EVALUATION OF BREEDING PERFORMANCE AND REARING OF BIGHEAD CARP (*Aristichthysnobilis*, Richardson 1845) USING LH-RHa SYNTHETIC HORMONE IN MANDAL FISH BREEDING CENTER (MFBC), PATHARDANDA RUPENDEHI, NEPAL

DEEPA SHARMA

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Submitted to

Central Department of Zoology

Institute of Science and Technology

Tribhuvan University

Kirtipur, Kathmandu

Nepal

September, 2019

DECLARATION

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

Date: 2076-5-22

Deepa Sharma

Deepa

TRIBHUVAN UNIVERSITY ☎ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

RECOMMENDATION

This is to recommend that the thesis entitled**"EVALUATION OF BREEDING PERFORMANCE AND REARING OF BIGHEAD CARP (*Aristichthysnobilis*, Richardson 1845) USING LH-RHa SYNTHETIC HORMONE IN MANDAL FISH BREEDING CENTER (MFBC), PATHARDANDA RUPENDEHI, NEPAL"**has been carried out by Mrs. Deepa Sharma for the partial fulfillment of Master's degree of Science in Zoology with special paper Fish Biology and Aquaculture. This is his 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 other degree in any institutions.

.....

Supervisor

Prof. Dr. Kumar Sapkota

Central Department of Zoology

Kirtipur, Kathmandu, Nepal

TRIBHUVAN UNIVERSITY ☎ 01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal.

Ref.No.:

LETTER OF APPROVAL

On the recommendation of supervisor **"Prof. Dr. Kumar Sapkota"** this dissertation submitted by **Mrs. Deepa Sharma entitled "EVALUATION OF BREEDING PERFORMANCE AND REARING OF BIGHEAD CARP (*Aristichthysnobilis*, Richardson 1845) USING LH-RHa SYNTHETIC HORMONE IN MANDAL FISH BREEDING CENTER (MFBC), PATHARDANDA RUPENDEHI, NEPAL"** is approved for the examination of the requirements for Master's degree of science with special paper Fish Biology and Aquaculture.

Date: .2076-5-22...

.....

Professor Dr. TejBahadurThapa
Head of Department
Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu, Nepal

TRIBHUVAN UNIVERSITY
01-4331896
CENTRAL DEPARTMENT OF ZOOLOGY
Kirtipur, Kathmandu, Nepal

Ref.No.:

CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Mrs. Deepa Sharma entitled **"EVALUATION OF BREEDING PERFORMANCE AND REARING OF BIGHEAD CARP (*Aristichthys nobilis*, Richardson 1845) USING LH-RHa SYNTHETIC HORMONE IN MANDAL FISH BREEDING CENTER (MFBC), PATHARDANDA RUPENDEHI, NEPAL"** has been approved as a partial fulfillment for the requirements of Master's degree of Science with special paper Fish Biology and Aquaculture.

EVALUATION COMMITTEE

.....	.....
Supervisor	**Head of Department**
Prof. Dr. Kumar Sapkota	**Prof. Dr. Tej Bahadur Thapa**
Central Department of Zoology	Central Department of Zoology
Kirtipur, Kathmandu, Nepal	Kirtipur, Kathmandu, Nepal
.....	.....
External Examiner	**Internal Examiner**

Date of examination: ...2076-06-05.....

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LIST OF ABBREVIATIONS

Abbreviated form	Details of abbreviations
^0C	Degree Celsius
ha	Hector
DoFD	Directorate of Fisheries Department
MoAC	Ministry of Agriculture and Co-operative
CBS	Central Bureau of Statistics
FAO	Food Agricultural Organization
AGDP	Agricultural Gross Domestic Production
BW	Body Weight
KM	Kilometer
Kg	Kilogram
Cm	Centimeter
Gm	Gram
Ml	Mili litre
Mg	Mini gram
Mt	Metric tons
Wt	Weight
HCG	Human Chorionic Gonadotropin
SnGnRH	Salmon Gonadotropin Releasing Hormone
LRH/LH	Luteinizing Releasing Hormone/ Luteinizing Hormone
LHRHa	Luteinizing Hormone Releasing Hormone and Its Analogues
CPE	Carp Pituitary Extract
GnRH	Gonadotropic Release Hormone
NGO	Non-Government Organization
INGO	International Non-Government Organization
BS	Bikram sambat
pH	Hydrogen Ion Concentration

ABSTARCT

The most significant advancement in the field of aquaculture during recent decade is the development of techniques to induce reproduction in fishes. Induced spawning is essential for species which do not breed in captivity. The process of fish breeding artificially is called induced breeding. Induced breeding is a technique where organism is stimulated by particular hormones or other synthetic agents. Hormone used in induced breeding is called synthetic agents. The present study was undertaken to evaluate the breeding performance and rearing of Bighead carp (*Aristichthys nobilis*, Richardson 1845) using LH-RHa synthetic hormone. During this experiment, Fecundity, Gonado Somatic Index (GSI), Fertility rate (%), Hatching rate (%), embryonic development and Growth rate of fingerlings of Bighead carp was studied. Experimented fish specimens were successfully spawned by administrating LH-RHa in two doses. Initial dose of 1.5ml/kg BW and final dose of 3ml/kg BW was given to experimented female brooders fish in the time interval of 11hrs. Male was injected by single dose of 3ml/kg BW at the second injection time of female. Ovulation usually occurs about 9-11hrs after the second injection. During the study period the range of water temperature in different ponds was 25-38^{0}C, pH of water showed it to be alkaline during whole study period i.e. 7.5-10, DO were recorded 5-9, CO2 were recorded 13.5-16. In this experiment relative fecundity of 64800-132900 in female fish of Bighead carp and GSI 8.64-9.73% was recorded. Fertility rate and hatching rate 61.5% and 52.15% respectively was recorded in experimented fish. The development of embryo was noted and studied. The development of embryo continued and hatching takes place after 12-14hrs of fertilization. Hatchlings after transferring to nursery pond, the fry were fed with artificially formulated feed with 40-45% protein regularly. The length and weight of hatchings were recorded gradually increased.

1 INTRODUCTION

1.1 Background

Aquaculture or Fisheries is an area which is associated with fish or other aquatic population which is harvested for its commercial value. Fisheries can be marine or fresh water. Fisheries also can be wild or farmed. Aquaculture is fairly a new activity in Nepal. It began in the 1940s with pond culture of Indian major carps. Over the years, carp polyculture in ponds has developed as the most viable and popular aquaculture production system in Nepal and in 2003/2004 accounted for over 90% of total aquaculture production (FAO 2005). The major part of the pond fish production takes place in the southern part of the country – the Terai plain – where 94% of the fish ponds are located. Fishing is traditional in Nepal but modern aquaculture techniques for fish production started with the introduction of exotic carp in the early 1950s. To utilize fish resources about fourteen state owned fish farms were established in different parts of the country during 1960-65, where spawning and seed production technologies of carp (*Cyprinus carpio, Ctenopharygodon idella, Aristichthys nobilis, Hypopthalmichthys molitrix, Labeo rohita, Cirrhinus mrigala and Catla catla*) were successfully developed in the warm southern region. At present, technology of subsistence carp farming in ponds has been widely disseminated in the southern part of the country (Banjade,2015). However, it is necessary to improve productivity by increasing our understanding and increasing inputs.

1.2 Water resources

Being second richest country in water resources, Nepal is gifted by Himalayan ranges in the north with ever flowing snow melted rivers and fresh water springs with huge power of producing energy and fetch out drinking water to the people. Abundant water resources originated from Himalaya and high geographical variation in Nepal are two factors which might be positive benefits to improve aquaculture in landlocked country. The aquatic ecosystem of Nepal offers excellent habitats to at least 230 indigenous, 11 exogenous and 16 endemic fish species of high economic, environmental and academic value (Shrestha 1995) of which cold water fishes exceed 90 species (Rajbanshi 2013). Among the recorded 230 species of freshwater fish approximately 59 species categorized as cold water fish (Peter and Swar et al. 2002) and 21 species are in the IUCN Red List. These

fishes are unique due to topography, geography structure and different climatic conditions. Nepal is a small country with fabulous geographical region viz. Himalayan region, elevated flatland and hilly region and Terai region accommodating 7%, 46%, 47% of the total population (CBS, 2001). Nepal is blessed with four major river systems (Koshi Gandaki, Karnali and Mahakali) fed by hundreds of small rivers originating in the Mahabharat and Siwalik mountain ranges. The total length of these rivers is estimated to be about 45,000 km with an estimated coverage area of about 395,000 hectares (FDD 1998). Nepal is endowed with several types of water resources arising from glaciers and the consequent types of rivers arising from the Himalaya. Collectively, these water bodies cover nearly 5.5% of Nepal's land area (Table 1).

S.N	Resources	Estimated area (ha)	Coverage (%)	Potential for fisheries (area in ha)
1	Natural waters	401500	49	-
	Rivers	395000	48.2	-
	Lakes	50000	0.61	-
	Reservoirs	1500	0.18	78000
2	Village ponds	7277	0.89	14000
3	Marginal swamps, irrigated fields	13300	1.6	
4	Irrigated paddy fields	398000	48.5	
	Total	820077		

Source: Directorate of Fisheries Development (DOFD, 2013/14)

1.3 Trends of Aquaculture production in Nepal

Aquaculture has a relatively short history in Nepal. It was initiated in the mid 1940 on a small scale in ponds with indigenous Indian major carp seed from India. Further development began in the 1950s with the introduction of the exotic species common carp (*Cyprinus carpio*). Its breeding success in the 1960s followed monoculture practices and gained considerable popularity in the private sector. More significant progress was seen in

the 1970s with the introduction and farming of three exotic Chinese carp species: silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idellus*). The phytoplankton feeder Silver carp was introduced in 1968 from Japan, zooplankton feeder Bighead carp in 1971 from Hungary for polyculture, herbivore Grass carp in 1966 from India and common carp was introduced from India in 1959 and Israel 1960 (Sah 2012). Their breeding success in captivity has been a major breakthrough in the development of aquaculture in Nepal. Similarly, the induced breeding of three commercially valuable indigenous major carps: rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and catla (*Catla catla*) were successfully established in the country. This success followed the polyculture system of production in ponds with seven species of fish with different feeding habits. These seven species are species commonly used by fish farmers for commercial production in Nepal.

The aquaculture production programmed in Nepal began in 1981/82 with the execution of the Aquaculture Development Project supported by the Asian Development Bank and the United Nations Development Programme. In 1981/1982 aquaculture production was estimated to be 750 tones. It reached 8,317 tons in 1992/93. This increase in production of over 11 times within 11 years of the project period was the remarkable accomplishment of the growth of the industry in the country. Aquaculture production continued to increase significantly by the end of the project and reached 20,000 tons in 2003/2004 (MOAC 2004). From the overall development of aquaculture production trends, pond fish culture was developed into the major production system and in 2003/2004 accounted for over 90% of production and area coverage in the country.

A compilation (FAO 2005 and DOFD 2005) indicated that in 2003/2004 silver carp accounted for the major share of (5,125 tons, 26 percent) of total aquaculture production of 20,000 tons. However, its price was reported to be the lowest among other cultured species. The production share of silver carp was reported to be high in all production systems. Common carp is a popular fish after silver carp, and fetches a higher price than silver carp. The cyprinids – three indigenous major carps (*L. rohita, C. mrigala and C. catla*) make up a significant share of the total aquaculture production in the country. These species are popular as a delicacy compared to other cultured exotic carp and accordingly fetch much higher prices. The fish seed industry is one of the important

production areas in the aquaculture sector. It has been estimated by DOFD, 2004 that a total of 8.22 million fish seed were produced in 2003/2004, of which 72 percent were provided by the private sector in the country.

1.3.1 Total Fish Production in Nepal

Fisheries and Aquaculture offers a great opportunity for self-employment and income generation. Total fish production of Nepal is 56000 metric tones annually among which 36,500 mt comes from aquaculture and 21,500 mt from capture fisheries, similarly the production distribution by geographic regions comprises 35% by Eastern, 40% Central, 15% Western, 7.5% Mid-western and 2.5% Far western. Private hatchery produced 81% fish seed and government hatchery can produced 19% fish seed (Shrestha et al. 2013). During 2003/2004 the fisheries sector, including aquaculture and capture fisheries, produced a total of 39 947 tons of aquatic products and contributed to over 2 percent of gross domestic production in the Agriculture sector in Nepal. Per capita fish production in Nepal for 2003/2004 reached 1.6 kg/year.

1.4 Chinese carps and their artificial breeding in Nepal

Chinese carps (Grass, Silver and Bighead carp) are natural inhabitants of the river systems Yangtze, West River, Kwangsi and Kwangtung in South and Central china. These species are major parts of Aquaculture and introduced into many countries for Aquaculture. Aquaculture in Nepal revolves the cultivation of exotic and carps. Carp aquaculture in Nepal is polyculture. Cultivable exotic fish species, Grass carp (*Ctenopharyngodon idella*) was introduced to Nepal from India in 1967, Silver carp (*Hypopthalmichthys molitrix*) from Japan in 1968 and Bighead carp (*Aristichthys nobilis*) from Hungary in 1971/72.

Economically these exotic carp are very convenient species for aquaculture because of their quick growth and less demanding condition for life. They are superior in their quality of flesh content and meat (8.6% fat and 16.5% protein). The weight ratios of edible and inedible parts of body of Grass, Silver and Bighead carps are given in table 4.0.

Table 2: Weight ratio of Edible and Inedible parts of different species

Types of fish	Average wt.(gm)	Edible parts(meat)	Head	Bones	Fins	Scales	Viscera
Grass carp	882.4	59.4	13.2	4.0	3.5	3.9	11.0
Silver carp	782.6	62.9	19.8	3.2	3.4	2.0	8.7
Bighead carp	703.0	51.1	23.2	14.6		3.5	7.6

Sources: Pond Fisheries by (Martyshev 1983)

1.4.1 Induced Breeding

The most significant advancement in the field of aquaculture during recent decade is the development of techniques to induce reproduction in fishes. Induced spawning is essential for species which do not breed in captivity (Sarkar et al. 2004). Some species do not readily breed in captivity due to environmental or cultural conditions which are different from those found in nature, such as water temperature and substrate type. Chinese carps are artificially breed in Nepal from April to September (Baidya, Shrestha and Yamada 1998) but the approximately breeding season of these carps are from March to May in Terai region of the country and from mid-May to June in Kathmandu valley (FAO, Fisheries Synopsis 1979). The process of fish breeding artificially is called induced breeding. Induced breeding is a technique where organism is stimulated by particular hormones or other synthetic agents. Hormone used in induced breeding is called synthetic agents. Hormone preparation for the artificial propagation of the carp has been used for many years. Hypophysation for spawning induction in fish have been employed in aquaculture in since 1930 (Yaron et al. 2001). The first success in induced spawning of Chinese carps by administering pituitary extract was made in the year 1972. Breeding fish with pituitary gland (hypophysis) extract is termed as Hypophysation. The technique of hypophysation was first introduced by Woynarovich (1969) for induced breeding of these carps. Later, by adopting the same technique, they were again successfully bred in 1979 (Jha 1979). After that this technique spread widely and plays a mandatory role in carp culture in government as well as private fish farm in the country.

After this major breakthrough, several efforts have been made to improve breeding and rearing of these carps. Several investigations were carried on to replace

pituitary hormone extract for induced spawning by using other inexpensive, convenient and dependable ovulating agents. Human Chorionic Gonadotropin (HCG), Luteinizing Hormone or Luteinizing Releasing Hormone (LH-RHa), Progesterone, Anti estrogens and Ovaprim are used to substitute pituitary extract in breeding performance of Chinese carps with satisfied results (Ali et al. 2016). In recent years, a combination of dopamine antagonists and LHRH analogue (LH-RHa) has been found to be successful in ovulation and induced breeding in some teleosts (De Leeuw et al; 1985; Fremin 1991). Carp pituitary extract (CPE) and luteinizing releasing hormone releasing hormone analogue (LH-RHa) are two known hormones for controlling ovulation in channel catfish (Fobes 2013). Since the 1990s, a drug known as spawning hormone has been used in fish breeding (Marte et al. 1987). Ovaprim, which is a combination of salmon GnRH analog combined with a dopamine antagonist Domperidone, has proved to be extremely successful in breeding of carps with a spawning rate of about 100%.

1.4.2 Necessity of Induced Breeding for Fish Culture

- Induced breeding gives pure spawn of certain species under cultivation. Spawn collected from natural water is not pure as because some undesirable wild species may come with them.

- Induced breeding assures timely available of pure seeds.

- It can fulfill the any quantity of demand in any time.

- The technique is very simple and does not need too much technical assistance or knowledge. It can be easily learnt by a layman without much training.

- The cost of expenditures is very low than the natural collection of spawns.

- Several carps attain sexual maturity in ponds but they do not breed in confined water. Such fish can be subjected to induced breeding and spawn can be collected.

1.4.3 Luteinizing Hormone or Luteinizing Releasing Hormone (LH-RHa)

The introduction of the synthetic decapeptide LH-RHa (luteinizing hormone releasing hormone) has made possible intervention in the endocrinological chain at a point one step removed from the application of gonadotropin itself: namely, at the hypothalamic-

pituitary interface. Work in this area is comparatively recent, and results obtained are largely experimental. The field is, however, a promising one, and future developments will almost certainly benefit the culturist. Among several inducing agents used in fish breeding, salmon gonadotropin releasing hormone (SnGnRH) or luteinizing hormone releasing hormone analogues in the combination of dopamine antagonists was found to effective in fish breeding.

LH-RHa is a synthetic decapeptide. It and its super active analogues serve as potential ovulating or spawning agents that induce gonadotropin secretion in fishes. Carps when injected with LH-RHa show greater success (78.5%) as against fish pituitary extract (75%) (Gupta et al. 2006). It is a synthetic analogue of its natural form i.e., salmon gonadotropin releasing hormone (SnGnRH). The secretion activities of the hypophysis are directly controlled by the hypothalamus, which secrets LH-RHa. In China it was refined from hypothalamus of sheep in the early 1971s. Artificially synthesized LH-RHa has a high biological activity for cows, sheep and humans. When it is used to induce spawning in Chinese carps, however, the dose must be 100 times higher than that for mammals because LH-RHa is easily destroyed by fish protease. In 1975 LH-RHa was synthesized which is more effective than natural form. From the experimental data available it has been observed that the naturally occurring releasing hormones are less effective than the synthetic analogues (Zhang et al., 2015). This is probably due to the releasing hormones being more rapidly metabolized or removed from the fish. In general, the synthetic analogues like LH-RHa are up to 1000-fold more potent. Analysis shown that LH-RHa to be a peptide of ten amino acid: pyroglutamic, histidine, tryptophan, serine, tyrosine, glutamic acid, leucine, arginine, proline and glycine amide; having molecular weight of 1182 (Lam. T. 1998). The LH-RHa available on the market is a white powder and is combined with mannite as a filter. It is soluble in water and should be stored in dry, shady, airtight environment.

Work on LH-RHa analogues has been carried furthest in China, where the ability of Ayerst 25205 to induce spawning in the silver carp *Hypophthalmichthys molitrix*, the bighead carp *Aristichthys nobilis*, the grass carp *Ctenopharyngodon idellus*, and the black carp *Mylopharyngodon piceus* has been extensively investigated since 1974 (Anonymous 1977). The compound (termed LH-RHa) is administered either intramuscularly or

intraperitoneally, with the minimum effective dosage found to be 1 µg/kg and 0.002 µg/kg respectively. In grass carp, which earlier experience had shown to be refractory to HCG alone, a single injection of 5-10 µg/kg LRH-A produced an ovulation rate of 86%, comparing with results obtained using carp pituitary. For silver and bighead carp the minimum dosages were 3 µg/kg and 1.4 µg/kg, delivered in two injections; for black carp a. dosage of 5200 µg/kg, followed by 0.5-2 mg pituitary extract, produced a spawning rate of 74.6% (Soin et al., 1972).

Reason of choosing LH-RHa Synthetic Hormone as An Inducing Agent

Typically, in hormone induced breeding of fish, effectiveness is more easily defined than cost, because the cost of a spawning hormone may only represent a small percentage of the overall cost of fingerling production. Though successful hormone induced breeding of fish has been widely reported more than a decade, the present trends is towards its standardization and reduction of cost.

In Nepal, three hormones namely, fish pituitary gonadotropins, HCG and LH-RHa has been proven effective in many situations. However, out of these, LH-RHa has been proven significantly cheaper because it is a simple molecule that can be easily synthesized and price will drop even further as more suppliers enter the Nepalese market. Another reason is that this hormone lasts longer, is not rapidly metabolized by fish protease and has potent stimulator effect on the ovulation to induce spawning and because it is active at such low concentrations, their use is economical. Yet another reason is that LH-RHa itself is not highly species specific (Dhakal 2003)

1.5 Bighead carp (*Aristichthys nobilis*, Richardson 1845)

1.5.1 Description of Bighead carp

Bighead Carps are native freshwater fish in China. It was introduced from Hungary in 1971 in Nepal. Bighead carp is a eurythermic fish, being able to tolerate water temperatures of 0.5-38°C. It is one of the most intensively exploited fishes in aquaculture, with an annual worldwide production of over three million tons in 2013, principally from china. This species seems to grow more rapidly than silver and grass carp and can reach 40-50 pounds in 5 years. Main producer countries of Bighead carp are china, Taiwan

province of China, the Islamic Republic of Iran, Nepal, Myanmar, Malaysia (FAO Fishery Statistics 2006). This species is basically zooplankton feeders throughout its life under natural conditions, which makes it a lucrative aquaculture fish. In culture bighead carp will also accept artificial feeds such as by products from grain processing and organic detritus, in addition to natural food. It inhabits lakes, rivers and reservoirs. Bighead carp normally dwell in the upper layer of the water column and prefers high fertility water with abundant natural food. Bighead carp is a synchronous and gonochoristic species that spawns annually for dozens of years during its life span. There is just one spawning season in a year, which takes place in early summer. Bighead is a semi-migratory fish. Flowing water and changes in water level are essential environmental stimuli for natural spawning.

Due to the natural features of Bighead carp, the systems used for its culture are rather limited. Extensive culture in open-waters and pond-based polyculture are the major systems used. Comparatively, it is more difficult to breed Bighead carp than other fish, due to their slow gonadal development. It is also more difficult to produce large-size fingerlings, due to their slow growth in the early developmental stage (Bitterlich 1985).

Semi-buoyant eggs are laid that suspend in the water column when there is a current. Bighead carp can reach sexual maturation in captivity but cannot spawn naturally. Hormone injection and environmental stimuli such as flowing water are essential for induced spawning. The bighead carp do not breed in still water or in small streams. Fish can be induced to spawn with hormone when it gets mature. It attains sexual maturity at the age of 2 years (Santiago et al. 2004). In Pakistan, Bighead carp was introduced for possible enhancement of species in aquaculture system (Afzal et al 2007). The embryonic development is closely dependent upon environmental factors such as water temperature, water current and oxygen supply.

1.5.2 Morphology of Bighead carp

Body laterally compressed, round abdomen before ventral fin, narrow abdominal edge between ventral fin and anus; standard length is 3.1-3.5 times of body height and 3.0-3.4 times of head length; head large; length of head is larger than body height; terminal

mouth and slanting upwards; lower jaw extends slightly over upper jaw; no palpus; gill rakes dense and large in number (more than 400), not connected; one row of pharyngeal teeth on each side, flat and smooth, formula 4-4; scales small, extreme 96-110 in lateral line, lateral line extends to caudal peduncle. Tip of ventral fin reaches and exceeds anus; dorsal fin ray: 3,7; pectoral fin ray: 1,17; ventral fin ray: 1,8; anal fin ray: 3, 12-13; body color: black in dorsal and upper lateral portion, silvery white in abdomen, irregular black spots on the lateral side of body; greyish color in fins (K.C. 1999).

1.6 OBJECTIVES

General objective

Evaluation of breeding performance of Bighead carp (*Aristichths nobilis*, Richardson 1845) using LH-RHa synthetic hormone.

Specific objectives

To investigate the fecundity, Gonadosomatic index (G.S.I), fertility rate and hatchlings rate of Bighead carp by administrating LH-RHa.

To assess the various stages of embryonic development of Bighead carp.

To examine growth rate of Bighead carp from fry to fingerlings stage.

1.7 Rationale

In Nepal, studies regarding induced breeding in birds, mammals, reptiles and environmental issues are conducted but such study on pieces are scanty. The study of fish like Bighead carp are highly successful as a food sources but their study about breeding and rearing is not done well. This study is one among the very few studies that describe the breeding and rearing of Bighead carp which help to fill to some extent the knowledge gap and research of these species.

2 LITERATURE REVIEW

2.1 State of World Aquaculture

Aquaculture is an ancient food production practice originally rooted from Asia. It is the fastest growing production sector with great potentials sector with great potentials to meet the expanding demand for aquatic food (FAO 2011). The growth rate of global aquaculture production in 2004 was estimated to be 45.5 million tons (FAO 2009). Aquaculture is considered one of the best options that involves given natural resources to bridge the gap between world fish demand and production deficit. Carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) are the main commercial fish species in aquaculture development. Large production of aquatic plants and mollusks, together with the fishery, complete the activity infrastructure. Such models of implementation can act as self-sustaining, mini natural ecosystems (FAO 2006). For a world that faces rapid population growths, aquaculture with its promising outlooks to reduce environmental pressure and maintain ecologically balanced of natural ecosystem. It is one of the best answers to increase aquatic food production and job opportunities to the rural communities.

According to (FAO 2006) report, out of the total amount of fish consumed in the world every year, almost half is produced in aquaculture farming. The remaining half is harvested from the oceans, natural lakes and rivers. Aquaculture has undergone development, expansion and intensification in almost all available regions of the world, except in sub-Saharan Africa. The global population demand for aquatic food products is expected to inevitable increased (FAO 2012).

2.2 Brief History of Induced Breeding

The concept of pituitary injection applied for successful spawning in fish was developed by (Houssay 1930) of Argentina. Brazil was the first country to develop a technique for hypophysation. Von Ihering and his co-workers, Mertins, Cardoso, de Aazevedo and others, conducted experiments with various hormones injections on the lined of Houssay (1930) and achieve success in 1934 (Rath 2000). Since then, Brazilian fish culturists have been employing this technique to obtain seed from indigenous fishes as a part of their routine pisciculture programme.

The Russians are considered the next to introduce hormone treatment in fish culture after the Brazilians. It was not until 1937 that (Gerbilskii 1938) could succeed and since then this method is utilized with advantages in the Soviet Union for production of sturgeon eggs in the farms situated along the lower Volga, Ural, Kuban and others rivers.

In India, the first attempt to induced fishes to spawn was made by (Khan 1938) in 1937, in which the employed mammalian pituitary hormones. He succeeded ovulation in *Cirrhinus mrigal*, Hussain (1945) administered 80-120 RU prolan and antuitrin S into female mrigal and rohu, which rendered them easy to strip but the eggs could not be fertilized. Chaudhari (1955) was the first to successfully induced *Esomus dandricus* to breed by intraperitoneal injection of catla pituitary gland extract. Ramaswamy and Sundararaj (1956, 1957) reported the successful breeding of catfishes, *Heteropneustes fossilis* and *clarias batrachus* during 1955 and 1956 respectively by hormone injection. Chaudahari and Alkunhi in 1957 succeeded in inducing *Labeo rohita, Cirrhinus mrigala, Cirrhinus reba, Labeo bata* and *Puntius sarana* to breed by injecting them with pituitary extracts.

The first hypophysial technique in China was adopted by aquaculture researchers of Gaungdong province by injecting the hypophysis of common carp into brood fish of Silver carp and Bighead carp cultured in pond. In 1960, artificial propagation of Grass carp in china succeeded by using pituitary gland of common carp. In 1963, the artificial propagation of black carp was done similarly by using common carp pituitary in China.

2.3 Role of Exogenous hormones/chemicals in induced spawning of carps

Ramos (1986) reported that LH-RHa analogue accelerated maturation of the ovaries in female fish Common sole. Peter et al. (1988) proposed linpe method of induced ovulations or spawning of culture fish consist of treatment with an analogue of Gonado tropin hormone plus a dopamine antagonist. The linpe method has proven to be a highly successful procedure for induced ovulation or spawning of cultured freshwater fish in China.

Afzal et al. (2008) studied the effect of Ovaprim-C and Ovatidae alone and in combination with Profasi in Pakistan on Bighead carp to compare weight of eggs (g), weight of eggs (percentage of female body wt.), number of eggs / spawning, fertilization

rate, percentage of hatching rate and number of three days old fry in four groups of fishes. Results were better in group II treated with a combination of Ovaprim + profasi. The results of present experiment clearly indicated that the Ovaprim + profasi could be used for better ovulation in females of bighead carp. Ali et al. (2015) studied the efficacy of synthetic hormones, Ovatide and Ovaprim in major Indian and Chinese carps viz *Ctenopharyngodon idella* (grass carp), *Hypophthalmichthys molitrix* (silver carp), *Labeo rohita* (Rohu), *Cirrhinus mrigala* (Mrigal). Ovatide induced maximum fecundity and fertilization in grass while ovaprim in silver carps.

Hawarry et al. (2012) found that, hCG, or mammalian LH-RHa together with dopamine inhibitors was more effective in induction of ovulation and increasing fecundity and hatching rate in Silver carp. They also stated that not only carp pituitary extract and human chorionic gonadotropin but also the mammalian LHRH analogue (i.e. Receptal) was effective to induce spawning in silver carp. Mammalian analogues are available more widely and their price is much more attractive. This would result in cost reduction of induced breeding by using mammalian LHRH analogues in combination with a dopamine antagonist or alone.

Aizen et al. (2016) present the novel findings to introduce the potential of utilizing recombinant gonadotropins in aquaculture. They produce carp (c) recombinant (r) Lh as a single chain in the methylotrophic yeast Pichia postoris. Currently, spawning is induced in carp species by carp pituitary extract (CPE) and a combination of synthetic agonist of GnRH combined with a dopamine antagonist. The main goal of this study was the production of recombinant gonadotropins (GtHs) on a large scale to serve as an alternative to currently used agents. rcLh tested in this work significantly enhanced both E2 and DHP secretion in a dose-dependent manner similar to the results obtained with CPE. At the highest dose of rcLh (350 µg/kg BW), the recombinant protein was more efficient than CPE in terms of both spawning success and fertilization rate. It is shown here that rcLh can elicit the secretion of DHP in vivo and actually trigger spawning.

Targońska et al. (2010) carried out the artificial reproduction of asp under controlled conditions by using two different spawning agents based on luteinizing hormone releasing hormone (LHRH) analogues and dopamine antagonists (Ovopel and Ovaprim), during two successive spawning seasons. The result suggest fish treated with Ovaprim

showed a higher percentage of ovulation (75–88%) and embryo survival to the eyed-egg-stage (69–78%) than those treated with Ovopel (ovulation 25–38%; embryo survival 36–47%). Similarly latency time was shorter in the groups where Ovopel was applied (40–42 h) than in Ovaprim groups (42–48 h). Similar results were obtained in out-off-season spawning. This indicates that Ovaprim might be successfully used for artificial reproduction of asp.

Khan et al. (2006) compared the efficacy of synthetic hormones ovaprim and ovatidae in *Labeo rohita*. Ovatide is cheap and less viscous as compared to Ovaprim. The result of this study suggested that ovatidae performed much better than ovaprim for fecundity, fertilization and hatching rate in Rohu.

2.4 Artificial Breeding of Catfishes

Sahoo et al. (2005) evaluated the different doses of ovatidae (0.5, 1.0, 1.5 and 2.0 ml·kg body weight of female) on breeding performance in C. batrachus. The results indicated that one ml of Ovatide per kg body weight was found optimum for best breeding performance.

Barrero et al. (2011) investigated the effect of Carp Pituitary extract and Luteinizing hormone releasing analog hormone on reproductive indices and spawning of 3-Year-Old Channel Catfish *Ictalurus punctatus*. The LHRHa-injected fish had a 14% greater incidence of spawning than non-injected fish. Fertilization rates were not significantly different among treatments. Of the treated fish, those that spawned had significantly higher plasma E2 concentrations at 20 h. Thus, LHRHa injection of early-egg-stage channel catfish could serve to increase the spawning rates of young adults without resorting to intensive spawning techniques.

Ali et al. (2016) performed an experiment on artificial breeding of *Ompk pabda, Heteropneustes fossilis* and *Pangasius hypophthalmus* using pituitary gland (PG). The average spawn production and body weight of fishes were Pabda, Shing and Pangus were 11500/kg and 120/gm, 21000/kg and 130/gm, 44500/kg and 3.5/kg, respectively. The average fertilization, hatching and survival rate of Pangus (80%, 73% and 64%), Shing (73%, 68% and 58%) and Pabda (78%, 65% and 60%) was noted respectively.

Sarkar et al. (2005) found that Wova FH at the dose of 0.3 ml kg1 body weight is more

effective on induced breeding of climbing perch, Anabas testudineus which might be considered for raising captive population.

2.5 Gonadal Development Spawning and Embryonic Development

Chen et al. (1969) reported that exclusive feeding on hydrilla could cause extensive mesenteric fat accumulation (even more than 6% of body mass) and can have a negative role on the gonadal maturation and breeding. Lin (1965) worked upon induced spawning of Chinese carps by pituitary injection and opined that Grass carps grows rapidly and accumulates fat with adipose tissues in vegetation rich ponds. He further commented that such growth acts adversely on the gonadal maturation and spawning of fish. Sinha et al. (1974) suggested that the sudden drop in the electrolyte level in the environment caused by heavy monsoon rains could bring about hydration of fish gonads results in the natural spawning.

Jadho (2007) studied the effect of continuous illumination on gonadal recruesence and spawning in an Indian Major Carps, *Labeo rohita* in field conditions. Results clearly indicated that the continuous photoperiod has a stimulatory effect on gonadal recrudescence of the major carp, *Labeo rohita* and such fishes were found to be 100% successful in breeding trails by artificial inductions.

Rath and Gupta (1997) stated that Non- stripping induced spawning and double spawning of Grass carp in a hatchery system with foliage-free brood diet stressed that non -foliage diet and improved hatchery management practices facilitates the non- stripping induced spawning of Grass carp to promote seed production. According to them the lower body mass gives better breeding response and the non-foliage diet broods are obviously of lesser body mass than those of the foliage diet broods. They further commented that on peak estruation, small, slender and healthy broods showed better coiling courtship over fatty and heavy broods. The inconsistent and low fertilization rate of fatty and heavy brood may be due to improper coiling courtship which might have reduced the chances of easy access of male and female gametes.

Baidya et al. (1998) worked upon effect of supplementary feeding on spawning success rate of Silver and Bighead Carp and concluded that proper nutrition and maintenance of brood fish enhance their breeding response. The spawning rate was increased to 70% in

silver carp and 95% in bighead carp by providing supplementary pellet feed. Wanatable (1985) indicated that vitamins like Vit. E could be effectively employed to increase fecundity and facilitate breeding of carps. The carps derived a substantial part of their nourishment from plankton, but the optimum dose of fertilizers for brood-stock ponds are also essential for spawning success. Somashekarappa et al. (1988) reported that installation of sprinklers in broodstock ponds brought down water temperature and created rainy conditions which helped in advancing the maturation of gonads of carps. Gupta et al., (1988) showed the possibility of using the anabolic steroid, winstrol as a potential inducer of final gonadal maturation and in enhancing fertilization rate in fish.

There are several methods used for the preservation of fish eggs for the study of embryonic development of fish. Basak and Basak (2014), Rath et al. (2002) used distilled water for observation of embryonic development of *Labeo rohita, Cirrhinus mrigala and Catla catla*. Tumbahanfe et al. (2014) used bouins fluid for embryonic development of *Catla catla*. Miah et al. 2009 used the mixture of 4gm NaCl and 3gm urea per liter water and Tannin solution (0.5gm/L water) for several times to remove stickiness *Labeo bata* eggs. Rahman et al. (2009) used Methylene blue for study of embryonic development of *Mastacembelus pancalus*.

Miah et al. (2009) reported that embryonic development of an endangered species of *Labeo bata*. They studied embryonic and larval development of *Labeo bata* under laboratory conditions.

2.6 Artificial Breeding of Fishes in Nepal

In Nepal, the first success in induced breeding of fish was made in the year 1972 through hypophysation technique introduced by Woynarovich (1969) in Chinese carp. Later by adopting the same technique they were also successfully breed in 1979. Baidya et al. (1998) worked on the effect of supplementary feeding on spawning success rate of silver and Bighead carp and concluded that proper nutrition and maintenance of brood fish enhance their breeding response. To overcome the problems and maintain a good yield, a new race of common carp i.e. Yugosalvin strains known as Nasice carp had been introduced in Nepal from Israeli in 1990 (Shrestha 2008). Wagle and Pradhan (2003) estimated effective population size and rate of inbreeding of common carp on the basis of

number of new individuals recruited as brood stock each year and the variance of the reproductive success.

Dhakal (2003) studied the effectiveness of LH-RHa in induced breeding of Grass carp (*Ctenopharyngodon Idella*) in order to find out the optimum size of brood for induced spawning. He injected broodfish with the uniform dose of LH-RHa of 20microgram per kg body weight in two successive doses. He reported the spawning success rate, average fecundity, average fertility rate and hatching rate as 89.3%, 235564, 66.4% and 61.4% respectively. He also stated optimum body length between 78cm and 83cm, optimum body grith between 50cm and 54cm and optimum body weight between 5.8-7.3 kg were taken as the optimum sizes of broods for the administration of LH-RHa synthetic hormone for induced spawning of Grass carp in mid-hilly region of Nepal

Banjade (2015) reported ovulation induction in Chinese carp (*Cyprinus carpio*) and Silver carp (*Hypopthalmichthys molitrix*) using ovaprim. He reported the percentage of fertilization in common carp (75-92.5) and silver carp (72.6-93.3) was found higher than ovaprim treatment. Similarly, the percentage of hatchlings in common carp was 44-58 and silver carp was 44-59 respectively. Cleavage of eggs was observed after 4 hours of fertilization. The development of embryo continued and hatching took place after 4 hours of fertilization. The length and weight of hatchlings was recorded gradually increasing. The fry were fed with artificially formulated feed with 45% protein at the rate of 5-10% body weight and growth check-up was done at weekly intervals.

Sah (2012) reported the induced breeding of common carp (*Cyprinus carpio*) through the application of ovaprim. She reported the total number of eggs spawned ranged from 201,000-333000. The fertilization and hatchlings rate were recorded 83% and 53.6% respectively. She reported that cleavage of eggs started after 4 hrs of fertilization. The development of embryo continued inside egg and the hatchling of larva took place 48 hrs after fertilization. Hatchlings were feed artificially formulated feed containing 30% protein at the rate of 5-10% body weight till one-month old fry stage. Length and weight of fry was noted gradually increasing from first week to fourth week.

Sah (2017) used ovulin on the induced breeding of *Labeo rohita* and *Cirrhinus mrigala*. Studied fish specimen was spawned successfully following a single dose of ovulin with 0.5 ml/kg for female and 0.3 ml/kg for male brooders. He reported the total number of

eggs spawned ranged from 233203-316384 in *Labeo rohita* and 423200-768000 in *Cirrhinus mrigala*. Fertilization and hatchings rate were recorded 77.77-88.33% and 75.29-82.68% respectively in *Labeo rohita.* Fertilization and hatchings rate were recorded 71.05-82.68% and 60.68-79.28% respectively in *Cirrhinus mrigala.* The development of embryo continued and hatching takes place after 8-10 hrs of fertilization. Fry were fed with artificially formulated feed with 40% protein regularly. The length and weight of hatchlings were recorded gradually increased.

3 MATERIALS AND METHODS

3.1 Study Site

The present work was carried in Mandal Fish Breeding Center (MFBC), Rupendehi of Western Nepal. MFBC is the private farm of Rupandehi was established in 2057 B.S. The total area occupied by the farm is about 5 hectares and out of which 3.5 hectares has water area. It lies approximately 13Km south of Butwal and 31Km North of Lumbini (PLATE-1, photo 1).

The main water source of farm is swallow and deep boring. The occasional supply of water is provided by canal from Tinau river. There is 1 hatchery along with

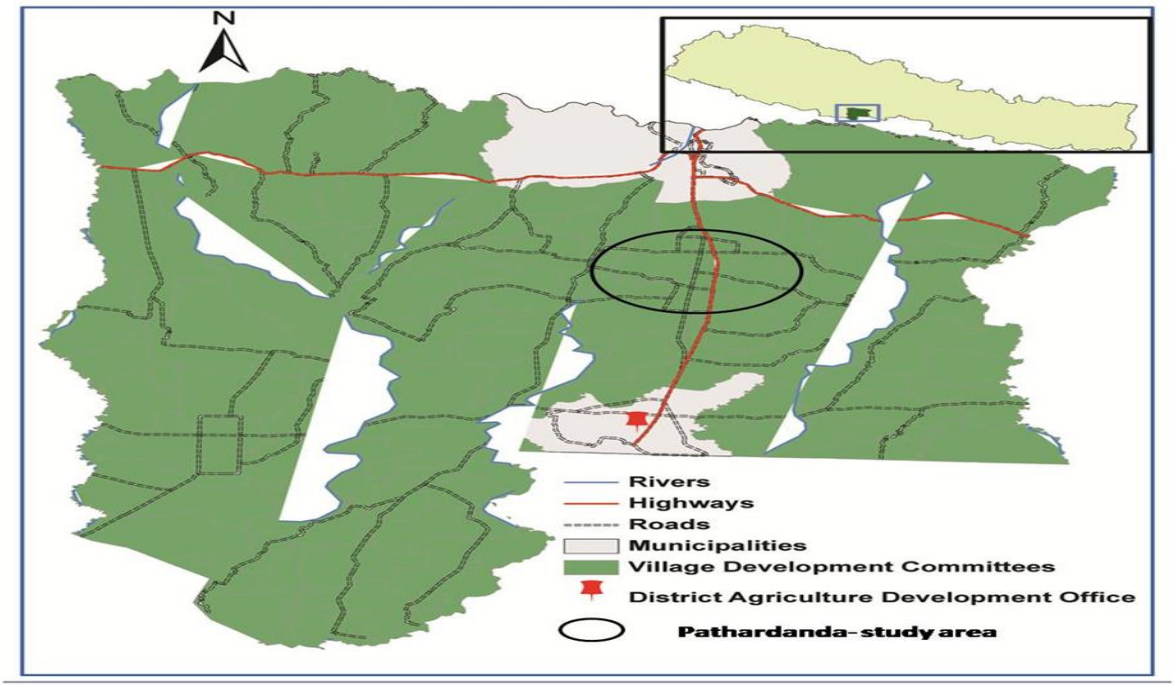
-18 nursery ponds

-6 brood fish spawning tanks

-2 early brood tanks.

-6 rearing tanks for fingerlings

-2 rearing tanks for hatch



Map of study site, Pathardanda 13 km North of Sidharthanagar

3.2 Study Period

The study was carried out from May 2019 to July 2019.

Breeding was conducted from May 08, 2019- May 17, 2019.

Embryological and growth check-up study of spawn was done from May 18 to 4th July, 2019. While larval rearing and growth check-up of fry was done from 17 May to 4th July 2019.

3.3 Physio-Chemical Parameters

3.3.1 Physical Parameters

The physical parameters of water were studied during experimental period was as follows:

Nature of day: It was recorded by visual observation.

Color of water: The pond water was taken in a petri dish over white paper and then the color of water was observed.

Temperature: The water temperature was measured by using a stand mercury thermometer graduated 0^0 – 35^0C with a precision of 0.1^0C. The surface temperature was measured directly by dipping the thermometer bulb onto the water body for two minutes and the reading was recorded.

3.3.2 Chemical Parameters

pH: The pH of water was measured by p^H meter (HANNA instruments HI 9025meter Kit.).

Dissolved Oxygen (DO): The dissolve oxygen was measured by using Winker's method.

Free carbon dioxide (CO2): Free carbon dioxide was determined by titration method.

3.4 Fish species

Bighead carp is one of the exotic carp cultured in different parts of Nepal in freshwater. In

this experiment Bighead carp was selected as major test fish. Extensive culture in open-waters and pond-based polyculture are the major systems used for production system.

3.5 collection and maintenance of brood fish

For conducting breeding experiment on Bighead carp, healthy and disease free, farmed reared brooders were selected. Broods refer to the mature male and female ready for spawning. Brood fish is prerequisite for all induced breeding programmes. Good brood stock have better breeding responses, increased fecundity, fertilization, hatching and larval survival rates and more viable fish seed. Two ponds – one for male brood fish and another for female broodfish were selected for experiment male and female were stocked in different ponds to avoid self-fertilization. Each of the pond had been prepared according to standard pond management practices for stocking and rearing of brood stock. The brooders of bighead carp were 3 to 5 years of age group and weighing 5kg to 10kg. The brooders were fed of 3-4% of their body weight for the first two months and later on, at the rate of 2% of the body weight till spawning (shown in table-3).

Table 3: The feed materials used for brood fish of Bighead carp

Ingredient	Percentage
Fish meal	25%
Soybean meal	05%
Wheat flour	20%
Rice bran	25%
Oil cake	25%

3.6 Identification of Male and Female Brooders

Sexual dimorphism is the phenomenon of distinguish males and females externally. In fish sexual dimorphism can be seen only at maturity. The male and female fishes can be identified on the basis of primary and secondary sexual characters. The primary sexual characteristics are physical characteristics directly involved in reproduction such as sex organs. The pectoral fins of males are generally large and rough where as in females these

are small and smooth. The abdomen is slender/ not swollen but little bit hard in males where as in females it is rounded or swollen and soft with eggs. The gills are rough in males and smooth in females. The genital aperture is in pushing/ inwards and not reddish in males whereas swollen outwards and reddish in color in females. The genital organ when pressed gently in front, milt (whitish milk- like fluid) comes out in males, but the genital organ when pressed in front, eggs are seen. At the same ages of males and females, the males are generally long or slender and thin whereas females are short and thick.

Whereas, secondary sexual characters are vital for understanding the behavior. The males of many Rays have clusters of strong sharp spine on their head and several rows along the upper outer surface of the pectoral fins. These are temporally developed during breeding seasons. The males fight for the possession of the females. In many species of fishes, the males are ornamented with bright colors than females. In majority of the fishes, males are brilliantly colored during breeding season. The females never willing to spawn except in the presence of the males & the males never fertilize the ova except in the presence of females.

3.7 Hormonal Dose and Administration

3.7.1 Luteinizing releasing hormone (LH-RHa)

Luteinizing hormone releasing hormone analogue or LHRH-A2 is a peptide that is similar in structure to native luteinizing hormone (LH-RHa). The LH-RHa available on the market is a white powder and combined with mannite as filler. White powder was dissolved by adding saline solution (0.7% NaCl) to adjust volume injection to 1 ml/kg BW.

3.7.2 Dose and Administration

Bighead carp was injected intramuscularly with different dosages of LH-RHa according to the body weight per kilogram i.e.

1.3 ml/kg BW for the female i.e. 1^{st} dose

3 ml/kg BW for the female i.e. 2^{nd} dose

3 ml/kg BW for the male

(Naeem and Salam, 2005)

3.8 Breeding Procedure

3.8.1 Collection of brooders for injection

Brood fish were collected from brood stock pond using seine net. Then the brood fish were transferred to hatchery. The brooders were than weight individually along using a digital balance machine.

3.8.2 Hormonal injection

For injecting the hormonal preparation the hypodermal syringe of 2Ml was used with the graduation of 0.1ml. The brooders in the hand net were then brought one by one for injection from the enclosure of the net and placed on soft towel. Two persons were required at the time of injection, one of them for hold the head of fish pressed gently against the towel, while the second injection on the body cavity. After injection the brooders were released inside the breeding tank.

The whole procedure of administration of the hormonal extract to the fish right from the bringing the brooders, weighing, injecting and releasing back to the breeding tank was done very quickly i.e. within 1-15 minutes. Time care was taken to hold the fish gently while injecting the hormone so that minimum stress is laid on the fish.

3.9 Breeding response

During the study, the breeding response of Bighead carp was observed with the use of LH-RHa. Fertilized eggs were located in chemically treated incubation tank. Malachite green (2-5 gram in 10liter water) was treated in water before transferring the eggs to prevent from attack of bacteria and fungus. Soon after the loading of eggs, water was treated with potassium permanganate (5-8 gm in 10liter water).

Fertilized eggs were kept in floating sieve in incubation tank for an experiment to study fertility rate and hatchling rate. The hatchings were given appropriate food just before total absorption of the yolk sac. Initially in hapa they were feed with eggs yolk mixed with water were given twice a day (one lakh fry fish for one eggs). In the same way

hatchlings were fed with milk powder by simply scattering it over the water slowly from the edge.

3.10 Response time

It is the latency period between injection of inducing hormone i.e. 8 to 10 hrs and the time of spawning in experimental fishes.

3.11 Determination of Fecundity and Gonado-Somatic Index (G.S.I)

The fecundity and Gonado-somatic index can be determined by using following formula given by Kaur and Dhawan in 1997.

Fecundity was estimated separately by sampling one gram of egg and multiplying with the total weight of brood female fish. One gram of the stripped out dry eggs were weighed on an electric weighing machine and counting eggs one by one with the help of brush.

Fecundity = No. of eggs per gm. X Wt. of total eggs (gm)

G.S.I expresses the weight of the gonads as the total weight of the body. Stage of the maturity is reflected by weight of gonads, which are related to the female fish. GSI increases with the maturation of fish being maximum during peak period of maturity decline abruptly after spawning. GSI (%) of all female fish of the respective carps was determined by the following formula.

Weight of gonads

GSI = Gonadal weight of brood/weight of brood x 100

3.12 Determination of Fertility Rate and Hatching Rate

Fertility rate (%) was calculated for every female separately by sampling eggs at the morula stage. It could be estimated properly after four hours of embryonic development. The eggs in the sieve were taken out in a plastic trough and checked for the fertility. Fertilized eggs were observed as a clear crystals balls whereas the unfertilized ones are dull and opaque. Its fertility was calculated in average of the total sample eggs. Fertility

rate was calculated by using formula given by (Kaur and Dhawan 1997).

Fertility Rate = Total number of fertilized eggs/ Total number of eggs x 100

Hatching rate (%) was determined by taking out the net bowl in a plastic trough and number of hatched ones and unfertilized eggs were counted to obtain the hatching rate.

Hatching rate = Total number of hatchling/ total number of fertilized eggs x 100

3.13 Study of embryonic development of fertilized eggs

The sample of fertilized eggs was collected for the study of the different embryonic stages of Bighead carp. The egg was taken and washed several times into distilled water. Various stages of eggs were preserved in 1% formalin solution and the eggs and larvae were examined under a Nikon SMZ 1500 dissecting microscope (7.5x-112.5x total magnification) noting developmental stages and photographed by using mobile phone camera (IOS, Iphone X's, 12-megapixel rear camera with dual optical image stabilization) in the laboratory of Butwal Multiple Campus, Butwal. The observation was continued from the time of fertilization till the completion of segmentation and till hatching.

3.14 Rearing of hatchlings till fry stage

After nursing of hatchlings for 5 days inside the hatchery in the incubation tank, one-week old hatchlings were transferred into the prepared nursery ponds in the farm. Collection of hatchlings was done through the outlet of the incubation tank by putting a piece of cotton cloth on the mouth of the exit of incubation tank outlet. The hatchlings were than measured in measuring cup (at the rate of 50,000 hatchlings per cup). The hatchlings were transferred to nursery pond for rearing in plastic bags containing sufficient amount of water. Fry were fed with two times a day with 40-45% crude protein feeds. This experiment was continued for 45 days.

Hatchlings after being transferred to the nursery pond, the growth checkup was done at weekly intervals. Hatchlings were scooped out from the pond with the help of scoop net and the length and weight of hatchlings were measured, up to 45 days i.e up to fry.

4 RESULTS

4.1 Physico-chemical parameters

4.1.1 Physical parameter

4.1.1.1 Nature of day

During the study period the nature of day was observed were cloudy, partly cloudy, rainy and rest days were sunny i.e. clear sky i.e. positive for the growth of fish.

4.1.1.2 Color of water

The water color at MFHF was noted greenish during the whole study period.

4.1.1.3 Temperature

The range of temperature of brood pond was 28-30^{0}c in the morning (6:30- 10a-m) and 34-36^{0}c during day (2-5 p-m.), that of incubation tank was 25-29^{0}c in the morning (6.30-10a-m) and 30-33^{0}c during day (2-5 p-m) from 8th May to 25th June and that of nursery pond was 29-33^{0}c during (7-10 a-m) and 35-38^{0}c during day (2-5 p-m) from 17th May to 4th July. The highest temperature 38^{0}c was recorded at nursery pond in June.

4.1.2 Chemical parameters

4.1.2.1 pH

The pH remained alkaline during whole study period. It ranged from 7.5 in May to 9.5 in July in brood pond. In incubation tank the pH was 7.5-8.5 in the morning (7-10 a-m) and 8.5-8.9 during day (2-5 p-m) from 8th May to 25th June and in nursery ponds it ranged from 8.5-10 from17th May to 4th July.

4.1.2.2 Dissolved Oxygen (DO)

The range of dissolved oxygen concentration of brood pond was 5.5-6.4 mg/l during 7-10 a-m and 6.5-7.4 mg/l during 2-5 p-m. and in incubation tank it was 7.5-8.4 mg/l in the morning (7-10 a-m) and 7.6-9 mg/l during day (2-5 p-m) from 8th May to 25th June. In the nursery pond it ranged from 5-7 mg/l during 7-10 a-m and 7.2-8.5 mg/l during 2-5 p-m from 17th May to 4th July.

4.1.2.3 Free CO2

The range of free CO2 (mg/l) of brood pond was 13.5-16mg/l and in incubation tank it was 14mg/l and in that of nursery pond it was 14-15.5mg/l.

Table 4: Physico-chemical parameters of Brood ponds, Incubation tank and Nursery ponds

Average data →	**Temperature (^{0}C)**		**pH**		**DO (mg/l)**		**Free CO2 (mg/l)**
	7-10a.m	2-5p.m	7-10a.m	2-5p.m	7-10a.m	2-5p.m	
Brood ponds	28-30	34-36	7.5-9.5		5.5-6.4	6.5-7.4	13.5-16
Incubation tanks	25-29	30-33	7.5-8.5	8.5-8.9	7.5-8.4	7.6-9	14
Nursery ponds	29-33	35-38	8.5-10		5-7	7.2-8.5	14-15.5

4.2 Fecundity and Gonado Somatic Index (GSI)

Table 5: Fecundity and GSI of Bighead carp by using LHRH-A in the different day at Mandal Fish Hatchery Farm

No. of female	**Weight of female (kg)**	**Fecundity**	**Weight of total eggs spawned (gm)**	**G.S.I 100%**
1	9.1	132900	886	9.73
2	8.4	118350	789	9.39
3	8.1	112500	750	9.25
4	7.3	106500	710	9.72
5	6.8	94500	630	9.26
6	5	64800	432	8.64

4.3 Fertility Rate and Hatching Rate of Bighead carp

Table 6: Fertility rate and Hatching rate of Bighead carp

The fertility rate was recorded 61.5%, out of total fertilized eggs, only 52.15% of them were successfully hatched (Table 6).

Number of sample eggs	No. of viable and fertilized eggs	Fertility Rate (%)	Hatching Rate (%)
30	13	43.33	33.33
37	27	72.97	67.56
34	16	47.05	47.05
18	11	61.11	41.17
14	10	71.42	57.14
42	30	71.42	66.66

4.4 Embryonic Development of Fertilized Eggs

Fertilized egg: The fertilized eggs were found floating, transparent, dispersal and light yellow in colour, 1-cell stage.

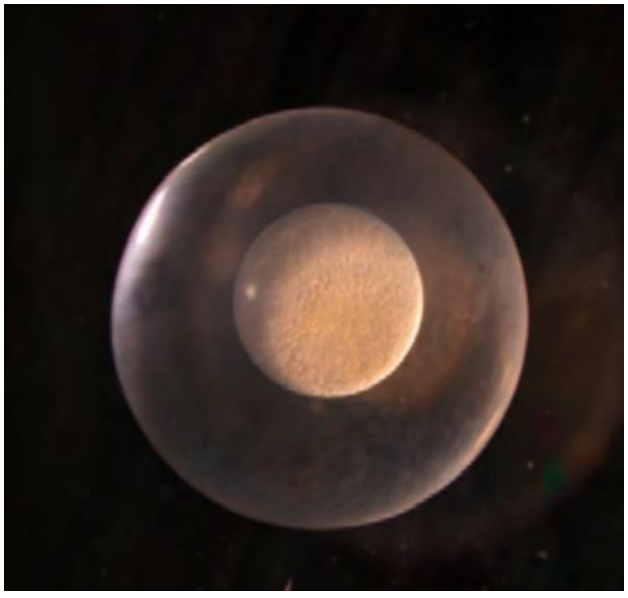


Figure 2: Fertilized egg

4hr Embryo: Cell divide vertically and Cytoplasm diminishes gradually.

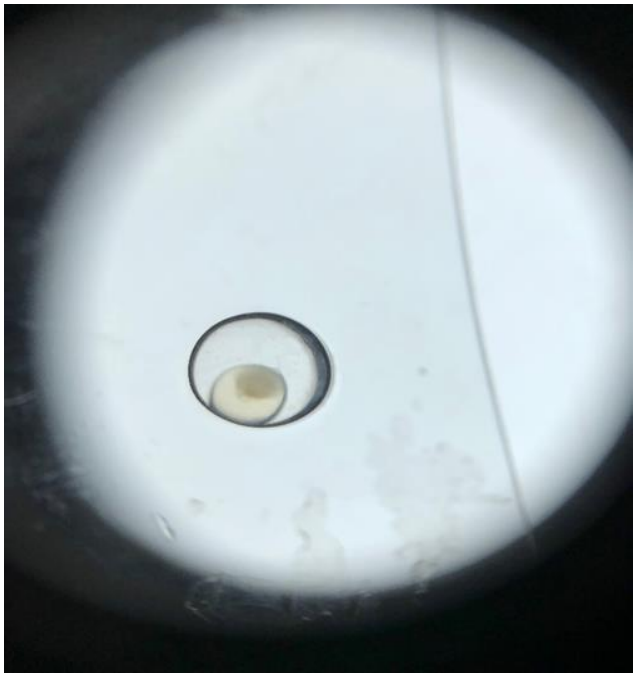


Figure 3: 4hr Embryo

6hr Embryo: Morula stage, the blastodisc is highly raised above the yolk.

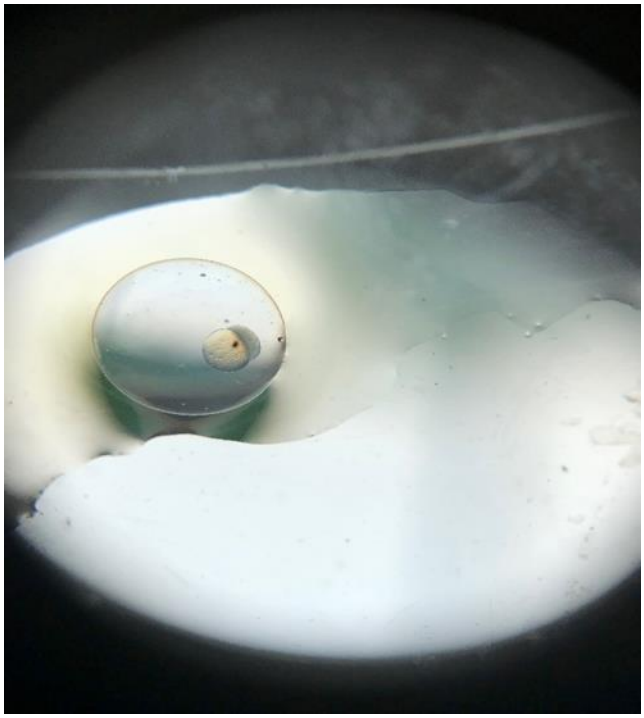


Figure 4: 6hr Embryo

8hr Embryo: Early blastula stage, blastodisc remains high, cells small

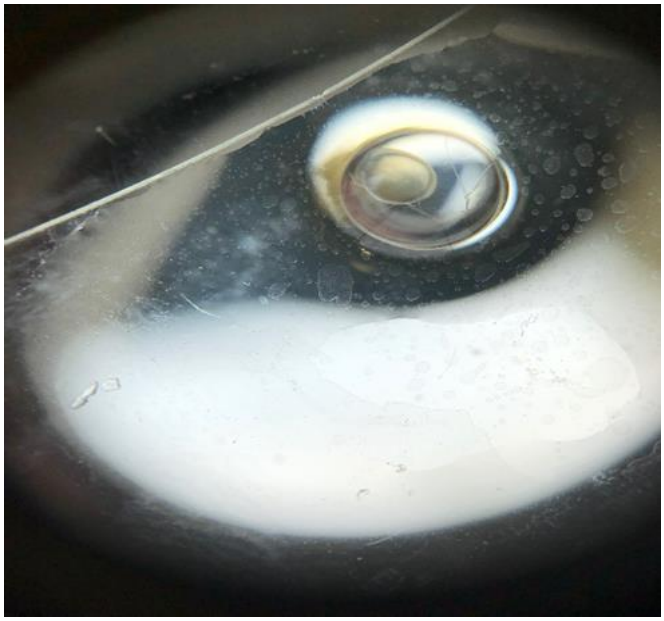


Figure 5: 8hr Embryo

10hr Embryo: Early gastrula stage, eggs looks round, dorsal lip appears, germ ring forms, blastoderm covers 1/3 of yolk.

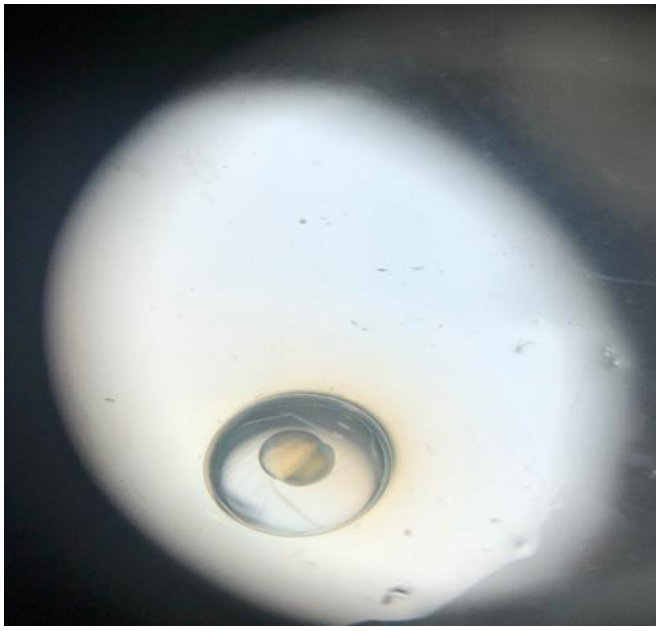


Figure 6: 10hr Embryo

12hr Embryo: Neurula stage, head enlarges, blastoderm covers almost entire yolk,

except for yolk plug.

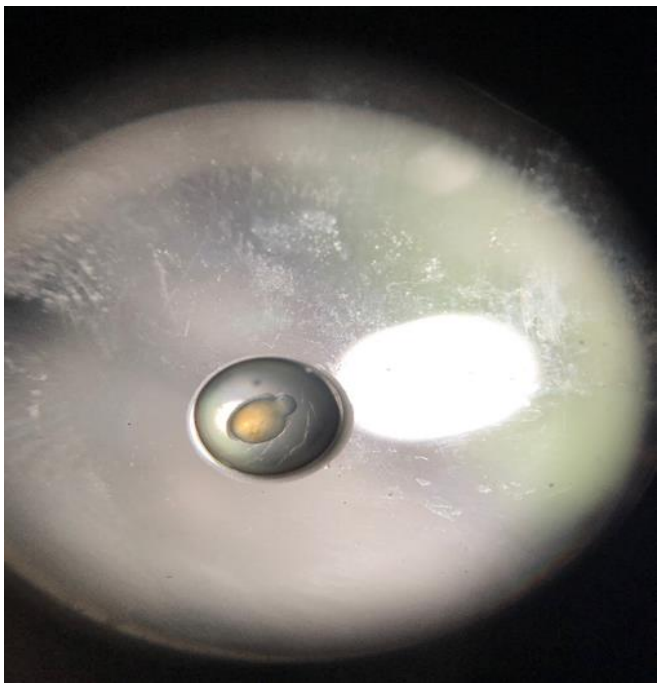


Figure 7: 12hr Embryo

New hatchling: Hatchling stage, larvae is approximately 7.0mm, tail length long, head extends straight forward, anterior part of yolk sac large and oval, posterior part of yolk sac narrow and lengthened tear drop shape, heart at the anterior edge of yolk sac, caudal vein large and flat.

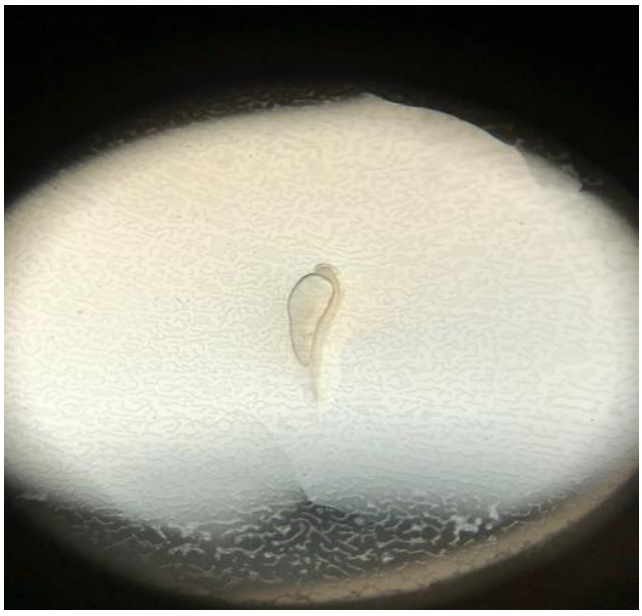


Figure 8: New hatchling of Bighead carp

2days hatchling: Xanthic eye stage, larvae is approximately 7.8mm, yellow pigmentation of eye appears, mouth open and can move, caudal vein wide and long.

Figure 9: 2days Hatchling

3days Hatchling: Melanoid eye stage, larvae is approximately 8.3mm, melanophores appears around the eye, caudal fin wide, long and dark yellow, normal swimming begins, gill filaments appear.

Figure 10: 3days hatchling

4days hatchling: Gas bladder emergence stage, larvae is approximately 9.2mm, blunt snout, initial gas bladder appear, gill filament extend, gut continuous, increasing pigmentation and swims normally.

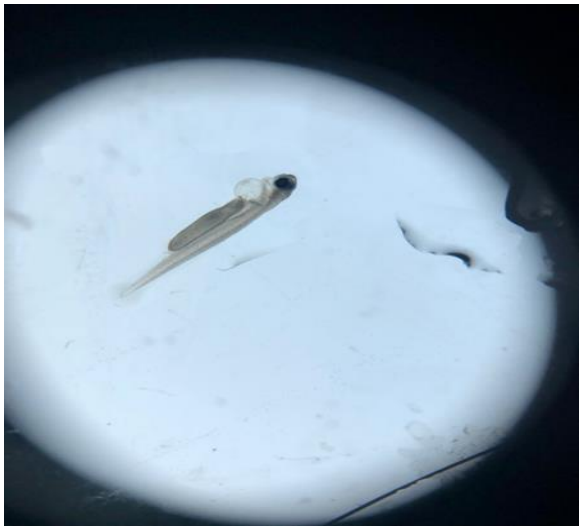


Figure 11: 4days hatchling

5days Hatchling: 1 chamber gas bladder stage, larvae is approximately 9.4mm, the gas bladder appear and is oval, inside of gut appears waxy, yolk sac shrinks to become a curved strip, upper jaw forms, pigment on head increases, pre anal fin fold extends forward to posterior margin of gas bladder.

Figure 12: 5days Hatchling

6days Hatchling: Yolk absorption stage, larvae is approximately 10 mm, yolk sac exhausted, dorsal fin further differentiates, posterior margin of caudal fin-fold become crenulated, pre anal fin-fold enlarges.

Figure 13: 6days hatchling

4.5 Growth of Bighead carp up to fry stage

4.5.1 Growth of length and weight of Hatchlings

After 5 days hatchlings were transferred to nursery pond. The fry were fed with artificially formulated feed with 45-50% protein at the rate of 5-10% body weight (Table 11) and the growth check-up of Bighead carp was measured at weekly intervals. Length and weight of fry was gradually increasing from first to fourth weeks.

Table:7 Growth of length and weight of Hatchlings

Hatchlings day	Length (Cm)	Weight (Mg)
15	2.5	204
20	2.8	239
30	3.6	675
45	5.4	1804

4.5.2 Growth performance of fry up to 45 days

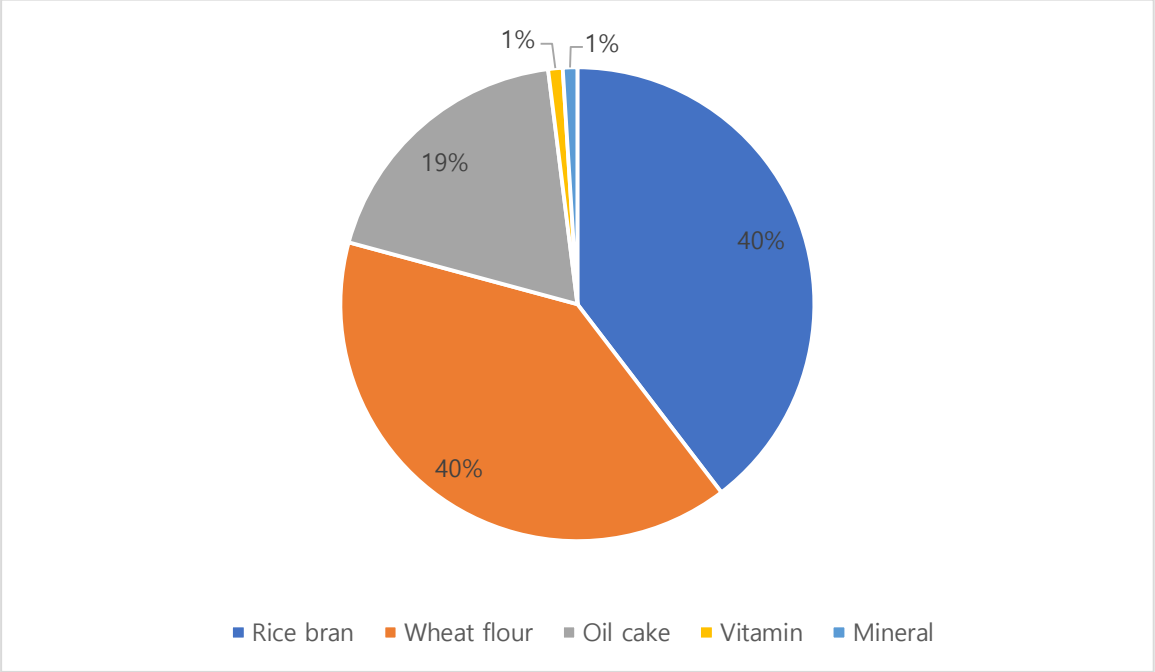


Figure 15: Composition of feed for fry rearing up to 45 days in percentage.

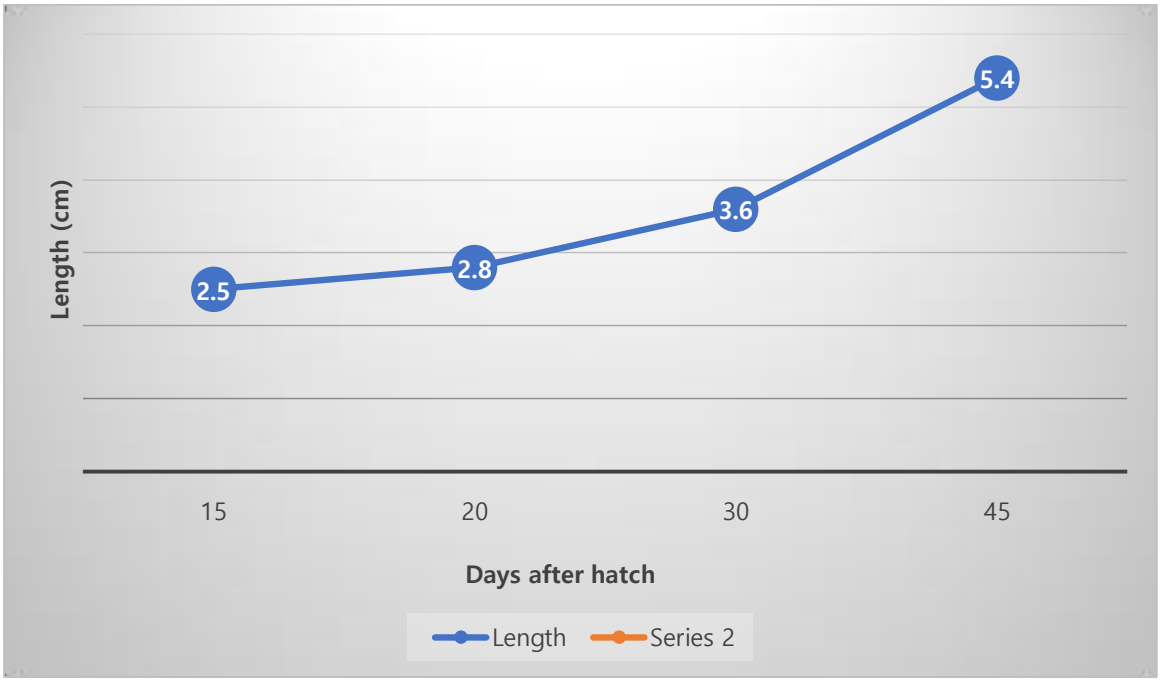


Figure 16: Growth performance (Length) of the fingerlings of Bighead carp under the

influence of commercial feed at regular intervals.

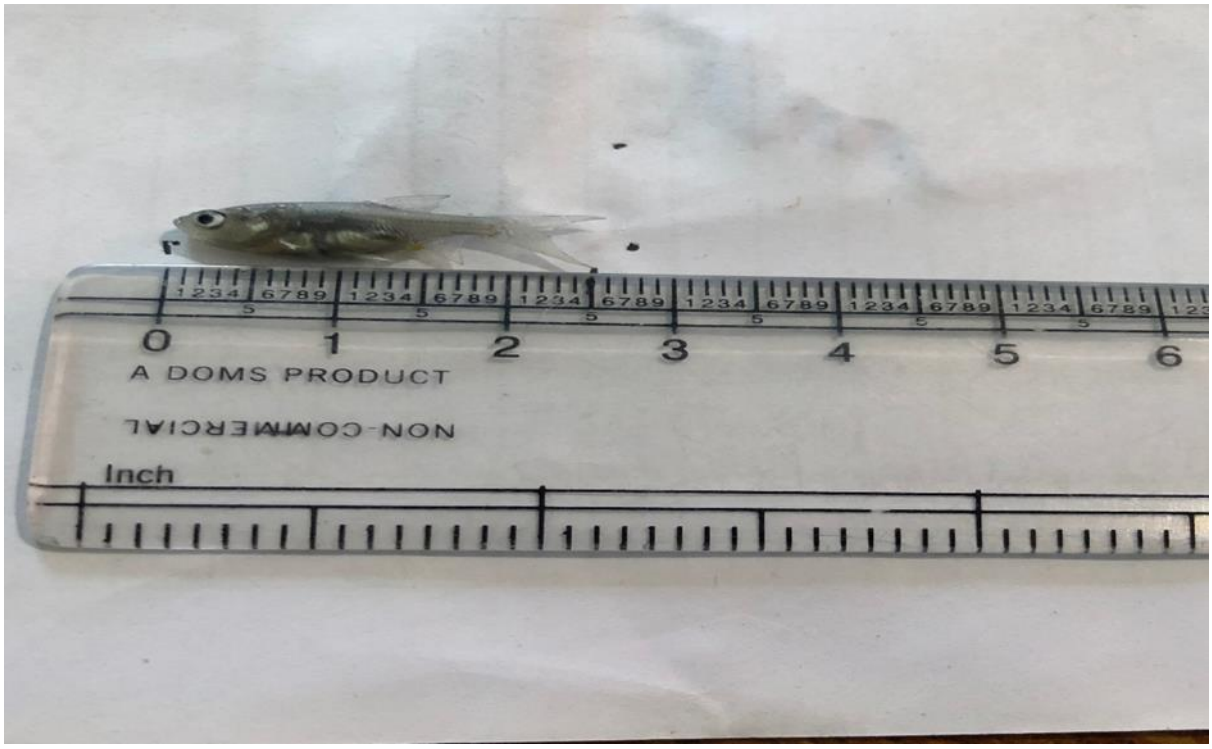


Figure 17: 15 days after hatch

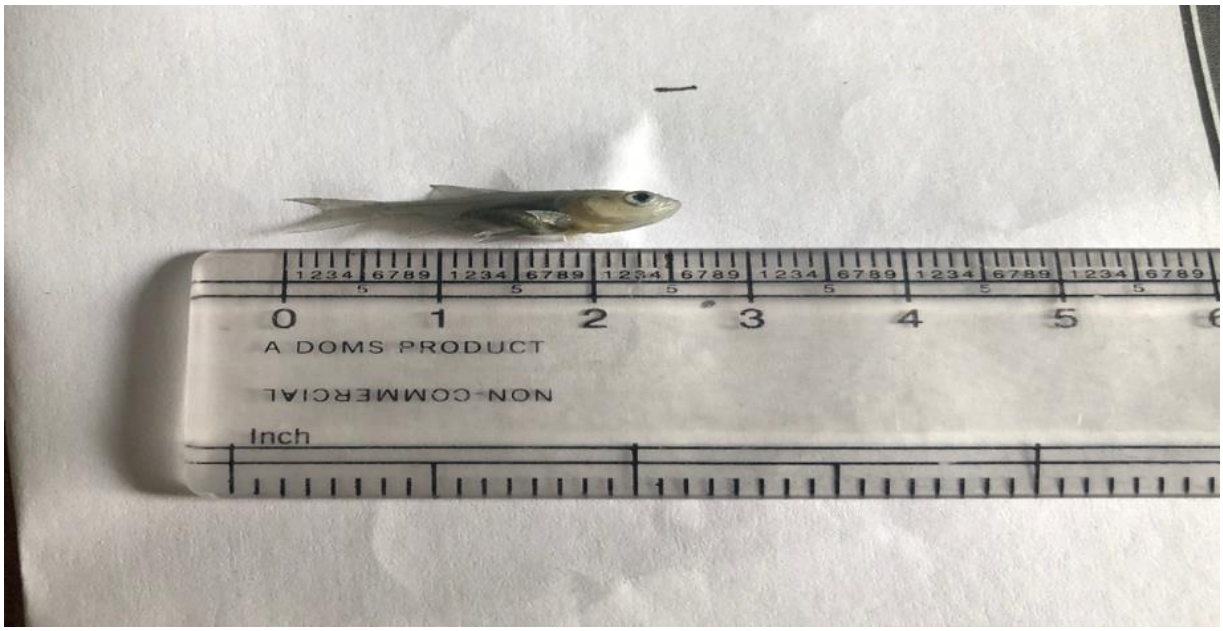


Figure 18: 20 days after hatch

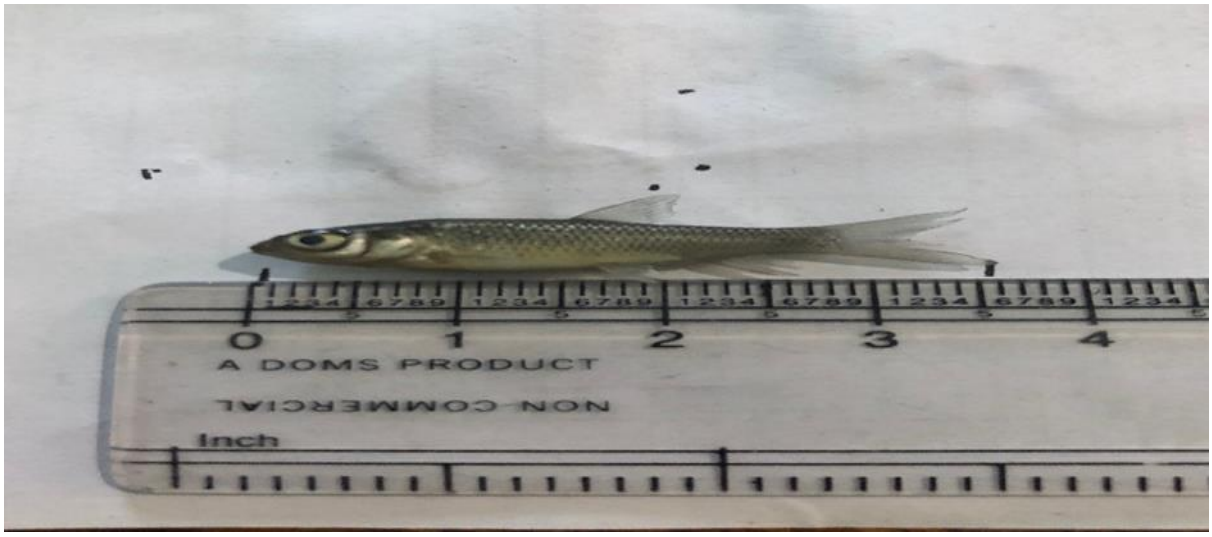


Figure 19: 30 days after hatch

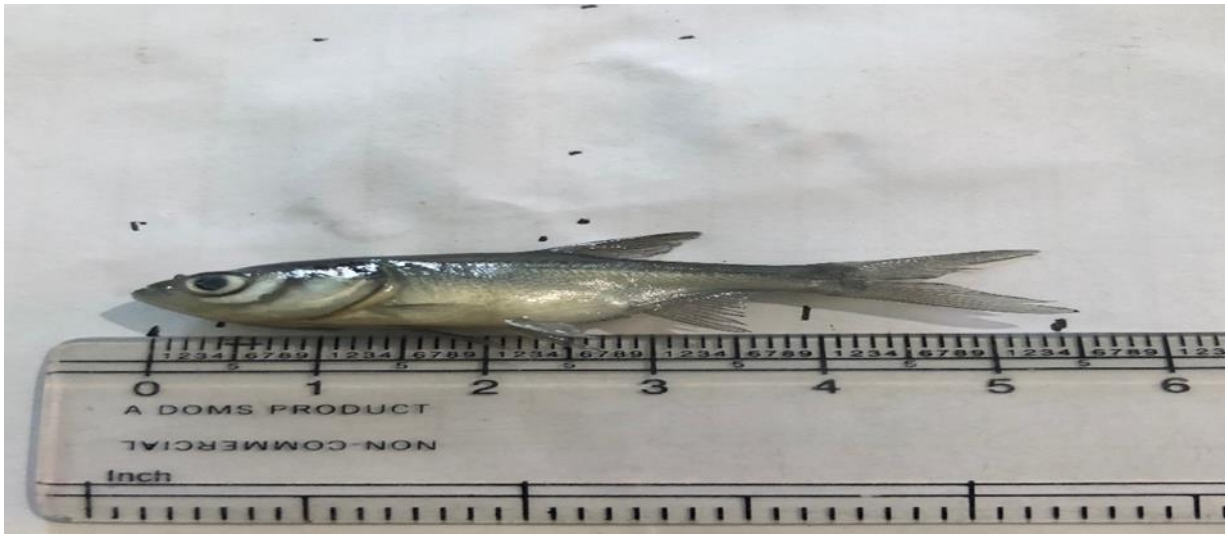


Figure 20: 45 days after hatch

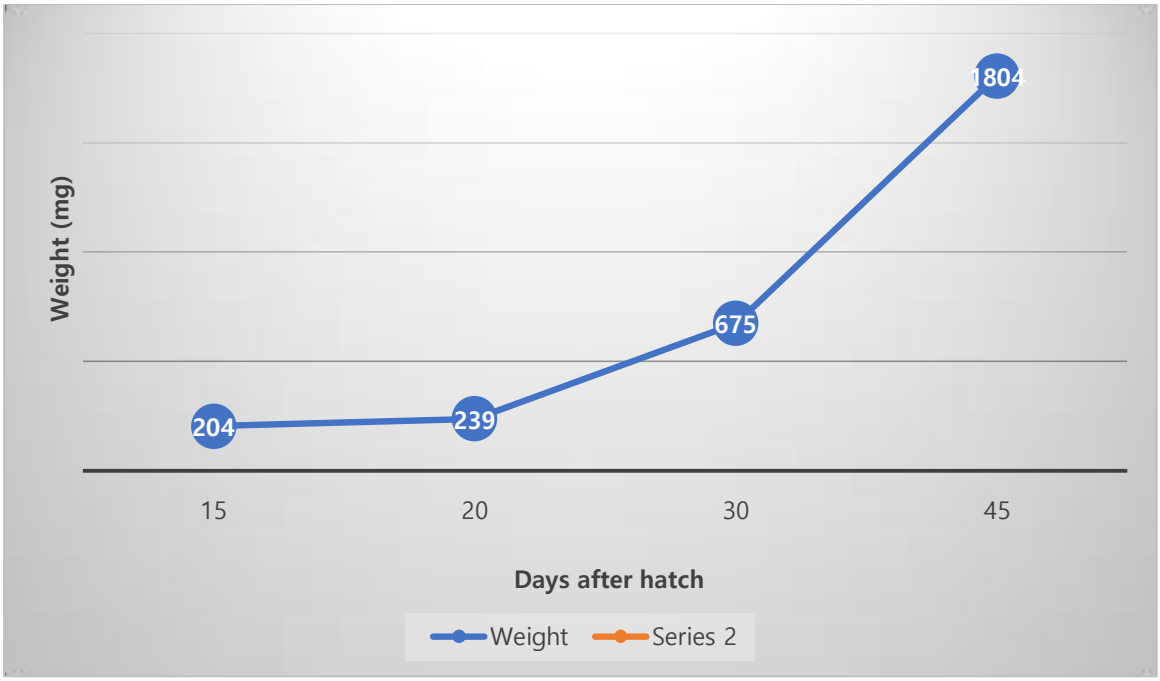


Figure 21: Growth performance (Weight) of the fingerlings of Bighead carp under the influence of commercial feed at regular intervals.

5 DISCUSSIONS

Physico-chemical parameter of water plays a significant role in the biology and physiology of fish. During the present investigation it was observed that water quality parameters of the brood fish pond, hatchery tank and nursery ponds were found to be within the suitable ranges as reported by (Jhingran 1991). Chapman et al. (2011) suggested that regardless of temperature treatment 24-26^{0}c, water temperature should remain within ranges compatible with egg and larvae development in bighead carp. The ranges of water quality parameters of the present study were nearly similar with that of the previous studies. These results are nearly same as the findings of present experiment. Water temperature is a significant factor affecting metabolic rate, age at first maturity and the developmental rate of the gonads. Mature silver carp, bighead carp and grass carp require approximately 1300–1400, 1400–1500, 1350–1450^{0}F days within a year for gonadal development and maturation, respectively (Woynarovich and Horváth, 1980).

5.1 Fecundity, Fertility, Hatchling and Embryology of Fish

Induced breeding were successfully done in carps by using various spawning chemicals also called synthetic reagents namely, Fish pituitary extract, Ovaprim, Ova-FH, Ovatidae, luteinizing releasing hormone etc. In present study, double dose of luteinizing releasing hormone (initial dose 1.5ml/kg BW only for female and second dose 3ml/kg for female in the time interval of 9-11hrs and a single dose of 3ml/kg for male) was used to induced final maturation and spawning in Bighead carp. The fecundity in present experiment ranged from 64800-132900 in Bighead carp according to their body weight. The fertility rate and hatching rate was recorded 61.5% and 52.15% respectively.

Afzal et al. (2008) attempted induced spawning of Bighead carp by using Ovatidae or Ovaprim alone or in combination with profasi. They reported the fertility rate and hatchlings rate was significantly higher when fish are induced by using 0.1 ml/kg ovaprim + profasi and found fertility rate and hatching rate 65.93% and 76.5% respectively. The fertility rate of their study was almost similar to the findings of present study but the hatchling rate of the current study was lower than the previous study. Low hatching might be due to deformities occurring during embryonic development.

In the present study, the GSI rate of Bighead carp was found to be 9.73+_8.64% by

applying the double dose of luteinizing releasing hormone. The result is comparable (10.1+_3.96%) with the findings of (Szabo et al. 2013) in Bighead carp induced with 4.5 mg/kg BW dry pituitary extract. Ovulation time was also exactly same with our study.

Silver carp, Grass carp and Bighead carp are Chinese major carp which are major fish for aquaculture. Rashid et al. (2014) reported a relative fecundity of 70000 and 80000 Grass carp and Silver carp respectively induced with Ovatidae at a single dose of 0.7 and 0.8-0.9 ml/kg body weight for female grass carp and silver carp and a single dose of 0.35 and 0.4-0.45 ml/kg body wt. for male fishes respectively. The fertilization percentage of Grass carp and Silver carp were recorded as 80.03% and 78.12% respectively whereas the hatchlings rate of Grass carp and Silver carp were recorded as 70.10% and 69.71% respectively. These results are in contrary to the findings of present study where Bighead carp was stimulated by LH-RHa. Naeem et al. (2015) observed a relative fecundity of 62542 eggs/kg fish induced with single intramuscular injection of Ovaprim-C with 0.6 ml/kg for female and 0.2 ml/kg for male Grass carp. This number similar when compared to the results of relative fecundity of Bighead carp in the present experiment. But the fertilization rate and hatchlings rate were recorded 80.36 and 79.49 respectively which is inconsistent with the present findings.

Dhawan and Kaur (2004) reported a relative fecundity of 62500-100805 in female fish of *Cirrhinus mrigala* induced with Ovaprim and Ovatidae respectively at the dose of 0.5 ml/kg. The fertilization percent was found to be 61.48% with Ovaprim which is exactly similar to the finding of the present study, however these results are contrary to the findings with ovatidae treatments where fertilization percent was recorded 83.65% which is significantly higher. Mishra et al. (2001) observed a relative fecundity of 85526 eggs/kg fish induced with 0.5 ml/kg of Ovatidae. This number is inconsistent when compared with the relative fecundity of Bighead carp in the present study.

Bighead carp eggs were generally larger than silver carp, but Chapman and Deters (2009) reported bighead carp eggs are slightly smaller. Egg size is highly variable and egg membrane began to swell immediately upon exposure to water and continued expansion for 3-4hrs, regardless of temperature, fertilization or developmental state. Bighead carp eggs were approximately 1.6mm in diameter before fertilization. Egg size is highly

variable and dependent on a number of factors, including environmental gradients and maternal effects (Johnston and Leggette 2002) and the ionic composition of rearing water (Fuiman and Trojnar 1980). Similar to the work of (Korwin and Kossakowski 2008) on grass carp and common carp there was evidence that rearing temperature affects the size of larvae at any given stage. Wv and Tan (2000) suggested that younger and smaller brood stock produce somewhat smaller eggs which produce smaller larvae. In the present experiment, embryonic development of Bighead carp was observed. The fertilized eggs were round, transparent, dispersal. The color of fertilized eggs was light yellow. The average diameter of fertilized egg is 4.70-5.22mm which was consistent with the finding of Soin and Sukhanova (1972). In this experiment about 1hr after fertilization, the blastodisc undergoes division and forms blastomeres and gastrulation begins at about 6hr as reported by (Anon 1970). Hatching of the embryo from the membrane begins about 1d after fertilization and may continue for several hours. Its duration depends upon water temperature and oxygen content. Character and development during larval phase were described by Sukhanova (1966), Anon (1970) and Soin and Sukhanova (1972). They reported newly hatch larvae is approximately 5.5-6.0mm but in this experiment the length of newly hatched larvae is 7.0mm which has nearly same with the previous findings.

5.2 Growth rate of Fingerlings

In the present experiment the growth rate of hatchlings was slow during 1st days but after 15 days of rearing the length and weight of hatchlings produced with the use of LH-RHa was increased gradually under the influence of 40-45% crude protein feeds. Comparatively, it is more difficult to breed Bighead carp than other fish, due to their slow gonadal development and also more difficult to produce large-size fingerlings, due to their slow growth in the early developmental stage (Peter et al. 2002). The hatchlings were scooped out from the pond with the help of scoop net and length weight of hatchlings was measured up-to 45 days. The 1st measurement was done in 15 days of rearing and length weight was recorded 2.5cm and 204mg respectively. Thoroughly, 2nd measurement was done at 2o days of rearing where hatchlings gain 2.8cm length and 239gm weight. After 20 days the length and weight was increased significantly, at day 30 of rearing they reach 3.6cm length and 675mg weight. The final measurement indicates the peak length and weight at 45 days of rearing under the influences of artificial feed and

reach 5.4cm in length and 1804mg in weight. This might be due to suitable physicochemical and environmental factors. High temperature influences the growth rate of Bighead carp. During that period the temperature was recorded 38^0C which one is the positive aspects for growth of Bighead carp. Contrary to the findings of the present study, (Saini 2001) observed a length of 3.0cm and gain weight of 9.82gm after 30 days of rearing *Cirrhinus mrigala* by induced breeding using Ovaprim. However, the fry produced with a use of ovatidae have shown relatively less growth having a length of 2.27cm and weight of 0.780gm after a rearing period of 30 days.

Organisms generally increase in size (length, weight) during development. The key factors that influence the growth rate of fish are the quantity of food available, the number of fish utilizing same food source, temperature, oxygen and other water quality factors besides the size, age and sexual maturity of fish. The length weight relationship provides the information on its growth and well being of fish in the surrounding environment. In the present experiment, a double dose of intra peritoneal injection of synthetic hormone, LH-RHa resulted in successful spawning of Bighead carp. The correlation coefficient (r) for Bighead carp was found to be 0.99, indicating a strong and highly correlated relation between length and weight of fish. This result was significantly higher than the previous studies (Wanner et al. 2011 – Schrank et al. 2001) and similar with (Kurbanov et al. 2015).

6 CONCLUSION AND RECOMMENDATION

The present experiment deals with induced breeding of Bighead carp by administrating the synthetic hormone LH-RHa.

6.1 CONCLUSION

From the present experiments on induced spawning of the Bighead carp by administrating LH-RHa synthetic hormone, conducted at Mandal Fish Breeding Center, Pathardanda Rupendehi, it can be concluded that this inducing hormone product can be used satisfactorily to induce Bighead carp. The hormone used in present study gave better economic benefits in terms of greater number of surviving span per unit weight of fish. LH-RHa was found more cost effective than other synthetic agents. The synthetic efficiency of LH-RHa has been revealed by significantly better response time and higher ovulation, number of eggs produced, relative fecundity, fertilization, hatching and survival percentage of spawn was recoded in experimental fishes, i.e. Bighead carp. By administrating LH-RHa significantly increased survival and growth of spawn during rearing period was recorded.

6.2 RECOMMENDATIONS

Induced spawning of bighead carp by administrating pure, more reliable and much stable exogenous hormones with predictable effectiveness are recorded to be used for reliable supply of quality fish seed.

It is very important to reduce stress from handling and recommend the use of sedatives during injection as much as possible.

It is mandatory to select fully mature, ripe and gravid brood fish for breeding.

The inbreeding should be discontinued to improve the fecundity and G.S.I.

The physico-chemical parameter of water should be maintained in suitable range for better breeding performance, survival and growth of spawn produced.

Nepal government and other organization like NGO, INGO should be support to such type of breeding center and training, seminar and workshop should be organized at national and international level to bring this industry globally competent.

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PHOTO PLATE: 1

Mandal Fish Breeding Center of Rupendehi

Photo:1 Farm visit with proprietor of MFBC

Photo: 2 Brood stock pond of MFBC

Photo: 3 Breeding hapa

Photo 4: Observing Hatchery with staff of MFBC

Photo 5: Rearing tank of MFBC Photo 6: Nursery pond of MFBC

PHOTO PLATE: 2

Methods of Induced Breeding at MFBC, Rupendehi

Photo 1: Adjusting net for collection of Brood fish Photo 2: Selection of Brood fish

Photo 3: Weighting of Broodfish

Photo 4: calculation of hormonal dosages for injection

Photo 5: Brood fish in spawning tank

Photo 6: Injecting fish with LHRHa

Photo 7: Hand stripping

Photo 8: Mixing of egg and milt

Photo 9: Fertile eggs in incubation tank

Photo 10: Transfer of Hatchlings in Nursery pond

PHOTO PLATE :3 Various activities at MFBC

Photo 1: water filling inside plastic bags

Photo 2: Analysing water quality parameter

Photo 3: Packing Oxygen

Photo 4: Transfer of hatchlings to Nursery pond

Photo 5: Counting fingerlings for sell

Photo 6: Removal of dead fish from tank