INDUCED BREEDING AND REARING PERFORMANCE OF INDIAN MAJOR CARPS (*LABEO ROHITA* AND *CIRRHINUS MRIGALA*) AT FISHERIES DEVELOPMENT CENTER, BHANDARA, CHITWAN

Deewa Khanal

T.U. Registration No: 5-2-19-413-2013

T.U. Examination Roll No: 615

Batch: 2074

A thesis submitted

In **partial fulfillment of the** requirements for the Degree of Masters of Science in **Zoology with special paper Fish Biology and Aquaculture**

Submitted to

Department of Zoology

Amrit Campus

Lainchaur, **Kathmandu, Nepal**

April, 2023

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DECLARATION

I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

Khanal

.....

Date: ..2023/04/09...

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RECOMMENDATION

This is recommended that the thesis entitled **"Induced breeding and rearing performance of Indian Major Carps (*Labeo rohita* and *Cirrhinus mrigala*) at Fisheries Development Center, Bhandara, Chitwan"** has been carried out by **Ms. Deewa Khanal** for the partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with a special paper Fish Biology and Aquaculture. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any degree in any institution.

.....

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Asst. Prof. Santoshi Shrestha

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LETTER OF APPROVAL

On the recommendation of supervisor **"Assistant Prof. Santoshi Shrestha"** this dissertation submitted by **Ms. Deewa Khanal** entitled **"Induced breeding and rearing performance of Indian Major Carps (*Labeo rohita* and *Cirrhinus mrigala*) at Fisheries Development Center, Bhandara, Chitwan"** is approved for the examination of the requirements for Master's degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by **Mrs. Deewa Khanal** entitled **"Induced breedingand rearing performance of Indian Major Carps (*Labeo rohita* and *Cirrhinus mrigala*) at Fisheries Development Center, Bhandara, Chitwan"** has been approved as a partial fulfillment of the requirements for the award of the degree of Master of Science in Zoology with a special paper Fish Biology and Aquaculture.

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LIST OF ABBREVIATIONS

BW	Body Weight
CPE	Carp Pituitary Extract
CPFCC	Central Fisheries Promotion and Conservation Center
CO_2	Carbon-dioxide
DO	Dissolved Oxygen
FDC	Fisheries Development Center
FAO	Food and Agricultural Organization
GnRH	Gonadotropin Releasing Hormone
GSI	Gonado Somatic Index
HCG	Human Chorionic Gonadotropin
IU HCG	International Unit Human Chorionic Gonadotropin
LH-RH-a	Luteinizing Hormone-Releasing Hormone analoque
pH	Potential of Hydrogen
PG	Pituitary Gland
PGE	Prostaglandin E
sGnRHa	Salmon Gonadotropin-Releasing Hormone Analogue

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ABSTRACT

The study was carried out in the induced breeding and rearing performance of Indian Major Carps (*Labeo rohita* and *Cirrhinus mrigala*) at Fisheries Development Center, Bhandara, Chitwan from June to September 2022. The embryonic development from egg to fingerlings as well as fecundity rate, GSI, fertility rate, hatching rate, survival rate, and growth of both species were studied. The Ovaprim hormone at a ratio of 0.25 ml/kg for male and 0.5 ml/kg for female of Rohu and Naini were administrated. The fecundity of Rohu and Naini was observed between 227,540 to 675,000 and 161,400 to 608,400 while GSI was between 10.33% to 19.74% in Rohu and 10.71% to 28.15% in Naini respectively. The latency period observed was 7-8 hrs whereas the hatching occurred after 17-18 hrs. A total of 6 hapas (3 for Rohu and 3 for Naini) of $1\times1\times1m^3$ were used to study the growth of fish and 100 hatchlings were kept in each hapa. The fertility, hatching, and survival rate were 82.28-87.27%, 73.91-83.33%, and 62-73% in Rohu and 79.63-87.88%, 77.90-83.90%, and 59-75% in Naini respectively. The correlation between the length and weight of fish was found near 1 which represents a similar relation and the value of regression coefficient 'b' ranged from 2.1 to 2.5 showing negative allometry. The condition factor (K) and relative condition factor (Kn) were found >1 in both fish which shows proper growth of the fishes.

Keywords: *Induced breeding, Length-weight relationship*, *Ovaprim, Fecundity*

CHAPTER-ONE

INTRODUCTION

Aquaculture is an employment and income-generating component which leads to sustainable rural pond fish farming (Sheikh and Sheikh, 2004). Fish has a high source of protein as compared to other animal products and an increase in health awareness has soared up fish consumption which led to the promotion of the aquaculture industry (Mishra, 2015). It is an important cash crop and an inexpensive source of protein in many regions of the world. In Nepal, one of the major agricultural activities is fisheries which play an important role in nutrition, income generation, and employment (Choudhary *et al.,* 2021). More than 6,000 rivers and streams are facilitating as a source of water for aquaculture where the terai plain with 94% of fish ponds of the country beings the major pond fish production area. Indian major carp are considered economically important food fish in Nepal. Around 95% of total fish production is occupied by the Carp in Nepal which can be extended up to rural areas and income will be generated up to four times (Mishra and Kunwar, 2014). The total fish production in Nepal is 91,832 mt. with 4.9 t/ha (CPFCC, 2018/19).

In Nepal during the mid-1940s, the culture fishery was initiated with Indian major carp seed on small scale (FAO, 2016). In 2003 B.S. (1946/47 AD), under Agriculture Council, the aquaculture program has been institutionalized with the development of a fisheries unit. In Nepal, the fisheries program was first started in 2004 B.S. (1947/47 AD) followed by aquaculture in the late 1950s with the introduction of Common carp (*Cyprinus carpio*) and successful breeding in the mid-1960s. In the early 1970s *Ctenopharyngdon idella*, *Hypophthalmichthys molitrix*, and *Aristichthys nobilis* were introduced which breed successfully in the mid-1970s. The breeding technique for Indian Major Carp was introduced in the late 1970s and led to the biggest achievement in the polyculture system in Nepal (Singh and Yadav, 1996).

Fisheries Development Center, Bhandara lies in the east of Chitwan and is the biggest supplier of fish seed in Chitwan and neighboring districts. They produce and distribute the fish seed of Indian major carp using different hormones like Ovaprim, and WOVA-FH.

1.1. Induced breeding

Induced breeding is a process of induction of breeding in mature parent fish after injection with exogenous hormones (Heggberget, 1996). It is a technique where the synthetic hormone is administered to ripe fish breeders to breed in captive conditions. The induced breeding technique was first evolved in the year 1930 in Argentina. Houssay produced pituitary extract and injected it into the viviparous fish to make premature birth. Brazilians succeeded in 1934 in induced breeding using pituitary extract. Later, America and Russia also succeeded in induced breeding with the use of the pituitary extract. The induced breeding of Indian major carp was succeeded in 1957 (Tiwana and Raman, 2012). Induced breeding is the technique done in ripe fish breeders through artificial stimulation of the pituitary hormone or any other synthetic hormone in captive conditions. Induced breeding has an important role in both the fish culture system i.e. intensive and semi-intensive. According to Seifi *et al.,* (2011) the artificial induction of maturation by Ovaprim enhances milt quality in common carp. Khan *et al.,* (1992) reported the successful spawning of Rohu and Mrigal using Ovaprim hormone. Induced breeding helps to overcome the problems of a short supply of quality seeds and dependency on wild seeds that are unreliable and time-consuming. This method is an alternative for quality seed supply and production (Sharma *et al.,* 2010).

Ovaprim, a combination of sGnRHa + Domperidone is used for the spawning of fish. Ovaprim hormone is present in the ready-to-inject form of solution. It was developed during research investigating hormonal triggers for ovulation and spermiation (Peter *et al.,* 1986). WOVA FH is a new, highly potent, and ready-to-use injectable formulation containing a synthetic peptide analog to the naturally occurring hormone salmon GnRH. GnRH analog stimulates the pituitary to release gonadotropins and trigger the process of reproduction and dopamine antagonist inhibits the release of dopamine and makes sure that secretion of gonadotropins is not inhibited. It is administered in a single dose without causing any adverse effect on brood fish after injection. Fishes injected with WOVA FH produce an increased number of eggs through complete spawning with high fertilization and hatching percentage. During the administration of hormones, body weight should be considered for female brood fish, 0.5 ml/kg body weight, and for male brood fish, 0.25 ml/kg body weight of hormone should be administered. More *et al.*, 2010 investigated the induced maturation of Rohu, Mrigal, and Catla using the Ovaprim hormone.

The freshwater fish species *Labeo rohita* is normally cultured in Asia, especially in the Indian subcontinent (Khan *et al.,* 2004). It is commonly known as Rohu, found in tropical freshwater of India and adjacent countries (Talwar and Jhingran, 1991). It is a column feeder and shows rapid growth so it is preferred widely for culture fishery. This fish can grow up to a maximum of 200 cm. total length (Frimodt, 1995). It feeds on detritus food and aquatic plants. It shows better growth performance in 25-35^{0}C temperatures and attains maturity at 3-4 years of age. They have two barbels, thick-lipped, sucking mouths on the underside of the head. Their body color is blue to brownish along the back and silvery on the sides and belly.

Cirrhinus mrigala is commonly known as Naini and an intensive component of a semi-intensive fish polyculture system (Beyers and Rice, 2002). It is a benthic feeder and feeds on left-over food from other surface feeders and detritus (Khan *et al.*, 2001). It looks similar to the grass carp and feeds on plants and other pellets. It shows better growth performance in 25-35 ^{0}C. It attains maturity at 2-4 years of age and can be 1.5-2 kg at 2 years. They have elongated bodies covered with cycloid cycles. They have dark grey color on the back of the body whereas silver on the belly and sides.

1.2 Objectives

1.2.1 General objectives

The main objective of this study is to study breeding performance in Indian Major Carp using the Ovaprim hormone.

1.2.2 Specific objectives

- To study the fecundity, fertilization rate, hatching rate, and survival rate of Indian major carp
- To study the embryological development of fertilized eggs
- To analyze the length and weight during the growth of hatchlings to fries

1.3 Significance of the study

This study signifies the comparison between the breeding performance of Rohu and Naini. It focuses on the comparative study of fecundity, G.S.I., fertility rate, hatching rate, survival rate, length, and weight of two Indian Major Carp.

CHAPTER-TWO

LITERATURE REVIEW

The effect of rainfall in the breeding of Indian major carp was studied in a semi-arid zone by *Jain et al. (*1985). The fish were treated with pituitary extract, where the air temperature was recorded to be 42^0C and the water temperature was 38^0C. There was a continuous supply of groundwater through the overhead tank so the DO level and the water temperature were maintained at 8.0 mg/l and 27 ± 1^0C respectively. In semi-arid conditions without rain, a high breeding success rate of 10% and an average hatching success rate of 82.3% were observed.

The Indian major carp, *Cirrhina mrigala* was induced spawned with LH-RH analog or Pimozide. The 15 experimental female fishes were divided into three lots where the first lot was injected with 5µg/kg of LH-RH-a, the second lot received 10 µg/kg, and the third lot was treated with distilled water only. More 15 fish were taken as experimental fish and again divided into 3 lots, but were treated with Pimozide. Lot 1 was injected with 5mg/kg, lot 2 with 10mg/kg, and lot 3 with acidified saline in a control medium. The fish injected with 10µg/kg of LH-RH-a spawned profusely whereas fish with 5µg/kg never spawned. Similarly, fish injected with 10mg/kg of Pimozide shows hatching success, while 5mg/kg neither spawned nor could be stripped. The controlled fish with the injection of both hormones did not spawn (Kaul and Rishi, 1986).

The induced spawning of Indian major carps using Ovaprim-C was carried out by Naik and Mirza (1992) where 11 female fishes of Catla and 20 female fishes of both Rohu and Mrigal experimented. With the injection of 0.3-0.5 ml/kg body weight, the total number of eggs obtained from Rohu, Mrigal, and Catla were 50.14 lacs, 30.30 lacs, and 47.39 lacs respectively. The number of hatchlings was 39.75 lacs, 22.16 lacs, and 39.51 lacs for Rohu, Mrigal, and Catla respectively which indicates the suitability of Ovaprim-C for Indian major carp's induced breeding.

The experiment using human chorionic gonadotropin and Ovaprim for induced spawning of *Channa punctatus* and *Heteropneustes fossilis* was carried out by Haniffa and Sridhar (2002). In *Channa punctatus*, the high fertilization rate (78%) was observed in high doses (3000 IU) of HCG hormones whereas the same result was observed in low dosage (1000 IU) of HCG hormone for *Heteropneustes fossilis*. The hatchling rate

was high in high doses (3000 IU) of HCG (70.5%) for *Channa punctatus* and for *Heteropneustes fossilis* in low doses (1000 IU) of HCG. The overall high survival rate (65%) was found in *Channa punctatus* at a high dose (3000 IU) of HCG hormone.

Reddy *et al.* (2002) cultured one farmed and five wild stocks of rohu in monoculture and polyculture systems with Catla and Mrigal to examine their growth and survival for two years. In 1993, the growth was found to be better in monoculture than polyculture system whereas the growth was similar in both systems in 1994. In the first year, there was no significant stock effect on body weight at harvest but it was found highly significant in the latter one.

Dhawan and Kaur (2004) studied the comparative efficacy of Ovatide and Ovaprim in the breeding of Indian Major Carps where the brood fishes were either injected with Ovaprim or with Ovatide. The result showed the efficacy of Ovaprim for the breeding of *Catla catla* and the efficacy of Ovatide for *Labeo rohita*.

The study on the induced spawning of *Labeo rohita* using synthetic hormones was carried out by Khan *et al.* (2006), where 8 females were treated with Ovaprim and another 8 females were treated with Ovatide. The high fecundity (0.58 lacs egg/kg) was observed in Ovaprim-treated fishes in comparison to Ovatide-treated fishes (0.49 lacs egg/kg). Similarly, the hatching rate was high for Ovaprim-treated fishes (42%) than for Ovatide-treated fishes (37%), whereas the fertilization rate was shown high for fishes treated with Ovatide (69%) than Ovaprim (54%). The post-larvae treatment obtained under Ovatide treatment was also high than Ovaprim. This result showed the better performance of Ovatide than Ovaprim hormone.

The comparative study on the effectiveness of the synthetic hormone Ovaprim and Carp pituitary gland extract (PGE) on the induced breeding of Stinging Catfish, *Heteropneusts fossilis* was carried out by Hossain *et al.* (2012). A group of three females and five males were injected with PGE and Ovaprim hormone where a higher ovulation rate was shown by Ovaprim (90%) and lesser by PGE (78.7%). The eggs treated with Ovaprim showed a higher fertilization rate (86.7%) than PGE (69.2%). The hatching rate of Ovaprim-treated fish eggs was 76.9% and that of PGE-treated fish eggs was 72.7% however hatching rate can be considered equally in both hormones-treated fish. The study concluded that Ovaprim-treated fishes showed better results than PGE-treated fishes.

Tiwana and Raman (2012) experimented economically viable approach for the induced breeding of *Labeo rohita* by injecting Ovatide, Ovaprim, and Carp Pituitary Extract (CPE). The result showed a better fecundity rate under Ovaprim treatment (0.38 lacs egg/kg) than Ovatide and CPE (0.37 lacs egg/kg and 0.35 lacs egg/kg) respectively. The fertilization and hatching rates were shown higher by Ovaprim (61.30% and 72.20%) followed by Ovatide and CPE. This study showed Ovaprim as a good inducing agent over CPE and Ovatide for induced breeding.

A study was carried out for the induced breeding of *Labeo rohita* through a single application of Ovaprim-C using 16 species of *Labeo rohita* of 1.6 to 3.0 kg. The study reveals the average number of eggs/kg as 63574 among which fertilized eggs/kg were 49067 and hatching/kg were 39952. The fertilization rate was found to be 77.50% and the hatching rate was 81.39% (Naeem *et al.* 2013).

The study on length-weight relationship and condition factor of the Indian major carp; Labeo rohita was studied by Ujjania *et al.* (2013). Each of the 180 specimens of *Labeo rohita* was collected from three water bodies Mahi Bajaj Sagar, Surawania Dam, and Aasana Pond of Southern Rajasthan India. The standard length, total length, and body weight of fish were measured which were then divided into different length groups and the condition factor was calculated. The condition factor was found to be 1.0 or >1.0 representing the good condition of fish in selected water bodies.

A study was carried out using the synthetic hormone drug WOVA-FH for off-season induced breeding of Indian major carp, *Labeo rohita* (Hamilton-Buchanan) by Pandey *et al.* (2015). 3 female and 6 male fish were used each in two groups. One group of fish was treated with CPE whereas another one was with WOVA-FH hormone. The group of brooder fish that were injected with WOVA-FH showed a high fertilization rate (95-100%), the yield of spawn (5.2 lakhs), and hatching success (90-95%) in comparison to the brooder fish which were injected with CPE (fertilization: 71-80%, yield of spawn: 3.0 lakhs, hatching success: 90-95%).

A study was carried out for the induced breeding of rohu and mrigal using ovatide. The spawning fecundity of Mrigal was found to be 1.25- 1.58 lakh and of Rohu was 1.3-1.82 lakh egg/kg body weight of female fish. The egg fertilization rate was calculated to be 90-100% in Rohu and Mrigal along with the 204 lakhs production of spawn (Mohapatra *et al.* 2016).

Ghanemi and Khodadadi (2017) studied the inducing effects of Ovaprim on the reproductive parameters of Shirbot, *Barbus grypus*, and Cyprinidae. In this study, 105 female fish were taken and divided into six experimental groups. They were injected with different doses of hormones, T1: 0.25 ml/kg.bw, T2: 0.5 ml/kg.bw, T3: 0.75 ml/kg.bw, T4: 1 ml/kg.bw, T5: 1.25 ml/kg.bw, and T6: 1.5 ml/kg.bw. Fishes of one control group were injected with 3 mg/kg.bw pituitary extract. 3 male fish were injected with 3 mg/kg.bw pituitary extract. The spawning rate, working fecundity, egg weight/gm.bw, and rate of larval survival rate observed. The fishes of T1, T2, and T6 did not spawn. The fishes of T4 and the Control group showed the highest spawning rate, working fecundity, and egg wt/gm.bw whereas the fishes of the T5 group showed a low spawning rate, egg wt/gm.bw, and working fecundity. The fertilization percent, hatching percent, and larval survival rate were found similar in experimental groups. This study suggests the better performance of pituitary extract to induce spawning in Shirbot.

The effect of the hormone ovulin on induced breeding in *Labeo rohita* and *Cirrhinus mrigala* was studied by Sah (2017). The hatching rate and GSI were the highest in rohu than in mrigal while the fertilization rate was almost the same in both of the fishes.

Verma and Mandal (2018) researched the growth performance of amur common carp and Mrigala where they found Common carp showed better growth than mrigal.

Yadav (2019) studied the use of LHRH-a hormone in the breeding of Silver carp showing the spawning rate ranging from 324996 to 606800, GSI 16.21 to 24.44%, fertility rate 72.5 to 92.5% and hatching rate 65.21 to 82.60%. This study also concluded the length of the hatchling to fry was positively correlated with the weight as length increased weight also increased.

The study on the impact of temperature variation on induced spawning breeding behavior of *Cirrhinus mrigala* using the Ovaprim hormone was carried out by Khan *et al.* (2021). The study was carried out in four different temperatures 26^0C, 29^0C, 32^0C, and 34^0C to investigate the maximum release of eggs, fertilization, and hatching rate. A high number of eggs were released at 29^0C in addition to high fertilization and hatching rate. This study showed less growth of larvae at 26 or 32^0C than at 29^0C.

CHAPTER-THREE

MATERIALS AND METHOD

3.1 Materials

pH meter (model no. HI98107), Secchi disk, thermometer, syringe, mahajaal, hapa, scoop net, weighing machine, Petri dish, glass watch, dissecting microscope, slides, burette, pipette, beaker, conical flask, titrating stand, dropper, measuring cylinder, and data sheet.

3.1.1 Chemicals

Ovaprim hormone, Saline water, Alcohol, Distilled water, Sulphuric acid, Potassium iodide, Sodium thiosulphate, Sodium hydroxide, Starch, and Phenolphthalein.

3.2 Methods

3.2.1 Study Area

The study was carried out in the Fisheries Development Center, Bhandara, Chitwan. It lies 28 km. east from Narayanghat in the Rapti Municipality. The total area of the farm is 28 hectares whereas the pond area is 14.5 hectares where only 9 hectares are used for pond culture. A total of 34 different ponds (breeding, rearing, producing, and nursery pond) are used for the culture of different fish species.

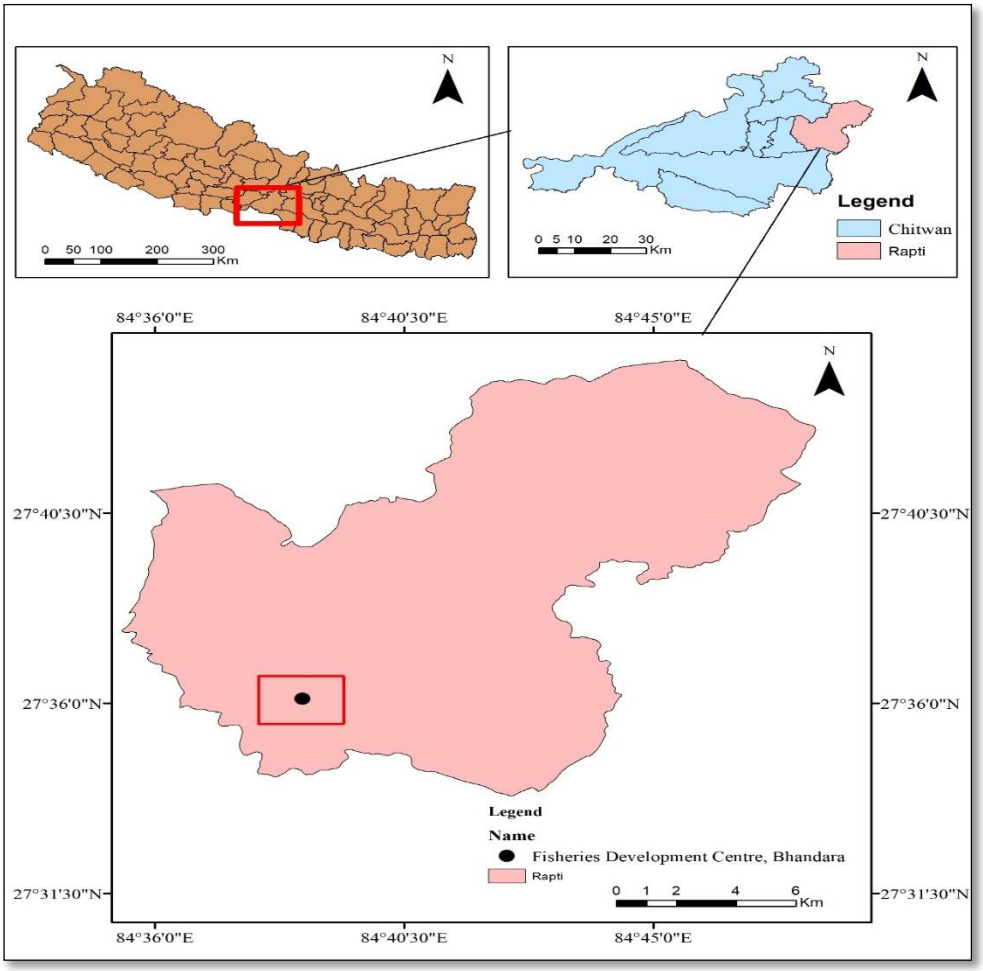
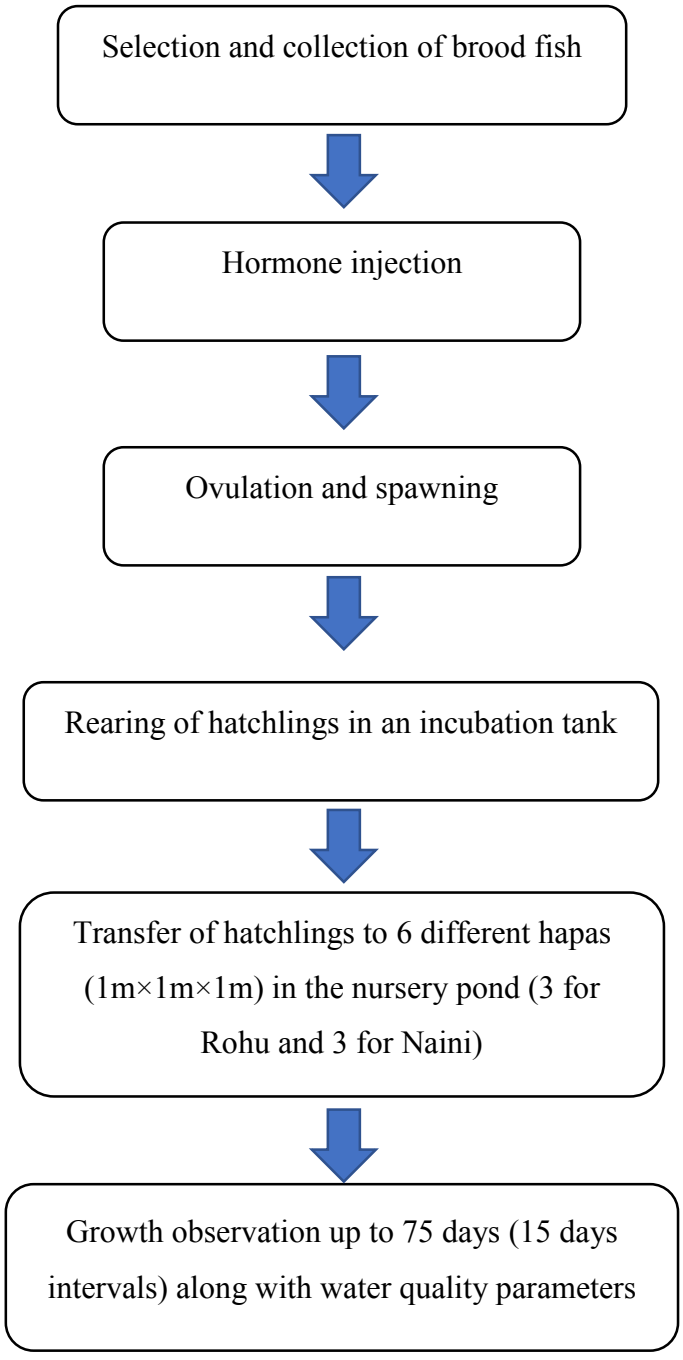


Figure 1: Study area of Fisheries Development Centre, Bhandara Chitwan

3.2.2 Study Period

The study was carried out for 90 days in the Fisheries Development Centre, Bhandara, Chitwan from June 15 to September 12, 2022.

3.2.3 Study Design



3.2.4 Physio-chemical parameters

3.2.4.1 Physical parameters

The following physical parameters were analyzed during the study period.

Nature of the day: The nature of the day was observed through visual sensation.

Color of water: The color of the water was observed by taking water in a watch glass where a piece of white paper was placed below it and the color was recorded.

Temperature: The temperature of the water was measured using a standard alcohol thermometer. It was dipped in the water and a reading was taken.

3.2.4.2 Chemical Parameters

The different chemical parameters studied were:

pH: The pH of the water was measured using a Hanna pH meter (HI98107).

Dissolved Oxygen (DO): The dissolved oxygen was calculated using Winkler's method. To calculate DO, a 300 ml. BOD bottle was brim-filled with a water sample where 2 ml. of $MnSO_4$ was added inside the bottle below the water surface so no water bubble formed. Similarly, 2 ml. of KI and NaOH reagent was added. The sample was mixed by inverting several times and 2 ml. of Conc. H_2SO_4 was added to fix the sample when floc (if any) also disappeared. A 100 ml. water sample was titrated against Sodium thiosulphate ($Na_2S_2O_3$) in which 1-2 drops of the starch indicator was added to the formation of blue color occurred. When the blue color disappeared and the sample was clear, the titration was stopped and the reading was noted. While titrating, three readings were noted and DO was calculated by using the formula:

$$\text{DO (mg/l)} = \frac{(ml \times N) of\ Na2S2O3 \times 44 \times 1000}{\frac{V2(V1-V)}{V1}}$$

Where,

V= Volume of $MnSo_4$ and KI added

V1= Volume of BOD bottle

V2= Volume of the part of the content titrated

N= Normality of $Na_2S_2O_3$

ml= Amount of $Na_2S_2O_3$ used

Free Carbon dioxide (CO_2)

The free carbon dioxide was calculated using the titration method. The water sample was collected and titrated. For titration 50ml. of water sample was taken in a conical flask and 2-3 drops of phenolphthalein indicator were added. It was found colorless

indicating the presence of free CO_2. The water sample was titrated against Sodium hydroxide (NaOH) solution. When pink color appeared in a water sample, the titration was stopped and the reading was noted. Similarly, three readings were recorded and free CO_2 was calculated using the formula:

$$\text{Free } CO_2 \text{ (as mg/l)} = \frac{(ml \times N) of\ NaOH \times 44 \times 1000}{V}$$

Where

V= Volume of a water sample taken (ml.)

3.3 Breeding and rearing of hatchlings, fries, and fingerlings of *Labeo rohita* and *Cirrhinus mrigala*

3.3.1 Experimental fish and their number

The study was carried out among Rohu (*Labeo rohita*) and Naini (*Cirrhinus mrigala*). For this study, 19 brood female fish, and 29 brood male fish of Naini and 8 brood female fish, and 12 male broods of Rohu were taken. The total weight of the female brood fish of Rohu and Naini was 26.1 kg. and 41.08 kg respectively whereas the weight of the male brood fish of Rohu and Naini was 27.5 kg. and 55.284 kg. respectively (Appendix I, II, III, and IV)

3.3.2 Selection and maintenance of brood fish

Brood fishes are mature males and females with healthy eggs and sperm which are free from any kind of diseases and deformities. The mature brood fish were selected by slightly pressing the abdomen and observing the scales in the fish's body. The breeders were kept in the breeding tank in the hatchery where they laid eggs and hatchlings were developed. The water quality parameters like pH and temperature were checked and maintained regularly. Brood fish along with natural feed were supplied with supplementary feeds provided by FDC, Bhandara, two times a day. This supplementary feed helps in further growth and maturation. The brood fishes were selected based on their external sexual characteristics. The mature female brood fish have bulged abdomen and slightly red abdominal vents whereas, the mature male brood fish have an elongated vent. Brood fishes were selected by pressing on their abdomen. When the abdomen of female brood fish was slightly pressed, they release eggs which confirms their maturity. Like-wise on pressing the abdomen of male brood fish, they ooze out

white milt. The male fish which oozes out milk was selected as the male brood fish. The pectoral fin of male brood fish is rough whereas that of the female is smooth. The ratio of male to female brood fish is 2:1. The selected 3 years old brood fish were transferred to the breeding tank present in the hatchery using a plastic transportation bag. They were kept gently in the oxygenated water-supplied circular tank so no deformities occur.

3.3.3 Hormone injection

Each brood fishes were weighed in the weighing machine. The amount of hormone to be injected was calculated according to the body weight. The Ovaprim hormone was used for induced breeding and before injection it was diluted so it won't burn brooders. The male brood fishes were injected with 0.25 ml/kg and female brood fishes were injected with 0.5 ml/kg of body weight. The hormone was injected intramuscularly in the area of the dorsal peduncle above the lateral line. Special care was taken to hold the brood fish at the head region during hormonal injection so they will not move and not harm anyone.

3.3.4 Latency period and estrus

The estrus time and latency period were noted by observation. When the administered hormone normally affects the induced fish, they will start to show estrus. Estrus phenomena occur after the injection of hormones into the brood fish. When a male brooder starts to chase a female brooder the intermittent splashing of the water surface can be seen.

3.3.5 Incubation of eggs and rearing of hatchlings

The eggs were transferred to the incubation tanks which were always supplied with water flowing from the overhead tank. The temperature and pH of the water were maintained in the incubation tank to ensure proper development and better survival. After 1 hour of transferring hatchlings to the incubation tank, Potassium permanganate ($KMnO_4$) was added, so the egg-shell become hard and fungus do not attack. The embryonic development was completed within the egg during the incubation period and hatchlings were hatched. After hatching, hatchlings started to show swirl movement downward to upward, and after absorption of the yolk sac, started to show horizontal swimming. The hatchlings were fed the yolk of the egg mixed with water twice a day.

3.3.6 Transfer of hatchlings

The hatchlings in the incubation tank get introduced to the new environment after being transferred to the nursery pond. On the fourth day, 2 buckets of nursery pond water were poured into the incubation tank to familiarize with the new environment. After 5 days, one-week-old hatchlings were transferred to the hapas in the nursery pond. A total of 6 hapas were set; 3 each for the hatchlings of Rohu and Naini. The hatchlings were scooped out with a netted cloth and collected. They were measured in a glass cup (at the rate of 50,000 hatchlings per cup) and transferred to a plastic bag containing a sufficient amount of water and oxygen. A total of 600 hatchlings (100 each of Rohu and Naini) were transferred to 6 different hapas. The transfer of hatchlings was done early morning when the water temperature was low.

3.3.7 Rearing of hatchlings till early fingerlings

The hatchlings, after being transferred to the hapa, were fed with water and milk (0.25l. of milk and 3l. of water) for 7 days. After 7 days they were fed with a mixture of different flour and vitamins as prescribed by FDC, Bhandara. The amount of feed was increased with an increase in their growth.

3.3.8 Growth rate till early fingerlings

The growth rate of the hatchlings was observed at 15 days intervals. The length and weight of hatchlings were measured and recorded up to 75 days i.e., up to early fingerlings. From each hapa, 10 fish were taken and weighed using a digital weighing machine. The length was recorded with the help of a measuring scale.

3.3.9 Determination of fecundity and gonadosomatic index (GSI)

The fecundity and Gonado Somatic Index (GSI) were calculated followed by Kaur and Dhawan, 1997.

Fecundity

Fecundity was estimated separately by sampling one gm. of egg and multiplying it with the total weight of eggs in brood female fish. One gram of stripped-out dry eggs was weighed on a weighing machine and eggs were counted one by one with the help of a brush and feather.

$$\text{Fecundity} = \text{Number of eggs per gm.} \times \text{wt. of total eggs (gm.)}$$

GSI

Gonado Somatic Index refers to the weight of gonads to the total weight of the brood fish. The study of the gonadal region helps to find the maturity of fish. GSI increases with the maturation of fish being maximum during the period of maturity and declines abruptly after spawning. The GSI % of all female fishes was determined by the following formula:

$$\text{GSI} = \frac{weight\ of\ gonads}{weight\ of\ brood\ fish} \times 100$$

3.3.10 Determination of fertility, hatching and survival rate

The fertility, hatching, and survival rate were determined by Kaur and Dhawan, 1997.

Fertilization rate

The rate of fertilization was calculated for every female separately. A properly washed glass cup was inserted into the incubation tank to scoop out egg samples. Three samples of eggs were scooped out randomly and observed. The fertilized eggs were observed as clear crystal balls and the unfertilized eggs were dull and opaque.

$$\text{Fertility rate} = \frac{total\ number\ of\ fertilized\ egg}{total\ number\ of\ eggs\ per\ female} \times 100$$

Hatching rate

The hatching number was determined by the volumetric method. Counting was done 4-5 days of hatching and for this one milliliter (ml.) of the scooped-out hatchlings was measured in a measuring glass tube. The sampled hatchlings were then immediately poured into a petri-dish containing water and counted with the help of a pipette.

$$\text{Hatching rate} = \frac{total\ number\ of\ hatching}{total\ number\ of\ fertilized\ eggs} \times 100$$

Survival rate

A hundred hatchlings of Rohu were placed in three hapas each and each of the hundred hatchlings of Mrigal was placed in another three hapas. The counting of fishes was carried out at an interval of 15 days and the number of surviving fishes from each hapa was counted. In addition to it, the length and weight of fries and fingerlings were also

measured and recorded. During regular checkups of the happa, the dead hatchlings were taken out and were noted for the mortality of fish.

$$\text{Survival rate} = \frac{final\ number\ of\ surviving\ fish}{initial\ number\ of\ fish} \times 100$$

3.3.11 Study of embryonic development

The sample of fertilized eggs of Rohu and Naini was collected separately from the embryonic stages. Eggs of various stages were preserved in a 70% alcohol solution. The eggs of Rohu and Naini were observed under the dissecting microscope at 10× magnification in the lab of the Zoology Department of Birendra Multiple Campus. The study was done under a dissecting microscope and photographed using Samsung Galaxy A12.

3.4 Length-weight relationship

The estimation of the length-weight relationship is very important to analyze their growth whether they are growing properly or not and to understand the condition of the fish. For this, the length and weight of 10 sampled fishes were noted from each hapa. The length-weight relationship was calculated by the formula;

$$W = aL^b$$

Where,

W= the weight of the fish in gm.

L= the total length of the fish

b= the exponent describing the rate of variation in weight to length

a= the coefficient of the length-weight relationship

The correlation coefficient was calculated using Pearson's coefficient method in excel. The two variables: length and weight of 50 fish each of all six hapas were used and the 'r' value was determined. The determination of condition factor (K) and relative condition factor (Kn) was carried out as suggested by LeCren (1951):

$$K = (W*100)/L^3$$

$$Kn = W/w$$

Where,

W= Weight of fish

L= Standard length of fish (cm.)

w= Calculated weight of fish (gm.)

CHAPTER-FOUR

RESULTS

4.1 Physicochemical parameters

4.1.1 Nature of the Day

During the study period, the day was observed mostly rainy, partly cloudy, and sunny.

4.1.2 Color of water

The color of the water was found greenish during the study period.

4.1.3 Temperature

The temperature of the brood pond of Rohu and Naini was observed from 27 ^{o}C to 34 ^{o}C and 28 ^{o}C to 34 ^{o}C respectively (Tables 1 and 2). The temperature of the incubation tank was maintained at 25.7 ^{o}C to 26.4 ^{o}C for Rohu and Naini was 25.8 ^{o}C to 26.6 ^{o}C (Appendix V and VI). The temperature of the nursery pond was observed to be 29.5 to 33.5 ^{o}C for Rohu and Naini (Appendix VII and VIII).

4.1.4 pH

The pH of the brood pond of Rohu and Naini was found to be ranged from 7.5-10 (Table 1). The pH of the incubation tank was maintained from 7-7.3 (Appendix V and VI), and of the nursery pond was from 7.1 to 9.8 (Appendix VII and VIII).

4.1.5 Dissolved Oxygen (DO)

The dissolved oxygen of the brood pond of Rohu was found to be ranged from 5-8.3 mg/l. The DO of the incubation tank ranged from 6.67-9 mg/l and of the nursery pond from 5.5-7.6 mg/l. Likewise, the DO of the brood pond of Naini was found between 5.7-8 mg/l. The DO of the incubation tank was found similar to that of Rohu however, the DO of the nursery pond of Naini ranged from 5-7.9 mg/l (Tables 1 and 2).

4.1.6 Free Carbon dioxide

The amount of free carbon dioxide in the breeding pond of Rohu was found to be ranged from 10-13.2 mg/l, and of the nursery pond was 7.9-11 mg/l. The amount of free CO_2 in the incubation tank of both Rohu and Naini was found to be ranged from 7.5-8.8

mg/l. The free CO_2 of the breeding and nursery pond of Naini was found to be 10-13.2 mg/l and 7.9-11 mg/l respectively (Tables 1 and 2).

Table 1: Physicochemical parameters of brood pond, Incubation tank, and Nursery pond of *Labeo rohita* at FDC, Bhandara, Chitwan

Average	Temperature (^{0} C)		pH		DO (mg/l)	Free CO_2 (mg/l)
	7-9 A.M	3-5 P.M	7-9 A.M	3-5 P.M		
Brood pond	27-34	30-33	7.5-8.5	8.5-10	8.3	13.2
Incubation tank	25.7-26.4		7-7.2		9	8.8
Nursery pond	29.5-33	31.4-33.5	7.2-9	8.3-9.5	5.5-7.6	7.9-11

Table 2: Physicochemical parameters of Brood pond, Incubation tank, and Nursery pond of *Cirrhinus mrigala* at FDC, Bhandara, Chitwan

Average	Temperature (^{0} C)		pH		DO (mg/l)	Free CO_2 (mg/l)
	7-9 A.M	3-5 P.M	7-9 A.M	3-5 P.M		
Brood pond	28-34	30.5-34	7.5-8.5	8.5-10	5.7	11
Incubation tank	25.8-26.6		7-7.3		6.67	7.5
Nursery pond	29.5-32	31.3-33	7.1-8.9	8.5-9.8	5-7.9	6.6-11

4.2 Latency period and estrus

The latency period and estrus started after 7 hrs. of hormonal injection.

4.3 Fecundity and Gonadosomatic index

The fecundity and G.S.I. of Rohu were found to be ranged from 227,540 to 675,000, and 10.33% to 19.74% respectively (Figure 2, Appendix IX) whereas the fecundity and

GSI of Naini ranged from 161,400 to 608,400 and 10.71% to 28.15% (Figure 3, Appendix X).

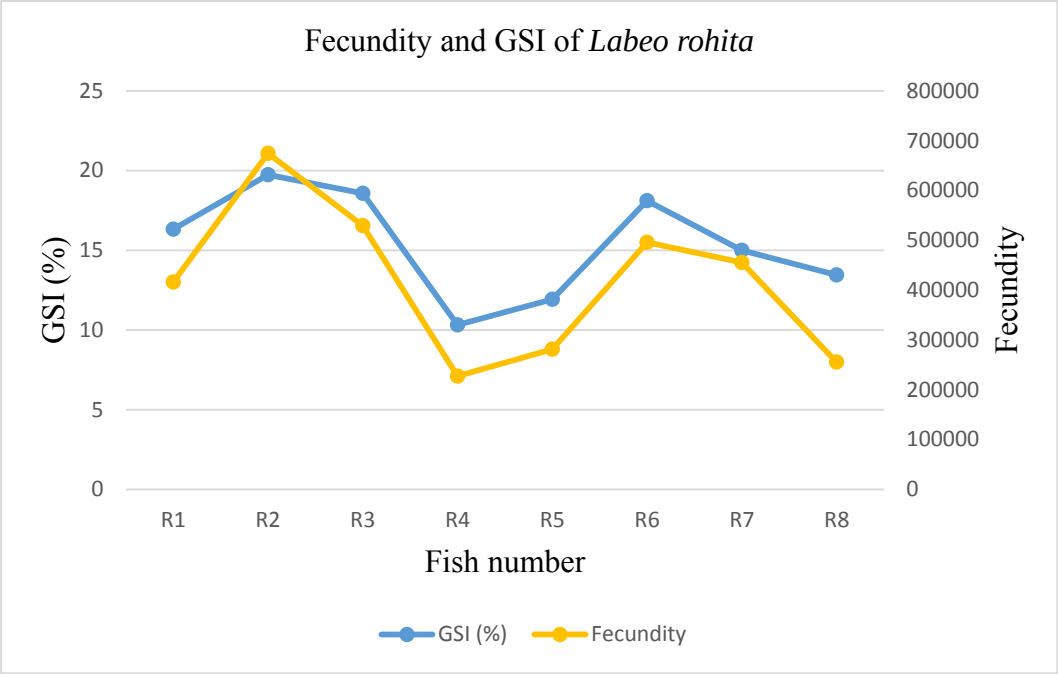


Figure 2: Fecundity and GSI of *Labeo rohita*

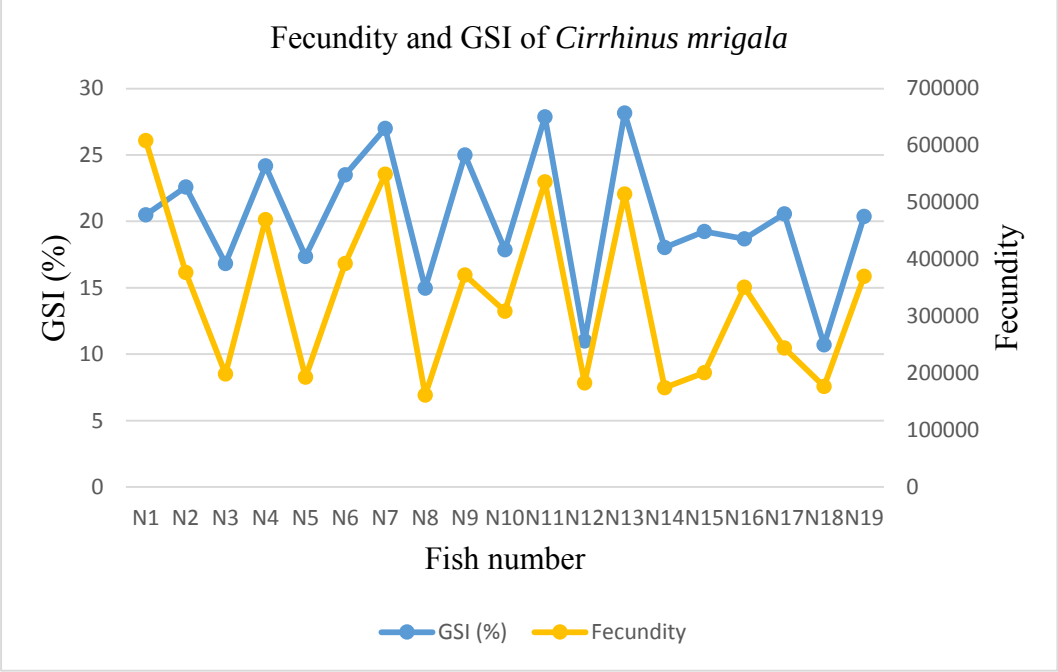


Figure 3: Fecundity and GSI of *Cirrhinus mrigala*

4.4 Fertility rate and hatching rate

The eggs of Rohu and Naini were analyzed whether they were fertilized or not. During the study, the unfertilized eggs were found opaque, dull, and unfused whereas fertilized ones were like a ball of a crystal. The fertility rate of Rohu ranged from 82.28% to 87.27% and the hatching rate from 73.91% to 83.33% (Figure 4, Appendix XI). Similarly, the fertility rate and hatching rate of Naini ranged from 79.63% to 87.88% and 77.90% to 83.90% respectively (Figure 5, Appendix XII).

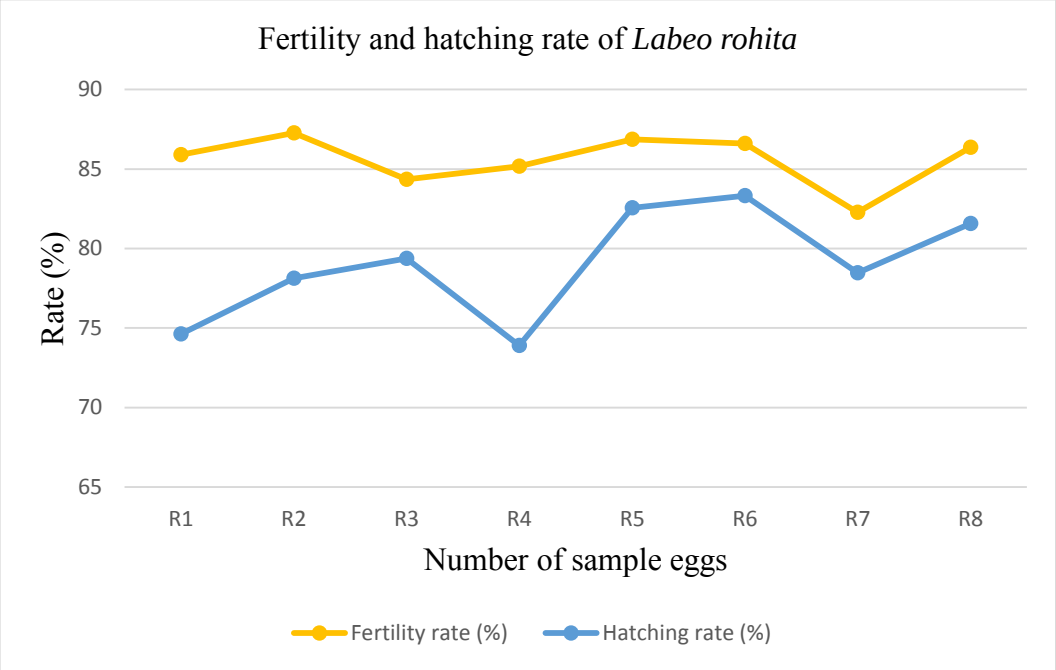


Figure 4: Fertility rate and hatching rate of *Labeo rohita*

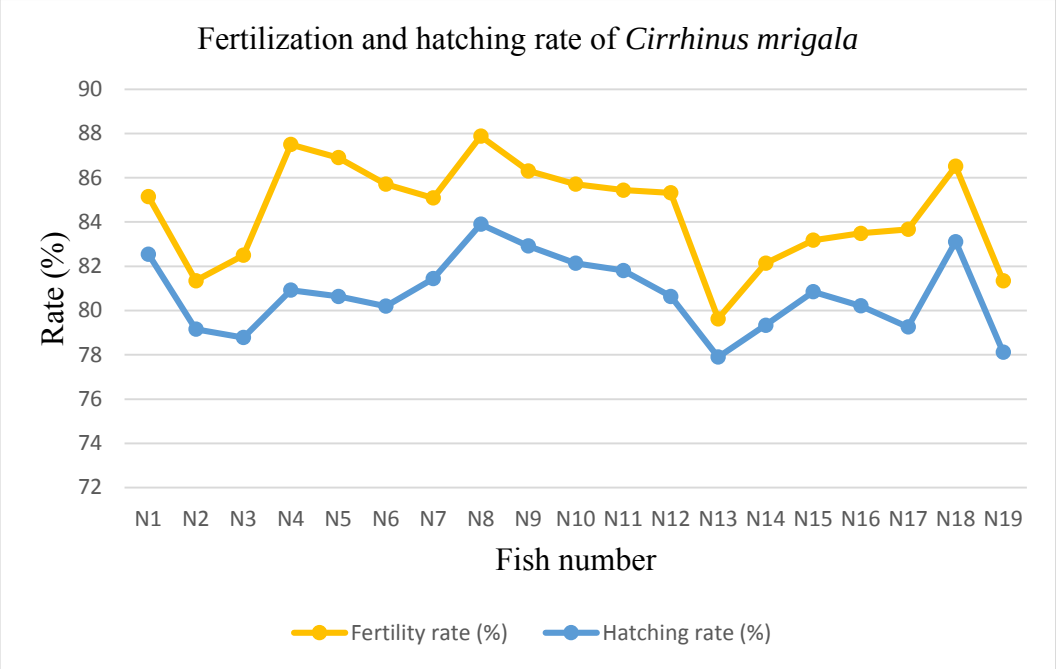


Figure 5: Fertility rate and hatching rate of *Cirrhinus mrigala*

4.5 Embryonic development of *Labeo rohita* and *Cirrhinus mrigala*

The different embryonic stages were observed from fertilization to the hatchling stage under microscope and some samples were preserved in a 70% ethanol solution for further study.

Unfertilized egg: The unfertilized eggs were found opaque which later diffused.

Fertilized egg: The fertilized eggs were observed floating, yellow-brown in color, and circular and bulged.

10 hrs. of fertilization: A comma-shaped embryo was observed. The appearance of optic vesicles, gill rudiments, pectoral fin bud were observed only in microscope. The occasional twitching movement started.

13 hrs. of fertilization: The twitching movement seems quite rapid. The constriction formed between the bulbous and elongated portion of the yolk sac started to disappear.

6 hr. of hatchling: The constriction formed between the bulbous portion and the elongated portion of the yolk sac was found disappeared. The posterior portion was slightly longer.

12 hr. of hatchling: The bulbous portion of the yolk sac was reduced in height. Eyes were slightly pigmented at the center. Notochord was seen as a spotted line.

24 hr. of hatchling: Eyes were more pigmented, particularly at the center, yolk sac got elongated. The mouth appeared as a slit.

48 hr. of hatchling: A few chromatophores were seen on the head. The caudal was nearly round, ventral fin-fold started from about the middle region of the air bladder. The eyes increased in size and pigmentation, pectoral and pelvic fins fold started to develop.

72 hr. of hatchling: The mouth was well developed and the yolk sac completely absorbed. The notochord was seen having a posterior end slightly upturned. A few chromatophores were seen around the tip of the notochord.

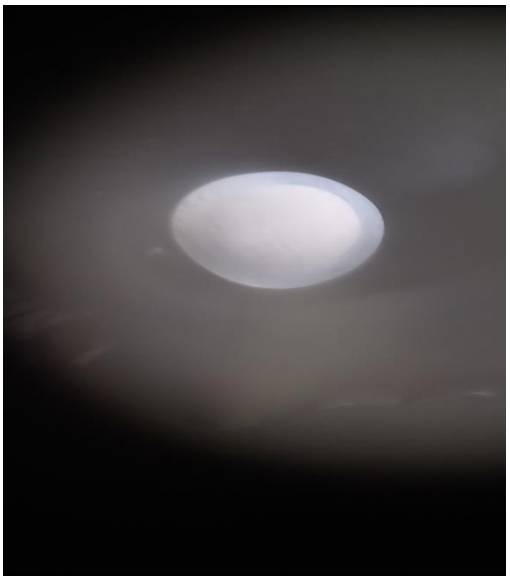


Photo 1: Unfertilized egg of Rohu

Photo 2: Unfertilized egg of Naini

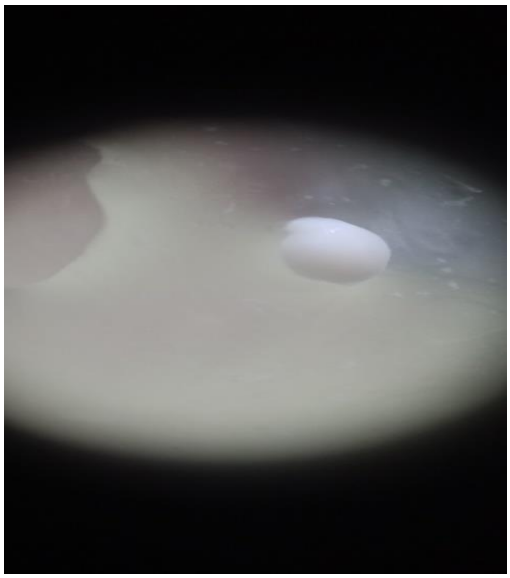


Photo 3: Fertilized egg of Rohu

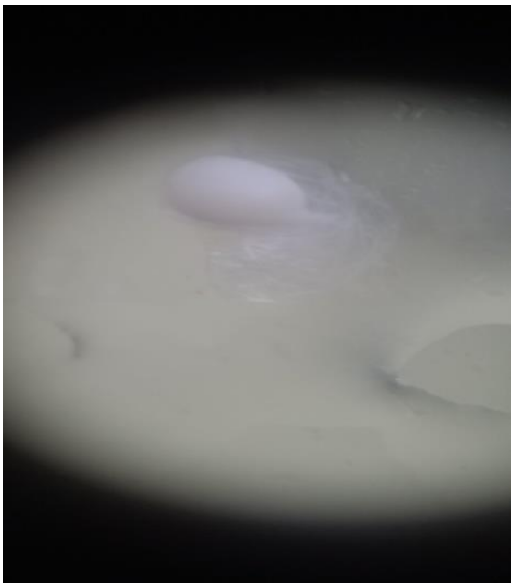


Photo 4: Fertilized egg of Naini

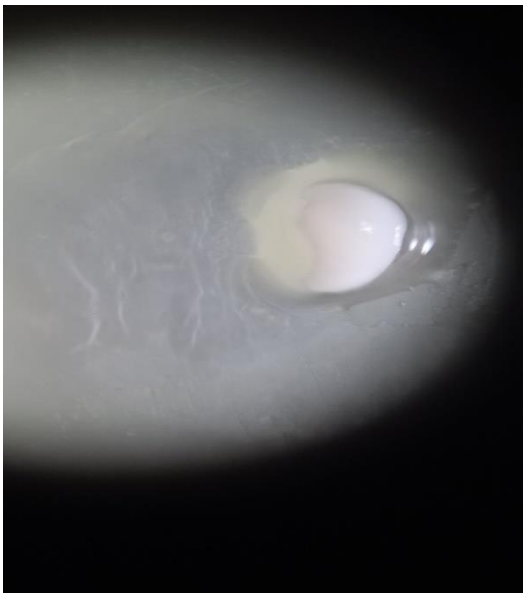


Photo 5: 10 hr. embryo of Rohu

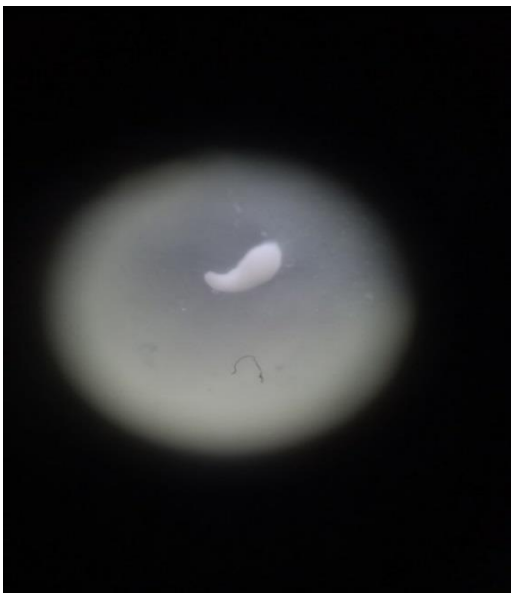


Photo 6: 10 hr. embryo of Naini

Photo 7: 13 hr. embryo of Rohu

Photo 8: 13 hr. embryo of Naini

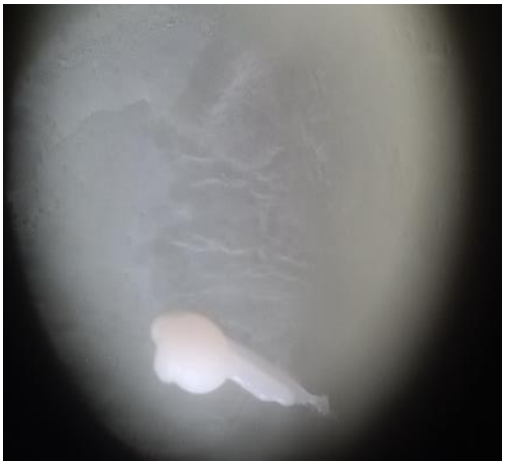


Photo 9: Hatchling of Rohu after 6 hrs.

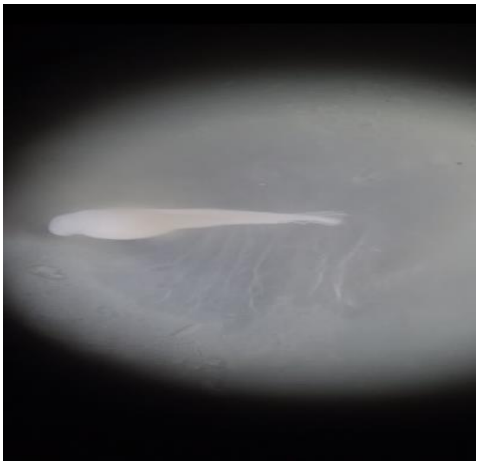


Photo 10: Hatchling of Naini after 6 hrs.

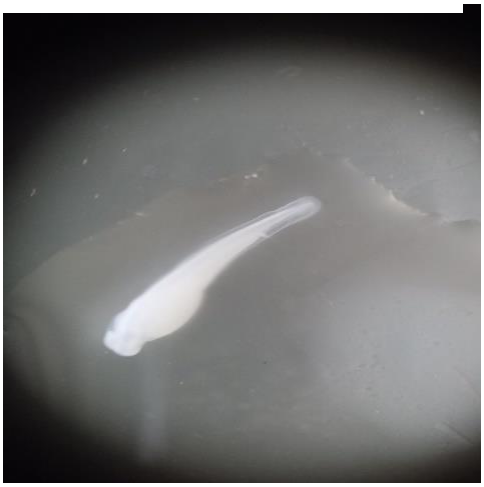


Photo 11: 12 hr. old hatchling of Rohu

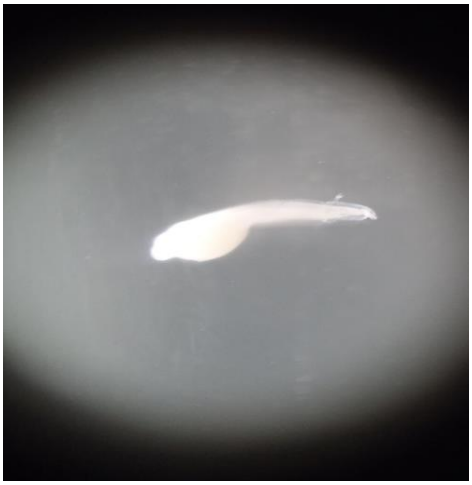


Photo 12: 12 hr. old hatchling of Naini

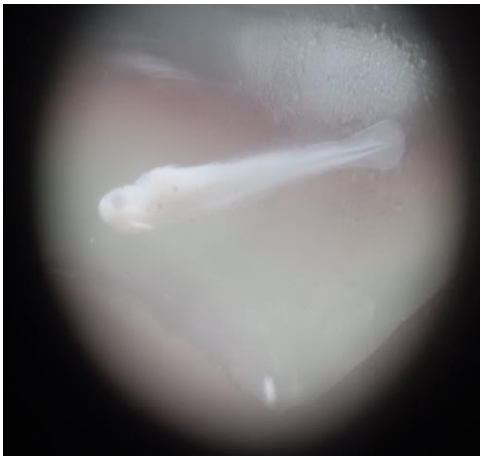


Photo 13: 24 hr. old hatchling of Rohu

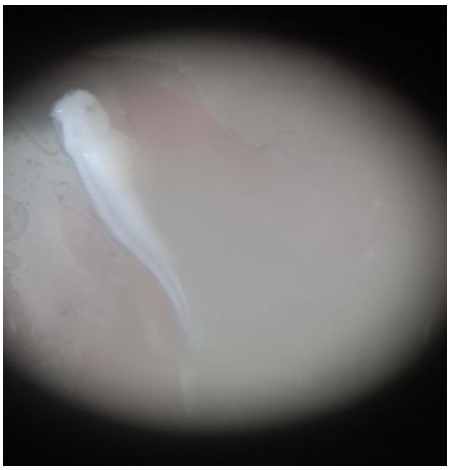


Photo 14: 24 hr. old hatchling of Naini

Photo 15: 48 hr. old hatchling of Rohu

Photo 16: 48 hr. old hatchling of Naini

Photo 17: 72 hr. old hatchling of Rohu

Photo 18: 72 hr. old hatchling of Naini

4.6 Growth of hatchling

A five-days old hatchlings of 5.7 mm. were transferred to the hapa (a total of 6; 3 hapa each for *Labeo rohita* and *Cirrhinus mrigala*) and reared for 75 days.

4.6.1 Growth of hatchlings of *Labeo rohita*

The growth of hatchlings of Rohu was obtained significantly in hapa HR1 followed by hapa HR2 and hapa HR3. The hatchlings of hapa HR1 gained 3.18 cm. to 7.2 cm. from the 15th to 75th day and weight from 0.59 gm. to 4.28 gm. The hatchlings of hapa HR2 attained 6.32 cm. length and 2.63 gm. weight during the 75th day. The fingerlings of length 6.17 cm. and weight 3.205 gm. were obtained during the 75th day in hapa HR3 (Appendix XVI, XVII, and XVIII).

Table 3: Mean length and mean weight of fries and fingerlings of *Labeo rohita*

Days	**Hapa R1**		**Hapa R2**		**Hapa R3**	
	Mean Length (cm.)	**Mean Weight (gm)**	**Mean Length (cm.)**	**Mean Weight (gm.)**	**Mean Length (cm.)**	**Mean Weight (gm.)**
15th day	3.18	0.592	3.07	0.559	3.21	0.549
30th day	4.21	1.158	4.23	1.029	4	0.967
45th day	5.27	1.765	4.81	1.264	4.64	1.307
60th day	5.97	2.53	5.74	2.081	5.53	1.937
75th day	7.2	4.283	6.32	2.663	6.17	3.205

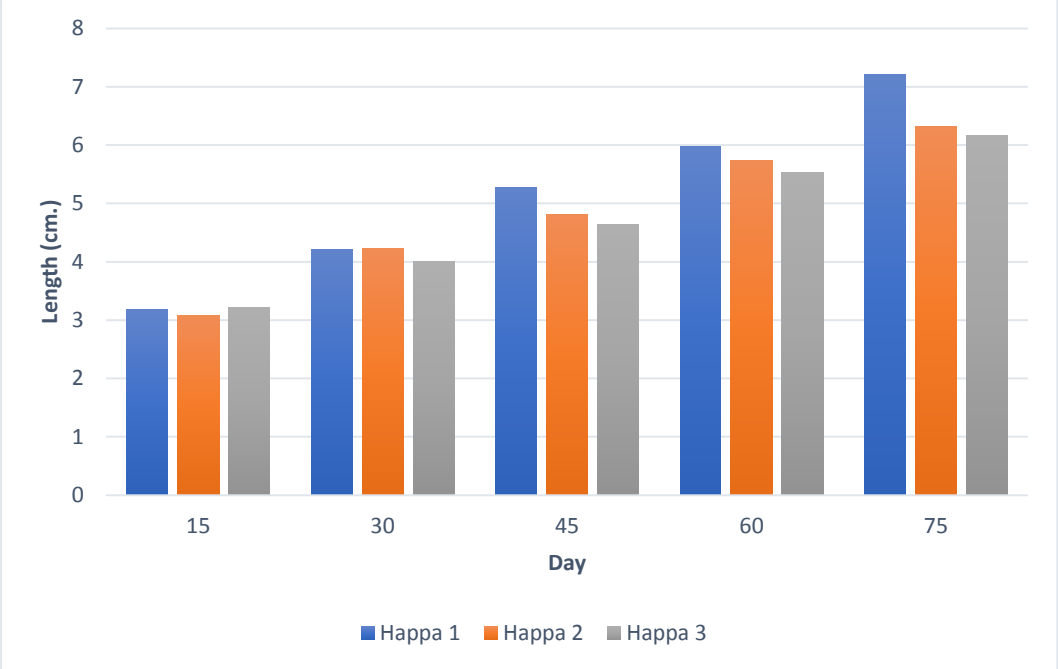


Figure 6: Bar diagram showing an increase in the length of *Labeo rohita* along with days

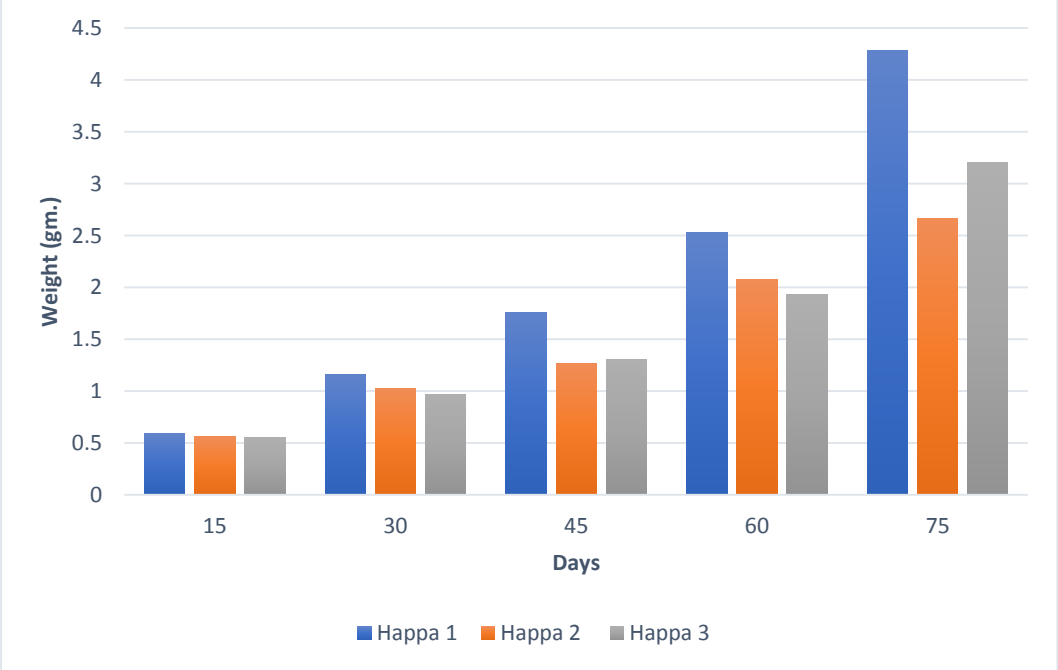


Figure 7: Bar diagram showing an increase in weight of *Labeo rohita* along with days

4.6.2 Growth of hatchlings of *Cirrhinus mrigala*

The highest growth of hatchlings of Naini was obtained in hapa HN1 followed by hapa HN3 and hapa HN2. The hatchlings of hapa HN1 showed significant growth from 3.84 cm. to 8.87 cm. length and 0.86 gm. to 5.96 gm. weight from the 15th to 75th day. The

fingerlings of length 8.41 cm. and 5.798 gm. were obtained during the 75th day in hapa HN3. The hatchling of hapa HN2 attained a growth of 7.76 cm. length and 4.895 gm. weight (Appendix XIX, XX, and XXI).

Table 4: Mean length and mean weight of fries and fingerlings of *Cirrhinus mrigala*

Days	Hapa N1		Hapa N2		Hapa 3	
	Mean Length (cm.)	**Mean Weight (gm.)**	**Mean Length (cm.)**	**Mean Weight (gm.)**	**Mean Length (cm.)**	**Mean Weight (gm.)**
15th	3.84	0.868	3.43	0.695	3.62	0.806
30th	5.14	1.712	4.99	1.896	5.71	1.945
45th	6.13	2.761	6.02	2.272	6.5	2.777
60th	7.3	4.354	6.89	3.815	7.26	3.412
75th	8.87	5.966	7.76	4.895	8.41	5.798

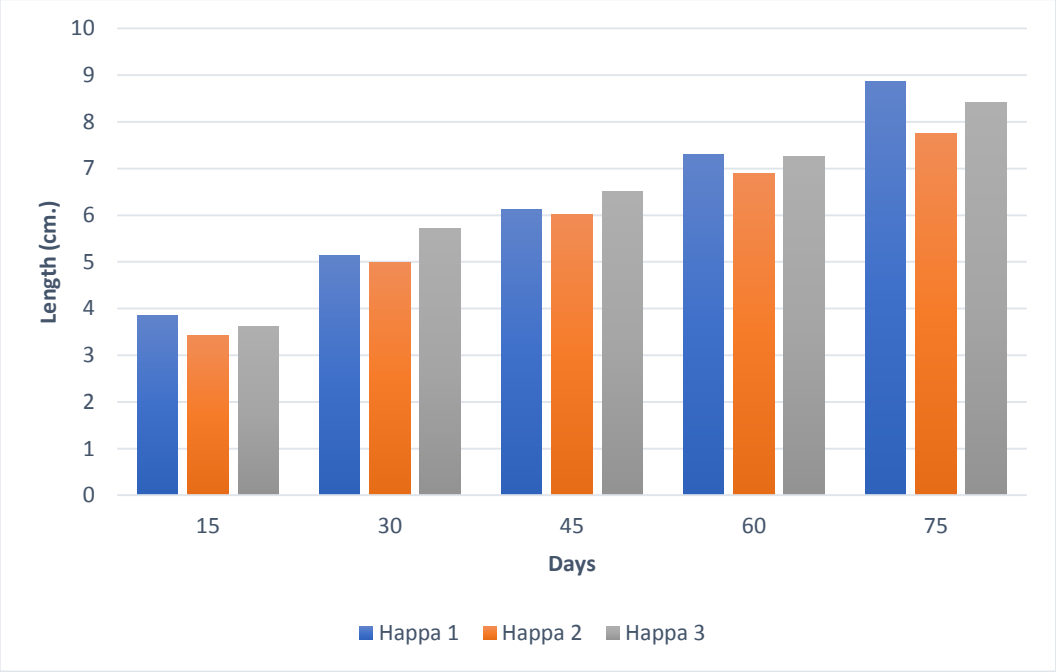


Figure 8: Bar diagram showing an increase in the length of *Cirrhinus mrigala* along with days

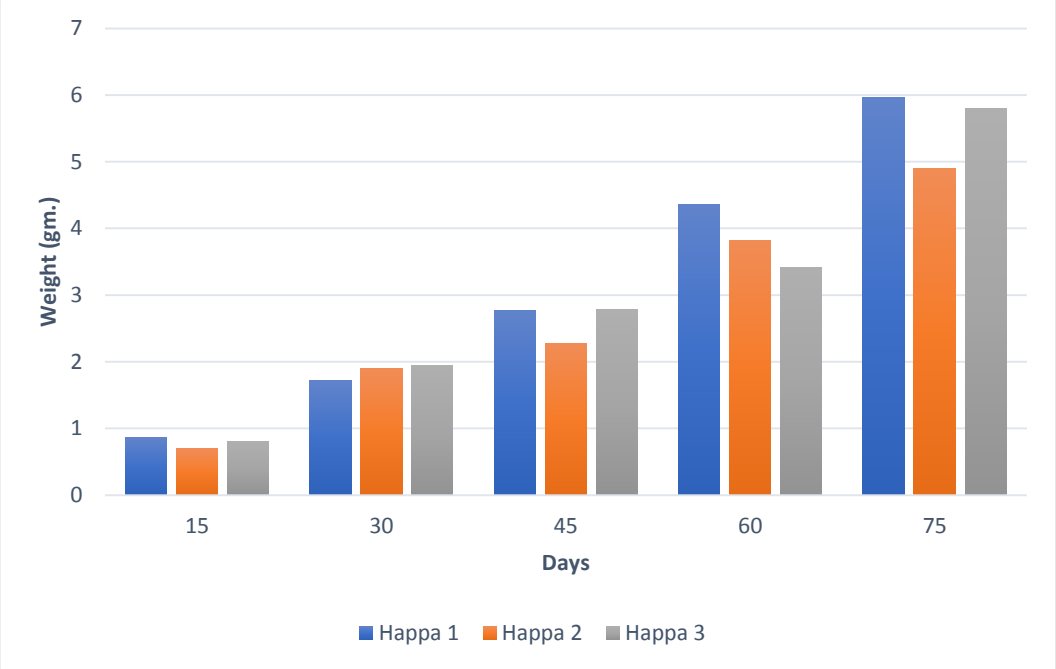


Figure 9: Bar diagram showing an increase in weight of *Cirrhinus mrigala* along with days

4.7 Length Weight relationship and condition factor

The correlation coefficient 'r' of different hapas were found as: 0.95 (HR1), 0.95 (HR2), 0.94 (HR3), 0.99 (HN1), 0.96 (HN2), and 0.94 (HN3). The condition factor (K) of different hapas were calculated and noted as 1.012 in HR1, 1.008 in HR2, 1.01 in HR3, 1.006 in HN1, 1.011 in HN2, and 1.01 in HN3. Similarly, the relative condition factor (Kn) obtained was 1.39 in HR1, 1.31 in HR2, 1.4 in HR3, 1.18 in HN1, 1.32 in HN2, and 1.13 in HN3. The value of regression coefficient 'b' was found to be 2.3218 in hapa HR1, 2.1269 in HR2, 2.5040 in HR3, 2.3497 in HN1, 2.2676 in HN2, and 2.2187 in HN3 which was less than 3 in all hapas. When the value of the 'b' is 3, then it shows isometric growth. When the 'b' value is less than 3 then growth is negative allometry which shows fish becoming slimmer and elongated with increasing length whereas, if the 'b' value is greater than 3, then fish shows positive allometric growth, and becomes heavier with a faster increase in height or breadth rather than length. In this study, the 'b' value of all six hapas was found less than 3 which ranged from 2.12 to 2.50 showing negative allometry and representing the elongation of fishes with increasing length. The correlation of fecundity and weight of female fishes was calculated and found to be 0.8722 for Rohu and 0.8555 for Naini.

4.8 Survival rate

Among the 600 fries, stocked 100 each in six different hapas, the highest survival rate was observed in hapa HR2 followed by hapa HR3 and HR1 (Table 5, Appendix XIII) and the highest survival rate of Naini was observed in hapa HN3 followed by hapa HN2 and HN1 (Table 6, Appendix XIV).

Table 5: Average survival rate (%) of *Labeo rohita* at FDC, Bhandara, Chitwan

Hapa	Size of hapa	No. of stocking fries	No. of days					Average survival rate (%)
			15	30	45	60	75	
HR1	1×1×1 m.	100	79	78	65	63	62	62
HR2	1×1×1 m.	100	87	85	76	75	73	73
HR3	1×1×1 m.	100	92	89	71	66	66	66

Table 6: Average survival rate (%) of *Cirrhinus mrigala* at FDC, Bhandara, Chitwan

Hapa	Size of hapa	No. of stocking fries	No. of days					Average survival rate (%)
			15	30	45	60	75	
HN1	1×1×1 m.	100	82	79	65	62	59	59
HN2	1×1×1 m.	100	88	86	76	74	71	71
HN3	1×1×1 m.	100	87	86	79	76	75	75

4.9 Mortality of hatchlings till fingerlings of *Labeo rohita* and *Cirrhinus mrigala*

During this study, the high mortality of hatchlings of Rohu was seen in hapa HR1 (21/100) followed by hapa HR2 (13/100) and hapa HR3 (8/100). There was no high mortality during the 30th day, i.e., hapa HR1 (1/79), hapa HR2 (2/87), and hapa HR3 (3/92). Significant mortality was seen on the 45th day in hapa HR3 (18/89), hapa HR1 (13/78), and hapa HR2 (9/85). Less mortality was observed during the 60th day, i.e., hapa HR1 (2/65), hapa HR2 (1/76), and hapa HR3 (5/71). Very less mortality among

fingerlings was observed during the 75th day. There was no mortality in hapa HR3 followed by hapa HR1 (1/63) and hapa HR2 (2/75).

During this study, the high mortality of hatchlings of Naini was recorded in hapa HN1 (18/100) followed by hapa HN3 (13/100) and hapa HN2 (12/100). There was no high mortality during the 30th day. In hapa HN1 (3/82), hapa HN2 (2/88), and hapa HN3 (1/87) mortality was observed. High mortality was observed on the 45th day. There was a high mortality in hapa HN1 (14/79) followed by hapa HN2 (10/86) and hapa HN3 (7/86). There was less mortality during the 60th day, i.e., hapa HN1 (3/65), hapa HN2 (2/76), and hapa HN3 (3/79). Very less mortality of fingerlings was observed during the 75th day. In hapa HN1 (3/62), hapa HN2 (3/74), and hapa HN3 (1/76) mortality was observed.

The highest mortality rate 21% in Rohu and 18% in Naini was found in the first 15 days which may be due to the transfer of hatchlings from the incubation tank to the nursery pond. After the subsequent days, the mortality rate was found decreased in both Rohu and Naini (Appendix XV).

CHAPTER- FIVE

DISCUSSION

The present study was conducted for the induced breeding of Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*). The fecundity and GSI of these two Indian major carp were calculated along with their fertilization and hatching rate. The length and weight relation of fish was also determined. However, the results may vary from place to place and depend upon the amount of hormone dosage.

The present study reveals the suitability of 25-34^0 C of water temperature for the breeding of Rohu and Naini which was similar as suggested by Sah (2017). According to Verma and Mandal (2018), the suitable water temperature ranged between 14-24.73^0 C for the breeding of Common carp. Similarly, Mohapatra *et al.* (2016) suggested that a temperature between 26.3-34.1^0 C is best for both *Labeo rohita* and *Cirrhinus mrigala*. According to Khan *et al.* (2021), the temperature range of 26-34^0 C is favorable for the growth of *Cirrhinus mrigala*, and Yadav (2019) found the water temperature between 24-36^0 C favorable for the Silver carp.

During this study, the pH of water ranged between 7-10 which was suitable for the growth and development of fish. A similar result of pH from 7-10.1 was observed in the study carried out by Sah (2017). In the experiment performed by Verma and Mandal (2018) in amur common carp and mrigal, the pH ranged between 7.23-8.42 which was favorable for the survival and growth of fish. In *Labeo rohita* and *Cirrhinus mrigala*, the pH range of 7-8 was observed as suitable by Mohapatra *et al.* (2016). Likewise, Khan *et al.* (2021) reported a pH of 7.5-7.7 good for the growth of *Cirrhinus mrigala*, and Yadav (2019) reported an 8-10.5 range pH is good for the growth of Silver carp.

The amount of DO plays significant role in the proper growth of a fish. In the present study, the DO limit of 5-9mg/l was recorded suitable as similar to Sah (2017) (5.0-9.1 mg/l). In the experiment carried out by Verma and Mandal (2018), the DO ranged between 5.01-6.61 mg/l in mrigal and amur common carp. According to the Khan *et al.* (2021) the DO range between 5.38-5.43 mg/l was good for the growth of *Cirrhinus mrigala* whereas in silver carp the DO range between 5-8.2 mg/l was obtained better (Yadav, 2019).

In the present study, the amount of free CO_2 was observed between 6.6-13.2 mg/l whereas Yadav (2019) obtained a free CO_2 range of 13.2-17 mg/l during the breeding of silver carp which was similar to Sah (2017) of the range limit 13.7-17.1 mg/l for Rohu and Naini.

In the present study, the female Rohu and Mrigal fishes were injected with 0.5 ml/kg body weight and males with 0.25 ml/kg body weight hormone. This hormone administration showed a great result. According to previous studies, the administration of 0.3-0.5 ml/kg body weight in Rohu, Mrigal, and Catla showed significant results (Naik and Mirza, 1992). According to Naem, *et al*. (2013) Catla when treated with 0.7 ml/kg for females, and males with 0.1 ml/kg Ovaprim hormone, obtained a better result. The low and high doses of Ovaprim showed no inducing effect in Shirbot nevertheless 1 ml/kg bw ovaprim and 3 mg/kg bw pituitary extract gave better results (Ghanemi and Khodadadi, 2017). The administration of 0.4 ml/kg bw and 0.2 ml/kg bw WOVA-FH hormone for females and males respectively in *Labeo rohita* was found successful than with CPE (Pandey *et al.,* 2015).

The latency period is the time after the hormone administration and before the initiation of spawning. During this study, the latency period was 7-8 hrs. and found similar to *Cirrhinus mrigala* 8 hrs. as reported by Kaul and Rishi, 1986. In *Labeo rohita*, 8.40 hrs. of latency period were observed with the introduction of 0.6 ml/kg of Ovaprim hormone in females (Khan *et al.,* 2006). According to Hossain (2012), *Heteropneustes fossilis* showed a latency period of 10 hrs. however, showed a latency period of 15 hrs. with PG. The latency period was found different in the experiment on *Channa punctatus* and *Heteropneustes fossilis*. It was found to be 28-34 hr. and 18-24 hr. for *Channa punctatus* and *Heteropneustes fossilis* respectively (Haniffa and Sridhar, 2002). According to Pandey *et al.* (2015), *Labeo rohita* in administration with CPE spawned after 10-12 hrs. whereas WOVA-FH spawned after 7-8 hrs.

In this study, the fecundity and GSI were found to be 227,540 to 675,000 and 10.33-19.74% respectively for Rohu whereas Mrigal showed a fecundity of 161,400-608,400 and GSI of 10.71-28.14%. Khan *et al.,* (2006) reported 0.64 million eggs in *Labeo rohita*. The amount of 0.38-lacs egg/kg was obtained during the breeding of *Labeo rohita* with the administration of Ovaprim hormone (Tiwana and Raman, 2012). After the spawning of *Cirrhina mrigala*, 120000 ova/kg body weight was reported by Kaul

and Rishi (1986). The spawning rate was observed to be 40-80% in *Barbus garypus* by Ghanemi and Khodadadi (2017). The *Heteropneustes fossilis* showed high ovulation rate of 90% with Ovaprim than 78.67% with PG (Hossain *et al.,* 2012). The spawning of *Labeo rohita* yields about 3.0 lakhs of eggs with the administration of CPE whilst 5.2 lakh eggs with WOVA-FH (Pandey *et al.,* 2015). In the experiment performed by Mohapatra (2016) using Ovatide hormone, the spawn obtained from Rohu and Mrigal were 125 lakh and 73 lakhs respectively, and the observed fecundity was 1.3-1.82 lakh egg/kg bw in Rohu and 1.25-1.58 lakh egg/kg bw in Mrigal. Sah (2017) performed a comparative experiment on *Labeo rohita* and *Cirrhinus mrigala* where the fecundity of 2.33-3.16 lac egg/kg bw in Rohu and 4.23-7.68 lac egg/kg bw in Mrigal was observed. The GSI was also found to be 11.25-15.45% and 10.52-12.52% in Rohu and Mrigal respectively.

In the present study, the fertilization and hatching rate of Rohu was found to be 82.28% to 87.27% and 73.91% to 83.33% respectively. The fertilization and hatching rates were observed to be 79.63% to 87.88% and 77.90% to 83.90% respectively in Mrigal. A similar result was obtained in *Cirrhina mrigala* by Kaul and Rishi (1986); 80-85% fertilization rate and 70% hatching rate. In *Labeo rohita*, a 53% fertilization rate was observed by Khan *et al.* (2006). Tiwana and Raman (2012) observed 61.30% as a fertilization rate and 72.20% as a hatching rate in *Labeo rohita*. The *Heteropneustes fossilis* when treated with ovaprim showed high fertilization and hatching rate of 86.67% and 76.92% respectively in comparison to 69.23% fertilization rate and 72.72% hatching rate with the administration of PG (Hossain *et al.,* 2012). The highest 78% fertilization rate was observed in *Channa punctatus* with a high dose and in *Heteropneustes fossilis* with the administration of a low dose (Haniffa and Sridhar, 2002). According to Pandey *et al.* (2015), the highest fertilization and hatching rate was observed in the *Labeo rohita* treated with WOVA-FH than in CPE. With the introduction of CPE, a 71-80% fertilization rate and 68-73% hatching rate were observed whereas with WOVA-FH the fertilization rate was 95-100% and the hatching rate was 90-95%. In the experiment carried out by Mohapatra *et al.* (2016), the fertilization rate was found in between 90-100% in both Rohu and Mrigal with the use of Ovatide hormone. Sah (2017) performed induced breeding in *Labeo rohita* and *Cirrhinus mrigala* where the fertilization rate of 77.77-88.33% and 71.05-82.24% were

obtained respectively. Likewise, the obtained hatching rate of Rohu was 75.29-82.68% and that of Mrigal was 60.86-79.28%.

The correlation coefficient 'r' value helps to determine if the linear relationship in the sample data can be used to analyze the relationship of the population. During the experiment, the 'r' value was found nearly 1 which represented the identical and similar relationship between the length and weight of growing fishes. A similar result was observed by Ujjania *et al.* (2013) in the breeding of Rohu. According to Sah (2017), the correlation coefficient of the length and weight of fish were calculated to be 0.966 and 0.961 for Rohu and Naini respectively.

In the present study, the regression coefficient b ranged from 2.12-2.50. The condition factor (K) of Rohu was observed greater than 1.0 in all six hapas which indicates a good condition of fish, furthermore, the relative condition factor (Kn) was calculated between 1.13-1.4. According to the study conducted by Ujjania *et al.* (2013) in Rohu, the regression coefficient b was found around 3, condition factor was observed similar (>1.0), whereas, the relative condition factor (Kn) was between 0.99 to 1.00. In this study, the correlation coefficient between the fecundity and weight of female Rohu was calculated 0.8722 which was less than computed by Sah (0.9582). Furthermore, the correlation coefficient of the weight of female fish and the fecundity of Naini was found to be 0.8555 and was less than that of Naini (0.9691) as performed by Sah (2017).

CHAPTER- SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The induced breeding performance of *Labeo rohita* and *Cirrhinus mrigala* using ovaprim hormone was found significant in the Fisheries Development Center, Bhandara, Chitwan. The fecundity of Rohu was observed 227,540-675,000 and GSI was 10.33-19.74% whilst that of Naini was 161,400-608,400 and 10.71-28.15% respectively. The fertilization and hatching rates were 82.28-87.27% and 73.91-83.33% in Rohu and 79.63-87.88% and 77.90-83.90% in Naini respectively. The survival rate of Naini was obtained higher 75% in HN3 whereas, Rohu with 73% in HR2. The length and growth performance of Naini was more remarkable than that of Rohu. The fingerling of Naini attain the highest weight of 5.966 gm. and length of 8.87 cm. whereas Rohu gained 4.283 gm. weight and 7.2 cm. length. It is observed that this hormone has similar effects on both fishes so it is very advantageous and economical for induced breeding of Indian Major Carps especially, *Labeo rohita* and *Cirrhinus mrigala*.

6.2 Recommendations

Based on the study, the following are the recommendations.

- Besides the temperature, pH, dissolved oxygen and free carbon dioxide other water parameters such as turbidity, hardness, alkalinity which could also influence the growth of the fish. Hence the influence of these parameters should be analyzed.

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APPENDICES

APPENDIX I: Weight of female brood fish of *Labeo rohita* and amount of hormone injected

S.N.	Weight of female brood fish (kg)	Hormone injected (ml/kg body wt)	Weight of female brood fish after breeding (kg)
1	3	1.5	2.51
2	3.8	1.9	3.05
3	3.5	1.75	2.85
4	3	1.5	2.69
5	3.1	1.55	2.73
6	3.7	1.85	3.03
7	3.4	1.7	2.89
8	2.6	1.3	2.25
Total	26.1	13.05	22

APPENDIX II: Weight of male brood fish of *Labeo rohita* and amount of hormone injected

S.N.	Weight of male brood fish (kg)	Hormone injected (ml/kg body wt)
1	2.2	0.55
2	3.1	0.775
3	3.6	0.9
4	1.9	0.475
5	2.1	0.525
6	2.4	0.6
7	2.7	0.675
8	2.5	0.625
9	2	0.5
10	1.9	0.475
11	1.7	0.425
12	1.4	0.35
Total	27.5	6.875

APPENDIX III: Weight of female brood fish of *Cirrhinus mrigala* and amount of hormone injected

S.N.	Weight of female brood fish (kg)	Hormone injected (ml/kg body wt)	Weight of female brood fish after breeding (kg)
1	4.39	2.195	3.44
2	2.126	1.063	1.646
3	1.604	0.802	1.334
4	2.73	1.365	2.07
5	1.382	0.691	1.192
6	2	1	1.53
7	2.816	1.408	2.006
8	1.336	0.668	1.186
9	2	1	1.5
10	2.126	1.063	1.746
11	2.8	1.4	1.94
12	2	1	1.78
13	2.7	1.35	1.94
14	1.22	0.61	1
15	1.3	0.65	1.13
16	2.3	1.15	1.87
17	1.41	0.705	1.12
18	2.24	1.12	2
19	2.6	1.3	2.07
Total	41.08	20.54	32.5

APPENDIX IV: Weight of male brood fishes of *Cirrhinus mrigala* and amount of hormone injected

S.N.	Weight of male brood fish (kg)	Hormone injected (ml/kg body wt)
1	3	0.75
2	1.9	0.475
3	2.5	0.625
4	2.7	0.675
5	1.9	0.475
6	1.6	0.4
7	1.5	0.375
8	2.472	0.618
9	2.312	0.578
10	1.5	0.375
11	1.8	0.45
12	2.4	0.6
13	1.7	0.425
14	1.5	0.375
15	1.8	0.45
16	2.2	0.55
17	2.5	0.625
18	2.2	0.55
19	2.2	0.55
20	1.7	0.425
21	2.1	0.525

22	1.7	0.425
23	2	0.5
24	2	0.5
25	1.6	0.4
26	1.1	0.275
27	1.1	0.275
28	1.3	0.325
29	1	0.25
Total	55.284	13.821

APPENDIX V: Data of water temperature and pH of incubation tank of *Labeo rohita*

Date	pH		Temperature (°C)	
	7 to 9 A.M	**3 to 5 P.M**	**7 to 9 A.M**	**3 to 5 P.M**
3/5/2079	7.2	7.1	25.8	26.1
3/6/2079	7	7.1	25.5	26
3/7/2079	7	7.1	25.7	26
3/8/2079	7	7.1	25.9	26

APPENDIX VI: Data of water temperature and pH of incubation tank of *Cirrhinus mrigala*

Date	pH		Temperature (^{0} C)	
	7 to 9 A.M	**3 to 5 P.M**	**7 to 9 A.M**	**3 to 5 P.M**
3/5/2079	7.3	7.1	26	26.6
3/6/2079	7.1	7.1	26	26.1
3/7/2079	7.1	7	25.8	26
3/8/2079	7	7.2	25.9	26

APPENDIX VII: Data of water temperature and pH of different hapas of *Labeo rohita*

Date	pH values			Temperature (^{0} C)		
	Hapa HR1	Hapa HR2	Hapa HR3	Hapa HR1	Hapa HR2	Hapa HR3
3/9/2079	8	8	8	34	34	34
3/10/2079	7.2	7.2	7.2	30.5	30.5	30.5
3/11/2079	7.5	7.5	7.4	32.5	32.2	32.2
3/12/2079	7.3	7.2	7.2	31	31.5	31.5
3/13/2079	7.5	7.4	7.5	31.5	31.5	31.5
3/14/2079	7.8	7.7	7.6	31	31	31.5
3/15/2079	7.4	7.5	7.5	29.5	29.5	29.5
3/16/2079	7.7	7.7	7.8	30	29.5	29.5
3/17/2079	8.1	7.9	8.1	30	30	30
3/18/2079	7.4	7.5	7.5	29.5	29.5	29.5
3/19/2079	7.9	7.8	7.9	30.5	30.5	30
3/20/2079	8	7.9	7.9	30.5	30.5	30.5
3/21/2079	8.2	8.1	8.1	31.5	31.5	31.5
3/22/2079	7.9	7.8	7.8	31.5	31.5	31.5
3/23/2079	8.2	8.2	8.1	32	32	31.5
3/24/2079	7.9	7.9	7.8	32	32	32
3/25/2079	7.9	7.9	7.9	31.5	31.5	31.5
3/26/2079	8	7.9	7.8	31	31	31
3/27/2079	8	8	7.9	31.5	31.5	31.5
3/28/2079	8.1	8	7.9	31.5	31.5	31.5

3/29/2079	8.1	8	7.9	32	32	32
3/30/2079	8.4	8.3	8.3	32	32	32
3/31/2079	8.3	8.2	8.1	33	32.5	33
2079/03/32	8.5	8.4	8.3	33	33	33
4/1/2079	8.3	8.3	8.2	33	33	33
4/2/2079	8.2	8.1	8.1	33	33	33
4/3/2079	8.4	8.4	8.4	31.5	31.5	31.5
4/4/2079	8.4	8.4	8.3	32	32	31.5
4/5/2079	8.4	8.4	8.4	31	31	31
4/6/2079	8.9	8.7	8.5	30.5	30.5	30.5
4/7/2079	8.9	8.9	8.8	31.5	31.5	31
4/8/2079	8.9	8.9	8.9	31	31	31
4/9/2079	9	8.7	8.7	30	30	30
4/10/2079	8.4	8.4	8.3	30.5	30.5	30.5
4/11/2079	8.9	8.8	8.7	30	30	30
4/12/2079	8.8	8.7	8.6	31	31	30.5
4/13/2079	8.7	8.6	8.4	29.5	30	30
4/14/2079	8.4	8.2	8.1	30.5	30	30
4/15/2079	8.4	8.2	8	29.5	30	30
4/16/2079	8.4	8.4	8	29.5	29.5	29.5
4/17/2079	8.3	8.3	8.3	29.5	29.5	29.5
4/18/2079	8.4	8.2	8.2	30	30	30.5
4/19/2079	8.3	8.3	8.3	30.5	30.5	30.5

4/20/2079	8.5	8.2	7.9	30	30	30
4/21/2079	8.3	8.3	8.3	31.5	31	31
4/22/2079	8.3	8.1	7.9	30.5	30.5	30.5
4/23/2079	8.5	8.4	8.1	31.5	31.5	31.5
4/24/2079	8.2	8.1	8	32	32	31.5
4/25/2079	8.2	8	8	31	31	31
4/26/2079	8	8	7.7	31.5	31.5	31
4/27/2079	8.5	8.5	8.2	30.5	30.5	30
4/28/2079	8.4	8.2	8	30.5	30.5	30.5
4/29/2079	8.4	8.2	8.1	30.5	30.5	30.5
4/30/2079	8.5	8.3	8	31	31	31
2079/04/31	8.6	8.3	8.3	31.5	31.5	31
5/1/2079	8.8	8	7.6	31.5	31	31
5/2/2079	8.6	8	7.9	31.5	31.5	31
5/3/2079	8.7	8.2	7.7	31.5	32	32
5/4/2079	8.6	8.4	8.3	31.5	31.5	31
5/5/2079	8.7	8.5	8.4	31.5	31.5	31.5
5/6/2079	8.3	8.2	8	30.5	30.5	30
5/7/2079	8.4	8.2	8.1	31	31	31
5/8/2079	8.5	8.4	8.1	31	31	30.5
5/9/2079	8.1	8.1	8	30	30	30
5/10/2079	8.3	8.1	8	30.5	30.5	30
5/11/2079	8.4	8.4	8.5	31	31	31

5/12/2079	8.7	8.5	8.1	31.5	31.5	31.5
5/13/2079	8.8	8.3	7.9	31	31	30.5
5/14/2079	8.6	8.5	8.1	30.5	30.5	30.5
5/15/2079	8.5	8.5	8.3	31	31	30.5
5/16/2079	8.3	8.3	8	31.5	31.5	31
5/17/2079	8.6	8.5	8.2	32	31.5	31.5
5/18/2079	8.5	8.4	8.3	33	32.5	32
5/19/2079	8.6	8.1	8.1	32	32	32
5/20/2079	8.4	8	7.7	29.5	29.5	29.5
5/21/2079	8.2	8.1	7.7	30	30	30
5/22/2079	7.4	7.7	7.7	29.5	29.5	30
5/23/2079	8	8	7.7	30	30	30

APPENDIX VIII: Data of water temperature and pH of different hapas of *Cirrhinus mrigala*

Date	pH values			Temperature (^{0} C)		
	Hapa HN1	Hapa HN2	Hapa HN3	Hapa HN1	Hapa HN2	Hapa HN3
3/9/2079	8	8	8	34	34	34
3/10/2079	7.2	7.1	7.1	30.5	30.5	30.5
3/11/2079	7.4	7.4	7.4	32	32	32
3/12/2079	7.3	7.3	7.4	31.5	31.5	32
3/13/2079	7.4	7.5	7.5	31.5	31.5	31.5
3/14/2079	7.6	7.6	7.6	31.5	31.5	31.5
3/15/2079	7.5	7.5	7.5	29.5	29.5	29.5
3/16/2079	7.8	7.8	7.8	29.5	29.5	29.2
3/17/2079	8.1	8.1	8	30	30	30
3/18/2079	7.5	7.5	7.4	29.5	29.5	29.5
3/19/2079	7.9	7.8	7.8	30	30	30
3/20/2079	7.9	7.8	7.7	30.5	30.5	30.5
3/21/2079	8.1	8.1	8	31.5	31.5	31.5
3/22/2079	7.7	7.8	7.8	31.5	31.5	31.5
3/23/2079	8.1	8.1	8	31.5	31.5	31.5
3/24/2079	7.8	7.8	7.8	32	32	32
3/25/2079	7.9	7.9	8	31.5	31.5	31.5
3/26/2079	7.7	7.7	7.7	31	31	31
3/27/2079	7.9	7.9	7.9	31.5	31.5	31.5

3/28/2079	7.9	7.8	7.8	31.5	32	32
3/29/2079	7.9	7.9	7.9	32	32	32
3/30/2079	8.1	8.1	8	32	32	32.5
3/31/2079	8.1	7.9	7.9	33	33	33
2079/03/32	8.2	8.2	8.2	33	33	33
4/1/2079	8.2	8.2	8.2	33	32.5	32.5
4/2/2079	8.1	8	8	32.5	32.5	32.5
4/3/2079	8.4	8.4	8.4	31.5	31.5	31.5
4/4/2079	8.3	8.3	8.3	31.5	31.5	31.5
4/5/2079	8.4	8.3	8.5	31	31.5	31.5
4/6/2079	8.4	8.3	8.3	30.5	30.5	30.5
4/7/2079	8.7	8.6	8.7	31	31	31
4/8/2079	8.9	8.8	8.2	31	31	31
4/9/2079	8.3	8.3	8.2	30.5	30	30
4/10/2079	8.3	8.3	8.3	30	30.5	30.5
4/11/2079	8.5	8.4	8.4	30.5	30.5	30.5
4/12/2079	8.6	8.6	8.5	30.5	30.5	30.5
4/13/2079	8	8	7.8	30	30	31
4/14/2079	7.9	7.7	7.7	30	30	30
4/15/2079	7.9	7.7	7.7	30	30	30
4/16/2079	8	7.7	7.7	30	30	30
4/17/2079	8.1	8	8	29.5	30	30
4/18/2079	8	7.9	7.9	30.5	30.5	30.5

4/19/2079	8.2	8.2	8.1	30	30	30
4/20/2079	7.9	7.7	7.6	30	30	30
4/21/2079	8.1	7.9	7.9	31	31	31
4/22/2079	7.8	7.7	7.5	30.5	30.5	30.5
4/23/2079	8	7.9	7.9	31.5	31.5	31.5
4/24/2079	8	8	8	31.5	31.5	31.5
4/25/2079	7.6	7.6	7.7	30.5	30	30
4/26/2079	7.7	7.6	7.4	31	31	31
4/27/2079	7.9	7.9	7.6	30	30	30
4/28/2079	7.9	7.9	7.7	30.5	30	30
4/29/2079	8	7.8	7.8	30	30	30
4/30/2079	8	7.8	7.8	31	31	31
2079/04/31	7.9	7.9	7.8	31	31	31
5/1/2079	7.6	7.4	7.4	31.5	31	31
5/2/2079	7.6	7.6	7.5	31	31	31
5/3/2079	7.7	7.6	7.6	32	32	32
5/4/2079	8	8	7.9	31	31	31
5/5/2079	8	7.8	7.8	31	31	31
5/6/2079	8	8	7.8	30	30	30
5/7/2079	7.9	7.6	7.6	31	30.5	30.5
5/8/2079	8.1	8	7.8	30.5	30.5	30.5
5/9/2079	7.7	7.7	7.6	30	30	30
5/10/2079	8	8	8	30	30	30

5/11/2079	8	8	7.9	30.5	30.5	30
5/12/2079	8.2	7.9	7.8	31	30.5	30.5
5/13/2079	7.8	7.6	7.5	30.5	30.5	30.5
5/14/2079	8.1	8	7.9	30.5	30	30
5/15/2079	8.1	7.9	7.7	30.5	30	30
5/16/2079	7.9	7.8	7.8	30.5	30.5	30.5
5/17/2079	8	8	7.9	31	31	30.5
5/18/2079	8	7.9	7.8	31	30.5	30.5
5/19/2079	7.9	7.7	7.6	31.5	31.5	31.5
5/20/2079	7.5	7.4	7.3	29.5	29.5	29.5
5/21/2079	7.6	7.4	7.2	30	30	30
5/22/2079	7.2	7.2	7.1	30	30	30
5/23/2079	7.6	7.2	7.2	30	29.5	29.5

APPENDIX IX: Fecundity and GSI (%) of *Labeo rohita*

S.N.	Brood fish	Wt. of female(kg)	Wt. of total egg spawned (gm)	No. of egg per gm.	Fecundity	GSI (%)
1	R1	3	490	850	416500	16.33
2	R2	3.8	750	900	675000	19.74
3	R3	3.5	650	815	529750	18.57
4	R4	3	310	734	227540	10.33
5	R5	3.1	370	760	281200	11.93
6	R6	3.7	670	740	495800	18.11
7	R7	3.4	510	893	455430	15.00
8	R8	2.6	350	730	255500	13.46

APPENDIX X Fecundity and GSI (%) of *Cirrhinus mrigala*

S.N.	Brood fish	Wt. of female (kg)	Wt. of total egg spawned (gm)	No. of egg per gm.	Fecundity	GSI (%)
1	N1	4.39	900	676	608400	20.50
2	N2	2.126	480	785	376800	22.58
3	N3	1.604	270	736	198720	16.83
4	N4	2.73	660	712	469920	24.17
5	N5	1.382	240	804	192960	17.37
6	N6	2	470	835	392450	23.50
7	N7	2.816	760	723	549480	26.99
8	N8	1.336	200	807	161400	14.97
9	N9	2	500	745	372500	25.00
10	N10	2.126	380	813	308940	17.87
11	N11	2.8	780	687	535860	27.86
12	N12	2	220	832	183040	11.00
13	N13	2.7	760	677	514520	28.15
14	N14	1.22	220	792	174240	18.03
15	N15	1.3	250	804	201000	19.23
16	N16	2.3	430	816	350880	18.69
17	N17	1.41	290	842	244180	20.57
18	N18	2.24	240	736	176640	10.71
19	N19	2.6	530	698	369940	20.38

APPENDIX XI: Fertility rate and hatching rate of *Labeo rohita*

S. N	Brood fish	No. of sample eggs	No. of viable and fertilized eggs	Fertility rate (%)	Hatching rate (%)
1	R1	78	67	85.90	74.62
2	R2	110	96	87.27	78.12
3	R3	115	97	84.35	79.38
4	R4	81	69	85.18	73.91
5	R5	99	86	86.87	82.55
6	R6	97	84	86.60	83.33
7	R7	79	65	82.28	78.46
8	R8	88	76	86.36	81.57

APPENDIX XII: Fertility rate and hatching rate of *Cirrhinus mrigala*

S.N.	Brood fish	No of sample eggs	No of viable and fertilized eggs	Fertility rate (%)	Hatching rate (%)
1	N1	101	86	85.15	82.55
2	N2	118	96	81.35	79.16
3	N3	120	99	82.50	78.78
4	N4	96	84	87.50	80.92
5	N5	107	93	86.91	80.64
6	N6	112	96	85.71	80.20
7	N7	114	97	85.09	81.44
8	N8	99	87	87.88	83.90
9	N9	95	82	86.31	82.92
10	N10	98	84	85.71	82.14
11	N11	103	88	85.44	81.81
12	N12	109	93	85.32	80.64
13	N13	108	86	79.63	77.90
14	N14	112	92	82.14	79.34
15	N15	113	94	83.18	80.85
16	N16	109	91	83.49	80.21
17	N17	98	82	83.67	79.26
18	N18	89	77	86.52	83.11
19	N19	118	96	81.35	78.12

APPENDIX XIII: Survival rate of *Labeo rohita*

Survival rate of *Labeo rohita*								
Happa	**Size of happa**	**No. of stocking fries**	**No. of days**					**Average survival rate (%)**
			15	**30**	**45**	**60**	**75**	
1	1×1×1 m.	100	79	78	65	63	62	62
2	1×1×1 m.	100	87	85	76	75	73	73
3	1×1×1 m.	100	92	89	71	66	66	66

APPENDIX XIV: Survival rate of *Cirrhinus mrigala*

Survival rate of *Cirrhinus mrigala*								
Happa	**Size of happa**	**No. of stocking fries**	**No. of days**					**Average survival rate (%)**
			15	**30**	**45**	**60**	**75**	
1	1×1×1 m.	100	82	79	65	62	59	59
2	1×1×1 m.	100	88	86	76	74	71	71
3	1×1×1 m.	100	87	86	79	76	75	75

APPENDIX XV: Mortality of *Labeo rohita* and *Cirrhinus mrigala*

Hapa	**Size of hapa**	**No. of stocking fries**	**No. of days**									
			15		**30**		**45**		**60**		**75**	
			R	**N**	**R**	**N**	**R**	**N**	**R**	**N**	**R**	**N**
1	1×1×1 m.	100	21	18	1	3	13	14	2	3	1	3
2	1×1×1 m.	100	13	12	2	2	9	10	1	2	2	3
3	1×1×1 m.	100	8	13	3	1	18	7	5	3	0	1

APPENDIX XVI: Length and weight data of fries and fingerlings of hapa HR1

HAPPA HR1 (*Labeo rohita*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3	0.54	4.5	1.44	5.6	2.8	6	2.24	6.5	3.49
2	2	3.1	0.61	4	0.82	5.5	1.53	4	1.7	7.4	4.59
3	3	3.6	0.72	4.4	1.22	5.5	1.8	5.5	1.83	7	3.55
4	4	2.8	0.46	4	0.91	5.6	1.78	6.3	3	6.5	3.28
5	5	3	0.56	4.1	1	5.3	2	6.7	3	7.8	5.62
6	6	2.9	0.48	4.5	1.8	5	1.53	6.3	2.75	7.2	4.41
7	7	3.4	0.65	4	1	5	1.33	6	2.51	7.2	4.39
8	8	3.4	0.65	4	1	5.2	1.74	6.7	3.15	7.2	3.65
9	9	3.1	0.56	4.1	1.11	5	1.7	6.6	3.12	7.5	4.7
10	10	3.5	0.69	4.5	1.28	5	1.44	5.6	2	7.7	5.15

APPENDIX XVII: Length and weight data of fries and fingerlings of hapa HR2

HAPPA HR2 (*Labeo rohita*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3.3	0.5	3.8	0.81	4.5	1	5.7	2	6.2	2.28
2	2	2.6	0.47	4.3	1	5.1	1.58	5.5	1.92	7.5	4.22
3	3	3	0.55	4.3	1	5	1	6.2	2.46	6	2.64
4	4	3.2	0.61	4.2	1	4.9	1	5.7	2	6	2.25
5	5	3.2	0.6	4	0.88	4.5	1.21	5.5	1.83	6.5	2.75
6	6	3.4	0.65	4	1	5	1.3	5.5	1.86	6.5	3
7	7	2.8	0.47	4.5	1.22	4.7	1.37	5.8	2.11	6	2.25
8	8	3	0.56	4.5	1	4.6	1.12	5.7	2.17	6	2
9	9	3.1	0.59	4	0.88	4.9	1.46	6	2.46	6.2	2.54
10	10	3.1	0.59	4.7	1.5	4.9	1.6	5.8	2	6.3	2.7

APPENDIX XVIII: Length and weight data of fries and fingerlings of hapa HR3

HAPPA HR3 (*Labeo rohita*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3	0.54	3.5	0.76	4.7	1.28	6	2.29	6.5	3.36
2	2	3.1	0.65	3.7	0.81	4.7	1.29	5.7	2.08	6.5	3.4
3	3	3.4	0.59	4.1	1.06	5.1	1.64	5.2	1.59	6.7	3.65
4	4	3.2	0.6	4.2	1.12	4.6	1.8	5.3	1.57	5.9	3.07
5	5	3.3	0.49	4	1	4.6	1.19	5.5	1.76	6.5	3.31
6	6	3.3	0.45	3.8	0.8	4.9	1.18	5.5	1.91	5.8	3
7	7	3.5	0.7	4.5	1.15	4.4	1	6.2	3	6.6	3.48
8	8	3.3	0.51	3.9	0.85	4.6	1	5.8	2.11	5.7	2.93
9	9	2.8	0.45	4.3	1.12	4.5	1.8	5.6	1.64	5.7	2.88
10	10	3.2	0.51	4	1	4.3	0.89	4.5	1.42	5.8	2.97

APPENDIX XIX: Length and weight data of fries and fingerlings of hapa HN1

HAPPA HN1 (*Cirrhinus mrigala*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3.3	0.71	5.2	1.92	6	2.88	7.6	4.41	9	6
2	2	3.5	0.78	5.7	2.6	6.7	3.32	6.6	3.59	8	5.17
3	3	3.6	0.79	5.9	2.21	6.5	3.19	8	4.9	7.5	4.83
4	4	3.9	0.88	5.4	2	7.5	4.32	9	6.5	9.5	6.58
5	5	3.5	0.78	4.6	1.11	5.6	2.12	8.2	5.73	9.4	6.37
6	6	4.1	0.96	4.6	1.16	6.6	3.21	5.2	1.64	9.5	6.62
7	7	4.5	1	5.8	2	6.5	3.15	7.2	5	9.3	6.21
8	8	3.9	0.87	5.2	1.62	6	2.45	6	2.5	9	5.96
9	9	4	0.93	4.5	1.42	4	0.76	7.2	4.17	10	6.92
10	10	4.1	0.98	4.5	1.08	5.9	2.21	8	5.1	7.5	5

APPENDIX XX: Length and weight of fries and fingerlings of hapa HN2

HAPPA HN2 (*Cirrhinus mrigala*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3	0.62	4.6	1.67	5.8	1.88	5.7	3.2	8.4	5.17
2	2	3.2	0.64	4.5	1.61	6	2.23	7.4	4.17	8	4.97
3	3	3.8	0.78	5.3	2	5.6	1.61	6.5	4.3	8.1	5
4	4	3.2	0.65	5	1.96	6.4	2.62	6.7	3.56	8	4.99
5	5	3.5	0.71	5.1	1.98	6.1	2.42	7.6	4.16	8.1	5
6	6	3.9	0.83	5.8	2.24	6.4	2.68	7.2	4	7.3	4.86
7	7	3.5	0.69	4.9	1.95	5	1.58	6.6	3.26	8.2	5.11
8	8	3	0.58	5.1	1.95	6.1	2.27	6.7	3.28	7.5	4.85
9	9	3.2	0.6	4.6	1.62	6.3	2.62	7.3	4.34	6.2	4.12
10	10	4	0.85	5	1.98	6.5	2.81	7.2	3.88	7.8	4.88

APPENDIX XXI: Length and weight data of fries and fingerlings of hapa HN3

HAPPA HN3 (*Cirrhinus mrigala*)											
DATE:		**3/24/2079**		**4/7/2079**		**4/22/2079**		**5/6/2079**		**5/22/2079**	
S.N.	**Fish number**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**	**Length (cm.)**	**Weight (gm.)**
1	1	3.5	0.78	6	2	6.6	2.89	6.2	3	9	6.1
2	2	3.6	0.81	5.3	1.62	7.5	3.9	8	3.31	8	5.72
3	3	3.5	0.75	6.1	2.32	5.5	2	8.2	4.44	8.8	5.96
4	4	3.2	0.71	5	1.38	7	3.26	8	4.24	8	5.35
5	5	3	0.67	6	2	7.4	3.65	7	3.08	8.6	5.85
6	6	3.5	0.75	6.3	2.45	5.6	1.84	6.2	2.55	8.4	5.8
7	7	4.1	0.96	5	1.22	6.5	2.83	8	4.25	8	5.69
8	8	3.9	0.85	5.7	2	6	2.36	7.7	3.72	8.9	6
9	9	3.9	0.83	5.7	2.15	6.6	2.79	6.5	2.53	7.8	5.63
10	10	4	0.95	6	2.31	6.3	2.25	6.8	3	8.6	5.88

PHOTO PLATES

Photo 19: Office building of FDC, Bhandara, Chitwan

Photo20: Figurative model showing area of FDC

Photo 21: Netting of brood fishes

Photo 22: Collection of brood fishes in breeding tank

Photo 23: Hormone injection to broods

Photo 24: Counting of eggs

Photo 25: Sample of fertilized and unfertilized eggs

Photo 26: Hatchlings in incubation tank

Photo 27: Setting of happas

Photo 28: Checking of pH and temperature

Photo29: Covering of happas for protection

Photo 30: Water sample collection in DO bottle

Photo 31: Observing samples through microscope

Photo 32: Water samples for titration of DO

Photo 33: Titration of CO_2

Photo 34: Titration of DO

Photo 35: Noting down of reading

Photo 36: Photo with field staffs

Photo 37: Photo with a field staff