BREEDING PERFORMANCE AND REARING OF BHAKUR (*CATLA CATLA*, Hamilton, 1822) USING OVAPRIM IN RUPANDEHI, NEPAL

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RECOMMENDATION

This is to recommend that the thesis entitled **"Breeding Performance and Rearing of Bhakur (*Catla catla*, Hamilton, 1822) using Ovaprim in Rupandehi, Nepal"** has been carried out by Soniya Gurung for partial fulfillment of Master's degree in Zoology with the 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 work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor Asst. Prof. Santoshi Shrestha this thesis submitted by Soniya Gurung entitled **"Breeding Performance and Rearing of Bhakur (*Catla catla*, Hamilton, 1822) using Ovaprim in Rupandehi, Nepal"** is approved for the examination and submitted to Tribhuvan University in partial fulfillment of the requirement for Master's Degree of Science in Zoology with special paper in Fish Biology and Aquaculture.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Soniya Gurung entitled **"Breeding Performance and Rearing of Bhakur (*Catla catla*, Hamilton, 1822) using Ovaprim in Rupandehi, Nepal"** has been accepted as a partial fulfillment for requirements of Master's Degree of Science in Zoology with special paper Fish Biology and Aquaculture.

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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).

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ABSTRACT

The study of breeding performance and rearing of *Catla catla* was done in Pure Line Fish Breed Conservation and Promotion Resource Centre, Rupendehi, Nepal from July to November 2020. Fishes were spawned successfully following a single dose of ovaprim with 0.5ml/kg for female and 0.25ml/kg for male. The spawning behaviour was observed after 8 hours of hormone treatment at temperature ranges from 29-31°C. The hatching occurred after 13 hours of fertilization. The experiment was conducted in three replicas of $1\times1\times1m^3$ hapa. The fecundity was 142,400 to 324,800 while Gonado somatic index was 5.12-8.88%. The fertilization, hatching and survival rate were 77.78%, 65.25% and 44.1% respectively. The pH ranges from 7.6 to 9.8, dissolved oxygen 5.0mg/lt to 8.6 mg/lt and temperature 25.8-37.1°C. The water quality parameters were suitable for breeding and rearing of *Catla catla* and the ideal weight of female was found 4.5 kg.

Keyword: *Induced breeding, Fecundity, Gonadosomatic index, Survival rate*

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LIST OF ABBREVIATIONS

ADB	Asian Development Bank
AGDP	Agricultural Gross Domestic Product
CFPCC	Central Fisheries Promotion and Conservation Center
DADO	District Agriculture Development Offices
DoFD	Directorate of Fisheries development
DFID	Department for International Development
FAO	Food and Agriculture Organization
GDP	Gross Domestic Production
IDRC	International Development Research Center
JICA	Japan International Cooperation Agency
UNDP	United Nations Development Programme
USAID	United States Agency for International Development

1. INTRODUCTION

1.1 Background

Presence of diverse agro-ecosystem zones that are suitable habitats for variety of fish species results in high fish diversity in the country (Gurung, 2003). Nepal, predominately a mountainous country is blessed with extensive water resources estimated 818500 ha, comprises of 5.5% of total area of country (Hausen, 2019) occupied by variety of freshwater aquatic habitat that includes snow fed rivers, lakes and torrential hill- streams and slow-moving rivers. Nepal having the vast water resources plays an important role in generating income for landless and marginal farmers by supporting several indigenous fish species (DoFD, 2013). The water surface area of Nepal covers 0.1 percent of the total world water systems and fish diversity accounts 0.21 percent of total global fish diversity (Shrestha, 1995). Out of 252 total fish species (236 indigenous and 16 are exotic) where 10 species are under commercial farming including 7 carp species, 1 *Pangasius*, 1 tilapia and 1 trout species (CFPCC, 2020).

Fishes are an integral component of streams which determine the distribution and abundance of other organisms in the ecosystem and are good indicators of the water quality and health of the ecosystem (McCormick *et al*. 2000). Nepal deprived of any oceanic resources, depends only in finfish farming. The diverse climatic condition of country favors both warm and cold-water species. Though fisheries are not a main agricultural activity, they have been practiced since long time and have strong tradition in Nepal. Aquaculture is the fastest growing subsector among all the agricultural sectors. The development of aquaculture in Nepal was slow even though institutional development of aquaculture was started seven decades ago. But in last decade the progress achieved in aquaculture is highly admirable (Kunwar and Adhikari, 2017).

Fish is a low- fat with high quality protein which consists of omega-3 fatty acids and loaded with essential micronutrients that are consumed by all the ethnic groups of Nepal. Fish consumption in Nepal is low when compared with poultry, buff and mutton but still increasing health awareness among people results in rise in consumption of fish. The annual production of fish in Nepal was increased from 45,425 metric tons to 97,271 metric tons in the time period of 2006 to 2020 (CFPCC, 2020). The fish consumption has remarkably increased from 0.330kg per person per year in 1982 to

3.11kg in 2018/2019 (CFPCC, 2019). The inclining rate of consumption of fish has made an impact on the demand of fish in the market. Nepal Agriculture Perspective Plan (APP) has labelled fisheries and aquaculture as a promising sub-sector of agriculture and an important supplement to the regular food in rural areas (Rai et al.,2008). Aquaculture contributes about 1.13% of Gross Domestic Production (GDP) and 4.18% of Agriculture Gross Domestic Production (AGDP) in Nepal (Rijal and Jha, 2020).

History of aquaculture in Nepal has a very short period compared to other aquaculture developed countries of south and south-east Asia. However, capture fisheries from natural water resources are practiced since ancient times. Even though fisheries programme in Nepal was commenced in 2004 B.S. (1947/48 A.D.) but aquaculture began from late 1950s. Fishing was carried out traditionally in Nepal, but the use of the modern aquaculture technique of fish production was initiated along with the introduction of exotic carp on the early 1950s (Gurung, 2003). Development started with the introduction of exotic species common carp (*Cyprinus carpio*) with breeding success in mid 1960s. Three cultivable exotic Chinese carp species- silver carp (*Hypophthalmicthys molitrix*), bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharyngodon idella*) introduced in early 1970s followed by successful breeding in mid 1970s resulted in more significant progress in aquaculture. Similarly, with the introduction and successful induced breeding of three commercially valuable indigenous major carps rohu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*) and catla (*Catla catla*) in late 1970s provided momentum to polyculture system in ponds with seven different species having different feeding habits (Kunwar and Adhikari, 2017). A great part of aquaculture production takes place in southern part of the country i.e., 94% of all the ponds located in terai plain.

The development of aquaculture had been supported by Asian Development Bank (ADB) and United Nations Development Programme (UNDP) since the beginning of 1980, with the execution of Agricultural Development Project. By the end of the project, the fish production was increased from 750 metric tons in 1981/1982, reached 8,317 tons in 1992/93 and 20,000 metric tons in 2003/2004 which was the remarkable accomplishment of the growth of the industry in the county. This expansion in aquaculture was due to the ministry of agriculture and a few international agencies such as FAO, UNDP, ADB, JICA, USAID, IDRC, DFID that provided financial and

technical support. The main government organizations that work under the Department of Agriculture (DoA) and Ministry of Agriculture Development (MoAD) for fisheries and aquaculture development are Directorate of Fisheries Development (DOFD) currently known as Fisheries Promotion and Conservation Center (CFPCC) and District Agriculture Development Offices (DADO). The supply of fingerlings, technical and extension support to the farmers and fisherman were achieved after the establishment of fishery centers and hatcheries in different places. For the aquaculture sector, breeding and culture technologies have been developed both in private and government sector. To fulfil the demand of farmers, the private sector contributes about 75% of total fingerlings and only 25% from government centers (Rai *et al*., 2008).

Different types of aquaculture practices such as pond fish culture, rice-fish culture, cage-fish culture, pen-fish culture, raceway culture, fish culture in ghols are used for fish production in Nepal. Among them, pond aquaculture alone contributes 87.46%, is the major contributor to production (Kunwar and Adhikari, 2016/17). Mrigal (29.2%), common carp (19.2%), rohu (12.2%) and bighead carp (12.2%) are the major fishes that contributes to total fish production in Nepal (Hausen, 2019). About 122,772 people are found involved directly or indirectly in aquaculture among which 67% is covered by male while 33% occupied by female. Aquaculture is growing and has expanded to 55 districts compared to 30 districts a decade ago out of 77 districts (Chaudhary and Jha, 2018). The increasing rate of aquaculture in Nepal is around 13% which is highest among SAARC nations (Rijal and Jha, 2020).

Different exploitations such as habitat destruction, many constructions of flood control structures, use of water for irrigation, intensive agriculture, pollution etc result poor production of fishes from natural waters such as rivers, floodplains and paddies. Due to this, the aquaculture practices picked up and had expanded rapidly over recent years as the demand for fish and fishery products are increasing, by different improved aquaculture technologies are being adopted and is a source of income generation (Hossain *et al*., 2012). The breeding of fish undertaken for pond culture is extremely important in the further development of fisheries.

Induced breeding is considered as a popular technique for qualitative and quantitative improvement of fish that expands the reproductive process of cultured fishes (Dhawan and Kaur, 2004). Induced breeding is the technique through artificial stimulation of the

pituitary hormone or any synthetic hormone in ripe breeders in captive condition. The method of induced breeding was explained by Houssay where the hormone was administered to viviparous fish for untimely birth after making pituitary extract in 1930 in Argentina (Tiwana and Raman, 2012). The stimulation of hormones promoted timely release of eggs and sperms and offered the best means of fish production within the shortest time in ponds. To overcome the problems, such as short supply of quality seeds and dependency on wild seeds, that are unreliable, time consuming, induced breeding is the alternative method for quality seed supply and production (Sharma *et al*. 2010). In India, the development of the technique of the induced breeding, using pituitary extract was the breakthrough in production of Indian major carp seed (Chaudhuri and Alikuni, 1957). The reproductive requirement of brood fish is not satisfied with the ecological condition of pond (Peter *et al*. 1998). Due to which, even after the gonads undergo normal growth and development, the final events of oocyte maturation and ovulation and spermatization do not occur in standing water (Donaldson and Hunter, 1983). Indexes in the process of selection for breeding include external appearance, weight and state of health, activity at time of spawning, degree of fecundity and presence or absence of deformities. The hormonal activity of pituitary and gonads are influenced by environmental parameters such as photoperiod, rain, temperature, current of water etc. The maturation of ovary is also affected by poor or insufficient natural foods, exposure to biocides and pollutions.

1.2 Catla catla (Hamilton, 1822)

The *Catla catla* is locally known as Bhakur in Nepal. The body is short, deep, moderately compressed and covered with large cycloid scales. Head is broad and devoid of scales. Mouth is wide, upper lip absent, lower lip is thick having a movable articulation at symphysis and without tubercle. Barbels are absent. Dorsal fin is long without any osseous ray, anal fin is short and caudal fin is forked. Lateral line is complete. The color of body is silvery alongside and abdomen and greyish on back and flanks. *Catla* is a fast-growing fish among the indigenous cultivated carps belonging to the carp family Cyprinidae. It is native to rivers of Northern India, Indus plain and adjoining hills of Pakistan, Bangladesh, Nepal and Myanmar. The natural distribution of this species depends on the temperature rather than latitude and longitude. *Catla* is a eurythermal species that grows faster at water temperature between 25-30℃ and can grow up to 1-1.2kg in first year if the condition is normal compared to 700-800g rohu

and 600-700g mrigal. It is a surface and mid-water feeder that feeds on planktons with preference to zooplankton using large gill rakers. It is a highly suitable fish for composite culture along with fishes that are column and bottom feeder.

Catla attains sexual maturity at an average of two years and an average weight of 2kg performing spawning migration during monsoon season towards upper stretches of rivers and breed in shallow marginal areas. It breeds in riverine ecosystem and the ready availability of seeds was the only source for culture until 1950s. Among the three Indian major carps (*Catla*, rohu and naini), *Catla* requires accurate environmental conditions for spawning so it is the most difficult species to breed. Depending on the length and weight of fish, the fecundity generally varies from 100,000-200,000 per kg body weight. This fish requires a riverine environment, so natural breeding is unable to occur in the stagnant water, even after attaining the maturity, therefore administration of hormone is needed. Although it has a great demand and contributes more in total production from polyculture system, the main disadvantage is its big head which reduces the portion of edible meat per unit weight (Bais, 2018).

A drug, Ovaprim, that contains an analogue of salmon gonadotropin releasing hormone (SGnRHa) and dopamine receptor antagonist, was first manufactured by Syndel Laboratories, Canada, was tested in three species of Indian major carps, viz. catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) for induced breeding by Dr. Lin and Dr. Peter. Dr. Peter found that the dopamine which is a neuromodulator of the hypothalamus acts as an inhibitor for the synthesis and release of gonadotropins from the fish pituitary. The inhibitory signal from dopamine neurons can be transmitted to GnRH neurons through the synaptic connections. Ovaprim is a stable solution that contains 20µg of SGnRHa and 10mg of Domperidone. It is an extensively used hormone available commercially since 1988. Ovaprim has been found to be highly effective and has the higher affinity for binding sites in the pituitary and can be stored at room temperature for more than a year even in the tropics and has been tested successfully with IMC and Chinese carp (Nandeesha *et al*. 1990) spotted mussel and catfish (Haniffa and Sridhar, 2002). It was observed better than the pituitary extract.

1.3 Objectives

General objectives

To study the Breeding performance and rearing of *Catla catla* using ovaprim in Rupandehi, Nepal.

Specific objectives

1. To study the physico-chemical parameters of different ponds.
2. To study the fecundity, fertilization rate, hatching rate and survival rate of *Catla catla*.
3. To study various stages of embryonic development of *Catla catla.*

1.4 Rationale

Catla has a great demand as food source and contribute more in total production of polyculture but only a very few studies have been done that describes the breeding and rearing of this species. The present study deals with the breeding, growth performances and growth rate of fries upto fingerlings. In order to improve the commercial fish production, it is essential to have adequate knowledge of spawning biology, breeding cycle and methodological development of breeding technique. To some extent, this study helps to provide knowledge and research of these species. Apart from this, this study might be useful to indicate some remedies to overcome the problem of low production of fish seed in Nepal along with the findings of most effective hormone for induced breeding of *Catla catla*.

1.5 Limitation of the study

Due to lack of sufficient time and Covid 19 lockdown situation the chemical was not available in time. Other chemical parameters such as free carbon-dioxide, alkalinity could not be analyzed. This also affected other microscopic studies and photography of samples which were planned to conduct in department laboratory.

2. LITERATURE REVIEW

Basic knowledge of the reproductive system is important to understand the action of hormones in fish reproduction. Reproduction is affected by both external and internal factors in fishes. In Nepal, the first success in induced breeding was made in 1972 through hypophysation technique introduced by Woynarovich (1969) in Chinese carps.

Kaul and Rishi (1986) performed an experiment on induced spawning of Indian major carp, *Cirrhinus mrigala* with LH-RH analogue or pimozide. The result shows the fishes with 10mg/kg spawned profusely, fishes with 5mg/kg spawned few eggs only through stripping whereas the control fish did not spawn. Similarly, fish received pimozide with dose 5mg/kg neither spawned nor could be stripped. Fish with 10mg/kg dose spawned by stripping. The control fish treated with acidified saline did not spawn at all.

Naik and Mirza (1992) injected the ovaprim-c for induced spawning of Indian major carps. Number of eggs obtained from 45.93kg wt. of rohu was 50.14 lacs with hatchlings of 39.75 lacs. 31.62kg wt. of mrigal 30.30 lacs of eggs and 22.16 lacs of hatchlings. The number of eggs count in 47.90kg wt. of *Catla* was 47.39 lacs and 39.51 lacs of hatchlings.

Nayak *et al.* (2001) tested the effects of LHRHa, pimozide (PIM), and ovaprim and suggested the successful inducing ovulation, breeding, achieving quality egg and larval production after the application of LHRHa+PIM and ovaprim in catfish cultivation. When LHRHa + PIM (0.05 ug + 5 ug/g body weight respectively) was administered, there was a high rate of ovulation and produced an average of 10±2.3 g eggs with hatching rate (93.5 ± 1.42%) and of normal larvae (87.3±3.3 %). A single dose (0.6 - 0.8 ml/kg) of ovaprim resulted in an average production of 13.75 ±2.9 g eggs having 96.3± 1.7% hatchability and yielded 92.5 ± 1.5% of normal larvae.

Das (2004) explained the evaluation of a new spawning agent, ovopel in induced breeding of Indian carps. The ovopel were compared with two other spawning agent viz. pituitary gland were given double dose to female brood and ovaprim with single dose. 100% complete spawning was achieved in ovopel treated fish whereas other two hormones viz. pituitary and ovaprim, the spawning responses varied widely.

The study on the induced spawning of major carp *Catla catla* by a single intramuscular injection of ovaprim-c and fecundity at fish hatchery Islamabad, Pakistan was done by

Naeem *et al*. (2005). Thirty females were injected with ovaprim-c of dose 0.7mg/kg. Total number of obtained eggs was 67670 per kg with 91.01% fertilization rate and 67.50% hatchling rate.

Naeem *et al*. (2005) studied the fecundity and induced spawning of silver carp, using ovaprim-c Fish spawned successfully with the single dose of injection of ovaprim (female-0.6ml/kg and male-0.2ml/kg). Ovulation of fish in these treatments was 100%. Total numbers of obtained eggs were 91778/kg, fertilization and hatching percentage was 72.56 and 71.09, respectively.

Rokade *et al*. (2006) conducted an experiment on the induced breeding of major carp, *Cirrhinus mrigala* using pituitary extract and ovaprim in July in Paithan fish farm in Chinese hatchery. The number of egg count in control fishes with pituitary extract was 14.00 lacs with overall fertilization 60% and with ovaprim injected fish's eggs were 21.54 lacs with overall fertilization of 91%.

Sarkar *et al*. (2006) published a document that described about the different doses of ovaprim (1.5, 1.0 and 0.5 ml/kg body weight) injected to endangered species, *Chitala chitala*, to determine the latency period, fertilization rate, egg output, hatching rate and hatching production which showed the fertilization rate varied from 48.86–80.2% and percentage survival of hatching varied from 42.2 to 65.60%.

Uthayakumar *et al*. (2011) tested ovaprim and LHRH for the successful captive breeding of *Oncorhynchus mykiss* and reported that the threatened and endemic species of rainbow trout can be successfully breed and can increase its population with immediate effects in captive breeding. The maximum hatchability rate percentage of Ovaprim was at the dose of 2.40ml/kg (84±0.640) whereas LHRH showed maximum performance at 0.0710 ml/kg dose (85±0.750).

Hossain *et al*. (2012) compared the carp pituitary gland (PG) extract and synthetic hormone ovaprim for induced breeding of stinging catfish, *Heteropneustes fossilis*. Ovulation rate was 90%, fertilization rate was 86.7% and hatching rate was 76.9% for ovaprim induced fish whereas in PGE injected fish, the ovulation rate was 78.7%, fertilization rate was 69.2% and hatching rate was 72.7%. The incubation period was 3.5h for eggs of ovaprim injected fish which was shorter in comparison to PGE induced fish eggs that require 5 hr incubation period.

Tiwana and Raman (2012) explained an economically viable approach for induced breeding of *Labeo rohita* by ovatide, ovaprim and carp pituitary extract. The fertilization rate was found 61.30% with ovaprim, 58.50% with ovatide and 55.96% with carp pituitary extract treatment. The hatchling rate was 72.20% with ovaprim, 66.37% with ovatide and 59.25% with carp pituitary extract.

Ghanemi and Khodadadi (2017) explained the inducing effects of ovaprim on reproductive parameters of shirbot (*Barbus grypus*). This study suggests that shirbot shows a better effect on reproductive parameters such as spawning rate, egg weight/g.bw and working fecundity when injected with 1ml/kg.bw dose of ovaprim and 3mg/kg.bw pituitary extract.

Chattopadhyay (2018) explained the comparative study of carp pituitary extract and ovaprim in pabda (*Ompok pabda*) for captive breeding. During the experiment ensured a high rate of fertilization (66.88 & 65.40) and hatching (78.40%), with highest survival rate was 59.8, 62.1 and 61.4% respectively with three treatments of Ovaprim (T1= male-0.5mg/kg, female-1mg/kg, T_2= male-0.5mg/kg, female-1.2mg/kg, T_3=male-0.5mg/kg, female-1.5mg/kg). Researcher presented that ovaprim can be better option for successful spawning production, fertilization, hatching and survival of pabda than carp pituitary extract.

Observations on the induced breeding in *Labeo rohita* by hormonal injection of pituitary gland extract and synthetic hormone ovaprim at FSPC, Paithan, Maharashtra state, India was done by Pawar *et al.* (2019). During the month of June in 2013, fertilization rate was 69.38% and hatching rate was 58.82% by 12 kg fish and in August, fertilization and hatching rate were 85.29% and 78.82% by 21.5kg when injected with pituitary extract. When fishes were administered with ovaprim in June, 19kg fishes showed 92% fertilization and 89.39% hatching rate and in August, 20.5kg fishes showed 95% fertilization and 94.21% hatching rate. In June 2014, fertilization and hatching rate were 73.07% and 66.06% by 10.5kg fish and in August, rate of fertilization and hatching rate were 80% and 71.83%by 12kg fishes when injected with pituitary extract. In case of ovaprim, rate of fertilization and hatching were 93.82% and 90.28%by 18kg in June and in fertilization and hatching rate were found 94.11% and 92.78% by 20.5kg fishes in August.

Availability of required quantity and quality food during reproductive cycle is essential for reproduction. The study on formulated diets with similar efficiencies as natural foods in an ongoing challenge for aquaculture. Among nutritional compounds, different unsaturated fatty acids were found to affect milt composition and sperm performance of European eel (Butts *et al*. 2015). Likewise, Teletchea *et al*. (2009) reported that high initial fat storage before the beginning of reproductive cycle was found to be a critical factor that influence semen quality of pink perch. 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. Wanatable (1985) indicated that vitamins like Vit. E could be effectively employed to increase fecundity and facilitate breeding of carps.

Swingle (1956) reported that the released excretory wastes of fish in water constituents' 'repressive factor', a hormone like substance inhibits reproduction in fishes. Similarly, Tang *et al*. (1963) observed *Cyprinus carpio, Carrassius auratus* and *Tiliapia mossambica* are unable to spawn at overcrowded habitat but spawn when transferred to fresh water and when the repressive factor is adequately diluted by flood water, spawning takes place in bunds or ponds. 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 resulting in the natural spawning.

Due to anthropogenic activity, the aquatic environment is contaminated with water pollutants which causes a serious threat for fish reproduction (Devaux *et al*. 2015). Rise in human pharmaceuticals as a source of aquatic environmental pollutants affects reproduction in fishes. Moreover, herbicides decrease sperm quality leads to decrease in fish reproductive potential (Silveira *et al*. 2019).

Wotton (1998) reported that high food levels support the growth of fishes that leads to larger body size. The intake of high-level food leads to high fecundity in female fishes. Better growth rate of fish also influences the size of ovary. Fecundity increases with the increase in total length, body weight and ovary weight. However, ovary weight is better index in comparison to other parameters to estimate fecundity (Iqbal and Kausar, 2009). A well grown healthy, well-proportioned and carefully selected fish should be used for artificial propagation (Bista *et al*. 1998).

Woynarovich and Horvath (1980) stated that the most suitable size of spawners in common carp, Chinese carps and Indian major carps is 3-5kg. They also stated that very large fishes require a large dosage of hormones so are less suitable for hormonal treatment and are also difficult in handling them. Larger specimens are convenient only when breeders spawn spontaneously without having to be stripped otherwise are rather difficult to handle and tiresome to be stripped.

3. MATERIALS AND METHODS

3.1 Materials

Dragnet, injection, ovaprim hormone, thermometer, pH meter, DO meter (HANNA instrument HI 9147), alcohol solution, $1\times1\times1m^3$ hapas, microscope, notebook, pencil, camera

3.2 Methods

3.2.1 Study area

The present work was conducted in Pure Line Fish Breed Conservation and Promotion Centre, Rupandehi, Province 5, Nepal. It is approximately 5 km north from Bhairahawa through Siddhartha highway. The total area occupied by the farm is 23 hectares and out of which 13.674 hectares has water area. The ponds are dependable on rainfall and deep tube well water. The hatchery produced the fish seed of common carp, silver carp, bighead carp, grass carp, rohu, naini and *Catla*. The farm is famous in western Nepal and is able to fulfil the demand of fish farmer.

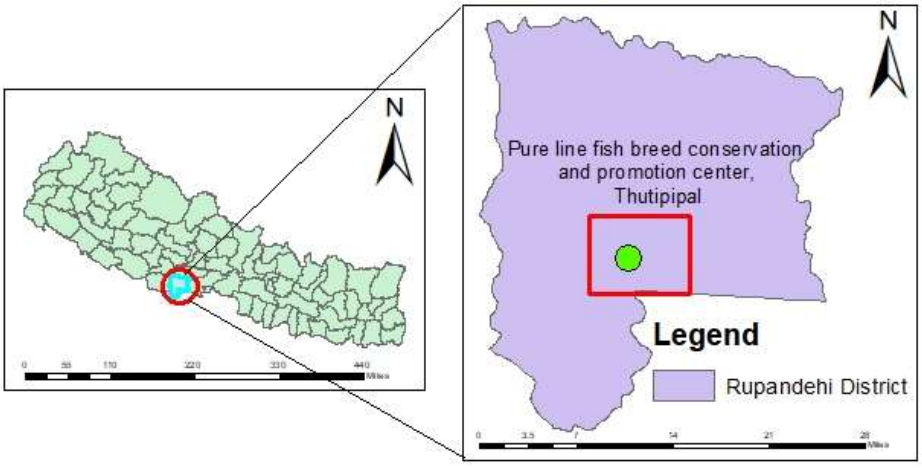


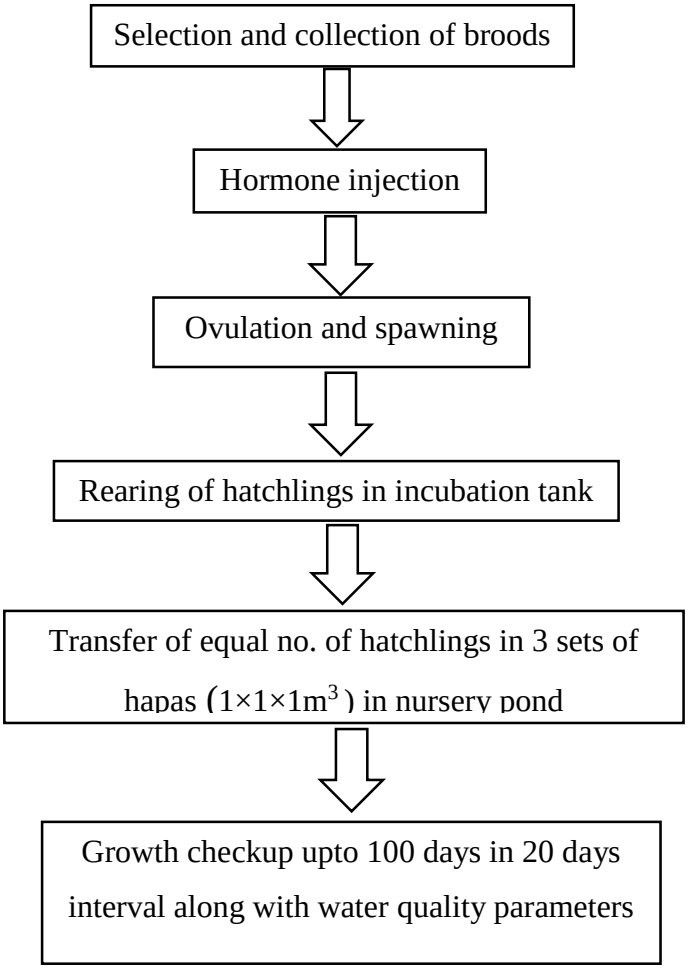
Fig 1: Map of study site

3.2.2 Study period

The study on the breeding of *Catla catla* was carried out in July, 2019. Even though the brood was injected with appropriate dose of ovaprim, the brood were unable to produce eggs that might be due to environmental factors. Therefore, at that time the study was

not able to continued and needs to stop as it was not possible to take further. Due to Covid-19 pandemic, the schedule of the field visit was also interrupted. So, later the field was carried out from July 2020 to November 2020. Breeding study was observed from 17th July to 6th November, 2020.

3.3 Study design



3.4 Physico-chemical parameters

3.4.1 Physical parameters

The following physical parameters were studied during experimental period.

Nature of the day: It was recorded by visual observation.

Colour of water: The colour of water was observed by taking pond water in the petri dish over white paper.

Temperature: The temperature was measured by standard mercury thermometer. The bulb of thermometer was dipped inside the surface of water and reading was taken.

3.4.2 Chemical parameters:

The different chemical parameters that analyzed during study period were;

pH: Digital pH meter was used to record the pH of water.

Dissolved oxygen (DO): Dissolved oxygen was measured by using HANNA instrument HI 9147 dissolved oxygen meter.

3.5 Breeding and rearing of hatchlings, fry and fingerlings of catla

3.5.1 Experimental fish and their number

One of the species of Indian major carp, *Catla catla* (Bhakur) were selected for the experiment. Altogether 4 mature females and 6 mature males were selected for the experiment. The average weight of selected female broods was 3.9kg to 4.9kg and that of male broods were 3.73kg to 5.5kg.

3.5.2 Maintenance of broods

Broodfish refers to those mature males and females that are developed enough to give eggs and sperm which are used in aquaculture for breeding purpose. The healthy broods without deformities were selected and stocked in separate brood stocking pond. The broods in brood pond were maintained by monitoring water parameters like dissolved oxygen, temperature and pH. In addition to natural feed, the fishes were fed with supplementary feeds containing shrimp, soybean, wheat flour, rice bran, oil cake, 2-3 times per day as nutrients that play an important role in maturation and fecundity.

3.5.3 Selection of brood

Matured male and female broods were selected based on external secondary sexual characters with the ratio 2:1. The females with enlarged abdominal vent, bulges slightly and turns reddish were selected. The profile being fuller due to the development of ripe ovaries and when pressed gently on the abdomen, eggs are released. The males whose vent elongates inwarding in the form of a narrow pale coloured slit and when abdomen is pressed gently milt oozes out were selected as brood fish. After selection, the free oozing males and ripe females were transferred to the circular breeding pond inside the

hatchery room from stocking pond by using hammocks with care and kept it there to acclimatize with the flow of water in tank.

3.5.4 Method of injection and dose

The body weight of each brooder was weighed. The hormone was administered by intramuscular injection on muscles beneath the dorsal fin slightly above the lateral line. The syringe needle was inserted at the angle of 45° laterally at the depth of about 1-1.5cm and the hormone was injected slowly. After injecting the fish, the injected site was massaged in order to prevent the suspension from running out. Ovaprim hormone was administered at the dose of 0.5ml/kg for female and 0.25ml/kg for male.

3.5.5 Estrus, spawning and fertilization

After injection, all the brooders were found to be ovulating if the hormone affects normally to the induced fish. This were indicated by the intermittent splashing of water surface as male chased the female, that process is called estrus. If the estrus was continued, the male broods chased female violently and the female began spawning. At the same time, the male nestled up to the female's abdomen and discharged milt. Spawning of *catla* was accomplished in natural way (self-spawning).

3.6 Determination of fecundity and Gonadosomatic index (G.S.I.)

The fecundity and gonadosomatic index were determined by following formula.

3.6.1 Determination of fecundity:

Fecundity was estimated separately by sampling one gram of egg and multiplying with the total weight of eggs in brood female fish. One gram of 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 × wt. of total eggs (gm) spawned by female

3.6.2 Gonadosomatic index (G.S.I)

Gonadosomatic index is the calculation of the gonad mass as a proportion of the total body mass. GSI of fish increase with the maturation of the fish highest at the peak of maturity and lowest during post spawning phase. G.S.I. (%) of all the female fish was calculated by Kaur and Dhawan,1997.

$$G.S.I. = \frac{weight\ of\ gonads}{weight\ of\ fish} \times 100$$

3.7 Incubation of eggs and rearing of hatchlings

The eggs were transferred to incubation tank. The fertilized eggs soon start to develop. The incubation tank was kept under favorable conditions to ensure proper development and better survival. The eggs complete their embryonic development within the egg shell during incubation and hatchlings hatched out by breaking through the egg shell. The hatchlings started to swim vertically downward to upward and soon started to swim horizontally after the absorption of yolk sac. The hatchlings were given appropriate food a little before total absorption of yolk sac. The hatchlings were provided with egg yolk mixed with water twice a day.

3.8 Determination of fertility and hatching rate

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

3.8.1 Determination of fertilization rate

Fertilization rate was calculated for every female separately. A properly washed beaker was inserted into the incubation tank to scoop out samples of eggs and 3 to 5 such samples were scooped out randomly and observed. Fertilized eggs were observed as clear crystal balls whereas the unfertilized eggs were dull and opaque shown in photo no. 1 and 2.

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

3.8.2 Determination of hatching rate

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

$$Hatching\ rate = \frac{Total\ number\ of\ hatchling}{Total\ number\ of\ fertilized\ eggs} \times 100$$

3.9 Study of embryonic development of fertilized eggs

The sample of fertilized eggs was collected of the different embryonic stages of catla. Eggs at various stages were preserved in 70% of alcohol solution and the study was done under microscope and photographed.

3.10 Transfer of hatchlings

The hatchlings of the incubation tank were transferred in the 3 hapas that are set in the prepared nursery pond after 5 days early at the morning when the water temperature was minimum. The piece of cotton cloth was kept on the mouth of exit of incubation tank outlet and the hatchlings were collected through the outlet of incubation tank. The hatchlings were measured in a measuring beaker (at the rate of 1,00,000 hatchlings per beaker). The hatchlings were transferred in plastic bags containing sufficient amount of water with oxygen. Fry were fed two times a day.

3.11 Growth checkup till advanced fingerlings

The growth checkup of the hatchlings was done at 20 days interval. The length and weight of hatchlings were measured up to four months i.e., up to advanced fingerlings.

3.12 Determination of survival rate

At initial and final stages of experiment, the fishes were counted from each replicate and their length and weight were recorded. For the sampling, 65 fishes from the incubation tank were collected and stocked in three different hapas in the nursery pond. Sampling was carried out at an interval of 20 days till advanced fingerlings to observe growth of fishes. The stocked fishes were fed with the prepared feed containing locally available feed ingredients., rice bran (35%), wheat flour (20%), oil cake (8%), soyabean meal (35%), minerals (1%) and vitamin (1%). At the time of sampling, 10 fishes from each hapa were collected randomly to record length and weight of fishes. At the end of experiment, the survival fishes from each hapa were counted that provides survival rate of fishes.

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

4. RESULTS

4.1 Physico-chemical parameters

4.1.1 Physical parameter

The physical parameters had been studied from the sub-surface of water bodies. In the period of study, the water color was noted greenish. The nature of day was observed sunny, rainy, cloudy and partly cloudy during study period. The range of temperature was found to be 25.8 -37.1℃ (Table2).

4.1.2 Chemical parameters

The pH ranges from 7.6 to 9.8 in brood pond, 7.6 to 8.5 in incubation tank and 8.1 to 9.8 in nursery ponds. The dissolved oxygen ranges from 5.0-8.6mg/l in three ponds. (Table 1).

Table 1: Physico-chemical parameters of Brood ponds, Incubation tank and Nursery ponds

Average data	**Temperature (℃)**		**pH**		**DO (mg/l)**	
	8-10a.m.	**3-5p.m.**	**8-10a.m.**	**3-5p.m.**	**8-10a.m.**	**3-5p.m.**
Brood ponds	28-31	34-37.1	7.6	9.8	5.0-6.3	5.6-7.4
Incubation tanks	25.8-27.6	29.7-32	7.6	8.5	7.0-8.1	8.0-8.6
Nursery ponds	29.3-33.4	34-36	8.1	9.8	5.2-6.6	7.1-8.0

4.2 Fecundity and Gonadosomatic index (G.S.I)

The sex ratio of one female to 1.5 male (4 female and 6 male brood) were used for experiment. The breeding was done in July 17th 2020. The weight of male was ranged from 3.3 to 5.5 kg and that of female was 3.9 to 4.9 kg. After 8 hours of hormonal treatment, the spawning behavior of catla was observed and breeding occurred. The total number of eggs spawned was ranged from 142,400 – 324,800 and G.S.I from 5.12 – 8.88% (Table 2).

Table 2: Fecundity and G.S.I. of *Catla catla*

S.N.	Weight of female (kg)	Weight of total eggs spawned (gm)	No. of egg per gram	Fecundity	G.S.I (100%)
1	4.4	300	768	230,400	6.81
2	4.5	400	812	324,800	8.88
3	3.9	200	712	142,400	5.13
4	4.9	300	752	225,600	6.12

4.3 Fertility rate and hatching rate

The rate of fertilization of eggs was recorded 77.78% and hatching rate was 65.25% (Table 3).

Table 3: Fertility rate (%) and Hatching rate (%) of *Catla catla*

S. N	No. of sample eggs	No. of viable and fertilized eggs	Fertility rate (%)	Hatching rate (%)
1	70	56	80	71.42
2	76	60	78.94	68.33
3	43	32	74.42	59.37
4	54	42	77.77	61.90
Total	243	190	78.78	65.25

4.4 Survival rate

The highest survival rate was recorded in Hapa 2 (49.23%) and lowest in Hapa 3 (38.46%). The average survival rate is 44.1% (Table 4).

Table 4: Survival rate (%) of *Catla catla*

Hapas	Size of hapa	No of stocking fries	No. of days					Survival rate (%)
			20	40	60	80	100	
1	$1\times1\times1m$	65	50	44	39	35	29	44.61
2	$1\times1\times1m$	65	59	51	47	41	32	49.23
3	$1\times1\times1m$	65	53	46	43	33	25	38.46
Total		195	162	141	129	109	86	44.10

4.5 Mortality of hatchlings till advanced fingerlings

In this experiment, the mortality of hatchlings was significantly higher in hapa 1 (15 out of 65) as compared to that of hapa 2 (6 out of 65) and hapa 3 (12 out of 65). The hapa 3 showed higher mortality than that of hapa 2 in the first 20 days. In the 40^{th}day, the mortality of fries did not differ much among the hapa 1(6 out of 50), hapa 2 (8 out of 59) and hapa 3 (7 out of 53). Similarly, much difference was not observed in the mortality of fingerlings in 60^{th} day, i.e., in hapa 1 (5 out of 44), hapa 2 (4 out of 51) and hapa 3 (3 out of 46). The mortality of fingerlings was higher in hapa 3 (10 out of 43) compared to that of hapa 1 (4 out of 39) and hapa 2 (6 out of 47) in 80^{th} day whereas there was very less mortality observed on 100^{th} day between the hapa 1 (6 out of 35), hapa 2 (9 out of 41) and hapa 3 (8 out of 33). The highest mortality of advanced fingerlings was observed in hapa 3.

4.6 Embryonic development of *Catla catla*

4.6.1 Structure of egg

The eggs are spherical, non-floating and non-adhesive. The eggs have an appearance like glassy bead, with a reddish tinge, a large perivitelline space between vitelline membrane and egg yolk in the center of the egg. The yolk is oval in shape with an elongation at the posterior end and is without oil globules. Soon after the shedding of eggs, there is no water in the space. Within fifteen minutes of discharge, the eggs get swollen by absorbing water through the vitelline membrane.

4.6.2 Different embryonic stage

During the process of development, the embryo within the fertilized egg of carp appears as a spot at first. Within approximately 4-5 hours, the embryo develops gradually as a pea-shaped structure. The embryo elongates and develops into a shape of comma within 7-8 hours. It starts a twitching movement within around 9-12 hours after fertilization. The newly hatched hatchlings are fine thread-like behind with an oval yolk sac at the anteroventral side that are absorbed by growing spawn within 3 days and the developing spawn attain hatchling stage.

Photo 1: unfertilized egg

Photo 2: fertilized egg

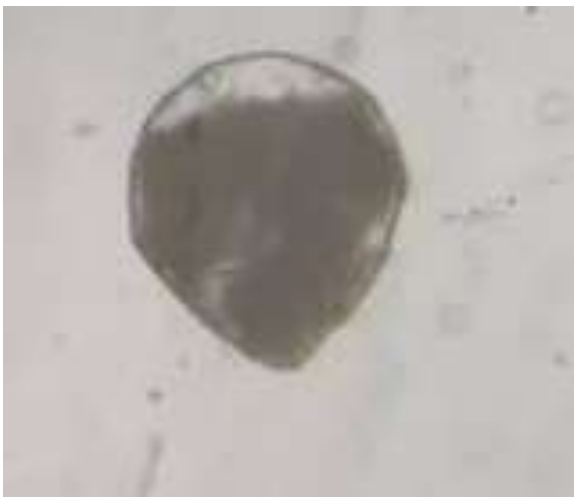


Photo 3: 4 hours of fertilization

Photo 4: 16 hours of fertilization

Photo 5: 24 hours of fertilization

Photo 6: 28 hours of fertilization

Photo 7: 3-day hatchling

Photo 8: 4-day hatchling

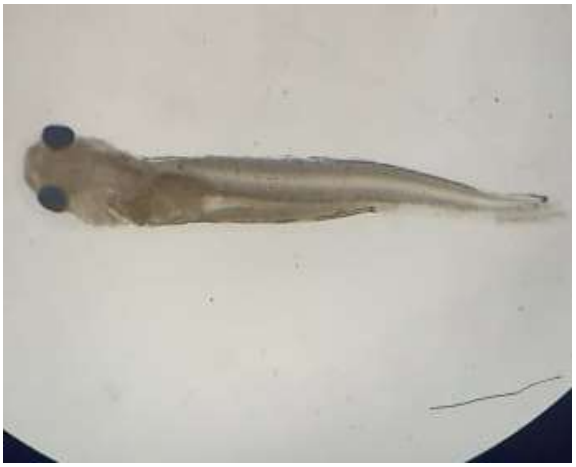


Photo 9: 5-day hatchling

Photo 10: 6-day hatchling

4.7 Growth of Hatchlings

The increase in length and weight was highest in hapa 3 followed by hapa 1 and hapa 2. The initial length and weight of hatchlings, stocked in the hapas were almost the same. The highest weight gain was in hapa 3 and lowest in hapa 2. The growth in hapa 3 was significantly higher than hapa 2, while hapa 1 was not significantly different from hapa 1 but was significantly different from hapa 3. The length and weight were increased satisfactory from 20 to 100 days observation period (table 5).

Table 5: Length and weight of fries and fingerlings

Days	Hapa 1		Hapa 2		Hapa 3	
	Length (cm)	**Weight (g)**	**Length (cm)**	**Weight (g)**	**Length (cm)**	**Weight (g)**
20th day	2.5	0.24	2.3	0.19	2.7	0.25
40th day	4.2	1.435	4.0	1.105	4.6	1.847
60th day	6.0	3.92	5.9	3.72	6.2	4.03
80th day	7.8	6.212	7.7	5.95	8.1	6.89
100th day	9.5	8.96	9.3	8.63	9.7	9.43

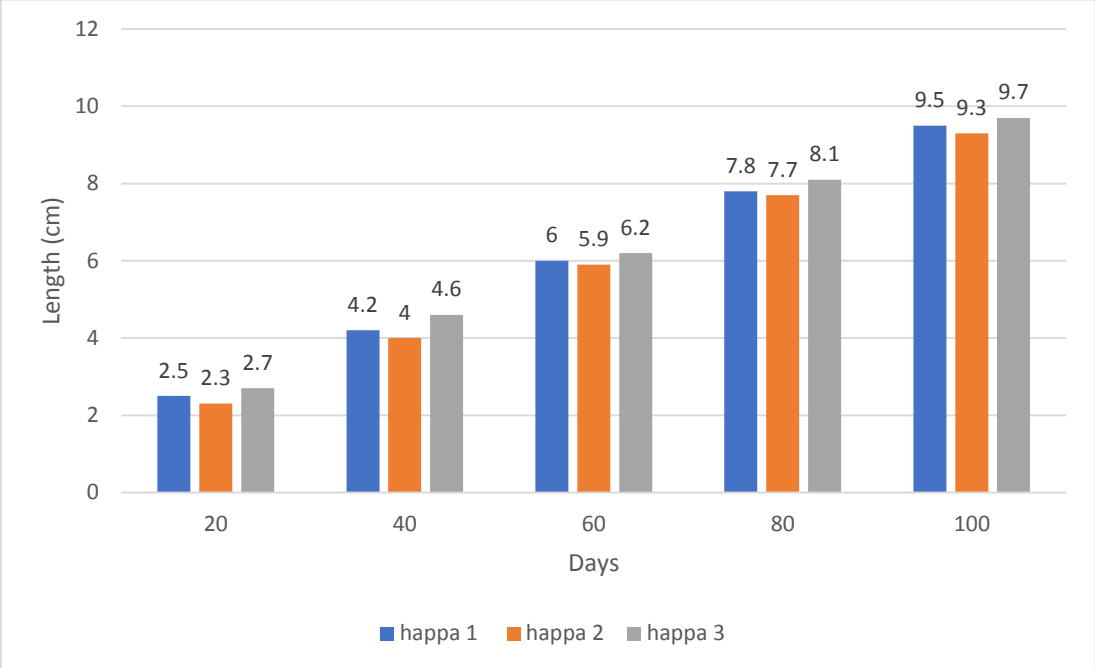


Fig 2: Bar diagram showing increasing in length along with days.

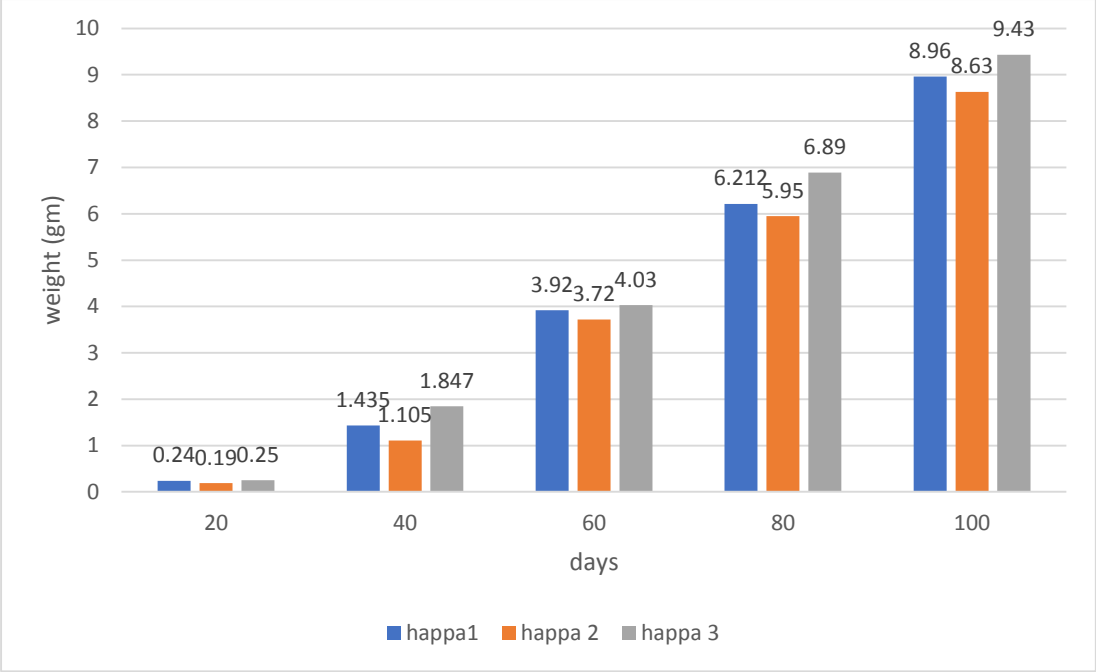


Fig 3: Bar diagram showing increase weight with days

5. DISCUSSION

The present study was conducted to study the effectiveness of ovaprim hormone and the growth upto fingerling stage in *Catla*. Experiment was also conducted to check the growth up to its fingerling stage. The fish life is governed by aquatic environment so, water quality should be suitable for fish culture. The productivity will be less if the physico-chemical parameters of water used for cultivation of fish are not optimal for fish and other aquatic organisms. All water quality parameters studied during the experimental period shows that all parameters were within the permissible limits in pond water for fish culture as reported by Jhingram (1991).

Temperature influences the maturation of gonads in fishes and appears to be a triggering factor for spawning in carps. The monthly values of water temperature throughout the experimental period were found between the temperature range from 25.8-37.1℃, the growth of fishes was high. Nazish and Mateen (2010) reported that the freshwater fish that have an optimum growing temperature in the range of 25-30℃ at which they grow quickly. In July, lowest temperature was recorded 29.3℃ in nursery pond while highest temperature (36℃) was noted in September. There is an optimum temperature for fish breeding, even below and above temperature than normal may not reproduce (Hoar and Robertson, 1959). Hontela and Stacy (1990) reported that in rose bitterling (*Rhodeus ocellatus*, cyprinidae), decreasing photoperiod and high-water temperature cause gonadal regression.

Low dissolved oxygen (5.2ppm) in nursery pond was found in the month of August while maximum dissolve oxygen (8.0ppm) was found in the month of July. The highest pH value (9.8) was recorded in the month of September and lowest pH (8.1) was recorded in the month of August from the experiment, it was observed that maximum growth of fish occurred at pH range of 7.6-9.8. Similar findings were reported by Bhatnagar and Singh (2010) that DO level greater than 5ppm is essential to support good fish production.

The doses of hormones used vary from species to species and situation to situation. In order to gain successful breeding of the fishes, appropriate dose of hormones need to be injected that stimulates the spawning. Even though the environmental conditions are favourable, Indian major carps are unable to breed normally in captivity so; induced breeding is an important aspect of aquaculture (Halder *et al*. 1991). Induced breeding

was successfully done in carps by using various spawning chemicals namely fish pituitary extract, ovaprim, LHRH, ovatide etc. Most of the induced breeding on carps was performed through the use of pituitary extracts and this method was introduced by Woynarovich (1969). However, increase in the number of carp seed farms in country results in shortage of fish pituitary glands, its varying potency and adulteration of glands leads to failure of spawning. This cause to introduction of other hormones like LH-RH, its analogue, ovaprim etc for breeding of cultured species throughout the country.

In present study, breeding of catla with ovaprim was assured at doses 0.5ml/kg body weight of female. According to previous studies, the cyprinid fishes show different responses after the administration of ovaprim. Indian major carps when injected with 0.3-0.4 ml/kg dose of ovaprim gives the best results in inducing spawning (Nandeesha *et al*. 1990). This indicates that reproductive responses to ovaprim are different on different species of fishes and are dose-dependent. Some studies explained about the failure in induction of fish spawning using hormones in low and high doses (Rotmann *et al*. 1991). In low doses, a reproductive hormone is unable to support maturation events like germinal vesicle migration and breakdown and ovulation (Rotmann *et al*. 1991). When high dose of hormones is used, due to physiological negative feedbacks on pituitary gland, the reproduction is impaired.

During this study, after 8 hours of hormonal treatment, the spawning behaviour of catla was observed and breeding occurred under the temperature range (29-31℃). Chaturvedi *et al.* (2015) also reported the ovulation of catla took place after 10-12 hours of drug administration under the optimum temperature (28-29℃). The hatching of catla was observed to be around 13 hours after the fertilization as reported by Tumbahangfe *et al*. (2014).

During experiment, the number of eggs before swelling were found to be ranged from 712 - 812 using ovaprim-c and the fecundity ranged from 142,400-324800 which is more than number of eggs per kg reported by Naeem *et al*. (2005). This may be due to the good nutritional status of brood fish as supplementary feed were given to them. Similarly, 47.39 lacs of eggs were counted in 47.90kg wt. by Naik and Mirza (1992).

In the present study, average fertility rate and hatching rate were found to be 77.78% and 65.25% respectively. Naeem *et al.* (2005) found average fertility rate and hatching

rate as 91.01% and 67.50% respectively. However, when shirbot were administered with 1ml/kg body weight ovaprim and 3mg/kg body weight pituitary extract, the fish shows highest value of spawning rate, working fecundity and egg weight per gram body weight (Ghanemi and Khodadadi, 2017). Similarly, when appropriate dose of ovaprim (2.5ml/kg in female, 2 ml/kg in male) and LHRH (60-70 µg/kg for females and 30-35 µg/kg for males) were stimulated in *Oncorhynchus mykiss*, high hatching rate and survival rate was obtained (Uthayakumar *et al*. 2011). In case of *Chhana punctatus* and *Heteropneustes fossilis,* spawning was completed when administered with medium dose 0.3ml and high dose 0.5ml of ovaprim but no spawning was observed in low (0.1ml) dosage of ovaprim (Haniffa and Sridhar, 2002). Similar observations were recorded by Sarkar *et al.* (2006) in *Chitala chitala* and *Labeo rohita* by Tiwana and Raman (2012) and Pawar *et al*. (2019). The ovaprim was found to be better than pituitary extract. Ovaprim-C being ready to use form simplify the breeding process and therefore can be easily adopted in carp seed production. Even though catla has a short spawning period and is a difficult species to breed with hypophysation technique (Bhowmick *et al*. 1977), with the administration of ovaprim, ovatide and gonopro-FH, it has been bred successfully.

The average survival rate during the study was recorded 44.1% which was not very good. Low survival rate might be due to the improper management of the nursery pond. Samad *et al*. (2017) reported the survival rate of *Catla catla* as 79.75% in T1 (123500 fry/ha), 85.13% in T2 (111150 fry/ha) and 89.63% in T3 (98800 fry/ha) respectively. Hossain *et al.* (2013) found the survival rate of silver carp 55.05-63.11%. Similarly, Priyadarshini *et al*. (2011) obtained survival rate of *Cyprinus carpio* fry 52.12% in manure applied pond. Hossain *et al*. (2014) reported the survival rate of silver carp as 38.60% and 66.48%, mirror carp as 31.20% and 45.50% and rui as 31.51% and 53.35% in treatment I (new made pond) and treatment II (renovated pond) respectively.

In carp seed production, many problems have been solved by using ovaprim such as saving considerable amount of time, lowers the post-spawning mortality since brood fish is needed to handle only once and has no adverse effects in fishes. Successful spawning has been reported in several species of fish through single dose of ovaprim (Nandeesha *et al*. 1990). In India, only 10 to 15% of fish breeders used pituitary extract due to its complex technique as most fish breeders preferred ovaprim (Dehadrai, 1986)

which might be considered as a best substitute over pituitary extract and ovatide during induced breeding.

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, dissolved oxygen and other water qualities 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

6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Although, most of the fishes are unable to reproduce under certain culture conditions the induced spawning can be of great value. The induced breeding of *Catla catla* in Pure line fish breed conservation and promotion centre, Rupandehi, with a single dose of ovaprim is sufficient for the successful spawning. The, fecundity was 142,200 – 324,800 and G.S.I was 5.12 – 8.88% of female brood fish which is less than *Labeo rohita*. Fertilization, hatching and survival percentage at spawn stage were 77.78%, 65.25% and 44.1 % respectively. The ideal weight of female brood fish was found 4.5 kg in this study. This synthetic hormone has been found advantageous for induced breeding of catla as it shows better response time and higher ovulation, number of eggs produced, fertilization, hatching and survival percentage of spawn.

6.2 RECOMMENDATIONS

On the basis of the study, some points are noted for improvement in coming days.

- Too much rough handling of fish should be avoided in order to reduce the stress while transporting the live fish.
- The physico-chemical parameter of water should be maintained in suitable range for better breeding performance, survival and growth of spawn produced.
- Trainings, workshops, seminars, Internship opportunities should be organized to learn more about fisheries development.

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PHOTO PLATES

Photo 11: Hatchery of fish farm

Photo 12: Netting of fingerlings to protect from predators

Photo 13: Netting of brood fish for breeding

Photo 14: Selection of brood fish

Photo 15: Injecting ovaprim hormone

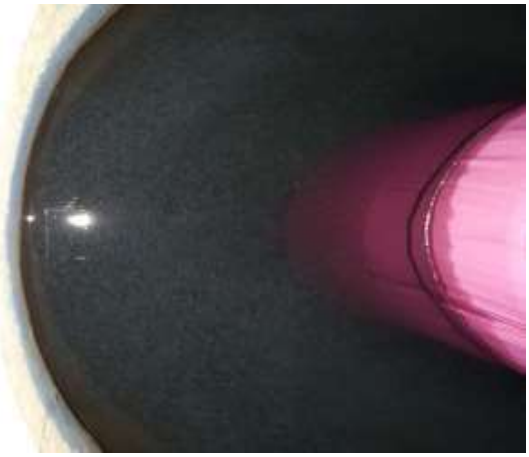

Photo 16: Eggs in incubation tank

Photo 17: Setting of Hapa

Photo 18:Measuring length of fingerlings

Photo 19: Pure Line Fish Breed Conservation

Photo 20: Main office building and Promotion centre

Photo 21: Staff members of fish farm