

1 INTRODUCTION

1.1 Background

Fresh water fish culture is an important sector of food production in Asia and throughout the world for raising the quality and quantity of domestic fish production for human consumption (Gjedrem *et al.*, 2012). During the last two decades, growth in human population, rising incomes and changes in commodity prices have changed human food consumption patterns with inclination towards eating meat, fish and other nutritious diet (Diouf 2009; Gjedrem *et al.*, 2012). Fishing traditional in Nepal but modern aquaculture techniques for fish production start with the introduction of exotic carp in the early 1950s. To utilize the 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*, *Hypophthalmichthys molitrix*, *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*) were successfully developed in the warm southern region (Banjade, 2015). At present, technology of subsistence carp farming in ponds has been widely disseminated in the southern part of the Country. However, it is necessary to improve productivity by increasing our understanding and increasing inputs. The 1960s, attempts to introduce trout in the country failed, perhaps due to inadequate technical and management skills (Gurung, *et al.*, 2016).

1.2 Water resources

Nepal is the second water richest country in the world measure against fresh water resources and possesses about 2.27% of the world fresh water reserves (CBS, 2005). Nepal is endowed with many forms of water resources scattered throughout the country. These water bodies are in the form of rivers and streams, lakes, reservoir, ponds, swamps, and paddy fields. On the basis of the resource availability, the rivers are of major importance representing about 49% of the total water area. Lakes and reservoirs cover 0.8%, and swamp and irrigated paddy fields share the rest of 50.2%. Open waters cover approximately 5.5% of the total area of Nepal.

Table 1 Estimated water surface area in Nepal.

Resource	Estimated area (ha)	Coverage percent	Potential for fisheries (area in ha)
Natural waters	401,500	48.9	-
Rivers	395,000	48.1	-
Lakes	5,000	0.6	3,500
Reservoirs	1,500	0.2	78,000
Village ponds	8,600	1.1	14,000
Marginal swamps	12,500	1.5	12,500
Irrigated paddy fields	398,000	48.4	100,000
Total	818,000	100.00	

(Sources: Directorate of Fisheries Development, 2014)

1.3 Trends of Aquaculture production in Nepal

Warm water fish species such as Rohu (*Labeo rohita*), Bhakur (*Catla catla*), and Naini (*Cirrhinus mrigala*) introduced in 1956 from India are main species cultured at a commercial scale in Nepal. The herbivore Grass carp (*Ptenopharyngodon idella*) was introduced in 1966 from India, phytoplankton feeder the Silver carp (*Hypophthalmichthys molitrix*) in 1968 from Japan, zooplankton feeder Bighead carp (*Aristichthys nobilis*) in 1971 from Hungary for polyculture and Common carp (*Cyprinus carpio*) was introduced from India in 1959 and Israel 1960 (Sah, 2012). These seven fish species are the main species commonly used by the farmers for commercial production.

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. Aquaculture production continued to increase significantly by the end of the project and reached 20,000 tones by 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 95 percent of production. A compilation (FAO, 2005 and DOFD, 2005) indicated that in 2003/2004 Silver carp accounted for the major share of (5125 tones, i.e. 26 percent) of total aquaculture production of 20,000 tones. However its price was reported to be the lowest among other cultured species. 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.

Table 2 Proposed Fish Production Trends during 2011-2022.

Year	Pond			Ghole			Other Production	Aquaculture Production
	Area	Productivity	Production	Area	Productivity	Production		
2010/11	7277	3.70	26924.90	2200	1.5	3300	725	30950
2011/12	7402	3.83	28349.66	2500	1.5	3750	757	32857
2012/13	7500	3.95	29625.00	2700	1.5	4050	825	34500
2013/14	7530	4.25	32002.50	3000	1.5	4500	825	37328
2014/15	8022	4.50	36099.00	3400	1.5	5100	825	42024
2015/16	8541	4.75	40569.75	3900	1.7	6630	825	48025
2016/17	9344	5.00	46720.00	4400	1.7	7480	825	55025
2017/18	9673	5.50	53201.50	5000	1.9	9500	825	63527
2018/19	10437	6.00	62622.00	5200	1.9	9880	825	73327
2019/20	11338	6.50	73697.00	5500	2.0	11000	825	85522
2020/21	12041	7.25	87297.25	5700	2.0	11400	825	99522
2021/22	13250	8.00	106000.0	6000	2.0	12000	825	118825

(Sources: DoFD, 2005)

1.3.1 Total Fish Production in Nepal

Fisheries and aquaculture sector play a key role in food security and employment generation. Nearly 3% of population is dependent on fisheries, aquaculture and allied activities for their livelihood sustenance and income. Besides, the sector also contributes nearly 2.7 of AGDP and 1% in National GDP.

Table 3 Fish production in Nepal, from Aquaculture and Capture fisheries.

Year	Fish Production from Aquaculture (mt.)	Fish production from capture Fishery (mt.)	Total Fish Production (mt.)
2005/06	22,500	20,000	44,525
2006/07	25,500	20,000	46,600
2007/08	28,000	21,000	49,000
2008/09	28,100	21,000	48,300
2009/10	28,400	21,000	49,900
2010/11	31,200	21,000	52,000
2011/12	34,500	21,500	56,000
2012/13	36,020	21,500	57,520
2013/14	43,400	21,500	64,900
2014/15	48,000	21,500	69,500

(Sources: DoFD, 2005 to 2015)

Over the 10 years from 2005/06 to 2014/15 the increase in fish production from aquaculture was 25,500 mt or 240 % thus averaging 21% annual.

Over the last 10 years from 2005/06 to 2014/15 the increase in fish production from capture fisheries was 1,500 mt.

1.3.2 Indigenous Species of Nepal

The richness and uniqueness of the fresh water fishes of Nepal are due to different topographic and climatic conditions of the country within a short distance of elevation from plain Terai to high altitude mountains as well as from tropical to alpine climate conditions. The ichthyology of Nepal is reported within varying account of total number of fish species recording in the country. In addition, various authors have reported new species and new records of fish from Nepal, which further increased the total number of species. The total number of fish is reported variable in different time periods starting from 120 (Shrestha, 1981) to more than 200 species till now.

The present list includes 232 indigenous species of Nepal (Shrestha, 2008). They belong to 11 orders, 32 families, 24 sub-families, 99 genera and 232 species including 15 endemic species. Their status as common is 71, insufficiently known 33, endangered 1, vulnerable 5, rare 36, fairly common 24, uncommon 49 and occasional 9. The main differences in reporting the total number of indigenous species of Nepal by different authors is due to the adoption of classifying taxonomic nomenclature by different authors (Shrestha, 1981; Shrestha 2008).

1.3.3 Exotic fish species in Nepal

According to Shrestha (2008) there are 15 exotic fish species recorded in Nepal. Common carp (*Cyprinus carpio*) was introduced from India in 1959 and Israel in 1960. Grass carp (*Ctenopharyngodon idella*) was introduced to Nepal from India in 1967, followed by Silver carp (*Hypophthalmichthys molitrix*) from Japan 1968 and Bighead carp (*Aristichthys nobilis*) from Hungary in 1971/72. Intensive commercial culture of Rainbow trout (*Onchorynchus mykiss*) is being practiced in cemented raceways from more than one decade in Nepal. Introduction of exotic fishes like Gold Fish (*Carassius auratus*), Silver Barbs (*Puntius gonionotus*), Tilapia (*Oreochromis niloticus*) and giant fresh water prawn (*Macrobrachium rosenbergii*) had started in culture practice recently as ornamental aquarium fish and in polyculture (Srivastava, 1999).

1.4 *Labeo rohita* (Hamilton, 1822)

1.4.1 Description of *Labeo rohita*

The herbivorous column feeder, preferring algal and submerged vegetation fish *Labeo rohita* was introduced in Nepal (1957-1970). This species has also been introduced in many other countries, including India, Sri Lanka, Pakistan, Bangladesh, Myanmar, the former USSR, Japan, China, Philippines, Malaysia and some countries of Africa. The traditional culture of this carp goes back hundreds of years in the small ponds of the eastern India states.

Rohu (*Labeo rohita*) is the natural inhabitant of fresh water sections of the rivers. Rohu thrives well in all fresh waters below an altitude of approximately 549 m. Rohu is a bottom feeder and prefers to feed on plant matter including decaying vegetation. Rohu attains maturity towards the end of the second year in ponds (Talwar and Jhingran, 1991). *Labeo rohita* comprises major portion of aquaculture production in our country. The fish can be profitably cultured in ponds, ditches and enclosure in combination with other carp species. A large number of government and private agencies have disseminated technology of *Labeo rohita* culture to the rural farmers as a low cost enterprise. Carp are herbivorous, with a high tendency towards the consumption of preferring algae and submerged vegetation. In its real life stages Rohu prefer zooplankton, mainly composed of rotifers and cladocerans, with phytoplankton forming the emergency food. In the fingerling stage, there is a strong positive selection for all the zooplanktonic organisms and for some smaller phytoplankters like desmids, phytoflagellates and algal spores. Pond farming of *Labeo rohita* is based on natural food with supplemental feeding of cereals. The practical technology includes (a) predatory and weeds fish control, (b) stocking of fingerlings at a combined density of 4,000-10,000/ha (30-40% Rohu), (c) pond fertilization with organic manures like cattle dung or poultry droppings and inorganic fertilizers, (d) the provision of a mixture of rice bran/wheat bran and groundnut/mustard oil cake as supplementary feed, (e) fish healthy monitoring and (f) water management. The growth-out period is normally one year, during which Rohu grows to about 700-800 gram. The usual harvestable size of Rohu is 1-1.5 kg and is achieved within a culture period of 12-18 months.

The spawning season of Rohu generally coincides with the south-west monsoon, extending from May to August. However breeding does not take place in such lentic pond environment, thus induced breeding becomes necessary. The fecundity varies from 226,000 to 2,794,000 depending upon fish size and ovary weight, in an average from 200,000-300,000 egg/kg body weight. The optimum temperature for spawning is 25-32°C.

These countries India, Nepal, Bangladesh, Myanmar, Laos, Thailand, Pakistan. (FAO fisheries statistic, 2006) are the major producing of *Labeo rohita*.

Classification (According to Berg, 1940)

Phylum- Vertebrata

Subphylum- craniate

Superclass- Gnathostomata

Series- Pisces

Class- Actinopterygii

Order- Ctrpriniformes

Division- Cyprini

Suborder- Cyprinoidei

Family- Cyprinidae

Subfamily- Cyprininae

Genus- *Labeo*

Species- *rohita*

1.4.2 Morphology of *Labeo rohita*

Labeo rohita has bilaterally symmetrical, moderately elongate, its dorsal profile more arched than the ventral profile body. Cycloid scales, covers the whole body except head. Snout is fairly depressed, projecting beyond mouth, without lateral lobe. Eyes dorsolateral in position, not visible from outside of head. Mouth small and inferior, with thick lips and fringed with a distinct inner fold to each lip lobate or entire. A pair of small maxillary barbels concealed in lateral groove; no teeth on jaws. Pharyngeal teeth in three rows, upper jaw not extending to front edge of eye. Simple (unbranched) dorsal fin rays three or four. Branched dorsal fin rays 12 to 14. Dorsal fin inserted midway between snout tip and base of caudal fin, pectoral and pelvic fins laterally inserted. Pectoral fin devoid of an osseous spine; caudal fin deeply forked. Lower lip usually joined to isthmus by a narrow or brood bridge, pre-dorsal scale 12-16. Lateral line distinct, complete and running along median line of the caudal peduncle. Lateral line is comprised of 40 to 44 scales. Snout not truncate, without any lateral lobe. Colour bluish on back, silvery on flanks and belly (Jhingran and Pulling, 1985).

1.5 *Cirrhinus mrigala* (Hamilton, 1822)

1.5.1 Description of *Cirrhinus mrigala*.

The bottom feeder, *Cirrhinus mrigala* was introduced in Nepal (1947). In addition, *Cirrhinus mrigala* has become an important component in the fish culture system of India, Bangladesh, Pakistan, Myanmar, the Lao people's Democratic Republic, Thailand, and Terai in Nepal. Mrigal has also been introduced in to Sri Lanka, Vietnam, China, Mauritius, Japan, Malaysia, Philippines and the former USSR.

Cirrhinus mrigala grow well to about 20 kg weight and at a length of about 150 cm (Shrestha, 2008). This fish live in rivers, lakes and reservoir, along canals of the riverine flood plain, feed on algae bottom living invertebrate insect larvae, crusteans and molluscs. Juveniles are omnivorous until they are about 5 cm. Adult are herbivorous. Adult eats almost everything from algae, bits of vegetation and various seed that have fallen into the water.

It matures sexually in the third or fourth year of its life. *Cirrhinus mrigala* was reported to survive as long as 12 years in the natural water. The growth-out period is usually one year, during which mrigal grows to about 600-700 gram. Bottom feeder, mrigal is usually stocked 20-30% Of the total species stocked in three-species culture, while in six-species culture Mrigal constitutes only about 15-20%.

As *Cirrhinus mrigala* needs a fluviatile environment for breeding, it does not bred in ponds. However, captive breeding in hatcheries has been made possible through induced breeding by hypophysation and the uses of synthetic hormone. Mrigal is a highly fecund fish, fecundity increases with age, and normally ranges from 100,000-150,000 egg/kg body weight. The spawning season depends upon the onset and duration of the south-west monsoon. Mrigal usually breeds at 24⁰C to 31⁰C.

These countries India, Nepal, Bangladesh, Myanmar, Laos, Thailand, Pakistan. (FAO fisheries statistic, 2006) are the major producing of *Labeo rohita*.

Classification (According to Berg, 1940)

Phylum- Vertebrata

Subphylum- Craniate

Superclass- Gnathostomata

Series- Pisces

Class- Actinopterygii

Order- Ctrpriniiformes

Division- Cyprini

Suborder- Cyprinoidei

Family- Cyprinidae

Subfamily- Cyprininae

Genus- *Cirrhinus*

Species- *mrigala*

1.5.2 Morphology of *Cirrhinus mrigala*

Body bilaterally symmetrical and streamlined. Its depth about equal to length of head, body with cycloid scales, head without scales, snout blunt, often with pores. Mouth broad, transverse, upper lip entire and not continuous with lower lip, lower lip most indistinct. Single pair of short rostral barbells. Pharyngeal teeth in three rows, 5.4.2/2.4.5 pattern. Lower jaw with a small post-symphysial knob or tubercle. Origin of dorsal fin nearer to end of snout than base of caudal. Dorsal fin as high as body with 12 or 13 branched rays, last unbranched rays of dorsal fin non-osseous and non-serrated. Pectoral fins shorter than head, caudal fin deeply forked, anal fin not extending to caudal fin. Lateral line with 40-50 scales (Jhingran and Pulling, 1985).

1.6 Induced breeding

Induced breeding is the highly reliable techniques to re-establish the declining natural as well as to meet the rising demand of seed to the farmers. Induced breeding is a technique by which the economically important fish (which generally do not breed in captive condition) are bred through artificial stimulation. Induced breeding is a technique where by ripe fish breeders are stimulated by pituitary hormone or any other synthetic hormone introduction to breed in captive condition. The stimulation promotes timely release of sperms and eggs. .

Induced breeding of Indian major carps has been carried out in Nepal since 1971, (Sah, 2012). Now a days the fisheries development center in public sectors and the private fish farmers are administering the synthetic hormone LH-RH and ovaprim instead of injecting pituitary extracts to Indian major carps.

1.6.1 Advantages of Induced breeding (Jain, 2003; Srivastava, 2005)

- The seed spawn is timely available, its availability from natural sources is quite uncertain.
- A pure spawn of a desired species is made available. The spawn obtained from the rivers are not pure. They are mixed with the spawns of other species and sorting of pure seed from the mixed spawn is not possible.
- Any quantity of pure spawn can be made available.
- 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.
- It is economical to obtain a spawn from induced breeding experiments in comparison to its collection from the riverine sources.
- The induced breeding technique is very simple and can be learnt even by a layman.

1.7 Objectives

1.7.1 General objective

TO STUDY THE EFFECT OF OVULIN ON INDUCED BREEDING IN ROHU (*Labeo rohita* Hamilton, 1822) AND NAINI (*Cirrhinus mrigala* Hamilton, 1822).

1.7.2 Specific objectives

- a) To investigate the effect of ovulin on the fecundity, fertilization and hatching rate of *Labeo rohita* and *Cirrhinus mrigala*.
- b) Study the embryonic development of *Labeo rohita* and *Cirrhinus mrigala*.
- c) Study the growth rate of *Labeo rohita* and *Cirrhinus mrigala* from fry to finger stage.

1.8 Rationale

In Nepal, studies, regarding Induced breeding in birds, mammals, reptiles and environmental related issues were conducted but such study on pisces are very scanty. The study of fish like *Labeo rohita* and *Cirrhinus mrigala* are highly successful as a food sources but their details 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 these two fishes which help to fill to some extent the knowledge gap and research of these species.

2 LITERATURE REVIEW

2.1 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 Azevedo and others, conducted experiments with various hormone injections on the lined of Houssay (1930) and achieved 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 piscicultural programme.

The Russians are considered the next to introduce hormone treatment in fish culture after the Brazilians. It was not until 1937 that Gerbilskaa (1938) could succeed, and since then this method is utilized with advantage 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 in induced ovulation in *Cirrhinus mrigala*, Hussain (1945) administered 80 to 120 RU prolactin 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 induce *Esomus danricus* to breed by intraperitoneal injection of catla pituitary gland extract. Ramaswamy and Sundorary (1956, 1957) reported successful breeding of the cat fishes, *Heteropneustes fossilis* and *Clarias batrachus* during 1955 and 1956, respectively by hormone injection. Chaudhari and Alikunhi in 1957 succeeded in inducing *Labeo rohita*, *Cirrhinus mrigala*, *Cirrhinus reba*, *Labeo bata* and *Puntius sarana* to breed by injecting them with carp pituitary extracts.

The first hypophysial technique in China was adopted in 1958 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) China, the artificial propagation of black carp was done similarly by using common carp pituitary.

2.2 Global context;

Balai *et al.*, (2017) reported the Length-Weight relationship and condition factor of a fish provide the information on its growth and well-being of fish in the surrounding aquatic environment. They reported these aspect have been investigated and elucidated with particular reference to the growth status and condition factor of three Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* in Jaisamand Lake India. The morphometric data were collected from 119 specimens of each species during May 2005 to 2006 and mean total length 54.081 ± 1.139 cm, 51.601 ± 0.754 cm and 51.723 ± 0.541 cm and mean weight 3197.971 ± 157.814 gm, 1853.333 ± 82.959 gm and 1701.250 ± 52.123 gm were observed for Catla, Rohu and Mrigal, respectively. A stronger linear relationship was observed between total length-weight variable. The value of regression coefficient depicted isometric growth in Mrigal, positive allometric growth in Rohu and Negative allometric growth in Catla. The condition and relative condition factors were observed >1.0 which indicates the good conditions of all the three fish species.

Naeem *et al.*, (2015) reported the induced breeding of *Labeo rohita* through single application of Ovaprim-c. They study fish specimens were spawned successfully following, a single dose of injection of ovaprin-c with 0.4 ml/kg for female and 0.05 ml/kg for male brooders. They reported the rates of fertilization 77.50% and hatchling 81.39%. They reported hatching occurred within 18-30 hr after fertilization. Wet body weight was observed to have a positive influence on absolute ($r=0.983$) and relative fecundity ($r=0.910$) in log-log scale.

Mir *et al.*, (2014) reported that Length-Weight relationships of the Indian major carp, *Labeo rohita*. The Indian major carp, *Labeo rohita* is a geographically widespread and economically important food fish species in tropical freshwater. They studied the Length-Weight relationships of 1033 specimens collected from the main channel of Ganga river and its five major drainages from March 2009 to July 2012. The length of male ranged from 16 to 92 cm and female 16 to 94 cm. The growth is allometric positive ($b>3$) for males, female and pooled sexes. The coefficient of determination (r^2) in Males ranged from 0.978 to 0.989 and Females from 0.958 to 0.985.

Ujjania *et al.*, (2012) reported that Length-Weight relationship and condition factors of Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Length-Weight relationships parameters are calculated for the three carp species and found significant. The correlation coefficient 'r' was significant and observed to be 0.818, 0.976 and 0.970 in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* respectively for pooled data. However these value ranged from 0.664-0.903, 0.741-0.880 and 0.819-0.967 for Catla, Rohu and Mrigala, respectively in the different length group.

There are several methods, preserve fish eggs for 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*. Tumbahangfe *et al.*, 2014 used Bouins fluid for embryonic development of *Catla catla*. Miah *et al.*, 2009 used (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*. In my study used distilled water for embryonic development study of *Labeo rohita* and *Cirrhinus mrigala*, which similar as Basak and Basak, 2014; Rath *et al.*, 2002.

Abid and Ahmed (2009) studied growth response of *Labeo rohita* fingerlings fed with different feeding regimes under intensive rearing for a period of six months. Stocking density was 25 fish/ glass aquarium/m² (280 liter water volume). Seven different diets were tested. Out of seven diets, three low cost based diets having (35%, 40% and 45% crude protein) and three high cost based diets having (35%, 40% and 45% crude protein) were evaluated against a control (rice polish) having (12% crude protein) in triplicate glass aquaria. Fish were feed daily @ 4% of body weight twice a day. The survival rate was 100% at all feeding levels. In aquaria fish fingerlings fed with 45% low cost based diet showed significantly higher ($p < 0.05$) weight gain (26.17gm) than other diets and highly significant to control diet (9.77 gm). Water quality such as Temperature, Dissolved Oxygen, total Hardness, were found approximately constant for rapid growth and survival for *Labeo rohita* fingerlings.

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. The eggs were obtained after reproductive induction. At fertilization the eggs were 0.7 mm in diameter. Samples were taken every 10 minutes interval till completion of morula and then every one hour interval up to hatching. After hatching, newly hatched larvae were observed daily until the attainment of the fingerling stage. The eggs presented brownish-yellow coloration. The stages of embryonic development observed with cleavage, followed by blastula, morula, and gastrula until hatching of non-pigmented larvae which displayed total average length of 2.0 ± 0.01 mm, 20 hours after fertilization. First cleavage was recorded within 45 minutes after fertilization and the embryonic rudiments of developing eggs appeared at 10 hours at 27.0°C - 31.0°C . The yolk sac was completely absorbed at 66 hours during embryonic development on attainment of 6.2 ± 0.02 mm total length. At the same time the digestive system became fully developed and the larvae searched for feeding.

Jadho (2007) studied effect of continuous illumination on gonadal recrudescence and spawning in an Indian major carp, *Labeo rohita* in field conditions. Results clearly indicate 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.

Gupta *et al.*, (2007) evaluated the changes in hormonal profile during early and normal breeding in Rohu in relation to breeding response parameters, such as percent spawning, egg quality, fertilization, hatching rate and spawn recovery. The female rohu were induced with ovaprim at 0.4ml/kg body weight. Breeding response parameter were recorded from each induced bred fish in different months. Higher breeding response were recorded in Rohu in normal season than early breeding. These findings suggested that lower breeding response in early induced bred rohu was mainly attributed to hormonal changes in relation to maturational status and maturational competence level of oocytes during different months in a season.

Rath *et al.*, (2007) induced the captive brood of *Labeo rohita* (Ham.) three times over a period of 4 months during April to August in an eco-hatchery system and the performance was compared with traditional breeding. The cumulative spawn production of multiple breeding was 3.11×10^5 per kg body weight, whereas same in traditional breeding was 1.27×10^5 during the same season. Unspawned oocysts exhibited quick changes and development in the inter spawning period of the multiple breeding than the traditional breeding system.

Denson *et al.*, (2007) compared Human Chorionic Gonadotropin (HCG) and Luteinizing hormone releasing hormone analogue (LHRHa) for ovulation induction in Black Sea Bass *Centropristis striata*. Matured Sea Bass (200-800g) captured during monsoon season were administered hormones. Females received a single intramuscular injection of HCG at the rate of 330 IU/kg (n=8) or two injections of HCG at 24 hrs. intervals. A significant difference in fertility was found between HCG (75.6 ± 11.4%) and LHRHa (55.6 ± 27.6%). The results of this study indicate that both HCG and LHRHa are effective for ovulation induction in prespawning Black Sea Bass.

Sial *et al.*, (2005) experimented on *Pangasius sutchi* (siluroid catfish) using carp pituitary extract, ovaprim and ovatide under captive conditions. Out of several doses of carp pituitary extract trail, a dose of 21.0 to 25.0 mg/kg body weight to female brooders induced successful spawning in *Pangasius sutchi* after a latency period of 10-11 hours. Similarly, in case of ovaprim and ovatide a dose of 0.8 to 1.0 ml/kg and 0.7 to 0.8 ml/kg body weight to female brooders respectively induced ovulation and spawning in the fishes with better fertilization and hatching rate. The result indicated that the response time, rate of fertilization and hatching were found to be better in group administered with ovatide followed by ovaprim and carp pituitary extract.

Dhawan and Kaur (2004) compared efficiency of ovaprim and ovatide (both at 0.5 mg/kg female and 0.25 mg/kg male) in carp breeding. The author's revealed the effectiveness of ovaprim over ovatide for the breeding of *Catla catla*. Ovatide resulted in higher fecundity and fertilization rate in *Labeo rohita* and *Cirrhinus mrigala* as compared to ovaprim.

Singh *et al.*, (2002) found that balanced diet during broodstock management and induced breeding using ovaprim showed very good gonadal maturation and breeding response in the Indian catfish, *Heteropheustes fossilis* (Bloch). The fishes were breed through striping after 10- 12 hours of ovaprim administration. Ovaprim was administered at the rate of 0.4-0.8 ml/kg body weight in female and 0.2- 0.5 ml/kg body weight in male fishes. The dry method of fertilization with milt from squeezed testes gave 80-90% success in fish breeding.

Jhahria (2002) compared efficacy of ovaprim and ovatide for induced spawning of Indian major and exotic carp. The experiment was conducted at 21.6⁰C to 27.8⁰C as optimum temperature by varying the injection time from 7:15 am to 4:00 pm. Inducement through ovatide yielded more spawn production (0.4895-1.4590 lakh). Studies indicated that ovatide is more convenient and less expensive, indigenous ovulating agent which required low dosage (0.3 ml/kg brooder) in carp breeding.

Pandey *et al.*, (2002) studied the induced breeding of various Indian major carps and *Cirrhinus mrigala*, Catfish *Heteropheustes fossilis* by employing the hormonal drug formulation-ovatide. The optimum doses required for successful induce spawning of these fishes have been evaluated and standardized.

Panday *et al.*, (2001) used ovatide for induced spawning in the Indian major carp *Labeo rohita* (Ham). Two years broodstock comprising six comprising six females (1.4 ± 0.5 to 3.9 ± 0.4 kg) and twelve males (weight 1.8 ± 0.3 to 3.0 ± 0.5 kg) were randomly segregated from the entire stock. The females were given priming injection of carp pituitary extract (CPE) at 2mg/kg body weight followed by resolving dose at 4 mg/kg body weight 12 hour later. Whereas, the males were given injection at 4mg/kg body weighed of CPE at time of resolving dose injection to the females. In another trial the females were administered ovatide at 0.4 mg/kg body weight and males at 0.1 mg/kg body weight. The result shows that natural spawning took place in CPE as well as ovatide administered *Labeo rohita* after 10-12 and 7-8 hours respectively. The fertilization rate (95-100%) as well as hatching success (90-98%) were better in ovatide-injected specimens as compare to those given CPE.

Ram *et al.*, (2001) studied induction of maturation in gonads of *Labeo rohita* in response to pituitary extract and ovaprim treatment. In specimens treated with pituitary extract, only fully grown yolky oocytes passing through the growth phase were induced to enter the maturational phase which led to subsequent succession in fully grown yolk bearing oocytes. The results suggests that pituitary preparation was capable of inducing terminal events in gametic maturation faster than ovaprim, whereas the activation of events related to sustainability in quality gametic production was of great magnitude in response to ovaprim resulting successful synchronization and breeding performance in this species.

Singh *et al.*, (2001) investigated the comparison of gonadotropic potency of pituitary glands collected from ovaprim and pituitary preparation induced bred specimens of *Labeo rohita* and assessed by inducing maturational and ovulatory responses in *Heteropneustes fossilis*. Both the low (0.1 mg/fish) and high (0.2 mg/fish) doses of ovaprim, induced new oogonial formation, oocyte growth as well as activated the vitellogenic yolk oocytes to enter the maturational phases but without resulting in ovulation. The ovulation percentage, number of ovulated egg/fish and ova diameter signified more effectiveness of PG collected from specimens induced breed by pituitary extract preparation over the ovaprim with corresponding more expensive at higher dose.

Sarkar *et al.*, (2001) carried out successful breeding of *Labeo dyocheilus* by intramuscular administration of synthetic hormonal drug Ovaprim. A 90-95 % fertilization and high rate of hatching was observed. After successful breeding of this hill stream fish is enabled to produce in the natural stream environment.

2.3 National context;

Banjade (2015) reported ovulation induction in Chinese carp Common carp (*Cyprinus carpio*) and Silver carp (*Hypophthalmichthys 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 with ovaprim treatment. Similarly the percentage of hatchling in common carp was 44-58 and Silver carp was 44-59 respectively. Cleavage of eggs was observed after 4 hrs of fertilization. The development of embryo continued and the hatching took place after 48 hrs of fertilization. The embryological study showed time for hatching of Common carp more than other carps because of its little thicker egg shell than other carps. 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 (*Ciprinus carpio*) through the application of ovaprim, at Mukhya fish farm Nanupati Dhanusha. She reported the total number of eggs spawned ranged from 201,000-333,000. The fertilization and hatching rate was 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.

3 MATERIALS AND METHODS

3.1 Study site

Janakpur is one of the historical town in Nepal. The environment condition in this area is ideal for the fish culture purpose. The fisheries Development and Training Centre (FDTC) lies in the southern region of the Janakpur municipality. The fish ponds and Chinese-ecotype hatchery of Janakpur fisheries development and training centre is selected for the present study. The Fisheries Development centre of Janakpur occupies 39.0 hectare in area with 92 Pond of various size for the purpose of nursing, rearing, stocking, brood management etc. The brood stock ponds are facilitated with inlet and outlet provision (PLATE–1, Photo-1).

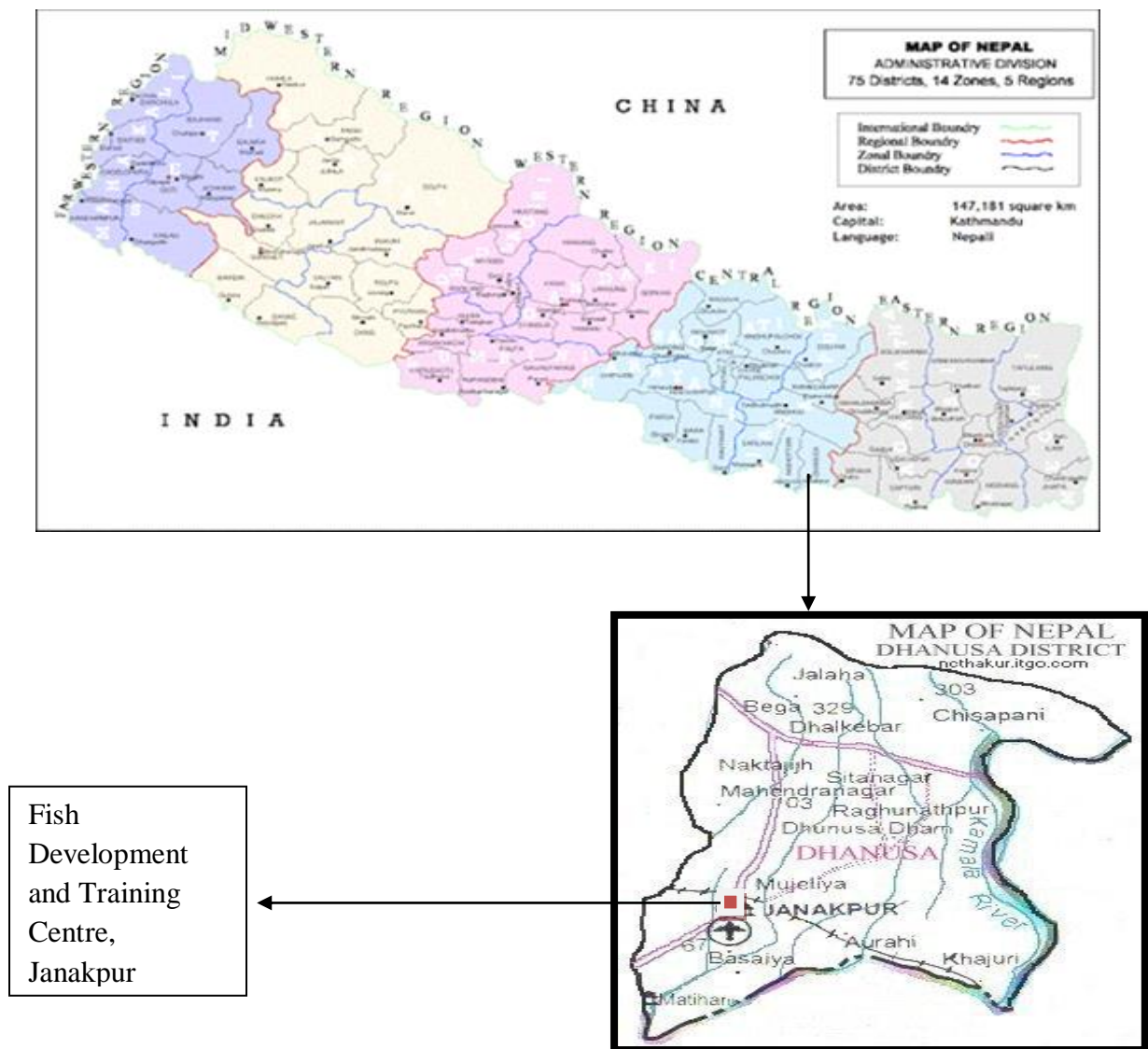


Figure1: Map of study site.

Table 4: The no. of pond and their area in Study site.

S. N.	Name of Ponds	Number	Area (ha)	Used Pond	Unused Pond
1	Brood stock pond	11	2.1	All	-
2	Nursing pond	16	1.0	All	-
3	Rearing pod	28	3.2	18	10
4	Production pond	23	6.5	19	4
5	Other pond	14	0.4	2	12
	Total pond	92	13.2	10.5	2.7

(Sources: FDTC annual progress report, 2071/72)

The brood stock ponds of fish farm ranges between 0.3 to 0.5 hectre sizes. The depth of pond varies between 1.0 to 1.5m (PLATE-1, Photo-2 to 6).

3.2 Study period

The field study was carried out for 4 months from May 2016 to August 2016.

Breeding was conducted from 10 May, 2016 – 24 July, 2016 (*Labeo rohita* and *Cirrhinus mrigala*).

Larval rearing and growth check-up fry was done from 27 May, 2016 – 20 August, 2016.

Table 5 Time table availability of fish seed at Fish Development and Training Centre, Janakpur.

S. N.	Types of Fish	Type of fish seed		
		Hatching	Fry	Fingerlings
1.	Common carp	March- May	April-May	May-June
2.	Silver carp	April-August	April-September	July-November
3.	Bighead carp	April-August	May-August	June-November
4.	Grass carp	April-May	May-June	July-August
5.	Rohu	May-August	June-September	August-February
6.	Naini	June-August	July-August	September-February
7.	Catla catla	July-September	August-September	September-December

(Sources: FDTC annual progress report, 2071/72)

3.3 Physio-chemical Parameters

3.3.1 Physical Parameters

The physio-chemical parameter of water studied during experimental period was as follows:

Nature of day: It was recorded in the field during working only 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° – 35° C with a precision of 0.1° C. The surface temperature was measured by directly dipping the thermometer bulb onto the water body for two minutes and the reading was recorded.

3.3.2 Chemical Parameters

The chemical parameters studied during present study were as follows:

pH: The pH of water in hatchery was measured in study sites by pH meter (HANNA instruments HI 9025 meter kit.). First pointer of pH meter was dipped into the water for two minutes and reading was recorded.

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

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

3.4 Fish species

Labeo rohita and *Cirrhinus mrigala* was selected as the major test fish. *Labeo rohita* popularly known as 'Rohu' and *Cirrhinus mrigala* popularly known as 'Nani' are well known Indian major carp. The fishes were widely cultured in fresh waters along with other carps.

3.5 Collection and Maintenance of brood fish

For conducting breeding experiments on *Labeo rohita* and *Cirrhinus mrigala*, healthy and disease free, farmed reared brooders were selected. The brooders of *Labeo rohita* were 2 to 3+ years of age group and weighing 1600 to 2700 gram. The brooders of *Cirrhinus mrigala* were in the age group of 2.5 to 3.5+ years and weighing 1700 to 4000 gram. For conducting the breeding trails preference was given to select male and female fishes from stock sources. The male and female brooders where stocked in segregation tanks where the stocking was done at 1500-2000 kg/ha. However, the brooders of both the species i.e. *Labeo rohita* and *Cirrhinus mrigala* were stocked. 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 their body weight till spawning (shown in table-6).

Table 6: The feed materials used for brood fish of *Labeo rohita* and *Cirrhinus mrigala*.

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

3.6 Identification of Male and Female Brooders

At the time of breeding the male and female brood fishes were identified on the basis of their secondary sexual characters. The ripe male were identified by roughness of the dorsal surface on the pectoral fin, which on the contrary was very smooth in the female. The roughness in pectoral fins was felt by touching the surface of fin close to the body. The mature male and female fishes were also distinguished from the shape of their body, condition of the vent and secretion of milt in males. The detailed key characters adopted for distinguishing and identification of the mature male and female brooders are explained in table-7.

Table 7 Details of the characteristic features of sexually mature male and female fishes used for induced breeding trials.

S. No.	Characters	Male	Female
1.	Scales	Rough with sandy texture, especially on the flanks and anterior dorsal side and nape.	Scale smooth and silky.
2.	Pectoral fin	Rough with sandy touch. Particularly on the dorsal side. Pectoral fins slightly stouter and longer.	Pectoral fins very smooth and slippery. Slightly smaller in length.
3.	Abdomen	Round and firm. Not very soft to touch.	Bulging out on both sides. Soft and palpable. A distinct cleavage is formed midventrally along the abdomen when fish is placed on its back.
4.	Vent	Elongated slit, introvert (concave), white in colour.	Round silt extrovert (convex) fleshy and pinkish in colour, papillae prominent.
5.	Putting pressure on abdomen	On slight pressure above the vent on the abdomen milky white fluid (milt) runs out through the vent.	pressure slight yellowish discharge or a few ova may come out through the vent. In very mature condition, ova in a chain would slip out on slight pressure.

(Sources: Sharma, 2008)

Usually the medium size fish were selected for breeding trails as they are easier to handle. At the time of selection the brooders were taken one by one in a hand net. After administration of sex hormone the breeding sets of matured brooders were made and kept in breeding tank. The breeding set of fishes comprised of one female and two male fishes of approximately similar size (Malik *et al.*, 2014). The breeding tank, size 2x1x1m was made of cemented having a lid cover over it.

3.7 Hormonal Dose and schedule of Administration

3.7.1 Ovulin

It's a liquid peptide supplement that is used to compress the spawning season, coordinate the spawning time and increase the milt production in males. It composes of each 10 ml contain domperidone 100mg and Salmon-gonadotropine 200µg. Ovulin contain an analogue of S-GnRH and a brain neurotransmitter (dopamine) inhibitor.

3.7.2 Dose and Administration

Injection of Ovulin delivered to the peritoneal (Abdominal cavity) using. Both *Labeo rohita* and *Cirrhinus mrigala* was injected intramuscularly with different dosages of Ovulin according to the body weight per kilogram i.e.

0.5 ml/kg for the female and

0.3 ml/kg of body weight at single dose to the male.

(Nandeeshya *et al.*, 1988)

3.8 Breeding procedure

3.8.1 Collection of brooders for Injection

For inducing the selected fish brooders they were collected from the breeding pond using a hand net made of 4-6 mm meshed net cloth stitched in the shape of a bag. The brooders were then weighed individually along with the hand net using a digital balance.

3.8.2 Hormonal Injection

For injecting the hormonal preparation a hypodermal syringe of 2ml was used with graduation of 0.1ml. The needle used in the syringe was BDH needle number 22. The brooders in the hand net were then brought one by one for injection from the enclosure of net and placed on a cushion. Two persons were required at the time of the injection, one of them held the head of the fish pressed gently against the cushion, while the second person pressed the tail of fish with one hand and with other hand gave the intramuscular injection on the caudal peduncle, above the lateral line (PLATE-II, Photo-3 and 4). After injecting, the brooders were immediately released inside the breeding tank (PLATE-I, Photo-6).

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 1-5 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 *Labeo rohita* and *Cirrhinus mrigala* was observed with the use of Ovulin extract. Fertilized eggs were located in chemically treated incubation tank. Malachite green (2-5 gram in 10 litre water) was treated in water before transferring 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 10 litre water).

Fertilized eggs were kept in floating sieves in the incubation tank for an experiment to study fertility rate and hatching rate. The hatching were given appropriate food just before total absorption of the yolk sacs. They were fed with milk powder by simply scattering it over the water slowly from the edge. This helps the food not be flushed out immediately through the outlet. In the same way, eggs yolk mixed with water were also given thrice a day (one lakh fry fish for one egg).

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, Gonado Somatic Index (GSI), Fertility and Hatching rate

The fecundity, fertility and hatching rate can be determined by following formula, (Kaur and Dhawan, 1997)

3.11.1 Determination of Fecundity rate (%): 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.)

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

Weight of gonads

$$GSI = \frac{\text{Gonadial weight of brood}}{\text{Weight of brood}} \times 100$$

3.11.2 Determination of fertility rate (%): Fertility rate was calculated for every female separately by sampling eggs at the early 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 clear crystals balls whereas the unfertilized ones were dull and opaque. Its fertility was calculated in average of the total sample eggs.

$$\text{Fertility rate}(\%) = \frac{\text{Total no. of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

3.11.3 Determination of hatching rate (%): Hatching number 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.

$$\text{Hatching rate}(\%) = \frac{\text{Total no. of hatching}}{\text{Total no. of fertilized eggs}} \times 100$$

3.12 Study of embryonic development of fertilized eggs

The sample of fertilized eggs was collected for the study of the different embryonic stages of the both fishes. The egg was taken and several time washed into the distilled water. Various stages of egg were preserved in 1% formalin solution and the study was done under a photographic microscope. The photos of different embryonic stages of *Labeo rohita* and *Cirrhinus mrigala* was taken by the microscope camera in the laboratory of Central Department of Zoology, Kirtipur.

3.13 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 were 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 then measured in a measuring cup (at the rate of 50,000 hatchlings per cup). The hatchlings were transferred to the nursery pond for rearing in plastic bags containing sufficient amount of water. Fry were fed 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 parameter

4.1.1 Physical Parameter

The physical parameters had been studied from the sub-surface of water bodies.

4.1.1.1 Nature of Day

During the study period the nature of day was observed, within the 4 months of study period, were recorded cloudy, partly cloudy, rainy and rest days were sunny (clear sky) i.e. positive for the growth of fish.

4.1.1.2 Color of water

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

4.1.1.3 Temperature

The range of temperature of brood pond was 25-30°C in the morning (7-10 a.m.) and 27-32.5°C during day (2-5 p.m.), that of incubation tank was 24- 28°C in the morning (7-10 a.m.) and 27-31°C during day (2-5p.m.) from 10th May to 23rd June and that of nursery ponds was 28-34°C during (7-10 a.m.) and 29-34.4°C during (2-5 p.m.) from May 27th to August 20th. The highest temperature (34.4°C) was recorded at nursery pond in June.

4.1.2 Chemical parameter

4.1.2.1 pH

The pH remained alkaline during whole study period. It ranged from 7 in May to 10.1 in July showing in brood pond. In incubation tank was 7.8-8.6 in the morning (7-10 a.m.) and 8.6 to 8.9 during day (2-5 p.m.) from 10th May to 23rd June and that of nursery ponds ranged from 8.6 to 10.1 from 27th May to 20th August.

4.1.2.2 Dissolved Oxygen (DO)

The range of dissolved oxygen concentration of brood pond was 5.0- 6.2 mg/l during 7-10 a.m. and 6.1 -7.6 mg/l during 2-5 p.m. In incubation tank was 7.5-8.7 in the morning (7-10 a.m.) and 7.8-9.1 during day (2-5 p.m.) from 10th May to 23rd June. In nursery ponds it ranged from 5.4-7.1 mg/l during 7-10 a.m. and 7.3-8.2 mg/l during 2-5 p.m. from 27th May to 20th August. The concentration of DO increased considerably from 7 a.m. to 5 p.m.

4.1.2.3 Free CO₂

The range of free CO₂ (mg/l) of brood pond was 14.2-17.1 mg/l in incubation tank it was 13.7 and that of nursery pond was 14.6-15.4 mg/l.

Table 8 Physico-chemical parameter of brood ponds, incubation tank and nursery ponds.

Average data →	Temperature (°C)		pH		DO (mg/l)		Free CO ₂ (mg/l)
	7-10 a.m	2-5 p.m	7-10 a.m	2-5 p.m	7-10 a.m	2-5 p.m	
Brood ponds	25.0-30.0	27.0-32.3	7 – 10.1		5.0-6.2	6.1-7.6	14.2-17.1
Incubation tanks	24-28	27-31	7.8-8.6	8.6-8.9	7.5-8.7	7.8-9.1	13.7
Nursery ponds	28-34	29-34.4	8.6 – 10.1		5.4-7.1	7.3-8.2	14.6-15.4

4.2 Fecundity and Gonado Somatic Index (GSI)

Table 9 Fecundity and GSI of *Labeo rohita* by using Ovuline Hormone in the different day at Janakpur Fish hatchery. The weight of each fish was measured.

S. N.	Weight of female (kg)	Fecundity	Weight of total eggs spawned (gm)	GSI (100%)
1	2.7	303696	304	11.25
2	1.9	270200	280	14.73
3	1.9	261226	286	14.11
4	2.5	316384	312	12.48
5	1.6	233203	247	15.45

Table 10 Fecundity and GSI of *Cirrhinus mrigala* by using Ovuline Hormone in the different day at Janakpur Fish hatchery. The weight of each fish was measured.

S. N.	Weight of female (kg)	Fecundity	Weight of total eggs spawned (gm)	GSI (100%)
1	3.5	609000	406	11.6
2	2.3	423200	288	12.52
3	4.0	768000	421	10.52
4	3.1	514600	332	10.70
5	2.8	492800	307	10.96

GSI showed little higher in *Labeo rohita* i.e. 15.45 than *Cirrhinus mrigala* i.e. 12.52.

4.3 Fertility Rate (%) and Hatching Rate (%) of *Labeo rohita* and *Cirrhinus mrigala*

Table 11 Fertility Rate (%) and Hatching Rate (%) of *Labeo rohita*

No. of sample eggs	No. of viable and fertilized eggs	Fertility rate (%)	Hatching (%)
208	179	86.05	82.68
212	170	82.07	75.29
72	56	77.77	80.35
120	106	88.33	78.30
137	117	85.40	80.34

Table 12 Fertility Rate (%) and Hatching Rate (%) of *Cirrhinus mrigala*

No. of sample eggs	No. of viable and fertilized eggs	Fertility rate (%)	Hatching (%)
205	168	81.95	72.61
214	176	82.24	67.04
76	54	71.05	70.37
115	92	80	60.86
183	140	76.05	79.28

Fertility rate almost same by hatching % was more in *Labeo rohita*.

4.4 Embryonic Development of *Labeo rohita* and *Cirrhinus mrigala* fertilized eggs

The eggs of *Labeo rohita* and *Cirrhinus mrigala* were observed under a light sources microscope X10 X10 magnifications and made in Japan by sony company 32 megapixel hand camera. The observations were continuous from the time of fertilization till the completion of segmentation and thereafter at 8hr intervals till hatching.

After hatching observation were made at 8hr, 24hr, 48hr,72hr, 96hr and 120hr for this specimens freshly preserved in 1% formalin were used (PLATE-IV, V, VI and VII).

Table 13 Embryonic development of fish.

Fertile egg	The fertilized eggs were found floating, dispersal and brownish-yellow in colour. The average diameter of the egg was 0.8 ± 0.01 mm. Slight swelling was observed immediately after the fertilized eggs.
New hatching	Newly hatched larvae were slender, straight and transparent, gradually tapering towards the tail. Larvae silver in colour and the yolk sac attached to the body. The length of larvae was 2.5 ± 0.01 mm in size.
Eight hrs post hatching	The yolk sac partially reduced. The tail become thickened. The length of larvae was 2.9 ± 0.02 mm in size.
24 hrs post hatching (One day)	Operculum becomes visible and dark eyes pigmented. The yolk sac become thinner prominent pectoral and pelvic fins fold. At this stage length of larvae was 4.4 ± 0.02 mm in size.
48 hrs post hatching (Two day)	Colour of larvae changed to silver-yellowish and mouth cleft formed. The eyes increase in size and pigmentation. Pectoral fin fold well developed. Yolk sac convex interiorly and air bladder distinct. Large black chromatophores observed on head. The length of larvae reached 5.3 ± 0.02 mm in size.

72 hrs post hatching (Three day)	The larvae were silver-blakish and transparent in colour. Larvae swim actively. Yolk sac completely disappeared and larvae started feeding. Eyes fully pigmented. The larvae reached to 6.0 ± 0.02 mm in size.
96 hrs post hatching (Four days)	Lips well developed, the lower one being thicker and slightly shorter. Ventral fin-fold originates from about $3/4^{\text{th}}$ the length of air bladder from the anterior end. Dorsal fin fold starts a little behind the air bladder. Total length 6.6 ± 0.02 mm in size.
120 hrs post hatching (Five day)	The upper jaw is slightly longer than the lower jaw. Differentiation of dorsal fin has started, two pigmented striations appear. A small patch of dark pigments is seen below the tip of the notochord. Caudal fin with 12 rays, three rays, on either side being unbranched. Total length 7.1 ± 0.02 mm in size.

4.5 Growth study up to fry stage

4.5.1 Growth of length and weight of Hatchlings

5 day after hatchlings transfer to the nursery pond, the fry were feed with artificially formulated feed with 40-45% protein at the rate of 5-10% body weight and the growth checkup of *Labeo rohita* and *Cirrhinus mrigala* was done at weekly intervals.

4.5.2 Growth check-up of fry up to 45 days

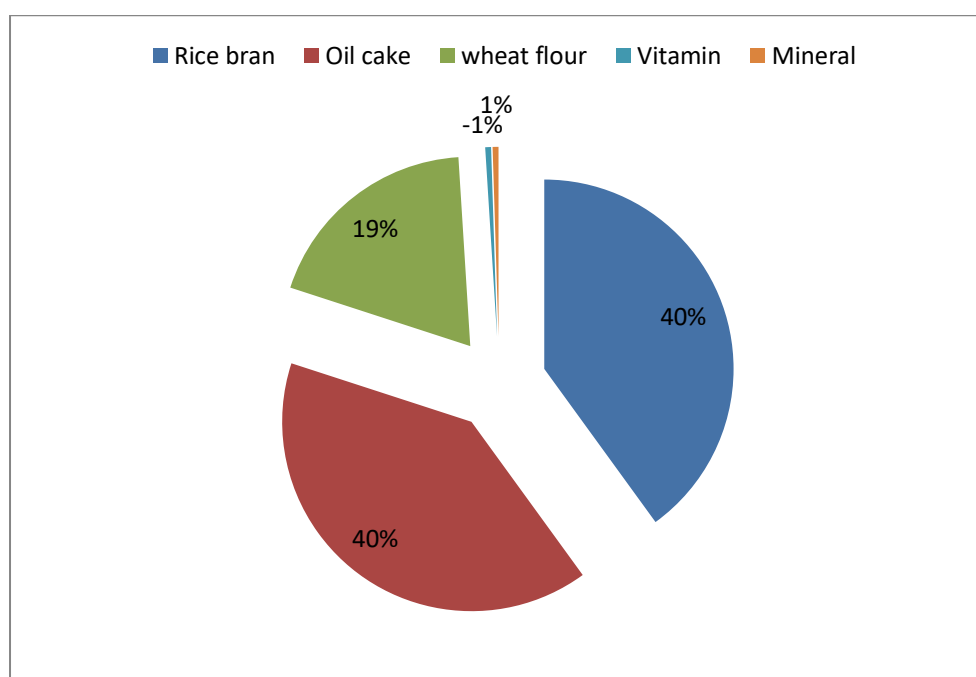


Figure 2 Composition of feed for fry rearing up to 45 days in percentage.

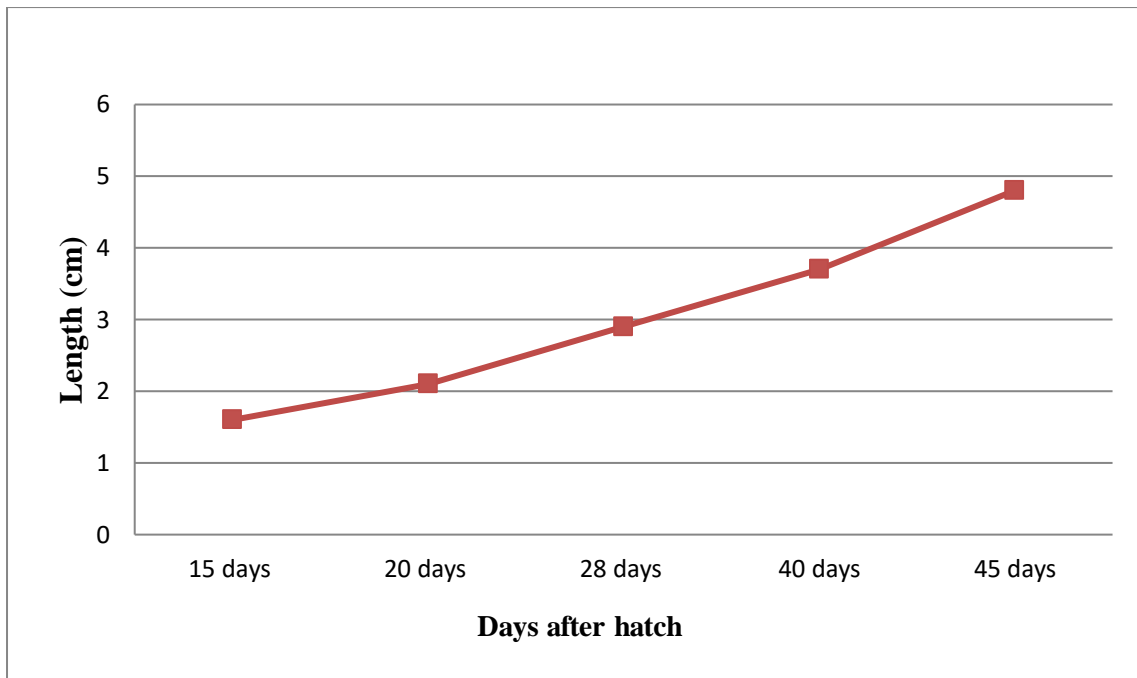


Figure 3 Growth Performance (Length) in the fingerlings of *Labeo rohita* under the influence of commercial feed at regular intervals (PLATE: VIII).

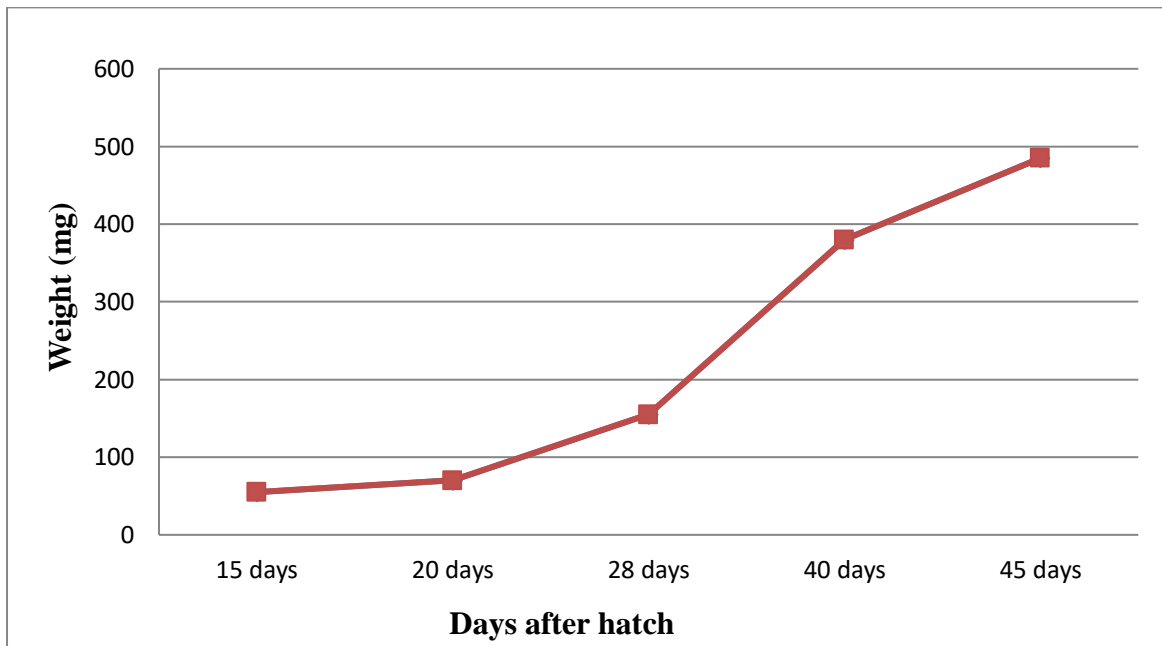


Figure 4 Growth Performance (Weight) in the fingerlings of *Labeo rohita* under the influence of commercial feed at regular intervals (PLATE: VIII).

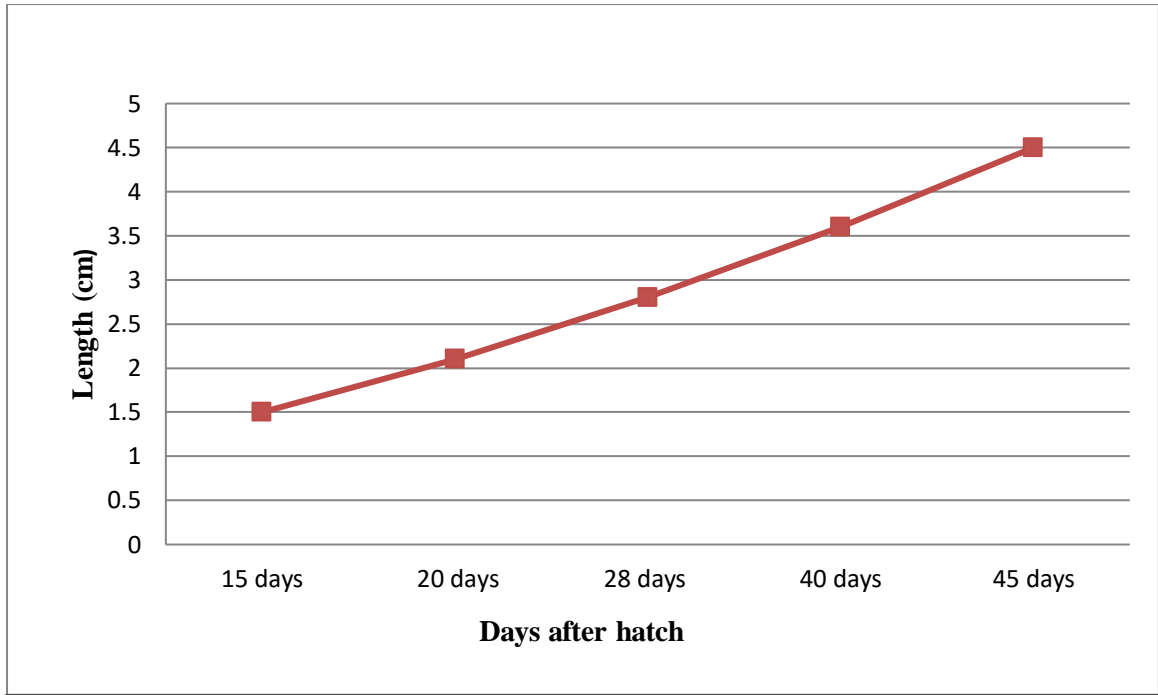


Figure 5 Growth Performance (Length) in the fingerlings of *Cirrhinus mrigala* under the influence of commercial feed at regular intervals (PLATE: IX).

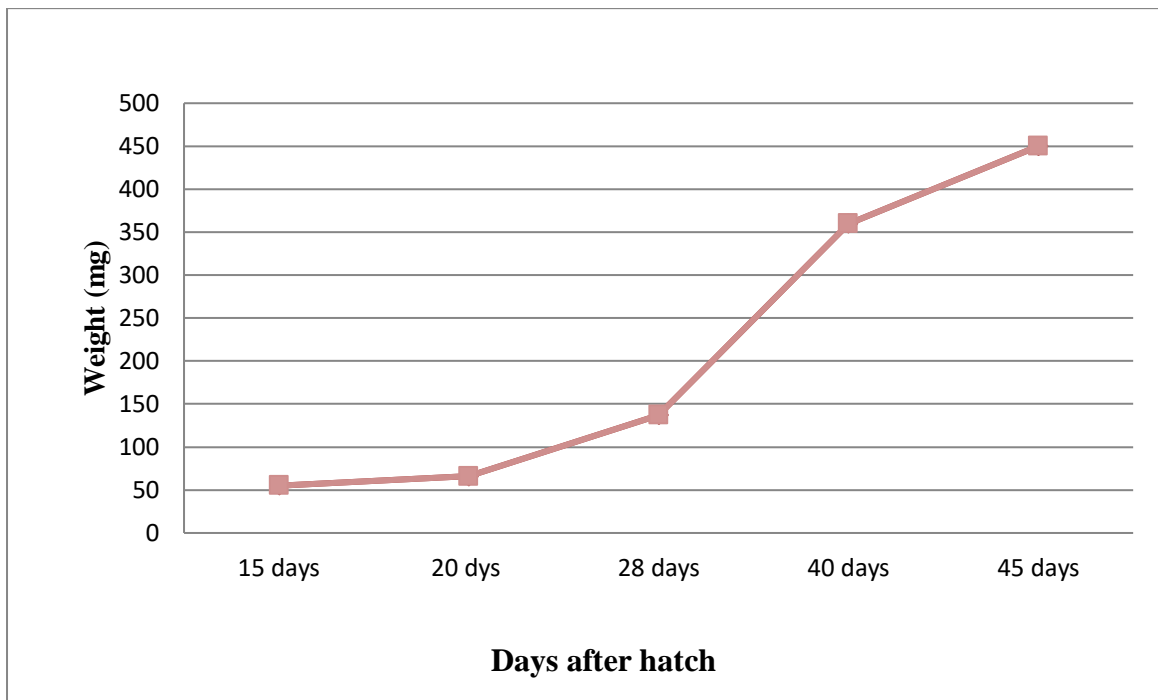


Figure 6 Growth Performance (Weight) in the fingerlings of *Cirrhinus mrigala* under the influence of commercial feed at regular intervals (PLATE: IX).

5 DISCUSSION

Physico-chemical parameters of water play a significance role in the biology and physiology of fish. During the present study 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.

5.1 Latency time

In my study the response (latency) time was recorded 8 to 10 hrs in both species (*Labeo rohita* and *Cirrhinus mrigala*) with 0.5 ml/kg of Ovulin, which was early than those of Ovatide, Ovaprim and Pituitary extract.

Saini (2001) observed a 9 hrs response time in *Cirrhinus mrigala* induced with ovaprim where induced with ovatide have shown a response time of 13hrs. Jhajhari (2002) reported a response time of 12:30 hrs and 25:10 to 70:00 hrs in *Cirrhinus mrigala* and *Cyprinus carpio*, respectively induced with ovaprim at a dose of 0.50 ml/kg during trails conducted in the year 2000. Mishra *et al.*, (2001) found that ovatide can be used successfully in a much more cost effective way for induced breeding of carps. In *Cirrhinus mrigala* the anothers noted an average response time of less than 6 hrs with 0.5 ml/kg of ovatide.

5.2 Fecundity, Fertility, Hatching and Embryology study of Fish

Induced breeding were successfully done in carps by using various spawning chemicals namely, Fish pituitary extract, Human chorionic gonadotropin, ovaprim (GnRHA) etc. In present study, Ovulin was used to induce final maturation and spawning in *Labeo rohita* and *Cirrhinus mrigala*. The fecundity, in present study, ranged from 233203 -316384 in *Labeo rohita* and 423200-768000 in *Cirrhinus mrigala* according to their body weight. The fertility rate was fairly good (77.77-88.33%) in *Labeo rohita* and (71.05-82.24%) in *Cirrhinus mrigala* but the hatching rate was recorded good (75.29-82.86%) in *Labeo rohita* and (60.86-79.28%) in *Cirrhinus mrigala* in present study.

The adhesive egg was reported in a single spawning season by each female 226000-794000 in *Labeo rohita* and 100000- 150000 in *Cirrhinus mrigala*, depending upon the fish size and ovary weight. (FAO, 2006). Naheem *et al.*, (2015) reported the induced breeding of *Labeo rohita* through single application of ovaprim-c. Fish species were induced with ovaprim at a dose of 0.4 ml/kg. They reported the percentage of fertilization 77.50% and hatchling 81.39% respectively, hatchling occurred within 18-30 hr after fertilization. These results were comparatively similar as compare to the finding of present study.

Pandey *et al.*, (2002a) attempted induced spawning in the Indian major carps *Labeo rohita* with carp pituitary extract in comparison to ovatide and found fertilization percentage of 81.25 and 97.00 respectively. These results were comparatively good as compare to the finding of present study. Pandey *et al.*, (2002b) reported a hatching success of 95 to 98% in *Cirrhinus mrigala* induced with ovatide at 0.4 ml/kg body weight. However, with the use of carp pituitary extract 2 mg/kg as primary dose and 4 mg/kg after 12 hrs, the hatching success 70 to 78% in *Labeo rohita* (pandey *et al.*, 2002) beside a hatching percentage of 90 to 98 % with the use of ovatide (0.4 ml/kg body weight of female).

More *et al.*, (2010) Compared the spawning response of ovaprim compared with pituitary extract in Indian major carps. They reported the percentage of fertilization (88.11-97.94%) was found higher with ovaprim treatment than with pituitary extract treatment (53.19-85.48%). Similarly, the percentage of hatching (74.70-95.92%) was reported higher with ovaprim treatment than with pituitary extract treatment (60-58.82%). The embryological study showed time for hatching of common carp more than other carps (Chinese carps and Indian Major carps) because of its little thicker egg shell than other carps.

Dhawan and Kaur (2004) reported a relative fecundity of 62500 and 100805 in female fish of *Cirrhinus mrigala* induced with ovaprim and ovatide respectively at a dose of 0.5 ml/kg. The fertilization percent was found to be 61.48% and 83.65% with ovaprim and ovatide respectively; however, these results are in contrary to the findings of present study where ovulin was found better inducing agents. Mishra *et al.*, (2001a) observed a relative fecundity of 85526 eggs/kg fish induced with 0.5 ml/kg of ovatide. This number similar when compared to the results of relative fecundity of *Cirrhinus mrigala* in the present study.

Jhajhria (2002) induced breed *Cirrhinus mrigala* using 0.5 ml/kg of ovaprim and found a hatchery percentage of 75-85% with the use of ovatide at 0.30 ml/kg for induced breeding of *Cirrhinus mrigla*, the hatchling percentage remained 88 to 90%. This fertilization percentage with ovatide was comparatively high in control of present study. Lakshman *et al.*, (1967) Found a survival percentage of 53.52% in *Cirrhinus mrigala* after 15 days rearing. Tripathi *et al.*, (1979) obtained a high survival of over 80% in carp spawn maintained on a mixture of fish meal, groundnut oil cake and rice bran in 1:1:1 ratio. Saini (2001) while rearing *Cirrhinus mrigala* spawn, produced with the help up ovaprim and ovatide found a survival of 76% in the seed which was reared in outdoor ponds whereas, 86% survival was noted in indoor cement cistern. He observed a length of 3.0 cm in *Cirrhinus mrigala* fry after 30 days of rearing with weight gain of 9.82 gm when the fry were produced by induced breeding using ovaprim. However, the fry produced with a use of ovatide have shown relatively less growth having a length of 2.27 cm and weight of 0.780 gm after a rearing period of 30 days.

In the present study, it is reported the embryonic development of *Labeo rohita* and *Cirrhinus mrigala*. The fertilized eggs were round, transparent, demersal. The colour of fertilized eggs was brownish-yellow. Average diameter of fertilized eggs of *Labeo rohita* was 0.8 ± 0.01 mm which was more or less similar to the finding of Miath *et al.*, 2009 who noted the diameter of fertilized eggs was 0.7mm. The length of the newly hatched larvae of *Labeo rohita* was found to be 2.5 ± 0.02 mm. But Maith *et al.*, (2009) found the length of newly hatched larvae of *Labeo bata* was 2.0 ± 0.01 mm which has little close with the present study. At larval stage, operculum appeared 24 hrs after hatching, pectoral fin was prominent and air bladder was visible which were similar to *Labeo bata* (Math *et al.* 2009) and *Cirrhinus mrigala* (Khan, 1943). The yolk sac of the *Labeo rohita* was completely disappeared 72 hrs after hatching, where as Maith *et al.*, (2009) and Chakraborty and Murty (1972) found same development in *Cirrhinus mrigala* within 72 hrs which has the similarity with the findings of this experiment.

5.3 Growth rate of Fingerlings

The length weight relationship of fishes provide the information on its growth and well-being of fish in the surrounding aquatic environment. In the present study, a single intramuscular injection of synthetic hormone, Ovulin resulted in successful spawning of *Labeo rohita* and *Cirrhinus mrigala*. The results of the current work in the hormonal stimulation are similar to the effectiveness of both fish. The length-weight relationship of correlation coefficient (r) for *Labeo rohita* (0.966) and for *Cirrhinus mrigala* (0.961). According to Mir *et al.*, (2014) length-weight relationship of experiment value of 0.978 in Male and 0.958 in Female for *Labeo rohita* from Ganga river. 0.664-0.903, 0.741-0.880 and 0.819-0.967 for Catla, Rohu and Mrigala, respectively in the different length-weight group by Ujjania *et al.*, (2012). The comparative increase in length-weight as compared to length-weight is therefore, most in *Labeo rohita* and least in *Cirrhinus mrigala*. Khan and Hussain (1945) also found that length-weight of *Labeo rohita* is heavier than *Cirrhinus mrigala*.

Sah (2012); Banjade (2015) was reported normal growth rate of *Cyprinus carpio* by using artificially formulated feed with 45% protein rate. The common carp and silver carp showed rapid growth in artificially formulated diet and similar outcome reaching 10 to 13 centimeters in length within the first year was reported by Steiner (2000), Spataru *et al.*, (1980) and Schroeder (1983) reported common carp and silver carp naturally depended on plankton and benthic macro invertebrates but when artificial food is applied, they will readily accept artificial feed. When artificial food was supplied with plankton and benthic macro invertebrates. The gut contents of common carp suggested that common carp preferred artificial feed to benthic macro invertebrates.

6. CONCLUSION AND RECOMMENDATIONS

The present work deals with induced breeding of *Labeo rohita* and *Cirrhinus mrigala* to study the synthetic efficiency of Ovulin.

6.1 CONCLUSION

The synthetic hormone ovulin has been found to be advantageous for induced breeding of *Labeo rohita* and *Cirrhinus mrigala*. The hormone used in the present study gave better economic benefits in terms of greater number of surviving span per unit weight of fish. Further, the ovulin was found more cost effective than the other hormones, in both fish. Using the ovulin resulted in significantly increased survival and growth of spawn during rearing period in *Labeo rohita* and *Cirrhinus mrigala*. The synthetic efficiency of ovulin has been revealed by best breeding efficiency i.e. significantly better response time and higher ovulation, number of eggs produced, relative fecundity, ova diameter, fertilization, hatching and survival percentage of spawn, were recorded in both fishes i.e. *Labeo rohita* as well as in *Cirrhinus mrigala*.

6.2 RECOMMENDATIONS

- The water quality should maintain within a congenial range to the best possible way for better breeding performance, survival and growth of the spawn produced.
- Induced breeding of *Labeo rohita* and *Cirrhinus mrigala* may be more beneficial preferably with the use of ovulin in combination of other hormone.
- The breeding should be discontinued to improve the fecundity and GSI rate.
- Further study on survival rate of hatching's in nursery ponds should be done.
- Training, workshop, seminar should be organized both at campus National and International level to bring this industry globally competent by GN, NGO, INGO.

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PLATE - I

Fish Development and Training Centre of Janakpur



Photo: 1 Gate of FDTC



Photo: 2 Hatchery of FDTC



Photo: 3 Brood stock pond of FDTC



Photo: 4 Incubation tank at FDTC



Photo5 Holding brood fish at spawning tank



Photo: 6 Brood fish in spawning tank

PLATE – II
Methods of Induced Breeding at FDTC, Janakpur.



Photo: 1 Selection of brood fish



Photo: 2 Weighting of brood fish



Photo: 3 Taking injection for fish



Photo: 4 Fish were injected



Photo: 5 Collection of fertile eggs



Photo: 6 Fertile eggs put in incubation tank

PLATE – III

Showing Feeding and Rearing at FDTC, Janakpur.



Photo: 1 Brood fish Naini



Photo: 2 Discussion during field



Photo: 3 Interaction with fish feed



Photo: 4 fingerlings in oxygenated air bag for transfer



Photo: 5 Oxygen packed fingerlings for selling.



Photo: 6 Solution of potassium permanganate.

PLATE - IV

Embryonic development of *Labeo rohita*.

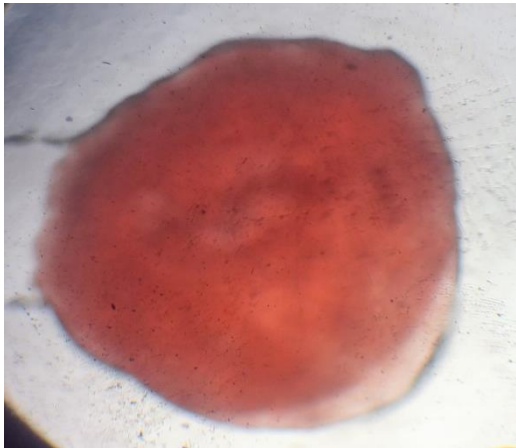


Photo: 1 Fertilized egg of Rohu. X100

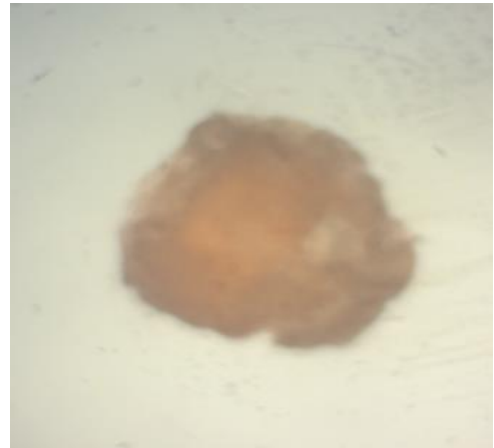


Photo: 2 Four hrs embryo of Rohu. X100



Photo: 3 New hatching of Rohu. X100



Photo: 4 Eight hrs hatching of Rohu. X100

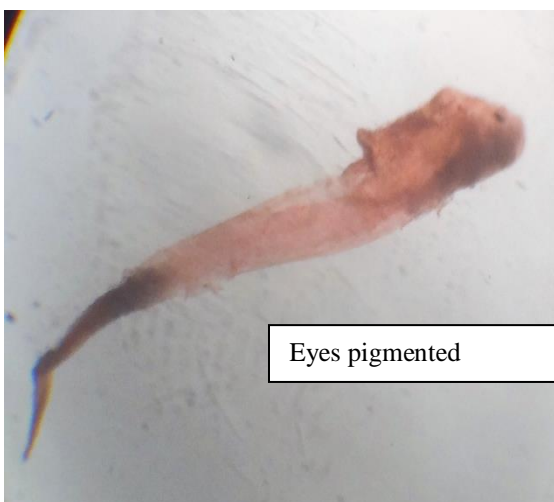


Photo: 5 One day hatching of Rohu. X100



Photo: 6 Two day hatching of Rohu. X100

PLATE - V
Embryonic development of *Labeo rohita*.

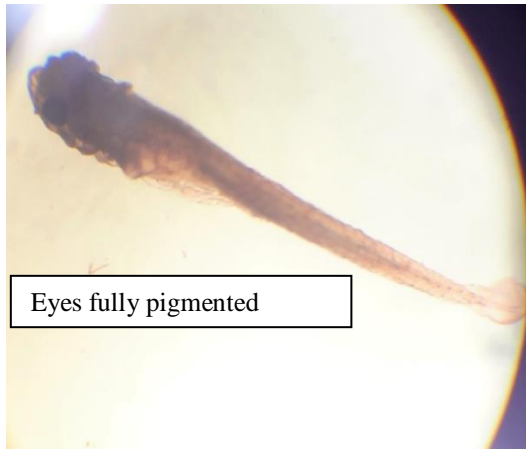


Photo: 1 Three day hatching of Rohu.X100

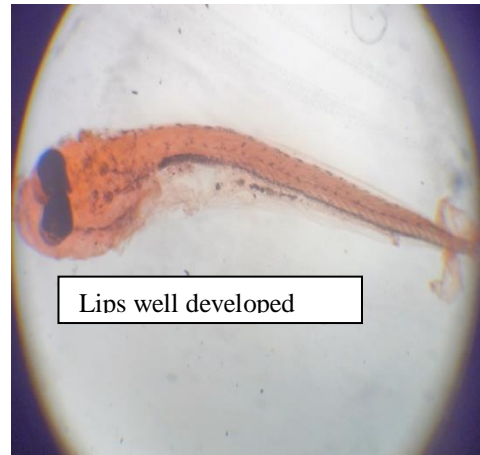


Photo: 2 Four day hatching of Rohu.X100



Photo: 3 Five day hatching of Rohu. X100

PLATE - VI
Embryonic development of *Cirrhinus mrigala*.

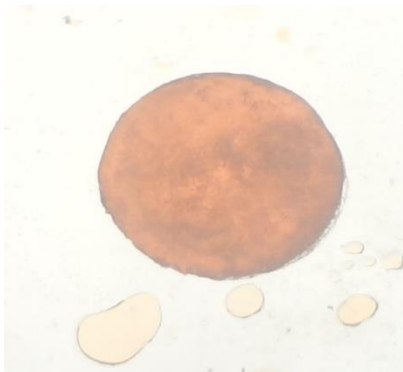


Photo: 1 Unfertilized egg of Naini. X100

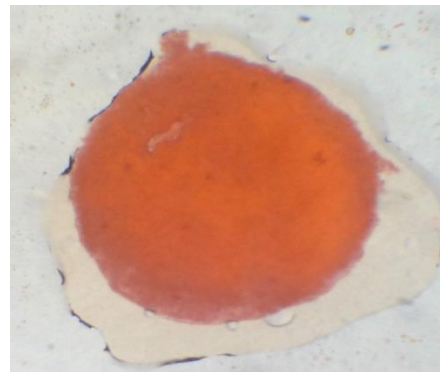


Photo: 2 Fertilized egg of Naini.X100



Photo: 3 Four hrs embryo of Naini. X100

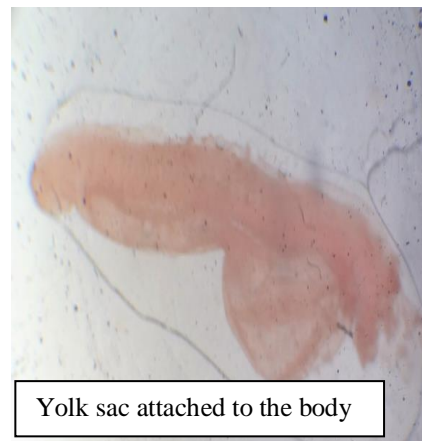


Photo: 4 New hatching of Naini. X100



Photo: 5 Eight hrs hatching of Naini. X100

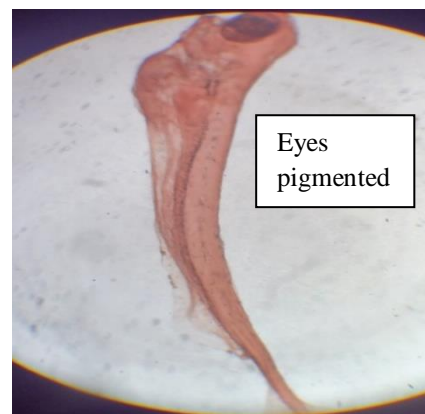


Photo: 6 One day hatching of Naini.X100

PLATE - VII

Embryonic development of *Cirrhinus mrigala*



Photo: 1 Two day hatching of Naini. X100



Photo: 2 Three day hatching of Naini.X100

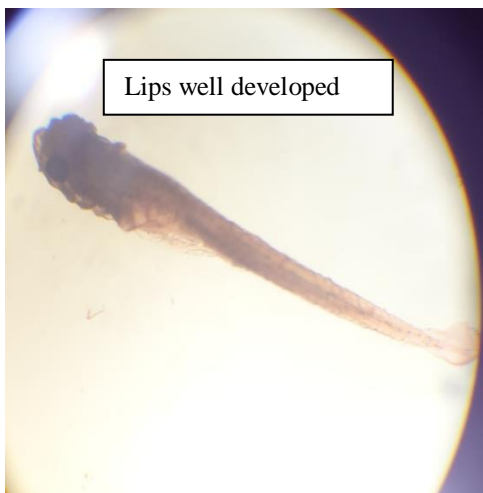


Photo: 3 Four day hatching of Naini.X100



Photo: 4 Five day hatching of Naini.X100

PLATE: VIII
Growth rate of *Labeo rohita*



Photo: 1 Twenty day after hatching.



Photo: 2 Twenty Eight day after hatching.



Photo: 3 Fourty day after hatching.



Photo: 4 Fourty Five day after hatching.

PLATE: IX
Growth rate of *Cirrhinus mrigala*



Photo: 1 Twenty day after hatching.



Photo: 2 Twenty eight day after hatching.



Photo: 3 Fourty day after hatching.



Photo: 4 Fourty Five day after hatching.