body weight in female, 1.0 ml/kg body weight in male. Hatchling percentage was 50%, 80% respectively.

Banik *et al.* (2011) during June- July 2009 and 2010 used altogether 52 individual, weight group of 4.05 kg (mean wt= 97.88g/individual) mature females and were bred and fertilized with spermatozoa suspension from the adult males. Hatching time was 23-24 hours after fertilization at about 27-32^{0}C. A total of 52 females were considered for breeding at of which about 87.90% responded and average fertilization rate was 72%. A total of 0.09 lakh spawn was grown and developed during 2010. Of this about 5450 individuals were reared into fry stage. During experiment the acclimatised brood stock were taken from the post-stocking pond and through repeated netting as well as segregation and then were transferred into breeding hapa for acclimatization further for about 7-8 hrs. For promoting gamete

production in both female and male as well as for the influencing fertilization, Ovaprim was applied, @ of 0.58-1.28 ml/kg body weight of the individuals. Females were stripped at 5-9 hrs after hormone injection. The number of eggs developed was varied from 1068-5892. The survival of the hatchling varied from 51-72% (Bhowmick *et al.,* 2000; Mukherjee and Das 2001; Chakrabarty *et al.,* 2006, 2007).

The breeding of butter fish (*Ompok malabaricus*) by hormone induction and environmental criteria were noticed. A successful spawning was observed by using a single intramuscular injection of ovaprim (dosage 0.5 ml/kg body weight). Spawning was observed at 12-13 hrs after injection. Averages egg production was 5584±100. Hatching occurred at 26 hrs after spawning. Hatchlings were reared to fingerlings size in 60 days. A total number of 1600 fingerlings were reintroduced into the upstream of Gadana River in Western Ghats, (Vijayakumar, 2010).

Sarkar *et al.* (2010) reported that synthetic hormone is injected (Ovaprim/Domperidone) at 0.3-0.4 ml/kg body weight for females and 0.1-0.2 ml/kg body weight for males. Males and females are kept separately from each other after injection. Hormone depends on climate. Monsoon season is the best for this, at a temperature between 25-30°C.

2.4.3. Ovatide

Hussain (2006) coducted breeding of *Pabda* in a masonry cistern in a hatchery by using Ovatide (dose 0.5-2.0 ml/kg of fish). The hatchlings obtained during the first phase were 428 nos. and a total of 1,000 nos. of fry were obtained for stocking purposes. They have highlighted the difficulties in the way of commercial production of Pabda seed due to small size of broodfishes, low fecundity and lack of technological knowledge of the breeding and seed rearing of Pabda.

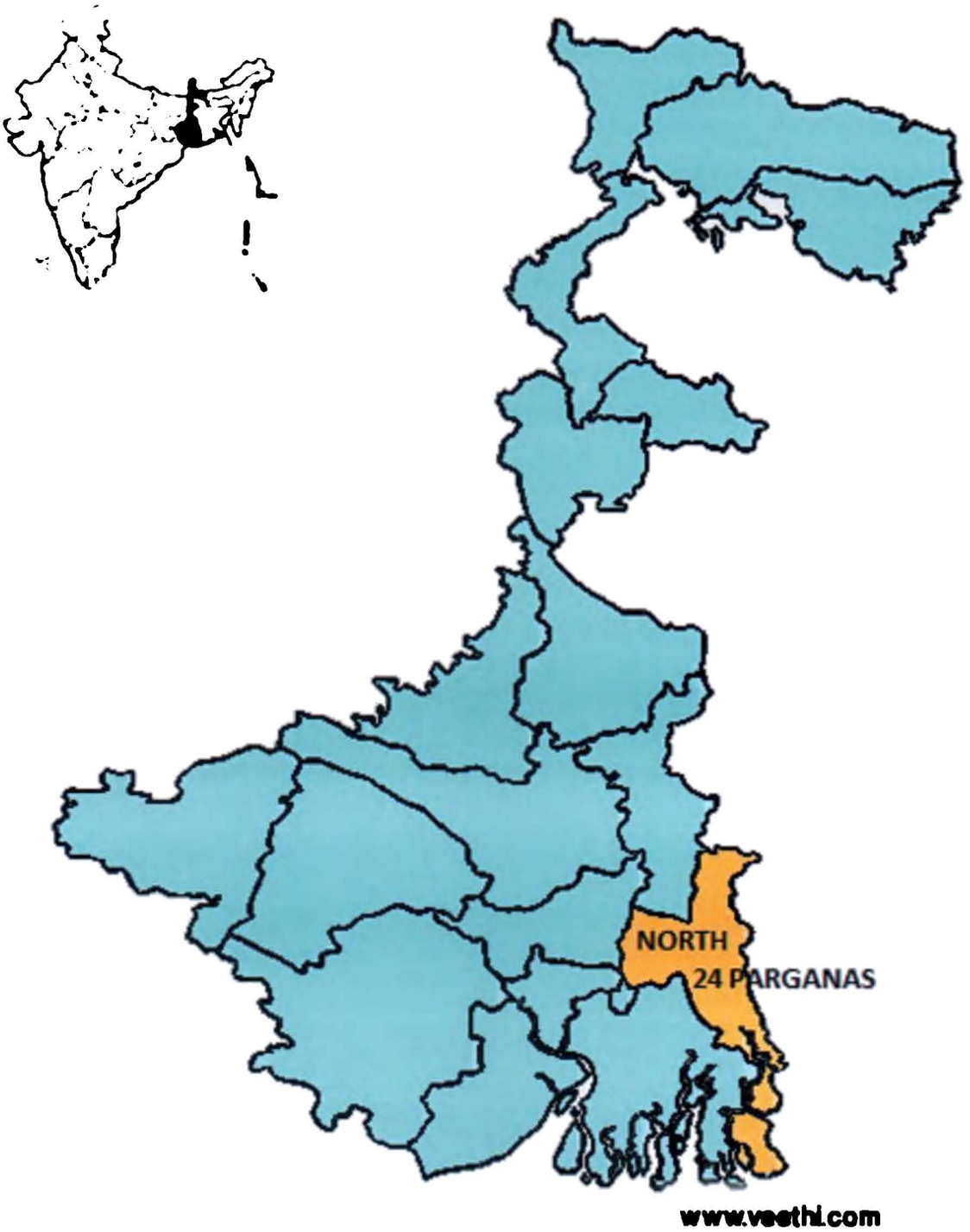
Goswami, *et al.* (2007) reported that induced spawning of *Pangasius pangasius* in Circular Eco Hatchery (CEH). Two breeding trials were conducted separately in altogether 18 fishes by administering synthetic ovatide hormone. Spawning response observed to a dose of 0.3-0.4 ml hormone per kg body weight (BW) in male and 0.6-0.7 ml per kg body weight in female. In the first breeding trial,

sex ratio the fish was 2:1 but in the succession trial, sex ratio was 1:1. Hatching took place within 24-26 hours at 28-31^{0}C. 22-28% hatching success was recorded due to outbreak of fungal infection in the fertilized eggs.

Sarma, *et al.* (2012) reported that *Pabda* fishes (male and female) can induced by intramuscular injection with respectively 0.5 ml and 0.6 ml of ovatide per kg body weight.

CHAPTER 3

MATERIALS AND METHODS



North 24 Parganas showing study area (West Bengal)

3. MATERIALS AND METHODS

Ompok pabda, popularly known as **butter fish**, belongs to the family **Siluridae**, is important for customer choice and fetches a high market value. Natural abundance of Pabda is becoming decreased day by day due to environmental degradation and destruction of breeding ground.

Present work has been conducted at Majumdar hatchery, located in Naihati, West Bengal. It is one of the leading hatchery in West Bengal and renowned for innovative Breeding Programmes. The entire breeding programme including brood stock management as well as nursery management was conducted in the said hatchery as the infrastructure facilities required are available in the said hatchery.

For the last 8 years huge amount of fry of Pabda has been produced by the owner of this hatchery for commercial purpose to increase the availability of this species in market. The species is recently prioritized as new candidate species for freshwater aquaculture. Recently IUCN (2000) has declared the *Pabda* as endangered fish. As of now various experiments were being conducted to protect this species from being extinct. Standardisation of breeding protocol and mass production of seed of this endangered fish species are important to save this unique species. Among cat fishes in India, *Ompok pabda* is considered a delicacy for taste and demand for this fish is very high for its palatability and high nutritive value.

3.1 Locality of the study area

The study area is located in Sibdaspur, near Naihati, North 24 parganas district of West Bengal. It is one of the leading hatchery in West Bengal and well known for innovative Breeding Programme. West Bengal is located between $23^0$00' N latitude and $87^0$00' E longitude and North 24 Parganas district is a district in Southern West Bengal, of Eastern India. This district extend in the (tropical zone) from latitude $22^0$11'6" N to $23^0$15'2" N and longitude $88^0$20' E to $89^0$5' E. This district is cover with population 10082852 (2011 census) and geographical area is 4094 sq. Kms. It is bordered by Nadia at north, to Bangladesh (Khulna Division) by north and east, to South 24 Parganas and Kolkata by South and to Kolkata, Howrah and

Hooghly by West. The Hooghly, Ichamati and Yamuna is the major river system in North 24 Parganas district (West Bengal). About 90% of the population of this region love to eat fish.

3.2. Materials required:-

- Brooder fish (male and female)
- Ovaprim (***VIRBAC*** ANIMAL HEALTH INDIA PVT. LTD., Hyderabad)
- Pituitary gland (Carp pituitary)
- Weighing Balance
- Tissue homogenizer
- Centrifuge machine
- Distilled water
- Glass Van (Syringe -1 & 2 ml)
- Insulin syringe
- No.24 needle
- Enamel or steel tray, Petri dish
- 0.9% Normal Saline Water
- Dissection Box
- Cotton cloth, Nylon cloth
- Beaker and Cylinder
- Record book, pen, pencil
- Camera, Emergency light
- Measuring tape, Scale
- Aerator
- Hand net, drag net
- Iron khancha (which is made up of 6 mm Iron rod in square or rectangular shape)

3.3. Brood stock collection and management

Male adults of *Ompok pabda* ranging from size 15.2-17.5 cm and female adults ranging from size 17.2-19.8 cm are collected from River Ganga in the vicinity of Murshidabad, West Bengal during November- February. They are stocked in a

pond about (0.5 ha) in a private hatchery. The breeding and larval rearing experiments were carried out at the same farm.

A monthly water exchange of 50% was made to maintain water quality parameters within favourable ranges. Proper management practices were initiated for rapid enhancement of growth and maturity of the brooders. Aquatic macrophytes like *Hydrilla verticillata* and *Eichhornia crassipes* etc. were introduced as these plants serve as ideal shelter for the fishes and also as source of periphytic food organisms. The depth of the pond was maintained between 1.0 – 1.5 m. After 1-2 month of rearing the fishes are considered for breeding.

3.4. Management of Water quality

The physiochemical characteristics of water of the brooder tank and hatching tank were studied periodically during the period of one year. The variations in water parameters such as temperatures, pH, dissolved oxygen, alkalinity and hardness were estimated following standard methods (APHA, 2000).

Parameter	Method	Reference
Temperature	Mercury Thermometer	Adoni *et al.*, 1985
pH	Digital pH meter	Adoni *et al.*, 1985
Dissolved oxygen	Winkler's idometric titration	APHA, 2000
Alkalinity	Acid-base titration	APHA, 2000
Hardness	Titrimetric	APHA, 2000

Three different readings were recorded for each tank. The depth of water was recorded by a measuring tape.

The surface water temperature was recorded by using a mercury thermometer. It was immersed at subsurface level and held for 2 minutes, and then the temperature is recorded (Adoni *et al.*, 1985).

The pH of water was measured by a digital pH meter (Systronics: model no. MK-VI) following the method described by Adoni *et al.*, (1985).

Dissolved Oxygen content of water (each tank) was estimated following the Winkler's iodometric method (APHA, 2000).

Alkalinity of water was calculated from the sum of bicarbonate and carbonate alkalinity following the acid-base titration method (APHA, 2000).

Examination of hardness of water was estimated following the titrimetric method (APHA, 2000).

Water hardness classifications

Classification	$CaCO_3$ equivalent (mg/L)
Soft	<75
Moderately	75-150
Hard	150-300

WATER QUALITY	
Temperature	29-31^{0}C
pH	7.5-8.5
DO (mg/l)	7-9 ppm
Total alkalinity (mg/l)	120-130
Total hardness (mg/l)	110-115 ppm

Pond	Temp (c)	pH	DO (ppm)	Alkalinity (ppm)	Hardness (ppm)
Brooders pond	29±1	7.5 – 7.8	6 – 6.5	130 ±10	110±15
Breeding tank	31±1	7.6 – 7.9	7 – 7.6	140±15	112±10
Incubation tank	29±1	7.5 – 8	6.9 – 7.2	135±15	107±15

Table 1: Water quality parameter

3.5 Selection of breeders

During breeding season (June-August), fully ripe females and males were identified with their secondary sexual character. These are comb like and swollen with milt but do not normally yield the contents with slight pressure on abdomen. Males have an elongated and pointed genital papilla. While females possess soft bulging abdomen for enlargement of the balloon shaped hard roes inside, reddish vent

and rounded genital papilla. The pectoral fin spine, relatively longer and thicker in male than female, becomes more prominent.

3.6. EXPERIMENTAL DESIGN

Two different hormones ie.CPE and OVAPRIM are used as inducing agent at varying doses. Total six experiments were conducted both for CPE (3 sets, which is denoted by R_1, R_2, and R_3) and OVAPRIM (3 sets, which was denoted by T_1, T_2 and T_3). All experiments were conducted in the well maintained hatchery for a period of one month.

At first all brooders were collected from the stocking pond and are stocked into two hapa (135 cm X 90 cm X 130 cm) fixed at the pond five hours before injection. The ratio was 9:6 (M:F) as given Table 16.

Hormone	**Treatment**		
CPE	R_1	R_2	R_3
OVAPRIM	T_1	T_2	T_3

3.6.1. USE OF DIFFERENT DOSES OF "CPE" IN THREE EXPERIMENT TANK

Treatment (Experiment tank)	**Sex Ratio**		**Dose of CPE hormone (mg/kg bw)**	
	Male	**Female**	**Male**	**Female**
R_1	9	6	3	12
R_2	9	6	4	14
R_3	9	6	5	16

Table 2: Different doses of "CPE" in three experiment tank

[A] EXPERIMENT TANK - R_1

According to size, fishes (average length-17.5-18.27 cm; weight-33.5 gm -36 gm) were injected accordingly. Fish require single dose of hormone and the dose provided was 12 mg/kg body weight for female and 3 mg/kg body weight for male which is administered generally at 7 AM (Plate I). Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for about 7 hrs at the

ratio of 9:6 =M: F (Plate J). Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. After that fishes were sex wise segregated in separate utensils (Plate K). Water temperature maintained at 29^0C and hardness at 110 ppm. After 9 hrs, injected females were stripped (Plate L). After stripping the female were released into the stocking pond giving dip treatment in Potassium permanganate solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt. Ltd., Thane) to prevent any infection and for speedy recover from stress condition. Dose depends upon the intensity of the infection/stress. The eggs are collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes (Plate M, N), kept in Petridish with 0.9% normal saline water (Plate O). Testes are then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution (Plate P (a), (b)). Sperm suspensions are then spreaded over the eggs and mixed gently with a feather for through mixing and fertilization. After 3-5 minutes clean water is added and washed thoroughly for complete removal of excess milt (Plate Q). After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour and swollen after water absorption (Plate R, S). These are fertilized eggs which are spreaded by hand on khancha, kept in pre-arranged in incubation tank with provision of aerator (Plate T, U). In the incubation tank, initially 4 inch water depth is maintained and after hatching, it was increased to 6 inch and maintained till 10-15 days with continuous aeration (Plate V). Water quality parameters of the tanks are recorded regularly (Table no.4) following the method given by APHA. After 18 hrs fertilized eggs were hatched out (Plate X) .After hatching is completed the Iron frame (khancha) and the unfertilized eggs are removed carefully from the incubation tank. The tank is washed throughly and filled with the fresh water up to 6 inch with *Hydrilla verticillata*. At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings are very active and moved very fast showing schooling behaviour and showed a tendency of lateral alignment due to yolk content on ventral side (Plate Y). The newly hatched larva possesses large yolk sac which is being absorbed by 3 days. After 3 days, barbells became prominent and mouth begins to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. After yolk sac absorption, grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter

to grown up hatchlings up to 15 days (Plate W). The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

[B] EXPERIMENT TANK – R_2

According to size, fishes (average length-16.5 cm -17.83 cm; weight-33.3gm -35.58 gm) were injected accordingly. Fish require single dose of hormone and the dose provided was 14 mg/kg body weight for female and 4 mg/kg body weight for male which is administered generally at 7 AM. Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for 7 hrs at the ratio of 9:6=M:F (Plate J) Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. Water temperature maintained at 31^0C and hardness at 112 ppm.

After 9 hours of injection females were stripped. After stripping the female were released into the stocking pond giving dip treatment in Potassium permanganate solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt. Ltd., Thane) to prevent any infection and for speedy recover from stress condition. Dose depends upon the intensity of the infection/stress. The eggs are collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes. Testes are then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution. Sperm suspensions are then spreaded over the eggs and mixed gently with a feather for through mixing and fertilization. After 3-5 minutes clean water is added and washed thoroughly for complete removal of excess milt. After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour and swollen after water absorption. These are fertilized eggs which are spreaded by hand on khancha, kept pre-arranged in incubation tank with provision are aerator. In the incubation tank, initially 4 inch water depth is maintained and after hatching it increased to 6 inch and maintained till 10-15 days with continuous aeration. Water quality parameters of the tanks are recorded regularly (Table no.4) following the method given by APHA. After hatching is completed the Iron frame (khancha) and the unfertilized eggs are removed carefully from the incubation tank. The tank is washed thoroughly and filled with the fresh water up to 6 inch with *Hydrilla verticillata*. At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings are very active and moved very fast showing schooling

behaviour and showed a tendency of lateral alignment due to yolk content on ventral side. The newly hatched larva possesses large yolk sac which is being absorbed by 3 days. After 3 days, barbels became prominent and mouth begins to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. As feed one grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter to grown up hatchlings up to 15 days. The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

[C] EXPERIMENT TANK – R_3

According to size, fishes (Average length-16.8 cm -18.06 cm; weight-34.035 gm -36.16 gm) were injected accordingly. Fish require single dose of hormone and the dose provided was 16 mg/kg body weight for female and 5 mg/kg body weight for male which is administered generally at 7 AM (Plate I). Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for 7 hrs at the ratio of 9:6=M:F (Plate J). Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. Water temperature maintained at 29^0C and hardness at 115 ppm.

After 9 hrs. injected females were stripped. After stripping the female were released into the stocking pond giving dip treatment in *Potassium permanganate* solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt Ltd, Thane) to prevent any infection and for speedy recover from stress condition. Dose depends upon the intensity of the infection/stress. The eggs are collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes. Testes are then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution. Sperm suspensions are then spreaded over the eggs and mixed gently with a feather for through mixing and fertilization. After 3-5 minutes clean water is added and washed thoroughly for complete removal of excess milt. After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour, and swollen after water absorption. These are fertilized eggs which are spreaded by hand on khancha, kept pre-arranged in incubation tank with provision of aerator. In the incubation tank, initially 4 inch water depth is maintained and after hatching it

increased to 6 inch and maintained till 10-15 days with continuous aeration. Water quality parameters of the tanks are recorded regularly (Table no.4) following the method given by APHA. After hatching is completed the Iron frame (khancha) and the unfertilized eggs are removed carefully from the incubation tank. The tank is washed thoroughly and filled with the fresh water up to 6 inch with *Hydrilla verticillata*. At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings are very active and moved very fast showing schooling behaviour and showed a tendency of lateral alignment due to yolk content on ventral side. The newly hatched larva possesses large yolk sac which is being absorbed by 3 days. After 3 days, barbells became prominent and mouth begins to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. As feed one grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter to grown up hatchlings up to 15 days. The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

3.6.2. USE OF DIFFERENT DOSES OF "OVAPRIM" IN THREE EXPERIMENT TANK

Treatment	Sex Ratio		Dose of Ovaprim hormone (ml/kg bw)	
	Male	Female	Male	Female
T_1	9	6	0.5	1
T_2	9	6	0.5	1.2
T_3	9	6	0.5	1.5

Table 3: Different doses of "OVAPRIM" in three experiment tank

[A] EXPERIMENT TANK No. - T_1

According to size, fishes (Average length-16.8 cm -18.8 cm; weight-28.85 gm -35.28 gm) were injected accordingly. Fish require single dose of hormone and the dose provided was 1 mg/kg body weight for female and 0.5 mg/kg body weight for male which is administered generally at 7 AM (Plate I). Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for 7 hrs at the

ratio of 9:6=M:F (Plate J) Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. Water temperature maintained at 29^0C and hardness at 107 ppm. After 9 hrs, injected females were stripped. After stripping the female were released into the stocking pond giving dip treatment in *Potassium permanganate* solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt. Ltd., Thane) to prevent any infection and for speedy recover from stress condition. Dose depends upon the intensity of the infection/stress. The eggs are collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes. Testes are then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution. Sperm suspensions are then spreaded over the eggs and mixed gently with a feather for through mixing and fertilization. After 3-5 minutes clean water is added and washed thoroughly for complete removal of excess milt. After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour, and swollen after water absorption. These are fertilized eggs which are spreaded by hand on khancha, kept pre-arranged in incubation tank with minimum provision of aerator. In the incubation tank, initially 4 inch water depth is maintained and after hatching it increased to 6 inch and maintained till 10-15 days with continuous aeration. Water quality parameters of the tanks are recorded regularly (Table no.4) following the method given by APHA. After hatching is completed the Iron frame (khancha) and the unfertilized eggs are removed carefully from the incubation tank. The tank is washed throughly and filled with the fresh water up to 6 inch with *Hydrilla verticillata.* At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings are very active and moved very fast showing schooling behaviour and showed a tendency of lateral alignment due to yolk content on ventral side. The newly hatched larva possesses large yolk sac which is being absorbed by 3 days. After 3 days, barbells became prominent and mouth begins to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. As feed one grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter to grown up hatchlings up to 15 days. The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

[B] EXPERIMENT TANK– T_2

According to size, fishes (average length-16.4 cm -18.8 cm; weight-28.26 gm -35.2 gm) were injected accordingly. Fish require single dose of hormone and the dose provided was 1.2 mg/kg body weight for female and 0.5 mg/kg body weight for male which is administered generally at 7 AM (Plate I). Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for 7 hrs at the ratio of 9:6=M:F (Plate J). Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. Water temperature maintained 29^0C and hardness at 113 ppm.

After 9 hrs injected females were stripped. After stripping the female were released into the stocking pond giving dip treatment in *Potassium permanganate* solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt. Ltd., Thane) to prevent any infection and for speedy recover from stress condition. Doses depend upon the intensity of the infection/stress. The eggs are collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes. Testes are then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution. Sperm suspensions are then spreaded over the eggs and mixed gently with a feather for through mixing and fertilization. After 3-5 minutes clean water is added and washed thoroughly for complete removal of excess milt. After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour, and swollen after water absorption. These are fertilized eggs which are spreaded by hand on khancha, kept pre-arranged in incubation tank with minimum provision of aerator. In the incubation tank, initially 4 inch water depth is maintained and after hatching it increased to 6 inch and maintained till 10-15 days with continuous aeration. Water quality parameters of the tanks are recorded regularly (Table no.4) following the method given by APHA. After hatching is completed the Iron frame (khancha) and the unfertilized eggs are removed carefully from the incubation tank. The tank is washed throughly and filled with the fresh water up to 6 inch with *Hydrilla verticillata.* At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings are very active and moved very fast showing schooling behaviour and showed a tendency of lateral alignment due to yolk content on ventral side. The newly hatched larva possesses large yolk sac which is being absorbed by 3

days. After 3 days, barbels become prominent and mouth begins to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. As feed one grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter to grown up hatchlings up to 15 days. The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

[C] EXPERIMENT TANK – T_3

According to size, fishes (average length-16.5 cm -18.9 cm; weight-28.24 gm - 37.35 gm) were injected accordingly. Fish requires single dose of hormone and the dose provided was 1.5 mg/kg body weight for female and 0.5 mg/kg body weight for male which is administered generally at morning 7 AM (Plate I). Both injected fish are then kept into a round cement tank (cistern) with *Eichhornia* for mating for 7 hrs. at the ratio of 9:6=M:F (Plate J). Slow flow rate is maintained. Before 9 hrs, eggs are checked at interval by small hand net for ovulatory response. Water temperature maintained at 30^0C and hardness at 110 ppm.

After 9 hrs injected females were stripped. After stripping the female were released into the stocking pond giving dip treatment in *Potassium permanganate* solution @ 0.1 ppm and in *Tetracycline hydrochloride* (water soluble antibiotic, Intervet India Pvt. Ltd., Thane) to prevent any infection and to recover from stress condition. Dose depends upon the intensity of the infection/stress. The eggs were collected in a clean dry enamel/steel/glass tray. The abdomens of induced males are dissected to remove the testes. Testes were then thoroughly macerated with the help of very fine nylon net and sperm suspensions are prepared with 0.9% saline solution. Sperm suspensions were then spreaded over the eggs and mixed gently with a feather through mixing and fertilization. After 3-5 minutes clean water was added and washed thoroughly for complete removal of excess milt. After a while, they were washed thoroughly with distilled water. The eggs were light brown in colour, and swollen after water absorption. These are fertilized eggs which were spreaded by hand on khancha, kept in pre-arranged incubation tank with provision of aerator. In the incubation tank, initially 4 inch water depth was maintained and after hatching it increased to 6 inch and maintained till 10-15 days with continuous aeration. Water quality parameters of the tanks were recorded regularly (Table no.4) following the method given by APHA. After hatching is completed the Iron frame (khancha) and

the unfertilized eggs are removed carefully from the incubation tank. The tank is washed thoroughly and filled with the fresh water up to 6 inch with *Hydrilla verticiellata*. At this stage the hatchlings had a tendency to gather at the margin of the tank. The hatchlings were very active and moved very fast showing schooling behaviour and showed a tendency of lateral alignment due to yolk content on ventral side. The newly hatched larva possesses large yolk sac which was being absorbed by 3 days. After 3 days, barbels became prominent and mouth began to develop and the larva starts accepting external feed. Initially hatchlings fed on egg yolk. As feed one grinded egg mixed with chopped tubifex is provided to each tank. After 10 days, *Hydrilla* is removed and in its place palm leaves is kept on the water surface for providing shelter to grown up hatchlings up to 15 days. The hatchlings now are ready to stock into the well-prepared nursery pond for further development.

3.7. Statistical Analysis

All the recorded data and observation were processed and analyzed keeping in view the objectives of the study. The following statistical methods were used in the study:

- Percentage analysis
- Mean
- Standard deviation
- Analysis of variance (ANOVA)

3.8. Analysis of variance (ANOVA)

The data generated from the investigation were tested for significance of variance of dose of inducing agents and its effect eggs released, fertilize percentage, hatching percentage, survival percentage during the period of study from May to July, 2012 through single factor analysis of variance. Statistical tests were performed using statistical software (MS-Excel 2007).

4. RESULTS

In the present study, the breeding of *Ompok pabda* was carried out following induced breeding in a private hatchery.

4.1. Status of fish and fish seed production

The seed production of *Pabda* is not yet standardized. Various attempts have been made by the State Govt., CIFA centre of Kolkata as well as private entrepreneurs to standardize the brood stock management and breeding technique for the last 3-4 years. Much improvement has been done towards understanding the same in the hatchery of our study. A lot more work is needed for captive breeding of *Pabda*. Previously, the entire process was carried out on an experiment basis, but now a day's hatchery owners are trying for commercial production of pabda seed in captivity. This year, 80% seed produced by induce breeding (Ovaprim) and 20% seed produced by CPE.

4.2. Pabda Hatchery

The hatchery is located in Shibdaspur, district North 24 Parganas, West Bengal, and is one of the pioneer hatchery for initiating breeding programme on *Pabda* since 2010. Scientists from CIFA also conducted some breeding programme in the last year with considerable success. We have started our programme from the breeding season of 2012. The name of the hatchery is "Mazumdar Fisheries". It started in 1986 with breeding of IMC, Common carp, and Grass carp. But now a day, this hatchery is one of the leading hatcheries in West Bengal.

4.3. Breeding season

Breeding season and maturation

The fish attained maturity at the end of the first year. Males matured earlier than female. Maturity cycle of the species was studied by examination of gonads at different months. During November-January the fishes were found to be in stage I and II of maturity. While they attained stages- III of maturity in March. Majority of the males were found to be matured during late March-April, while the bulk of females were only in stage- IV. Fully ripe females were

observed during May to the end of July. Breeding season extends from early June to late Aug., sometime extends up to September.

The fry price varies with the season, during early days in June the price is Rs. 5.00/fry, which slowly begins to decline to Rs. 4.00/fry as the season reaches its peak during July (monsoon). Later part of July and up to August-September then there is again a price hike (Table 4 and Fig. 1).

Sl. No.	Month	Price (Rs.)/fry
1.	June	4.00-5.00
2.	July	3.00-4.00
3.	Aug-Sept	5.00-6.00

Table 4: Breeding season and price variation

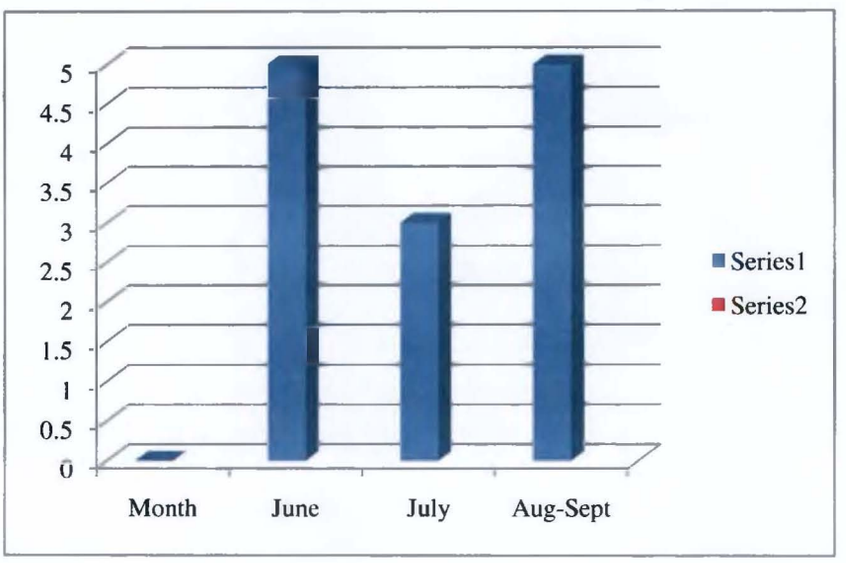


Fig 1: Price variation with month

4.4. Breeding Practice

4.4.1. Identification of male and female fish

Adult fish exhibit sexual dimorphisms. At maturity dorsal side of the pectoral fin of **male** is rough to touch on dorsal surface, body elongated, the

genital papilla pointed and narrow with freely oozing milt slight pressure on the abdomen.

In case of female, the pectoral fin is smooth, genital papilla is found swollen with slightly pink colour. The abdomen is bulging and soft.

Plate A: Male and female brooder of *Pabda*

4.4.2. Age and size of brooders

Table 5 depicts the average age and size of the brooders was used for breeding. The average age of brooder was 1year+ and average wt. and size was 28.24-37.35 g, 16.4-18.9 cm respectively (both male and female).

Actors involved in fish seed production	Variables
Brooders age	1 year+
Size (Length)	16.4-18.9 cm.
Weight	28.24-37.75 gm.
Sex ratio (M:F)	9:6

Table 5: Age and size of brooders

Plate B: Selected brooders of *Pabda*

4.4.3. Sex ratio

The average sex ratio considered for breeding in the hatchery was found to be 9:6=M:F (Table no. 16). It is observed that three fully mature and healthy males are sufficient to fertilize the eggs of two or more numbers of female. Moreover; there is a dire scarcity of mature and sufficient number of healthy brooders in the hatcheries. For this the farmers often use the skewed sex ratio for achieving the targeted production not aware of the consequences.

4.4.4. Restocking of brooders:

Restocking of brooders after spawning is a common practice adopted by hatchery owner .The hatchery owners restock the brooders and kept as potential brood stock for the ensuing season and the subsequent years.

4.5. Inducing agent

Mainly two types of inducing agents are used for induce breeding of fish. One is the natural induce agent called Carp Pituitary Extract (CPE) (Plate G) and other is synthetic inducing agent like OVAPRIM (Plate H). The hatchery owners of Bengal still use CPE as the sole inducing agent, as the same are easy available at a rate of Rs. 2-3 and may be due to organic in origin. But due to uncertainty in procuring the pituitary gland and for some advantages, now a day, pituitary is being replaced by OVAPRIM.

At present 80% of the farmers are using OVAPRIM for induce breeding programme against 20% of CPE users.

4.5.1. CPE protocol used in the experiment

During experiment, pituitary protocol as followed for each experimental tank is given in Table no. 6. It was observed that pre-monsoon and post-monsoon breeding required higher dose, may be due to non availability of congenial environmental condition.

TREATMENT (TANK)	Mean ± SD MALE (cm)		Mean ± SD FEMALE (cm)		DOSE OF HORMONE CPE(mg/kg b.wt.)	
	LENGTH	WEIGHT	LENGTH	WEIGHT	MALE	FEMALE
R_1	17.14±0.23	28.08±1.00	18.80±0.47	34.88±1.13	3	12
R_2	17.02±0.20	29.11±0.80	19.23±0.36	35.33±0.33	4	14
R_3	17.39±0.37	28.72±0.67	19.12±0.19	35.40±0.32	5	16

Table 6 : CPE protocol use for experiment

4.5.2. OVAPRIM protocol used in the experiment

OVAPRIM protocol as applied for fishes in experimental tank is given in table no. 7. It was observed that the dose of OVAPRIM did not vary much with the breeding season as seen with the pituitary gland. As single dose was given to every male and female, ovaprim was highly successful and advantageous compared to CPE, as it increases spawning, fecundity, fertilization and hatching rate.

TREATMENT (TANK)	Mean ± SD MALE (cm)		Mean ± SD FEMALE (cm)		DOSE OF HORMONE (ml/kg b.wt.)	
	LENGTH	WEIGHT	LENGTH	WEIGHT	MALE	FEMALE
T_1	16.83±0.69	28.85±1.73	18.81±0.70	35.28±2.72	0.5	1
T_2	16.42±0.27	28.26±1.79	18.88±0.89	35.18±3.44	0.5	1.2
T_3	16.47±0.22	28.24±1.75	18.90±0.96	37.35±2.40	0.5	1.5

Table 7: OVAPRIM protocol used in the experiment

4.5.3. Effect of CPE and OVAPRIM on Latency period

The mode of fish breeding operation was closely observed to study the latency period by using Carp Pituitary Extract and OVAPRIM. The latency period recorded with CPE was 18 hrs in R_1, 17 hrs in R_2, 16 hrs in R_3 while 12 hrs in T_1, 11 hrs in T_2, 9 hrs in T_3 with OVAPRIM (Table no. 16).

4.5.4. Number of eggs released and fertilized eggs with CPE and OVAPRIM

Number of eggs released and the eggs fertilized with CPE and OVAPRIM during the study was recorded and compared (Table 8). It was found that number of eggs released with the OVAPRIM (T_3- 0.5 ml/kg body weight in male and 1.5 ml/kg body weight in female) was higher (108565 numbers eggs) than the CPE (R_3- 5 mg/kg body weight in male and 16 mg/kg body weight in female) (84475 numbers eggs). It was observed that maximum number of fertilized eggs (93691numbers) were with the OVAPRIM, which was higher than the CPE (67748 fertilized eggs).

Hormone	Treatment	No. of eggs released	No. of fertilized eggs
CPE	R_1	68765	51230
	R_2	79577	60876
	R_3	84475	67748
OVAPRIM	T_1	64355	52771
	T_2	88760	74114
	T_3	108565	93691

Table 8: Number of eggs release and fertilized eggs with CPE and OVAPRIM

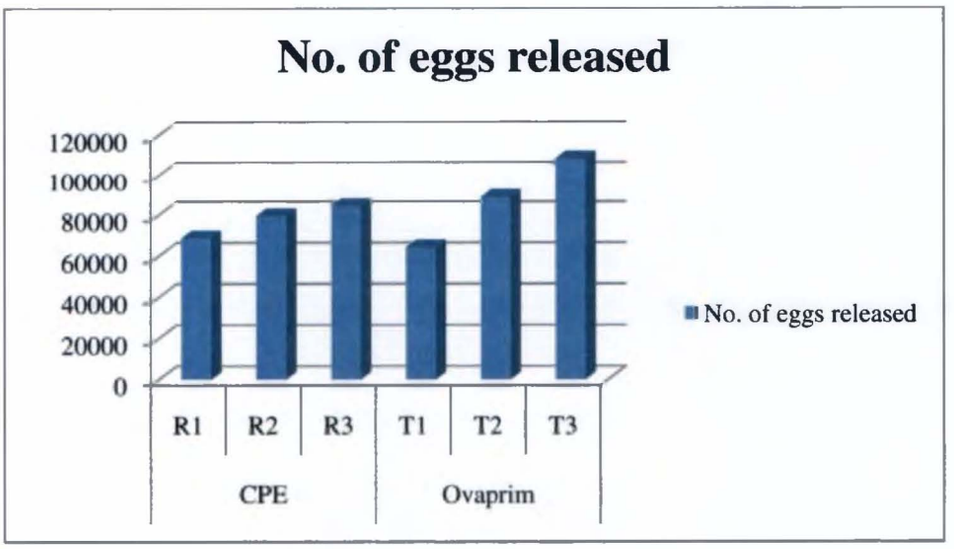


Fig 2: Number of eggs released with CPE and OVAPRIM

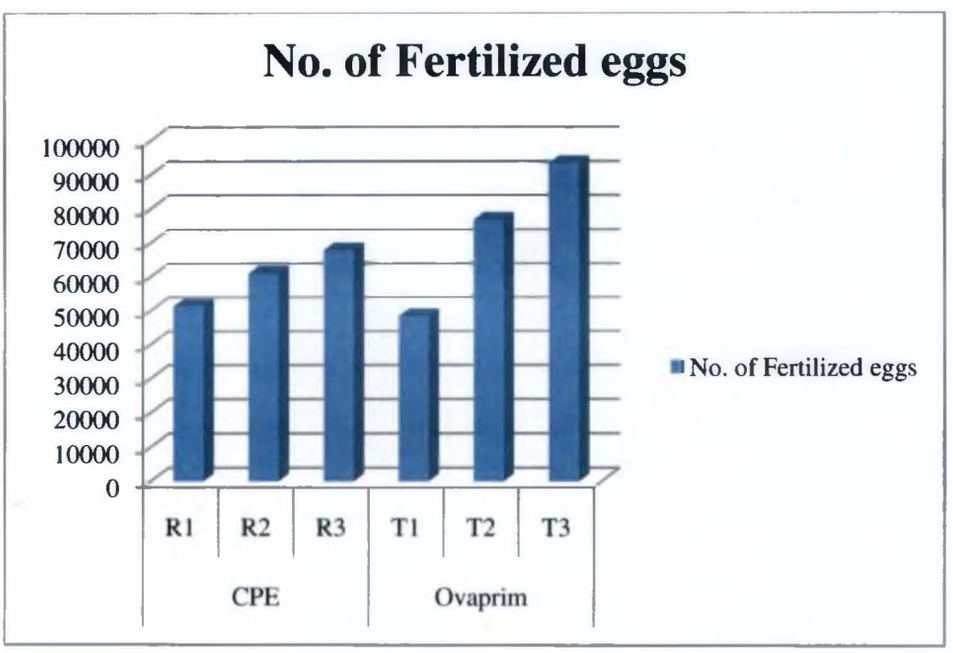


Fig 3: Number of fertilized eggs with CPE and OVAPRIM

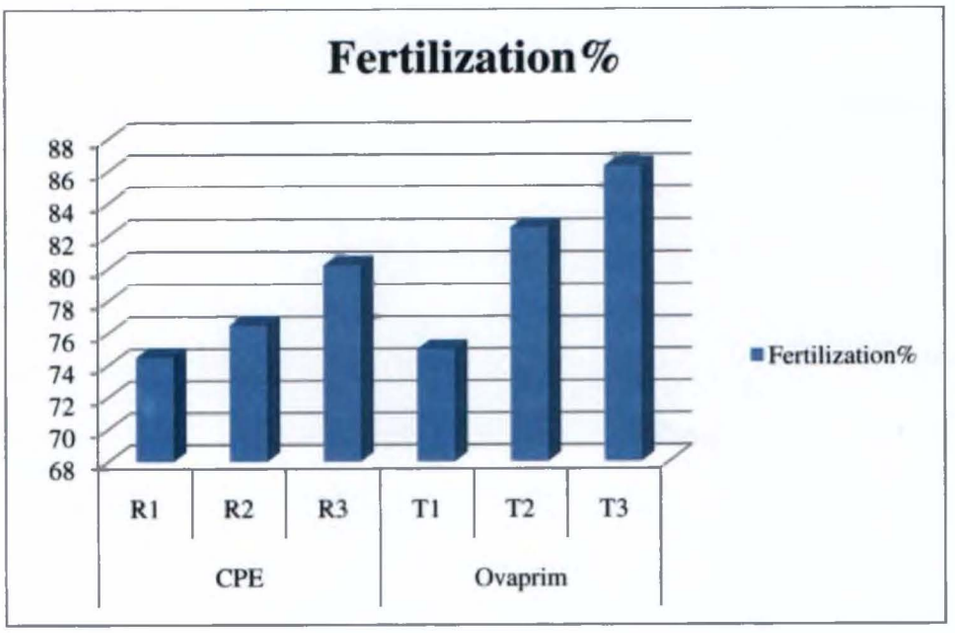


Fig 4: Number of fertilization percentage with CPE and OVAPRIM

4.5.5. Number of hatchlings with CPE and OVAPRIM

Highest numbers of hatchling (73453 hatchlings) were obtained in the T_3, in which male and female are treated with single dose of OVAPRIM at the rate of 1.5 ml/kg body weight in female and 0.5 ml/kg body weight in male.

Relative lesser numbers of hatching were obtained in the treatments T_1 and T_2 with 3521 and 48470 numbers of hatchling respectively.

Whereas, the highest numbers of hatchling were obtained in the R_3 of CPE treatment with 42003. Lesser numbers of hatchling were obtained in R_1 (26229 numbers) and R_2 (34699 numbers) (Table 9).

Hormone	Treatment	No. of hatchlings
CPE	R_1	26229
	R_2	34699
	R_3	42003
OVAPRIM	T_1	35251
	T_2	48470
	T_3	73453

Table 9: Number of hatchlings with CPE and Ovaprim

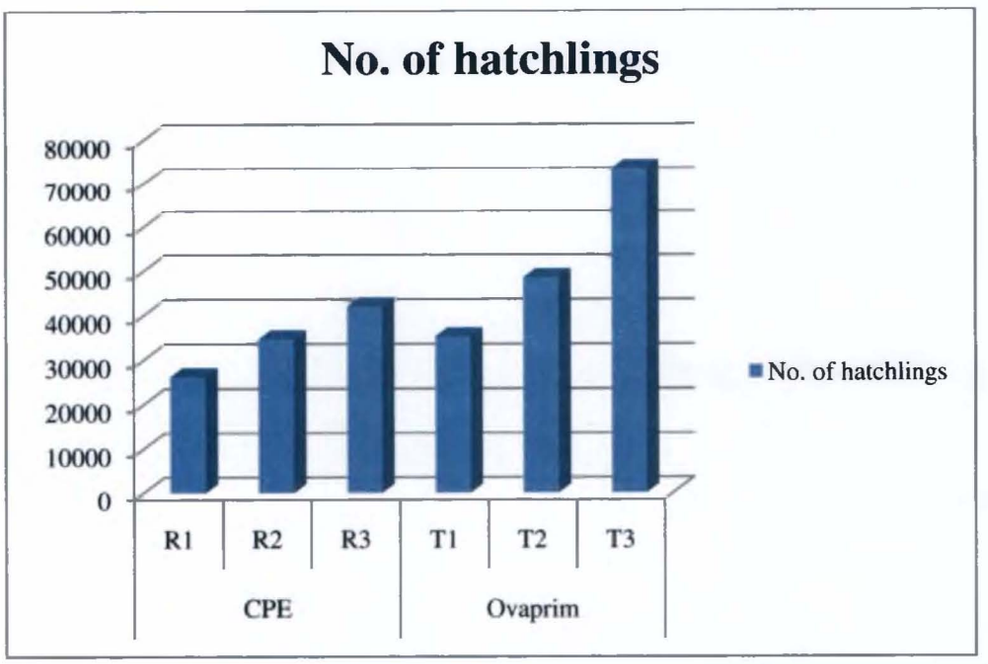


Fig 6: Number of hatchlings with CPE and Ovaprim

4.5.6. Hatching percentage with CPE and OVAPRIM

During experiment period, hatching percentage with the CPE and OVAPRIM was different with dose variation. In this experiment, ovaprim yield the higher percentage (78.40%, respect to dose of 0.5 ml/kg body weight to female and 0.5 ml/kg body weight to male) than that of CPE (62.2% respect to dose of 16 mg/kg body weight to female and 5 mg/kg body weight to male) as given Table 10.

Treatment (Experiment)	DOSE OF CPE (mg/kg bw)		DOSE OF OVAPRIM (ml/kg bw)		Hatching %
	Male	Female	Male	Female	
R_1	3	12	-	-	51.2
R_2	4	14	-	-	57
R_3	5	16	-	-	62.2
T_1	-	-	0.5	1	66.80
T_2	-	-	0.5	1.2	65.40
T_3	-	-	0.5	1.5	78.40

Table 10: Hatching percentage with CPE and OVAPRIM

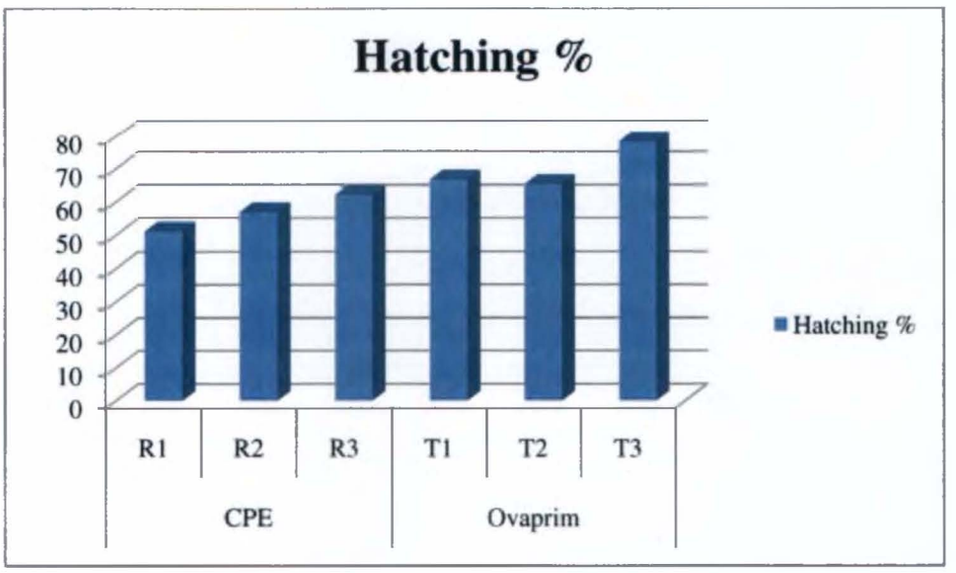


Fig 8: Hatching percentage with CPE and OVAPRIM

4.5.7. Number of Survival, Survival percentage with CPE and OVAPRIM

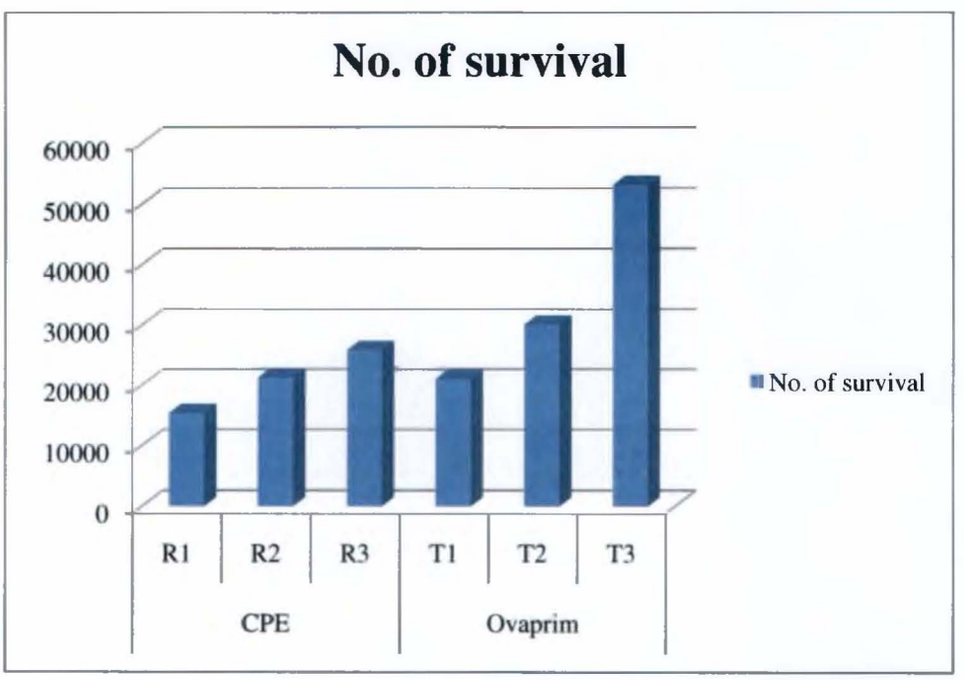


Fig 9: Number of Survival with CPE and OVAPRIM

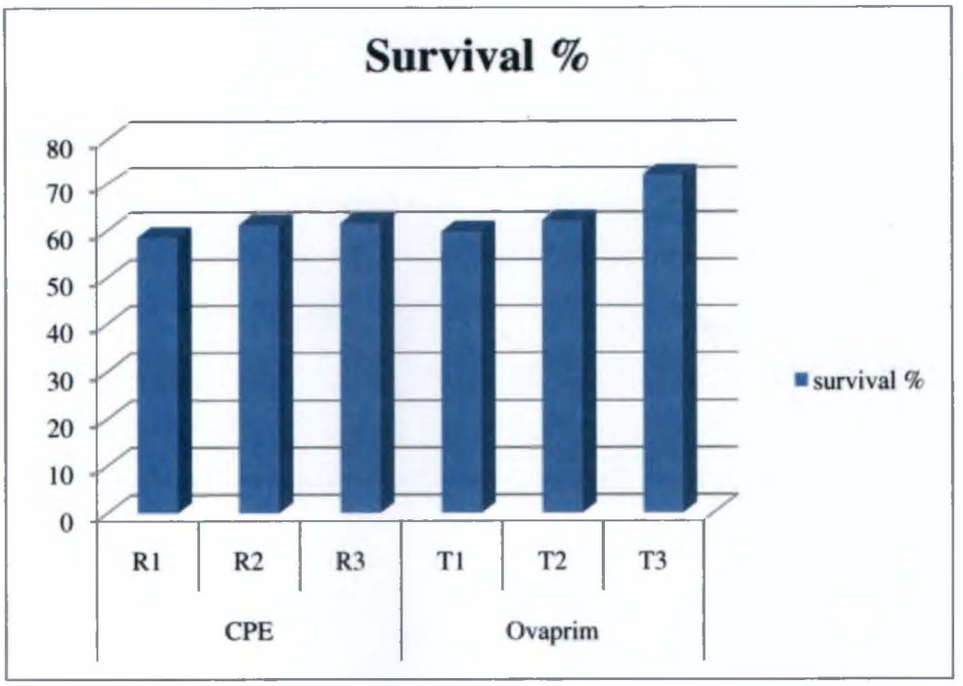


Fig 10: Survival percentage with CPE and OVAPRIM

Table 11: One way analysis of variance (ANOVA) for eggs released after injects the CPE and ovaprim hormone doses.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	1	2.82E+08	2.82E+08	0.619378*
Within Treatments	4	1.82E+09	4.55E+08	_
Total	5	2.1E+09	_	_

*Not significant

Table 12: One way analysis of variance (ANOVA) for fertilized percentage after injects the CPE and ovaprim hormone doses.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	1	8715.757	8715.757	2084.169**
Within Treatments	4	16.72754	4.181886	_
Total	5	8732.485	_	_

**Significant at P<0.01

Table13: One way analysis of variance (ANOVA) for hatchling percentage after injects the CPE and ovaprim hormone doses.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	1	4720.478	4720.478	311.7361**
Within Treatments	4	60.57018	15.14255	_
Total	5	4781.049	_	_

**Significant at P<0.01

Table14: One way analysis of variance (ANOVA) for survival percentage after injects the CPE and ovaprim hormone doses.

Source of Variation	*Degrees of freedom(df)*	Total sum of square	Mean sum of square	F ratio
Between Treatments	1	0.601667	0.601667	0.297609*
Within Treatments	4	8.086667	2.021667	_
Total	5	8.688333	_	_

*Not significant

4.5.8. USE OF DIFFERENT DOSES OF "CPE" IN THREE EXPERIMENT TANK

The latency period after the administration of Carp Pituitary Extract (CPE) at different doses in the three different treatments (R_1, R_2 and R_3) were observed to be 18 hours, 17 hours and 16 hours respectively. The corresponding numbers of eggs released in these three different treatments tanks were 68765, 79577 and 84475 respectively. The maximum rate of fertilization was observed in R_3 (80.2%) while the minimum was observed in R_1 (74.5%) and intermediate value of 76.5% in R_2. The numbers of eggs fertilized were estimated at 51230, 60876 and 67748 respectively in the treatments R_1, R_2 and R_3. The maximum numbers of hatchling was observed in R_3 (42003) identically a percentage of 62.2%, followed by R_2 (34699 numbers) with a percentage of 57%, while the minimum numbers of hatchling were observed in R_1 26229 numbers and a percentage of 51.2%.

4.5.9. USE OF DIFFERENT DOSES OF "OVAPRIM" IN THREE EXPERIMENT TANKS

The latency period after the administration of Ovaprim at different doses in the three different treatments (T_1, T_2 and T_3) were observed to be 12 hours, 11 hours and 9 hours respectively. The corresponding numbers of eggs released in these three different treatment tanks were 64355, 88760 and 108565 respectively. The maximum fertilization rate was observed in T_3 (86.3%) while the minimum was observed in T_1 (82.0%) and intermediate value (83.5%) in T_2. The numbers of eggs fertilized were estimated at 52771, 74114 and 93691 respectively in the treatments T_1, T_2 and T_3. The maximum number of hatchlings was observed in the treatment T_3 (73453 numbers) indicating a percentage of 78.4%, followed by T_2 (48470 numbers) with a percentage of 65.4%, while the minimum numbers of hatchling were in T_1 (35251 numbers) with 66.8%.

4.5.10. Maximum number of hatching percentage of *Pabda* with CPE and OVAPRIM

Table 15

Treatment	Hormone used	No. Of Fish (M:F)	Dose of hormone (mg/ kg)	No. Of eggs released	Hatching (%)
Maximum in R_3	CPE	9:6	F-16mg M-5mg	84475	62.2
Maximum in T_3	Ovaprim	9:6	F-1.5 ml M-0.5 ml	108565	78.40

The synthetic hormone (**Ovaprim**) yield better result, as the number of hatchlings (78.40%) was higher than that of pituitary extract (62.2%) (Table 6).

Table No.16: Induce breeding experiment of *Ompok pabda* by using CPE and OVAPRIM hormone

Hormone	CPE													
Experiment Tank	No. of individuals (M:F)	Male L (cm)	Male wt (g)	Female L (cm)	Femal e wt (g)	Dose of hormone (mg/ml) in MALE	Dose of hormone (mg/ml) in FEMALE	Latency period (hrs)	No. of eggs released	Fertilizatio n (%)	No. of Fertilized eggs	Hatching (%)	No. of hatchlings	Survival rate (%)
R1	M-9, F-6	17.14± 0.23	28.0± 1.00	18.80± 0.47	34.88± 1.13	3	12	18	68765	74.5	51230	51.2	26229	58.6
R2	M-9, F-6	17.02± 0.20	29.11± 0.80	19.23± 0.36	35.33± 0.33	4	14	17	79577	76.5	60876	57	34699	61.2
R3	M-9, F-6	17.39± 0.37	28.72± 0.67	19.12± 0.19	35.40± 0.32	5	16	16	84475	80.2	67748	62.2	42003	61.6
Hormone	OVAPRIM													
T1	M-9, F-6	16.83± 0.69	28.85± 1.73	18.81± 0.70	35.28± 2.72	0.5	1ml	12	64355	82	52771	66.80	35251	59.8
T2	M-9, F-6	16.42± 0.27	28.26± 1.79	18.88± 0.89	35.18± 3.44	0.5	1.2ml	11	88760	83.50	74114	65.40	48470	62.1
T3	M-9, F-6	16.47± 0.22	28.24± 1.75	18.90± 0.96	37.35± 2.40	0.5	1.5ml	9	108565	86.30	93691	78.40	73453	61.4

4.6. Brood stock management

4.6.1. Brood stock collection and management

Brood fishes were collected from River Ganga in the vicinity of Murshidabad, West Bengal during November- February. They were stocked in a pond about (0.5 ha) at the hatchery. The breeding and larval rearing experiments were carried out at the same farm.

A monthly water exchange of 50% was made to maintain water quality parameters within favorable ranges. Proper management practices were initiated for rapid enhancement of growth and maturity of the brooders. Aquatic macrophytes like *Hydrilla verticillata* and *Eichhornia crassipes* etc. were introduced as these plants serve as ideal shelter for the fishes and also as source of periphytic food organisms. The depth of the pond was maintained between 1.0 – 1.5 m. After 1-2 month of rearing the fishes were considered for breeding.

4.6.2. Species and Stocking Density

Initially *Ompok pabda* were collected from natural sources and are stocked in brooders pond. Different age groups and sizes are maintained at very low stocking density of 25 kg/bigha (Table5 and Plate C) and all the fishes survived, because of their hardy nature. This in Cates the positive side of *Pabda* culture with a high production per unit area.

Plate C: Stocking pond of *Pabda* brooder

4.6.3. Feed and Fertilizer management

- **For Brooders :-**

The fishes were fed with supplementary feed made up of mustard oil cake, rice bran (1:1 ratio), chopped earth worms, small live prawns and small fishes like *Puntius* sp. @5% of average body weight per day.

In the present study they were fed with trash fish (Bombay duck) and boiled chicken viscera and boiled gram (Plate D) at about 5- 10% of total body weight per day. Trash fish /boiled chicken viscera kept hanging (3 inch under water from water surface level) by thread at an interval of two inch.

Fishes were reared for a period of 6 months and feeding schedule was maintained throughout the culture period. The body weight of the fish ranged from 30-45g.

Plate D: Boiled feed (Gram) for *Pabda*

Plate E: Tubifex feed for spawn

- **For spawn :-**

24 hrs after hatching the spawns were fed with grinded/chopped tubifex worms at morning (7 am) and evening (4-5 pm) for two days (Plate E). Again some grinded tubifex worms were given for two days; then again light grinded worms were given to hatchlings for 7 days till half inch fry. The fry attained 5-6 cm/3-4.5 g in a rearing period of 40-50 days with a survival of 72.4%. Fingerlings of this size are considered fit for stocking in grow-out farming ponds.

Hatchery owner have applied proper feeding schedule for the brooders or nursery in their farms. They have provided feed and fertilizer on monthly basis. It is just before the start of the breeding season there is a change in feeding schedule. From February onwards the brooders are fed with boiled pea/ chicken viscera. Just after the onset of breeding season the feed is given regularly to spent brooders only early maturation. During pond preparation for brooders they apply 9 kg (1 bag) of lime (CaO) and 0.1 MT of raw cow dung per 0.13 ha pond.

4.6.4. Source of brood stock:

The hatchery owner depends entirely on outside source for collection of brood fish prior to breeding programme. The entire brood stock collected from The Ganga River near Murshidabad, West- Bengal, and reared in the pond.

Plate F: *Pabda* Brooders were kept in hapa before injection

Plate G (a): Pituitary gland

Plate G (b): Preparation of CPE hormone by pituitary gland

Plate H: OVAPRIM

Plate I (a): Hormone administration to female *Pabda*

Plate I (b): Hormone administration to male *Pabda*

Plate J: Injected fishes in cistern

Plate K: Segregation of fish (Male and Female) for stripping

Plate L: Stripping of female fish to collect egg

Plate M: Sacrificing the male to collect the testes

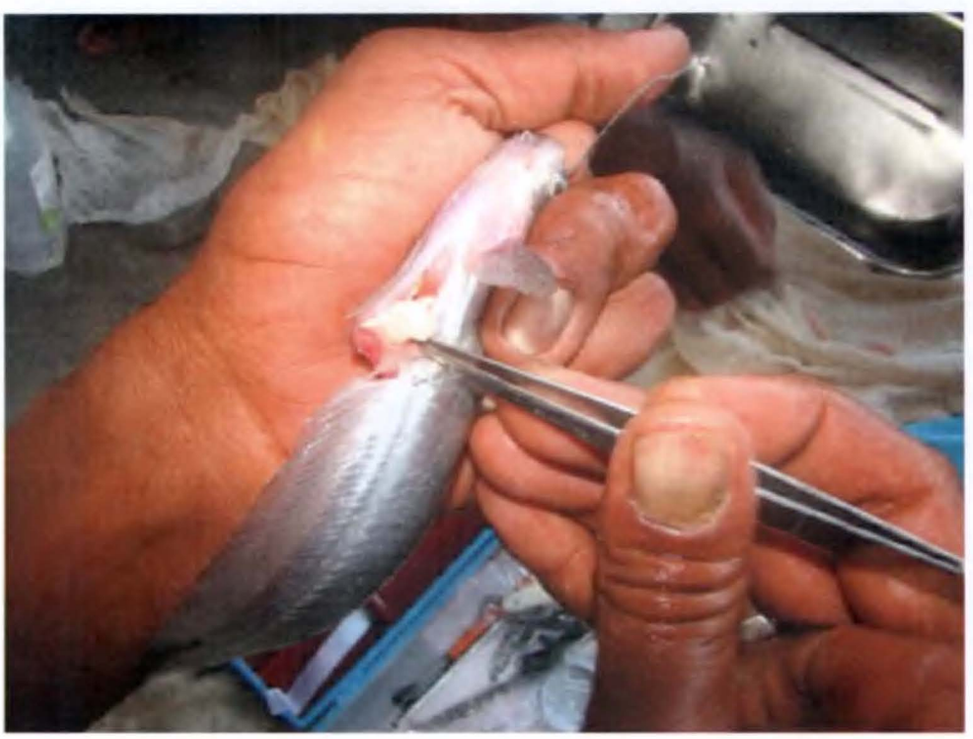


Plate N: Removal of testes from male *Pabda*

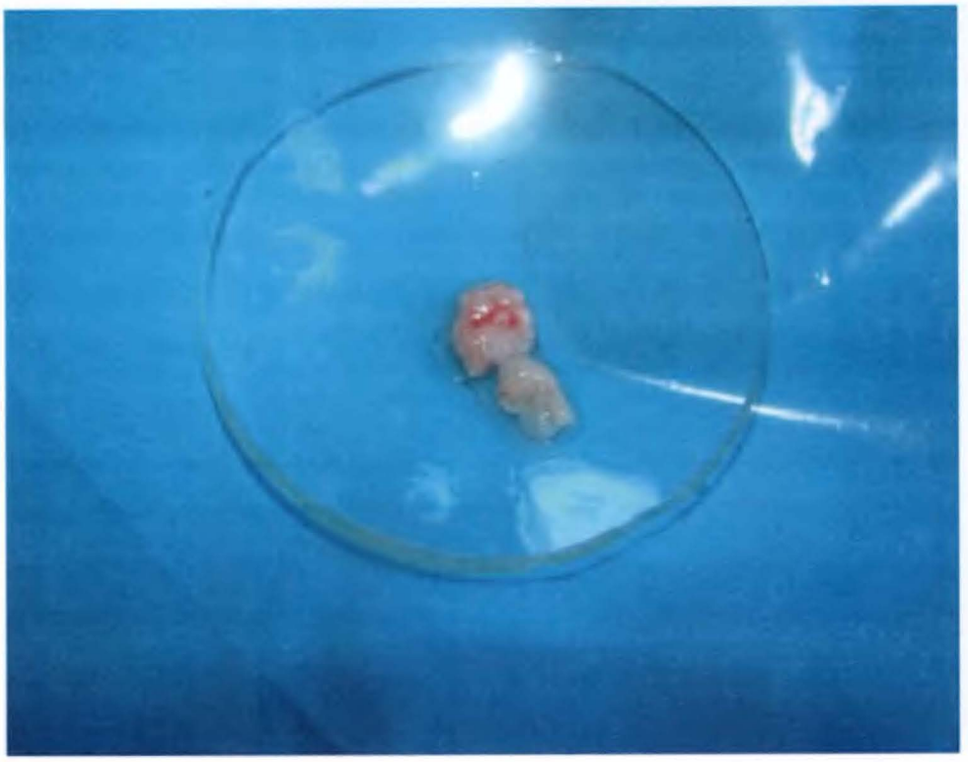


Plate O: Testes of male collected in 0.9% Normal Saline Water

Plate P: (a) Release of milt by squeezing process of Testes

Plate P: (b) Preparation of milt suspensions

Plate Q : Administration of milt suspensions over the eggs and mixing gently with a feather for fertilization

Plate R: Fertilized eggs mixed with distilled water

Plate S: Swollen eggs

Plate T: Stretched net over the iron frame (KHANCHA)

Plate U: Netting ready for incubation of fertilized eggs

Plate V: Spreading of fertilized eggs over the net for hatching

Plate W: Use of palm leaves for shelter of newly hatchlings

Plate X: spawn of *Pabda*

Plate Y: Juvenile of *Pabda*

Plate Z: Fry of *Pabda*

CHAPTER 5

DISCUSSION

5. DISCUSSION

5.1. Status of fish and fish seed production

In the present study efforts were initiated for commercial production of *Pabda* seed in captivity. This year (2012) 80% seed was produced through application of breeding (Ovaprim) and 20% by CPE. Due to, highly market value hatchery owners are very interested for large scale seed production. Mukherjee *et al.* (2002). reported that many fish species has declined already and some have become endangered due to combination of factors like over-exploitation, pesticide and aquatic pollution, spread of disease, uncontrolled introduction of exotic fishes, and habitat modification due to industrialization, river-valley projects, excessive water abstraction and siltation. The Department of Fisheries, Government of West Bengal is trying to conserve these species through artificial breeding and restocking in their natural habitat and to establish gene banks using cryopreservation techniques.

5.2. Pabda Hatchery

Pabda hatchery has not yet been standardized in the study area, which deals with carps and some exotic species hatchery. Three years ago, efforts were initiated for induce breeding through ovaprim on trialed basis. Previous to the several attempts were initiated for breeding of *Pabda* since 2010. Regional Research center of CIFA at Kalyani, West Bengal made a major breakthrough in captive breeding of *O.pabda* first time during the year 1999 (Chakraborty *et al.* 2006). According to Khanam (2005), *Pabda* is declining fast due to climatic change and destruction of breeding ground. Recently IUCN (2000) has declared the *pabda* as endangered fish. Till then various experiments were conducted to protect this species from being extinct. In 2002 first success were made to produce huge amount of fry commercially by the farm owner in West Bengal.

5.3. Breeding season

Matured fishes (1 year+) were selected for induce breeding. Females mature during May – July. According to Khanam (2005) *Pabda* become sexually mature at the age of 10-11 months and the long breeding season extends up to mid April to mid August. (Banik *et al.*, 2011) reported that male *O. bimaculatus* was become mature

during late March-April and female during May to the end of July and breeding also observed in June- August. It was observed that price of fry fluctuate in the breeding season with a varying rate Rs. 4.00-5.00 in June, Rs.3.00-4.00 in July and Rs.5.00-6.00 in August-September. Compare to Bangladesh (Rs. 2.00/piece) the rate is high in Bengal.

5.4. Breeding Practice

5.4.1. Age and size of brooders

The average age of brooder was 1 year+ and average wt. and size was 28.24-37.35 g, 16.4-18.9 cm respectively. Mukherjee *et al.* (2002) reported that the brooders attained maturity after one year while the average body weight was 85gm. Both male and female broodstocks were found to be mature in May.

5.4.2. Sex ratio

A sex ratio of 9:6 (M:F) was used in each treatment. The average length and weight of fishes used are given Table 16. Sarkar *et al.* (2005) also used same ratios. Khanam (2005) a ratio of 1:1, However maximum eggs were obtained when male and female ratio is maintained 1.5:1. Fertilization rate was also higher at the said ratio.

5.4.3. Stripping

It was observed that maximum breeders used the stripping procedure in hatchery (Plate L) as it ensures higher fertilization. Instead of that the most of the fish breeders still use natural breeding. One of the constrain may be the lack of expertise in this field. In the modern hatcheries of Bengal stripping is mainly practiced for exotic carps and cat fishes. For IMC it is done for hybridization and acute cases of male brooders shortage. The farmers practice without considering the negative consequences like damage to internal organs, stress to the brooders, mortality, etc. (Plate L - Q).

5.4.4. Restocking of brooders

Restocking of brooders after spawning is a common practice adopted by hatchery owners. This practice allows the seed producer to reap the harvest repeatedly with the same to numbers of brooders for a longer period of time without replacing

the stock. It is quite interesting to note that the breeders in some hatcheries reported that they continue with the breeding of same set of brooders till it sustains. The hatchery owners restock the brooders and kept as potential brood stock for the ensuing season. There are few who sales the brooders partially after breeding. There are also some of them who sale the non performing ones, old aged, etc. This blocks the supplementation of genetic material into the breeding population.

5.5. Inducing agent

5.5.1. CPE protocol used in the experiment

The different pituitary doses were used in the each treatment, which is given in the Table 7. It was observed that the dose was higher in R_3 treatment. Best results were obtained in $R_1$and R_2 treatment. Mukharjee *et al.* (2002) advocated a single dose of hormone 16 mg/kg BW as pituitary for female, which yielded around 4,200 and hatching percentage was 50%. Khanam (2005) reported a double dose. Female was given a dose of 2-3mgPG/kg body weight, then after 6 hr again female was induced with 4-6 mg PG/kg body weight, at the same time male was given a dose of 4-6 mg PG/kg body weight.

5.5.2. OVAPRIM protocol used in the experiment

The ovaprim protocol was used for each treatment are presented in Table 8. It was observed that the dose did not vary much with the long breeding season as observed with the pituitary gland.

5.5.3. Effect of CPE and OVAPRIM on Latency period

The latency period was different in each treatment as recorded in Table 16, Which was highest in R_1 (18 hr) and lowest in R_3(16 hr) in respect of CPE, while highest in T_1 (12), lowest in T_3 (9 hr) in respect of ovaprim. Latency period fluctuate depending on various factors such as temperature, pH, hardness and preparation of hormone etc. Chakrabarty *et al.*, (2009) reported a 23 hr latency period against single dose of ovaprim (1-1.5 ml/kg BW for female and 0.5-1.0 ml/kg BW). Mukharjee *et al.*, (2002) repoted a latency period of 22 hr while Khanam (2005) a latency period of 18-24 hr. Chakrabarty *et al.*, (2006) also reported latency period of 20-26 hr in response to ovaprim. Sarkar *et al.*, (2005), Sarma *et al.*, (2012) reported a hatching

time 22 hr while Vijayakumar (2010) reported a hatching time of 26 hr after spawning in case of *Ompok malabaricus.*

5.5.4. Number of eggs release and fertilized eggs with CPE and OVAPRIM

According to protocol of CPE and OVAPRIM which was used in the two sets, consisting of each set of three treatments, for each treatment different hormone doses were used for CPE, maximum numbers of eggs was 84475 released, and The number of fertilized eggs was 67748 With ovaprim was eggs released was108565 and fertilized eggs 93691 compare to CPE. Chakraborty *et al.,* (2007) reported that the fecundity was high as 8400-13800 nos./100 gm of fish. Sarkar *et al.,* (2005) reported that the fecundity ranged from 84-138 eggs gm^{-1} body weight.

5.5.5. Number of hatchlings with CPE and OVAPRIM

With CPE hormone the number of hatchlings was 26229 in R_1, 34699 in R_2 and 42003 in R_3, while with ovaprim the hatchling numbers was 35251 in T_1, 48470 in T_2 and 73453 in T_3.

5.5.6. Hatching percentage with CPE and OVAPRIM

Hatching percentage was 51.2% in R_1, 57% in R_2 and 62.2% in R_3 against CPE and was less than that of ovaprim. Hatching percentage of 66.80% in T_1, 65.40% in T_2 and 78.40% in T_3 was noticed with ovaprim. The dose of ovaprim applied was 0.6 ml for male, 0.5 ml for female ratio maintaining a sex of 9:6=M:F, 8:5=M:F with a maximum hatching percentage of 65.2%, 66.5% against sex ratio of 9:6 and 67.5% ,62.3% against 8:6 as recorded.

5.5.7. Survival number, Survival percentage with CPE and OVAPRIM

It was observed that number of survival was 15370 in R_1, 21235 in R_2 and 25873 in R_3 and the percentage was 58.6%, 61.2% and 61.6% respectively in response to CPE. While with ovaprim the survival number was 21080 in T_1, 30099 in T_2 and 53179 in T_3 and the percentage 59.8%, 62.1% and 74.4% respectively. Sarkar *et al.,* (2005) reported survival percentage of 64.9-65.88% while applied a dose of 0.6 ml (male) and 0.5 ml (female) respectively.

5.6. Brood stock management

5.6.1. Species and Stocking Density

The hatchery owner have stocked multi species brood fishes like *Pangas, Pacu, Pabda, Chitala* and IMC of different age groups and sizes with high stocking density except *Pabda* fish. Stocking density is considered to be one of the vital criteria to be taken into account in brood stock management programme. During my study period it was recorded that the hatchery owner maintained a stocking density of *Pabda* 25 kg/bigha. Mukharjee *et al.,* (2002) reported that *Pabda* juveniles were collected from river, can be stocked in a polyculture tank with IMC in a 0.5 ha pond. .Khanam (2005) reported a stock migrate of 70-80 broods per decimal with a sex ratio of 1:1.

5.6.2. Feed and Fertilizer management

During study period the fish stock were fed with conventional feed consisting of mustard oil cake and rice bran @ 5% of average body weight per day in the ratio of 1:1. Sometimes trash fish like Bombay duck and boiled chicken viscera are used at about 5- 10% of total body weight per day. Just after the one set of breeding season formulated feed is given regularly to spent fish to achieve early maturation. With this change in feeding schedule the brood fish attain maturity stage quickly. The body weight of the fish ranged from 30-45g. The maximum numbers of farmers provide feed on weekly basis but fertilizer provided on monthly basis. Just before the start of the breeding season there is a change in feeding schedule. According to Khanam (2005) brood fish were fed with good quality feed at a rate of 5% BW which was prepared with 30% fish meal, 30% soybean meal, 30% wheat flour and 10% rice bran and vitamin. Purkayastha *et al.,* (2012) used the same feeding schedule while body weight of the fish were ranged between 40-50 gm. Chakrabarty *et al.,* (2009) reported the use of trash fish and boiled chicken viscera as feed at the rate of 5-10% of total body weight per day.

For pond preparation lime (250 kg/acre), cattle dung (500 kg/acre), urea (15 kg/acre), TSP (Triple Super Phosphate) (7-10 kg/acre) and MP (Murate of Potash) at 2 kg/acre, are applied. Fertilizer applied 5-7 days after application of lime.

The newly hatched larva after four days accept grinded tubifex and egg custard at the rate of 100% of total body weight. Most farmers use tubifex worm and egg yolk. According to Hecht and Appelbaum (1987), the juveniles of *Clarias gariepinus* preferred live feed rather than formulated feed. Live earth worm is better to tubifex, because of its high protein content, the percentage composition of protein in earth worm is 67.68% (Kumar *et al.*, 2007); while in live tubifex worm it is 32.13% (Sarowar *et al.*, 2010) for *Ompok pabda*, formulated feed with the addition of piscimix helps to combat mortality, enhances survival rates and allows larger growth (Mukherjee *et al.*, 2002).

CHAPTER 6

SUMMARY

6. SUMMARY

The technology of breeding fish in captivity was developed by the scientists to produce quality seeds of desired species when it was understood that natural source is declining fast due to pollution, destruction of spawning ground, habitats and related anthropogenic activities. The species has been listed as one of the endangered fishes. The reason for this may be attributed to indiscriminate fishing of the brooders of this fish during the breeding season, wide use of pesticides and insecticide in agriculture field causing pollution as well as siltation. Considering the shortfall. It was implemented to the farming situation to produce quality seed and the increasing demand of culture sector with the application of remarkable technology of fish farming.

The study was undertaken towards of standardization of breeding protocol and brood stock development. While comparing the effect of dosages on induced breeding by CPE and ovaprim on *O.pabda* it was found that both the dosages were quite effective. However, respect to CPE, in R_3 the dose @ 5mg/kg bw of male and @ 16mg/kg bw of female indicated less fertilization & less hatchlings. As compared to that of ovaprim in T_3 @ 0.5 ml/kg bw of male and @ 1.5 ml/kg bw of female. Different synthetic hormone were used for effective induced spawning and seed production of cat fishes by Mukharjee *et al.,* (2002), Chakraborty *et al.,* (2007), Khanam *et al.,* (2005). As compared to the conventional hypopysation, which requires high quantity of carp pituitary extract (CPE), ovaprim is quite effective at low quantity for inducing breeding under captivity. Vijayakumar (2010) reported induce spawning of *O.malabaricus* with single dose ovaprim @ 0.5 ml/kg bw. Banik *et al.,* (2011) also reported induce spawning of *O.bimaculatus* with single dose ovaprim @ 0.5 ml/kg bw of male and 1.5 ml/kg bw of female. Haniffa *et al.,* (2001) advocated three different dosage of ovaprim (0.3ml,0.5ml and 0.7ml) for induce breeding of *O.malabaricus* where the variations in fertilization rate were 70,87 and 91% and survival rate was 58,62 and 70% respectively. During this study complete spawning, high rate of fertilization and hatching were 66.88, 65.40 and 78.40% while survival rate was 59.8, 62.1 and 61.4% respectively.

From results it can be concluded that for induced breeding with an aim to achieve CPE should be substituted by ovaprim. Because ovaprim not only induce complete spawning but the fertilization, hatching and survival is ensured. Further study should be conducted for better management and understanding about the reproductive physiology, breeding behavior and larval rearing the fishes to save this unique fish in their natural habitat.

CHAPTER 7

BIBLIOGRAPHY

BIBLIOGRAPHY

Adoni, A., D.G. Joshi, K. Ghosh, S.K. Chourasia, A.K. Vaishya, Manoj yadav and H.G. Verma.1985. Work book on limnology. Pratibha publisher, Sagar: 1-166.

Alok, D., Krishnan, T., Talwar, G.P. and Garg, L.C.1993. Induced spawning of cat fish *Heteropneustes fossilis* (Bloch), using D-Lyse super (6) salmon gondotropin releasing hormone analog. *Aquaculture* **115**: 159-167.

Andersen, O., and Hayes, H., 2005. Population Genetics. In T. Gjedrem (*ed.*), Selection and Breeding Programs in Aquaculture: 23-33.

APHA (2000). Standard methods for analysis of water and waste water American Public Health Associated. Washington, D.C.

Ayyappan, S., Raizada, S. and Reddy, A.K. 2001. Captive breeding and culture of new species of aquaculture.pp 1-20. *In* Ponniah, A.G., Lal, K.K., Baseer, V.S (eds.), *Captive Breeding for Aquaculture and Fish Germplasm Conservation* NBFGR-NATP Publ.3.

Ayyappan, S. 2006. Hand Book of Fisheries and Aquaculture. ICAR Publication, New Delhi: 248-264.

Banik, S., Som, S., Roy, N., Nath, S., and Choudhary, S. 2002. Threatened fish fauna of Tripura: Strategies for conservation. In: Banik, S. Ed. **Proceedings of National Seminar on Conservation of Aquaculture Resources, March, 2002, Department of Life Science, Tripura University, India. Tripura.**Tripura University: 62-67.

Banik, S., Goswami, Pritamand Malla, Samir. 2011. Ex-situ studies of captive breeding of *Ompok bimaculatus* (BLOCH, 1794) in Tripura., Journal of advanced laboratory research in biology, vol. II Issue III July: 133-137.

Banik, S., and Malla, Samir. 2011. Habitat Maping for *Ompok pabda* (Hamilton-Buchanan, 1822) Gomati River of Tripura. **Proceedings of the 10th Agricultural Science Congress, February 10-12, 2011, Lucknow India.** Lucknow, National Bureau of Fish Genetic Resources (ICAR):388.

Banik, S., Goswami, Pritam. Acharjee, T., and Malla, Samir., 2012. *Ompok pabda* (Hamilton-Buchanan, 1822): An endangered Catfish of Tripura, India: Reproductive Physiology Related to Freshwater Lotic Environment. Journal of Environment (2012). Vol. 01, Issue 02:45-55.

Banik, S., and Bhattacharya, P. 2012.*Ompok pabda* (Hamilton, 1822) of Tripura, India: an endangered fish species in relation to some biological parameters. **Res. J.Biol.** 2 (3): 91-97.

Bhowmick, M.L., Mondal, S.C., Chakrabarti, P.P., Das, N.K., Saha, R.N., and Ayyapan. S. 2000. Captive breeding and rearing of *Ompok pabda.* Sarkar, A.G. ed. In: **Fish biodiversity of North East India.** NBFGR Lucknow, NATP Publishers.

Bondari, K. and Dunham, R.A. 1983. Effects of inbreeding of economic traits of Channel catfish.Theor.*App/. Genet.* **74:** 1-9.on of a Silurid fish Pabo, *Ompok pabo* (Ham) Fishing Chimes Vol. 21. No.1, 2001:75-78.

Bondari, K. 1983. Response to bidirectional selection for body weight in channel catfish. Aquaculture 33:73-81.

Bornbusch, Alan H. 1995. " Phylogenetic relationship within the Eurasian catfish Family Siluridae, with comments on genetic validities and biogeography". Zoological Journal of the Linnean Society 115:1-46.

CAMP (1998) Report of the Workshop on Conservation Assessment and Management Plan (CAMP) for Freshwater Fishes of India. Zoo Out Reach Organization and NBFGR, Lucknow, India: 156.

Chondar, S. L. 1994. Induce Carp Breeding. CBS Publishers and Distributors, New Delhi **(3):** 24-86.

Chakraborty, N.M., Chakraborty, P.P. and Mondal, S.C. 2006. Mass seed production of *Pabda* and their farming- a challenging step for sustainable utilisation of this vulnerable fish species. Fishing Chimes, vol.26 No.1:133-135.

Chakraborty, N.M., Mondal, S.C., and Chakraborty, P.P., 2007(a). Artificial Breeding, Seed Production, and Rearing of Butter fish (*Ompok pabda*)-asignificant milestone in technology advancement. Fishing Chimes, vol. 26No.10:134-136.

Chakrabarty, N.M., Chakraborty, P.P., and Mondal, S.C. 2007 (b). On Breeding of Tangra (*Mystus vittatus*), and production of its post larvae/Fry. Fishing Chimes Vol.27.No. 4:16-18.

Chakraborty, N.M., Mondal, S.C. and Chakrabarty, P.P. 2007 (c). Captive breeding, Early Life History and Rearing of Hatchling of *Ompok pabda* (Hamilton). *Environment & Ecology* 25(1):58-61.

Chakraborty, P.P., Chakraborty, N.M. and Mondal, S.C., 2009. Breeding and seed production of butter catfish, *ompok pabda* (Siluridae) at Kalyani Centre of CIFA, India, Aquaculture Asia Magazine, January-March: 33-35.

Choudhury, H. 1956. Effect of pituitary injections and other factors on reproduction of pond fishes. *Ala. Pol. Inst. Bull.* (MS Dissertation).

Choudhury, H., and Alikunhi, K. H. 1957. Observations on the spawning in Indian major carps by hormone injection; *Curr. Sci.*, **26:** 381-382.

Demska-Zakes, K., and Dlugosz, M. 1995. Fecundity of vend ace from two lakes of Mazurian district. **Rybna**, 31: 37-50.

DoF. 2008. Annual Report 2007-2008. Department of Fisheries, Bangladesh, Ministry of Fisheries and Livestock: 111.

Dutta, O.K. 2000. An Overview on the Status of Fish Seed Production in Assam, p.1-4. In S.K.Das (ed.) proceedings of the first state level workshop on current

status and future of the fish seed production industry in the state of Assam. April 8-9, 1999. *Spec. Publ.* ***3,*** 39pp. College of Fisheries, Assam Agricultural University, Raha, Nagaon, Assam, India.

Fagede, S. O., Adebesi, A.A., and Aatanda, A.N. 1984. The breeding cycle of tilapia, *Sarotherodon galilaeus* in the IITA Lake, Ibadan, Nigeria. **Hydrobiologia,** 100: 493-500.

Gupta, S.D; Rath, S.C. and Dasgupta, S. 1995. Fertilization efficiency of cryopreserved spermatozoa over four years at different time intervals after thawing. *Geobios* **22**: 208-211.

Goswami, M.M., Hazarika, R. And Saud, B.J., 2007. Iinduced spawning, embryonic and development of *Pangasius pangasius* (Hamilton-Buchanan). *J.Inland Fish.Soc. India,* 39(2): 1-9.

Haniffa, A.M., Thangarose, M., and Junaith, S.M. 2000. Induced spawning of the stripped Murrel Channa striatus using pituitary extracts, Human chorionic Gonadototropin, Luteinizing Hormone Releasing Hormone Analogue, and Ovaprim. *Actalcht. Piscat.*, **30** (1): 53-60.

Haniffa, A.M., Jesu Arokiya Raj A and Verma, T.M.A. 2001. Optimum rearing conditions for successful artificial propagation of catfish. NBFGR-NATP Publ.3:1-11.

Hecht, T and Appelbaum, S. (1987). Notes on growth of *clarias gariepinus* during the primary nurshing phase. Aquaculture, 63, 1987: 195-204.

Hussain, A., 2006. Seed production of *Ompok pabda* (Ham) in hatcheries of C.G.Co-operative fish federation. Fishing Chimes 2006, Vol. 26 No.1: 136-139.

Hussain, M.G. and Mazid, M.A. 1997. Problems of inbreeding and cross breeding in hatchery and their remedial mitigating measure, pp. 7-11.In M.R. Hasan, M.M. Rahman& M.A. Sattar (eds). *Quality assurance in* induced breeding.Jessore, Bangladesh, Department of Fisheries.

IUCN (2000) Red book of threatened fishes of Bangladesh, IUCN- The World Conservation Unian. Xii: 116.

Jhingran, V.G. 2004. Fish and Fisheries of India. Reprinted. Delhi, Daya Publisher.

Khanam, T., Mollah, F.A. and Nurul Haque, A.K.M., 2005. Study on seed production Technique of Indigenous Magur (*Clarias batrachus*), Singhi (*Heteropneustes fossilis*), and Pabda (*Ompok pabda*) through induced breeding.

Khan, H. 1938. Ovulation in fish (effect of administration of anterior lobe of pituitary gland). *Current. Sci.*, **7 (50):233-234.**

Kumar, P. Sagar, V.Choudhary A.K and Kumar, N. 2007. Vermiculture Boon for fish farmers. Fishing Chimes, vol.27, No.1: 40-42.

Lin, S. Y. 1940. Fish culture in the New Territories of Hong Kong Fisheries Research Station 1 (2).

Little, D.C., Satapornvanit, A. & Edwards, P. 2002. Freshwater fish seed quality in Asia: 185-193. . In P. Edwards, D.C. Little & H. Demaine (eds). Rural Aquaculture.Oxon, United Kingdom, CABI Publishing.

Mijkherjee, M., Praharaj, Aloke., and Das, Shamik., 2002. Conservation of endangered fish stocks through artificial propagation and larval rearing technique in West Bengal, India. Aquaculture Asia, April-june 2002: 8-11.

Mukherjee, M., and Das, S., 2001. .Artificial propagation of a Silurid fish Pabo, *Ompok pabo* (Ham) *Fishing chimes* Vol 21. No. 1, 2001: 75-78.

Nandeesha M.C., Das, S.K., Nathaniel, D.E. and Varghese, T.J. 1990. Project report on breeding of carps with ovaprim in India. *Special publication* **4**, Asian Fisheries society, Indian Branch, Mangalore, India.

Pillay, T.V.R. 2000.Aquaculture – Principles and Practices. Cambridge, Fishing News Books.

Ponniah, A. G. and Sarkar, U. K. 2000. Fish Biodiversity of North-East India., *NBFGR. NATP Publ*: 228 p.

Purkayastha, S., Sarma, S., Gupta, S., Santosh kumar singh, A., and Biswas, S.P., 2012. Captive breeding of an endangered fish *Ompok pabda* (Hamilton-Buchanan) with ovatide from Guwahati, Assam. Asian Journal Exp. Biol. Sci. Vol. 3(2): 267-271.

Ramaswamy, L. S. and Sundararaj, B. 1956. Induce spawning in the Indian catfish, *Science* **123** (3207): 1080.

Sarkar, U.K., Deepak, P.K., Negi, R.S., Poul, S.K. and Singh, S.P. 2005. Captive breeding of an endangered fish *Ompok pabda* (Hamilton-Buchanan) using different doses of ovaprim J. Inland Fish Soc.India, 37(2): 37-42.

Sarkar, S., Kuri, Kartik Chandra, Rezowan, M., Chowdhury, S.M., Rahman, T. 2010. Farming and Broodstock Management of Pangus in Bangladesh. Fishing Chimes., Vol. 30 No. 2.

Sarower, M.N, Jewel, M.Z.H, Sayeed, M.A and Mollah, M.F.A. 2010. Impact of different diets on growth and survival of *Channa striatus* fry. Int.J.Bio Res. 1(3): 08-12.

Sridhar, S., Vijyakumar, C. And Haniffa, M.A. 1998. Induced spawning and establishment of a captive population for an endangered fish *Ompok bimaculatus.* India. Curr. Sci., 75:1066-1068.

Sarma, D., Das, J., Dutta, A & Goswami, U.C. 2011. Captive breeding and larval rearing of an endangered fish, *Ompok pabo* (Hamilton-Buchanan) through low cost technology. International Journal of Biological Technology (2012) 3(1): 1-4.

Sarma, D., Das, J., Dutta, A & Goswami, U.C. 2012. Early embryonic and larval development of *Ompok pabo* with notes on its nursery rearing. European Journal of Experimental Biology, 2012, 2 (1): 253-260.

Sahoo, C.D., Bhakat, P.B., Gupta, S.D., and Rath, S.C. 2001. Design and Cost Estimation of a Medium Scale Carp Hatchery for Indian Farmers. *Fishing Chimes 20* (12): 51-52.

Thomas, P.C, Rath, S.C, and Mohapatra, K.D. 2003. Breeding and Seed Production of Fin Fish & Shell Fish.Daya Publishing House, Delhi: 40-105.

Vijayakumar,C. 2010. Conservation of an Endangered Indian Catfish *Ompok malabaricus* through captive breeding and Establishment of captive population. International Journal of Biological Technology (2010):1(2):131-133.

Weber, M.And L.F. de Beaufort, 1913: The fishes of the Indo-Australian Archipelago.II.-Malacopterygii, Mycotophoidea, Ostariophysi :I.-Siluroidea. E.J.Brill, Leiden: 404.