CHAPTER-1

INTRODUCTION

In the recent years, the alacrity of rearing and fish keeping in aquarium has become a conspicuous passion and major hobby among millions of people around the globe. To many enthusiasts, the view of these beautiful creatures swimming peacefully in the aquarium is a pure sense of enjoyment and mental satisfaction and also adds the aesthetic scenario of a house (Arindam et al., 2018). Dating back to several centuries this hobby has a long history, started from the introduction of civil aviation and after the Second World War expanded the hobby to a global industry (Tissera, 2010). It is considered as one of the important commercial activity and this sector is a small even though the vital part of an international fish trades, contributing positively to rural development in many developing countries of this glove (FAO, 2010). The ornamental trade also contributes multimillion-dollar industry in aquarium business such as tanks, filter systems and other accessories (Monticini et al., 2010). To a great expanse, the network of the ornamental fish trade is highly complex and dynamic, associating of two million people worldwide, collectors to hobbyists including governments, airlines etc. According to FAO (2006), the ornamental species trade constitutes only 0.5% of the international fish trade. However, its significance goes beyond to share the international market. The export of live fishaccelerated in value from USD 21.5 million in 1976 to USD 315 million in 2007 (Monticini et al., 2010). However, this sector contributes to provide income and employment in developing countries in the world (Domínguez and Botella, 2014).Though the ornamental fish trade is dominated especially by freshwater species, the increasing popularity of coral reef fishes has become a leading circumstance since the late 1980's and also the prices have become increasingly affordable for European and American markets (Olivier et al., 2001). Although the popularity of household aquariums is increasing, ornamental fishes belongs less than 1% to public aquaria sector and the rest remain confined to hobbyist (Dey, 2016). Organized trade in ornamental fish depends on assured and adequate supply of demand, which is possible only by mass breeding (FAO, 2000). The earning potential of this sector has hardly been understood and the same is not being exploited in a technology driven manner. Considering the relatively simple

technique involved, this activity has the potential to create substantial job opportunities, besides helping export earnings (FAO, 2000). The present economic crisis may lead to a major drop in this business, which until now has been growing and developing rapidly (Domínguez and Botella, 2014). Southeast Asia in particular Singapore shares with a 20% of the aquarium fish trade where Europe shares 29% and the biggest importers are USA, Japan, Germany, Netherlands and Italy (Monticini et al., 2010).The economic crisis in the sector arrived from late 2008, with a great decrease in the volume of trade and its value also. The socio-economic consequences in exporting and importing countries remain unrevealed and should be important, especially for rural communities in exporting countries (Monticini et al., 2010).

In Bangladesh, the trade of ornamental fish is limited to its own territory till now. Moreover, it seems costing a lot of money each year importing ornamental fish to meet the demand of the country. There are lots of scopes of domestic production of ornamental fish which may save this money and apparently add the export earnings (Mahmud et al., 2012). The present study was undertaken to develop the induced breeding technique by using synthetic ovulin hormone for oranda goldfish (*Carassius auratus*) in context of Bangladeshi environment. Now-a-days the availability of fish fry is pre-requisite for fish culture in our country. Without the artificial propagation of fish culture is hardly possible. Once, our country was totally dependent on fish seed production from the natural water resources but the production of fish seed in natural water-bodies has been reduced gradually due to natural and anthropogenic effects. Hypophysation technique of carps was initiated in 1967 along with the collection of spawn from river (Sarder, 2007). The induced breeding technique is an appropriate way for artificial propagation and mass breeding and the main objectives of induced breeding is to ripening fish gonad to breed in captive condition using stimulating agent and during off spawning season. Administration of the pituitary extract from mammal and/or fish or use of synthetic hormone is a common practice of fish induced breeding (Sudha, 2012). Administration of hormonal stimulation in domesticated cyprinids and in some other fish, such as perch or burbot, increases the rate of ovulation in females, helps synchronize spawning and increases the amount of eggs produced by a female (Krejszeff et al., 2009, 2010). Along with the succession of fish seed production through artificial propagation techniques, the

Government of Bangladesh (GoB) established a number of carp hatcheries in the public sector in different parts of the country with the course of time and a remarkable number of hatcheries has been established in private sector (Mahmud et al., 2012), but it is a matter of sorrow that very few ornamental hatcheries has been established in public sector yet although the ornamental fish demand is increasing day by day.

The knowledge of artificial breeding is a key attribute as it allows intensive seed production of a given species in controlled conditions. Only a reliable induced breeding and larvae rearing technique can supply the quality fish seeds (Mollah et al., 2008). According to Balon (2004), goldfish (*Carassius auratus*) is a domesticated fish. It is a medium-sized fish and regarded as a model representative of cyprinids (Sokołowska et al., 1934; Bandyopadhyay et al., 2005) for assay regarding with its reproductive biology (Stacey et al. 2001), genetics (Yuen et al., 1997), physiology (Kobayashi et al., 2002), toxicity (Szczerbik et al., 2006) and immunology (Wang et al., 2006). The goldfish is becoming important aspects in fisheries science rapidly not only as an ornamental fish trade but also as experimental test animals because of its adaptive capability to fluctuating environmental conditions (Helen and Battle, 1940). Goldfish were originally reared and developed by the Chinese and by the 1500's; goldfish were first traded to Japan. By the 1600's and 1800's goldfish traded in Europe and America. The fancy goldfish were developed by Asian breeders (David, 2005). According to Mohanta et al., (2008), there are more than 100 varieties of goldfish appeals to a wide range of aquarium fish lovers. The oranda goldfish (*C. auratus*) is one of the oldest and captive bred fancy goldfish variety belongs to cyprinidae family as there are no wild populations of this fish (Mohanta et al., 2008). It is not listed in the IUCN red list so that, without proper management this variety of gold fish may become extinct as a result of inbreeding depression or other problems (Andrews, 2002). It is one of the most popular goldfish in the world and valued for its egg-shaped body and hood, a fleshy growth on the top of its head called the wen which starts to show at about 3-4 months, but only really begins to form at about 1-2 years and finally developed when the fish gets to be about 2-2.5 years old (Geoff and Nick, 2004). This beautiful goldfish has a large, round shape, shimmering scales, all of their fins are paired except the dorsal fin and a long flowing split caudal fin.

They are available in a wide variety of colors, including red, black, calico, chocolate, red/white combinations (Andrews, 2002). They prefer temperature and pH for survival ranged 18.3-22.2°C and (6.0-8.0) respectively. Generally, they reach about 5-18cm in 1 year and usually mature in their second year but this varies with diet, water temperature, and other environmental influences (Street, 2002). They are batch spawners, spawn more than 2000-10,000 eggs per spawning season and majority of spawning occurred March to October (Marshall, 2003).

Plate 1: Oranda goldfish in aquaria

Hormonesused in induced breeding of fishesinclude pituitary extract, deoxycorticosterone acetate (DOCA), ovaprim, ovulin, ovatide, human chorionic gonadotropin (HCG), ovopel, dagin and dquaspawn (Brzuska 2004, 2005; Zohar & Mylonas, 2001). Among them ovaprim, ovopel and aqua spawn are becoming popular day by day and considered to be efficient in successful spawning infishes because these hormones containing especially GnRH and dopamine blocker receptor (Hassan et al., 2018). GnRH stimulates the synthesis and release of pituitary gonadotropins, follicle stimulating hormone and luteinizing hormone to control gametogenesis and sex steroid hormone production (Zohar et al., 2010). To date, 15 different forms of GnRH have been identified from vertebrates (Zhao et al., 2017) and two or three (Hypophysiotropic GnRH or GnRH1, chicken GnRH-II or cGnRH2 and salmon GnRH or sGnRH3) from a single vertebrate species (Shahjahan et al., 2010). Ovulin is a newly available synthetic inducing

agent that is in ready-made form manufactured by Ningbo Sansheng Pharmaceutical Company Ltd. China. Each 10 ml vial contain 100mg domperidone and 0.2mgs GnRH analogue. It is a peptide supplement that is used to compress the spawning season. sGnRHa contains an analogue of sGnRH and a brain neurotransmitter (dopamine) inhibitor. Dopamine antagonists are used for stopping of dopamine activity which acts as an inhibitory factor for the synthesis of gonadotropin (Naeem et al., 2005). Chang et al., (1983) and Chang and Peter (1983a) manifested that, dopamine has GTH release-inhibitory activity in goldfish to control spontaneous release of GTH, and to directly prevent the action of GTH-releasing hormone. Chang and Peter (1983b) also reported that pimozide, a dopamine antagonist, having significant potential effect on the GTH releasing activity of LRH-Aa in goldfish, causing both oocyte maturation and ovulation in goldfish. The GnRH in compound sGnRHa elicits the release of stored gonadotrophins from pituitary. It is injected to the peritoneal cavity using standard needle and syringe. The manufacturer's recommended dose of ovulin is 0.5mg/kg body weight in female and half dose in male fishes.

Plate 2: Ovulin hormone in vial

There have been wide varieties of research works done on various aspects of *Carassius* species worldwide (Spieler et al., 1977; Peňáz et al., 1979; Salas et al., 2006; Tsoumani et al., 2006). In Bangladesh, there only single work has been done so far which focused only on induced breeding, embryonic and larval development of comet goldfish *Carassius auratus* (Mahmud et al., 2012). As there are no reports regarding induced

breeding of oranda goldfish. Some important facts need to be considered in the artificial propagation of goldfish, such as brood stock development, larval management, environmental conditions, water management, larval nutrition and disease control (Pramod et al., 2017). This study is an attempt to establish appropriate technique of induced breeding by administrating synthetic ovulin hormone.

1.1 Aims and objectives of the study:

- To establish artificial propagation technique of oranda goldfish, *C. auratus*
- To determine the optimal dose of ovulin hormone for oranda goldfish (*C. auratus*) breeding
- To determine fertilization rate, hatching rate and larval survival rate of hormone treated oranda goldfish (*C. auratus*) induced breeding

CHAPTER- 2

LITERATURE REVIEW

Prior to conduct any experimental study, it is essential to know about the previous related research work which helps to gain a better understanding of the study while assaying. There are no published research work related with induced breeding of oranda goldfish (*C.auratus)* and use of ovulin hormone in ornamental fish breeding. However, the following information was reviewed in favor of the study which undertaken around the world and similar to research.

In our country, most of the aquarium fishes are exotic and only a few native fish species e.g. rani fish (*Botia* sp.) considered as ornamental fish. Among the exotic species only common goldfish and comets successful induced breeding are being managed in Rakamari Hatchery of Feni district, under Chattogram division (Rezwanul, 2013).

Ruhul et al., (2013) investigated over 136 females for 12 months to determine the presence of year around goldfish gravid female and egg diameter. Highest percentage of gravid female was found in December (87.5%) where lowest in November (69.23%).The mean of the month wise average egg diameter except June to August, varied (0.03- 1.55 mm) from October to March. Their study stated that, goldfish (*Carassius auratus*), breeds within September to March in freshwater and laboratory condition and spent fish first appears from April to May and no gravid female found from June and July.

Helen and Battle (1940) found that, the eggs of the goldfish are spherical pale cream-colored globules with 1.25 to 1.46 mm. diameter and slightly flattened along the margin of attachment side. At the initial of laid eggs, the whole surface was adhesive, but that quality was lost due to water hardened and attachment to aquatic plants. The incubation period of egg was varied with the temperature variations. Eggs hatched in 3 to 4 days which were kept in temperature range of 18.5°- 29.5° C while at 24°-28°C required only 64 to 72 hours to hatch and at a constant temperature of 25° C, hatching took place in 76 hours.

Ukwe and Abu (2016) conducted an experimental study to evaluate the efficiency of ovulin and ovaprim hormones (0.25 and 0.375 ml/kg body weight) in the induced breeding of the African catfish (*Clarias gariepinus*). Fertilization rate of the eggs of (81, 48.6 and 40.66%), ovulation period of (13, 12 and 10 hours), hatching rate were (78.35%, 32.63% and 4%) and survival rate of (27, 18.67 and 6%) were found in different concentrations (50, 75 and 100%) of ovulin hormone. Fertilization rate of the eggs were (58.33, 49.30 and 41.70%), ovulation period (14, 12 and 11 h), hatching rate of (40.27%, 31.13% and 18.1%) and survival rate of (14.62, 33.02 and 9.2%) found in different concentrations (50, 75 and 100%) of ovaprim hormone. The result concluded that, ovulin hormone performed significantly better ($p<0.05$) in all the parameters investigated except in survival rate.

Ovulin hormone was used in induced breeding of twelve brood-stocks (9 females and 3males) of *Clarias gariepinus* (Maradun et al., 2018) to determine the effectiveness of different doses (0.3, 0.5 and 0.7 ml/kg body weight). They found 0.7 ml/kg BW dose had the highest fertilization rate (88.12%) and higher hatching rate (82.07%) which was significantly different from the other doses. Highest survival rate was found in 0.5ml/Kg dose (85.20%), where other treatments (0.3 and 0.7ml) mean values were 84.23% and 83.60% respectively. They concluded that, catfish like C. *gariepinus* can successfully be induced to spawn at 0.3ml Ovulin per kg body weight, lower than the recommended 0.5ml/kg dosage which assures high quality eggs and more normal and healthy larvae.

According to Mamun and Basudev (2016), under the creation of artificial breeding environment the female goldfish endlessly chased by male goldfish, so that, goldfish become tired and release her eggs, sometimes by the hundreds or thousands, all over the tank. Most of the eggs stick to the plants. After the release of eggs, the male goldfish spray his milt over the eggs and the tank gets cloudy. At the age of 1st year goldfish released 850-1200number of eggs which increase in 2nd year (3000-3800) approximately. They also found that, goldfish egg needed 84-95 hours to hatch with a hatching rate of 80- 87%.

Sokolowaska et al., (1934) conducted a study on investigation of the effects of LRH-Aa injections, implantation of pellets consisting an analogue of LRH-At, and the combination of these treatments with injection of pimozide on GTH secretion and ovulation in goldfish at 18-20°C in a trial, to develop a process for reliably inducing ovulation within 24h duration. Low ovulation rate of goldfish was found (26%) at the administration of LRH-Aa (0.1 µg/g body weight) or pellet implants of LRH-At (25 and 125 µg/fish) and 87% was observed while pimozide was injected precede to or with the first of two injections of LRH-Aa, or following implantations of LRH-At pellets.

Mahmud et al., (2012) administrated synthetic ovaprim hormone to mature comet gold fish brooders (31.37-72.90 g) at a dosage of 0.5, 0.7, 1.0 and 1.2 ml/kg body weight maintaining (18-22^{o}C) temperature. Spawning was occurred 6 hours after the second dose of injection and fertilization rate of eggs were found 50.34, 51.47, 47.30 and 48.24 % respectively and the hatching rate were found 44.35, 43.79, 39.83 and 36.00 % respectively within the doses of 0.5, 0.7, 1.0 and 1.2 ml/kg of ovaprim. They also demonstrated that, the incubation of eggs needed 76 hours at the temperature range of 15-20 ^{0}C and 100% hatching was occurred in approximately 90 hours after hatching and the hatchlings were measured 1.3- 1.7 mm of total length.

Lam et al., (1976) administrated different doses of synthetic (LHRH) hormone at the intra-peritoneal area of gravid goldfish *Carrasius auratus* (L.) and found highest rate of ovulation (63.4 %) at the dose of 500 ng/g and lowest ovulation rate (34.2%) was found at 100ng/g while goldfish were kept at 12^{0}C.

Cejko and Kucharczyk (2015) administrated ovopel and metochlopramide (MET) with a dose of 20mg/kg body weight at 17^{o}C ambient temperature in crucian carp, *Carassius carassius* (L.) to evaluate the ovulation and hatching rate. Ovulation and hatching rate found higher in MET(90% and 84.4%) and lower in ovopel hormone (80% and 76.2%) respectively.

According to Jagtab et al., (2011), four different synthetic inducing agents (ovaprim, PGF2α, cloprostenol and tirapose) with the doses of (0.5µl, 1µl and 1 µl/g body weight in goldfish female and half doses in male) were administrated to spawners weighing 20-50 g at a temperature ranged 20-22^{o}C to determine eggs number, fertilization rate, hatching

rate and ovulation period. The mean eggs number, fertilization rate and hatching rate of goldfish were found (1000, 75% and 78.33%) in ovaprim; (200, 20% and 0%) in PGF2α; (2300, 80% and 91.66%) in cloprostenol and (3333, 81.66% and 91.66%) in tirapose respectively. Ovulation period was occurred after 10-12 hours except tirapose (72 hours).Their study concluded that, cloprostetenol and tiaprost is more effective as inducing agent in goldfish than all other prostaglandins used.

Khan et al., (2013), conducted an experiment to compare induced breeding efficiency of *Catla catla, Cirrhinu scirrhosus, Labeo rohita, Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* by injecting 0.4 and 0.45 ml/kg body weight of ovatide and ovaprim. They observed 100% spawning was occurred in ovatide hormone while fertilization rate and hatching rate both were higher in ovatide (80% and 64%) than ovaprim hormone administration (77% and 58.66%) respectively. They also reported that, ovatide hormone is cost effective than ovaprim especially in carp induced breeding.

Ayoola et al., (2012) used 0.5ml/kg BW dose of natural piscine hormone (*Clarias gariepinus* pituitary extract and frog *Haplobutrachus occipitalis* pituitary extracts) and artificial hormone (ovulin) to compare the breeding performance of African catfish (*Clarias gariepinus*). Fertilization rate, hatching rate and survival rate were found significantly better ($p< 0.05$) in ovulin hormone administration (67%, 90.61% and 69.33%) rather than catfish pituitary hormone (60.70%, 56.26% and 61.67%) and frog pituitary hormone (56.25%, 69.33% and 47.67%).

Ibrahim et al., (2019) injected ovulin hormone in African catfish (*Clarias gariepinus*) where fertilization, hatching and survival rate were found lower (34%, 36.03% and 37.67%) than other hormones (dry pituitary and fresh pituitary) used (80.47%, 75.46% and 50% ; 51.27, 46.03 and 58.20%).

Clarias gariepinus brood stocks were administrated synthetic ovulin hormone intramuscularly at different doses of 0.10 ml, 0.30 ml and 0.50 ml where stripped eggs, fertilization rate, hatching rate and larval survival rate were found (9876,15.27%, 23.77% and 25.87% respectively; 7553,15.07%,16.80% and 35.42% respectively; 12051, 13.04%, 12.67% and 34.22% respectively). Latency period was 10 hour and incubation temperature was maintained at 27-30^{o}C. They concluded that, *Clarias*

gariepinus can successfully be induced by using 0.3 and 0.1 ml/kg bodyweight of ovulin, which was lower than the manufacturers recommended dosage of 0.5 ml/kg bodyweight if other water quality parameters are well monitored (Uruku et al., 2018).

Paulo and Antonio (2005) conducted an experiment over survival rate of goldfish for three weeks. After injecting carp crude pituitary intra-peritoneal area spawning was induced and eggs were incubated at 20^{o}C. After the hatching they observed the survival rate of goldfish fry and found 90% survival rate in first week which was 80% at the end of the experiment.

Zainal et al., (2014) examined the performances of ovaprim, oxytocin and chicken-pituitary-gland extracts on induced breeding of seurukan fish (*Osteochilus vittatus*). Their study showed that fishes injected with ovaprim gave 7 hours of ovulation period with 93.33% of fertilization, 82.33% hatching and 80.66% survival rates while chicken pituitary gland extracts gave 13 hours of ovulation period with 82.33% of fertilization, 66.66% of hatching and 45.66% of survival rates and no hatching and survival rates observed for the oxytocin treatment.

A comparative study was carried out by Mosha (2018) to evaluate the breeding performances of synthetic hormone (ovaprim) and pituitary hormone ACPE (African catfish pituitary extract) at the dose of 0.5ml/kg in *Clarias gariepinus* broods. Relative fecundity, hatching rate, survival rate, hatching period and latency period were found (30.95, 46.30%, 50.14%, 48 hours and 9.05 hours) at ovaprim administration while (78.33, 25.99%, 82.89%, 48 hours and 9.05 hours) were found in ACPE. The result concluded that, ovaprim had lower performances than ACPE hormone in case of relative fecundity and survival rate while higher hatching rate was found in ovaprim.

According to Arindam et al., (2018), single dose of synthetic ovatide was administrated to goldfish broods at 0.5ml/kg body weight for female and 0.2ml/kg body weight for male for induced breeding while the non-administrated groups were exposed to natural breeding and temperature ranged 22-30^{o}C during experimental period. Fecundity and hatching rate were found to be significantly higher (6400 and 59.50%) than the non-injected groups (5950 and 54%). Hatching period was observed to vary from 18-20 hours at 29^{o}C. Fertilized eggs were adhesive and transparent with diameter ranging between 0.9

mm and 1.10 mm. Also, the survival rate was found to be higher (93%) in induced bred fish than natural breeding (87%).

Targon´ska et al., (2012) conducted an experiment to compare different synthetic hormones performances in induced breeding of crucian carp *Carassius carassius* (L.). Relative fecundity, ovulation period and survival rate were found (166eggs g−1 female BW, 16 hour and 83.4%) at 0.2 pellets/kg ovopel; (170eggs g−1 female BW, 14 hours and 87.3%) at 0.5ml/kg crude pituitary hormone (CPH) and (163eggs g−1 female BW,16 hours and 92.4%) at 0.5ml/kg ovaprim . No ovulation was observed at control group (treated with a 0.9% solution of NaCl).They also conducted another trial to observe the effects of different temperature (17, 21 and 25^{o}C) on relative fecundity, ovulation period and survival rate of the larvae. Relative fecundity, ovulation period and survival rate were found (155eggs g−1 female BW, 24 hours and 78.2%) at 17^{o}C; (159eggs g−1 female BW, 16 hours and 87.5%) at 21^{o}C and (86eggs g−1 female BW, 12 hours and 30.2%) at 25^{o}C which concludes that, a temperature regime of 21°C can be considered as the optimum for controlled reproduction of the crucian carp.

According to Ali et al., (2015), crucian carp broods (*Carassius carassius*) with the doses of 1.0, 1.5 and 2.0 mg PG kg-1 body weight, were administrated to evaluate and compare the breeding efficiency between doses. At the doses of 1.0, 1.5 and 2.0 mg PG kg-1 bw, ovulation rate, fertilization rate and hatching rate were found 96.52±2.35, 88.37±3.52 and 71.44±5.93% respectively; 90.35±3.15, 56.88±4.89 and 55.00±3.45% respectively and 78.65±2.84, 45.23±3.99 and 36.89±2.13% respectively. Fertilized eggs needed 44 to 72 hours to hatch out within at temperature ranged from 20 to 22°C.

Mahadevi et al., (2018) conducted an experiment to evaluate the induced breeding performance of Telescopic eyed gold fish (*Carassius auratus auratus*) broods (20-32 g) in captive condition by injecting WOVA-FH hormone at different doses (0.5, 0.7, 1.0 and 1.2 ml/ kg body weight) including control (non-injected broods). The average ovulation period, fecundity fertilization rate and hatching rate were found (12 hours, 767.33 eggs, 46.6% and 52.66%) at non administrated group;(9 hours, 868 eggs, 65.3% and 69%) at 0.5ml/kg body weight dose;(8 hours, 1189 eggs, 74% and 82%) at 0.7ml/kg body weight dose respectively; (7 hours, 982.33 eggs, 73% and 79.3%) at 1.0ml/kg body weight dose

respectively and (5 hours, 670.33 eggs, 52.3% and 63.6%) at 1.2 ml/kg body weight dose respectively and egg diameter ranged 0.8-1.1 mm and hatching was completed within 50-56hours at 22.8^{o}C. Their results indicated that, WOVA-FH at a dose of 0.7ml/kg and 1.2ml/Kg can be considered as effective doses for Telescopic eyed gold fish (*Carassius auratus auratus*) induced breeding.

According to Vahid et al., (2012), 96% of goldfish egg hatched in spring season and the 94.33% of larval survival rate was found at day five. They estimated the egg diameters of goldfish eggs ranged 1.15 and 1.32 mm and relative fecundity was estimated 14.96%.

According to Helen and Battle (1940), hatched larvae of goldfish estimated an average length of 4.5 mm which were restricted into movement due to yolk sac weight. Day two larvae attained the length of 5.8mm and the yolk sac reduced into narrow tubular band and enormous increase of yellow pigmentation was appeared. The mouth was distinct and an opercular membrane was on growing stage posteriorly over the gills and swim-bladder was appeared and developed at day 3-6. At day seven larvae attained 6.8 mm in length and yolk materials completely disappeared and rudiment of caudal fin rays found distinct. After 9 weeks, the fish looked alike adults with body covering and total length estimated 15.7 mm.

Takao (1960) described about the development of goldfish for three weeks. Pigmentation was started after 50 hours and heart beating and circulation started at 60 hours of fertilization and larvae still unmovable state due yolk sac weight and yolk sac started to reduce after 70hr and completely reduced within 100hr. Fin bud formation was observed after 80 hr of development and pectoral fin bud appeared first. Two days after hatching swim bladder developed completely and mouth completely developed within day 3-5. After first week of hatching, caudal fin rays were developed and dorsal and anal fin rays developed within three weeks.

As oranda goldfish is a newly introduces aquarium species in our country, information of breeding of this species is still unknown. Addressing this fact, the present study was conducted to evolve the all possible attributes of artificial propagation of oranda goldfish by using synthetic ovulin hormone.

CHAPTER-3

MATERIALS AND METHODS

3.1 Experimental site:

The present study was conducted in the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. All the preparations associated with the induced breeding of oranda goldfish (*C. auratus)* were taken in the laboratory and brood conditioning tank, breeding bowl setup, water supply facilities, working space etc. were assured before the breeding program. This experiment was conducted in compliance with the guideline of the animal care and use committee of the Chattogram Veterinary and Animal Sciences University.

3.2 Collection of oranda brood fish:

Oranda goldfish broods weighing 30-44g were procured from Katabon fish market, Dhaka. Before transferring fish into conditioning tank, weight and length measurement of the spawners were done by using electric balance and centimeter scale respectively (Table 1).

Table 1: Mean ± SD of Standard length (SL), Total Length (TL) and Body Weight (BW) of purchased oranda goldfish broods.

Treatments (ml ovulin/kg)	Male TL (cm)	Male BW (g)	Female TL (cm)	Female BW (g)
T_0	12.86 ± 0.38	30.80 ± 0.96	11.21 ± 0.69	32.91 ± 2.60
T_1 (0.2)	13.07 ± 0.83	31.47 ± 1.31	11.17 ± 0.63	36.53 ± 3.83
T_2 (0.4)	13.06 ± 0.55	32.14 ± 1.14	11.16 ± 0.72	33.26 ± 2.88
T_3 (0.6)	12.70 ± 0.92	31.08 ± 0.86	10.26 ± 1.08	34.41 ± 3.39
T_4 (0.8)	13.10 ± 0.44	32.33 ± 1.54	12.52 ± 0.14	35.38 ± 7.05

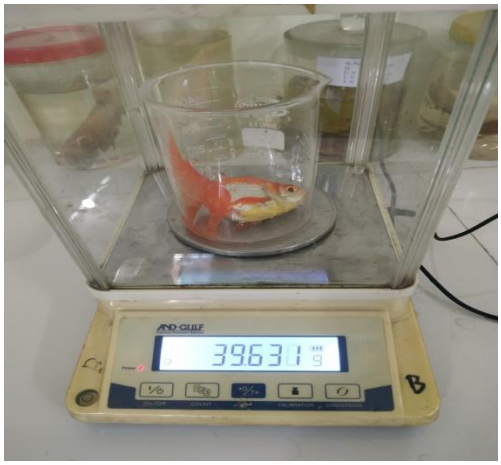

Plate 3: Weight Measurement

Plate 4: Length Measurement

3.3 Conditioning of broods:

Fishes were stressed enough and moving very slowly inside the poly-bag because it took about 5 hours to bring the fish from Dhaka to experimental laboratory. After that, the brood carrying poly-packs were submerged into the aquarium water for 10-20 minutes. The procured brood fishes were kept in the glass aquaria (60 x 30 x 45 cm) having 70 liter of water holding capacity. Then the brood were unpacked and released into the aquaria and kept for one week before the hormone administration. The vital water quality parameters maintained properly (Table 3) and dried tubifex was supplied twice in a day.

Plate 5: Conditioning of brood fish

3.4 Selection of broods:

Gold fish of both sexes (Plate 6) having 10-14 cm size (Table 1) was used in the present study. Characteristics used to select the mature goldfish for breeding program is presented in table 3.

Table 2: Characteristics of broods during breeding season considered in this study:

Characters	Male	Female
Tubercles	Appear on head, operculum, pectoral fins	Do not show
Abdomen	Smaller, slender and firm	Large and circular in outline
Genital opening	Long, concave and smaller opening	Convex and protruding out side
Pectoral fin	Thicker edge and more pointed	Thinner edge and rounded
Body shape	Thinner, longer	Fatter, shorter
Behavior	Chase the female	Chased and harassed by male

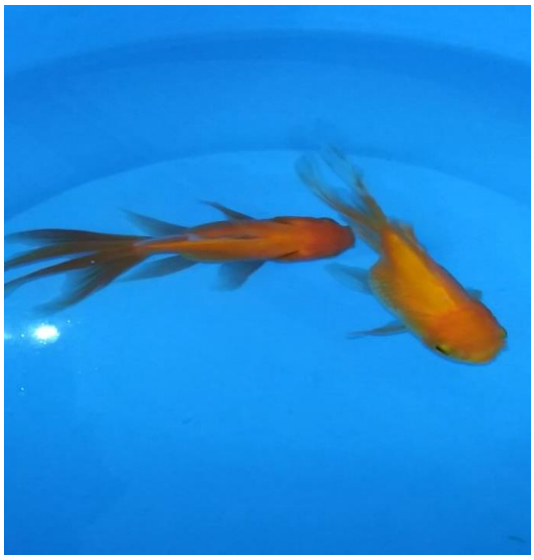

Plate 6: Male and female oranda goldfish

3.5 Experimental Setup:

To evaluate the breeding performance of oranda goldfish (*C. auratus*) 15 plastic bowls were used as breeding bowl with 20litre water holding capacity. The broods were kept in each bowl with male and female in 2:1ratio. Tape water was de-chlorinated with anti-chorine agent (1drop/liter) before transferring the spawners. To maintain the constant temperature submersible water heater was provided in each breeding bowl and continuous aeration was assured. Before hormone administration fishes were kept 24 hours in breeding bowl and water quality parameters maintained properly**.**

Table 3: Physico chemical parameters of water during acclimatization:

Parameters	Results
Temperature (^{o} C)	26.2 ± 0.17
Dissolved oxygen (DO) mg/l	4.69 ± 0.65
pH	7.6 ± 0.48

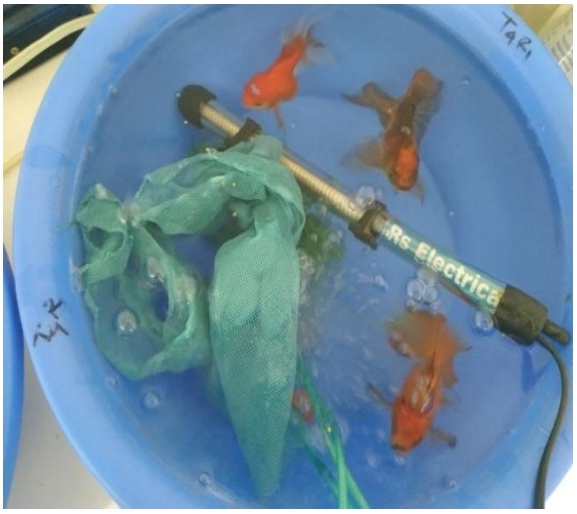

Plate 7: Broods in breeding bowl

3.6 Hormone administration:

Synthetic ovulin hormone was purchased from local trader for experimental study which preserved in 10 ml vial. The comparative study of different doses of ovulin hormone was conducted in two phases. First one was carried out during May – June of 2019 to evaluate the performances of induced breeding of oranda goldfish weighted (20-25 g). Induced breeding was performed by intramuscular administration of different doses (single for male and double dose for female) of synthetic ovulin hormone such as 0.3ml, 0.5 ml, 0.7ml and 0.9ml/kg body weight with three replicates and no hormone was administrated in control group for better comparison. Second experimental trial was carried out in October –November, 2019 where doses were (0.2, 0.4 , 0.6 and 0.8ml/kg body weight) with three replicates each respectively (R_1, R_2, R_3), administrated at intra-peritoneal area by using standard 1ml IM syringe at 45^o angle of fish body (Shinkafi and Ilesanmi, 2014) and no hormone injected in control groups**.** The doses were selected on the basis of previous studies (Ibrahim et al., 2019; Uruku et al., 2018; Maradun et al., 2018; Ukwe and Abu 2016 and Ayoola et al., 2012; Cejko and Kucharczyk , 2015; Mahmud et al., 2012; Paulo and Antonio ,2005; Jagtab et al., 2000; Lam et al., 1976 and Sokolowaska et al., 1934). Female was injected two doses and male was injected a single dose of injection and male was injected at the time of second dose given to the female. The hormone doses administrated in the two different sexes are shown in (Table 4 and Table 5). After injection, light rubbing was done in injected area, so that; the hormones could spread to the body muscle.

Plate 8: Hormone injection in fish

Table 4: Doses of ovulin used in the induced breeding of oranda goldfish (*C. auratus*) carried out in May-June, 2019:

Treatment	Sex	Ovulin (ml/kg body weight)		Time interval between the doses
		1st dose	2nd dose	
T_1	Female	0.3	0.3	6 hours
	Male	-	0.3	
T_2	Female	0.5	0.5	
	Male	-	0.5	
T_3	Female	0.7	0.7	
	Male	-	0.7	
T_4	Female	0.9	0.9	
	Male	-	0.9	

Table 5: Doses of ovulin used in the induced breeding of oranda goldfish (*C. auratus*) carried out in October-November, 2019:

Treatment	Sex	Ovulin (ml/kg body weight)		Time interval between the doses
		1st dose	2nd dose	
T_1	Female	0.2	0.2	6 hours
	Male	-	0.2	
T_2	Female	0.4	0.4	
	Male	-	0.4	
T_3	Female	0.6	0.6	
	Male	-	0.6	
T_4	Female	0.8	0.8	
	Male	-	0.8	

3.7 Stripping and counting the eggs:

After the observation of ovulation period stripping was done by slight pressure at the posterior ventral side of the spawners. At first, the turbid water due to ovulation, was washed out and replaced with de-chlorinated water to the bowl. De-chlorination was done

by using anti-chlorine agent to save the eggs from fungal attack. After that, female was stripped and eggs were collected in the breeding bowl and then respective male was stripped in the same breeding bowl and mixed well with previously collected eggs. Eggs were adhesive and attached all around the breeding bowl. After the stripping, egg were counted from each replicates with visual observation and waited for fertilization.

3.8 Determination of relative fecundity:

Relative fecundity of the oranda females of the present study was determined by the following formula described by Kahkesh et al., (2010) as follows:

$$\text{Relative fecundity (\%)} = \frac{\text{Number of stripped eggs}}{\text{Body weight of female}} \times 100$$

3.9 Determination of fertilization rate:

After a period of 01-02 hours the eggs were examined to observe the fertilization. For this purpose, egg samples from each bowl were taken into a slide and observed under the microscope (Optica B-192) and the fertilized eggs were counted with the help of a soft thin brush. The fertilized eggs were easily separated from the unfertilized eggs by the presence of transparent shell with black eye spot within the egg shell, while, the unfertilized eggs were whitish color with no black spot. The fertilization rate was calculated by using the following formula (Adebayo and Popoola, 2008):

$$\text{Fertilization (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs counted}} \times 100$$

Plate 9: Fertilized eggs

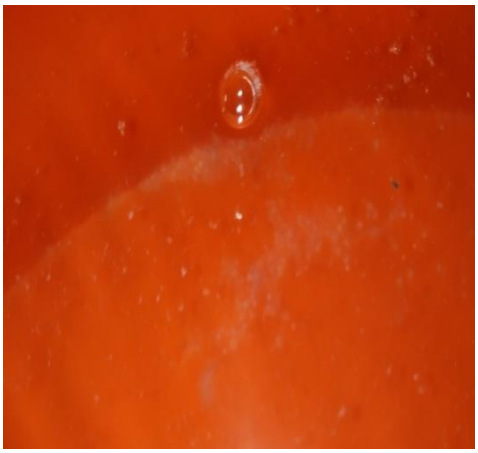

Plate 10: Unfertilized eggs

3.10 Determination of hatching rate:

Microscopic observation determines the hatching of the larvae. Hatching was occurred after 36-50 hours of fertilization (Appendix 1). After that certain period hatching rate was determined by the following formula (Haniffa and Sridhar, 2002).

$$\text{Hatching (\%)} = \frac{\text{Number of eggs hatched}}{\text{Number of fertilized eggs}} \times 100$$

3.11 Observation of the development of hatchlings:

The development of hatchlings was studied by examining live specimens under the microscope and microphotographs were taken simultaneously. No staining agent was used during microscopic observation. 5-10 specimens from each treatment were used to describe the development.

3.12 Determination of survival rate:

Data of survived larvae was collected for one week and the survival rate of the oranda goldfish larvae was determined by the following formula used by Ayinla and Akande (1988).

$$\text{Survival rate (\%)} = \frac{\text{Number of larvae at the end of the experiment}}{\text{Number of larvae hatched}} \times 100$$

3.13 Statistical analysis

Statistical analysis of obtained data was carried out using SPSS software (version 22 for windows). Data are expressed as means ± standard deviation (SD). Significant differences between treatments were compared by One-way ANOVA method. To describe significant differences within and between treatments Tukey's-b multiple range tests ($p<0.05$) was used.

CHAPTER-4

RESULTS

4.1. Fish response to different doses of hormone administration:

Different doses of hormones were administrated intra-peritoneally and response was found within 8-13 hours of ovulation while no response or ovulation observed in control group. Control group kept in natural breeding condition to breed. No significant differences (*p>0.05*) were found in ovulation and hatching period corresponding to the different hormone administration (Table 6).

Table 6: Response in ovulation and hatching period in the study period**:**

Treatment	Ovulation Period (hour)	Hatching Period (hour)
T_0	-	-
T_1	10.00 ± 2.00	40.33 ± 3.84
T_2	10.33 ± 1.52	43.66 ± 2.96
T_3	11.67 ± 0.57	40.00 ± 2.64
T_4	11.33 ± 0.28	48.66 ± 1.15

4.2. Reproductive performance of synthetic ovulin hormone:

Reproductive performance of ovulin hormone in oranda goldfish were performed with four different doses viz., 0.2, 0.4, 0.6 and 0.8ml ovulin/kg body weight. Data representing the effects of ovulin doses on stripped eggs, relative fecundity, fertilization rate, hatching rate and larval survival rate of Oranda goldfish are shown in (Table 7).

Table 7: Performance of stripped eggs, relative fecundity, fertilization rate, hatching rate and larval survival rate corresponding to the different doses of ovulin hormone in oranda goldfish:

Dose of ovulin (ml/kg BW)	Stripped Eggs/ Females	Relative Fecundity (%)	Fertilization Rate (%)	Hatching Rate (%)	Survival Rate (%)
0.2	360.67±47.04^{a}	11.01±2.42^{a}	34.67±3.51^{a}	38.5±3.90^{ab}	31.92±7.56^{a}
0.4	717.67±87.12^{a}	21.74±3.68^{b}	73.00±4.58^{c}	71.07±4.02^{c}	69.64±6.65^{c}
0.6	427.67±105^{a}	12.33±2.18^{ab}	56.33±9.07^{b}	47.12±5.53^{b}	51.74±6.65^{b}
0.8	380±288^{a}	10.08±5.99^{a}	30.67±2.08^{a}	31.50±4.22^{a}	27.42±2.50^{a}

*Mean values with same superscript did not show any significant difference (P>0.05).

4.2.1 Ovulation rate and period:

Ovulation was occurred among all the injected broods and no ovulation was found in control group. It is a fruitful finding that 100% ovulation occurred in all the different doses of synthetic ovulin hormone. After the 2nd doses of ovulin administration according with different doses ovulation was occurred at 8-13 hours of injection (Appendix 1).

Plate 11: Observation of ovulation

4.2.2 Stripped eggs:

Eggs were counted by direct observation after stripping. Number of stripped eggs was recorded as 360, 717, 427 and 380 in the treatments of T_1, T_2, T_3 and T_4 respectively. The highest numbers of stripped eggs were recorded 717 in T_2 and lowest eggs were found 360 in T_1. There were no significant differences found among the four doses of ovulin hormone (Table 7).

4.2.3 Relative fecundity:

The highest relative fecundity was estimated 21.74 % in T_2 and the lowest was 10.08% in T_4. There was a significant difference found among the four doses of ovulin hormone whereas T_2 was significantly (p<0.05) better than that of T_1 and T_4 (Figure 1 and Table 7).

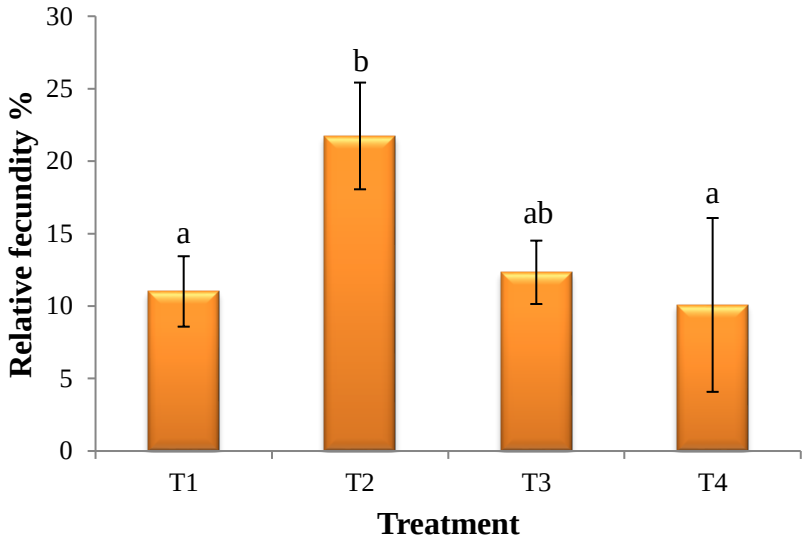

Figure 1: Comparison of relative fecundity (%) of *C. auratus* during induced breeding with the administration of different doses of synthetic ovulin hormone. Values are presented as mean ± SEM and are statistically significant at p< 0.05.

4.2.4 Fertilization rate:

The fertilization rate was found to vary between different doses of hormone administration. The highest fertilization rate was found 73% in T_2 and lowest fertilization rate were found 30.66% in T_4. There was a significant difference found among the four doses of ovulin hormone whereas T_2 was significantly (p<0.001) higher than that of T_1, T_2 and T_3 (Figure 2 and Table 7).

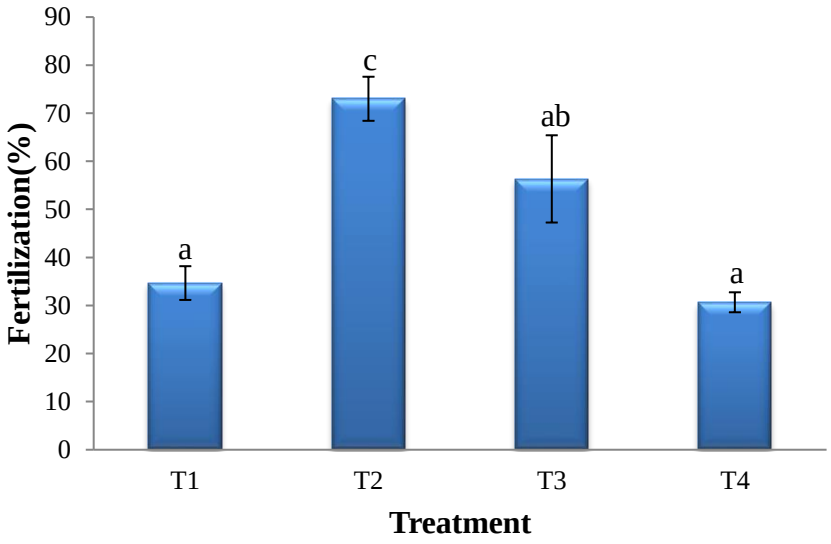

Figure 2: Comparison of fertilization rate (%) of *C. auratus* during induced breeding with the administration of different doses of synthetic ovulin hormone. Values are presented as mean ± SEM and are statistically significant at p< 0.001.

4.2.5 Hatching period and rate:

Oranda goldfish eggs hatched within 36-50 hours of fertilization at the temperature of 25-27ºC temperature among all the treatments applied .From the experimental study, the hatching rate of oranda goldfish eggs were recorded as 38.57, 71.07, 47.00 and 31.50% in the administration of 0.2, 0.4. 0.6 and 0.8 ml/kg of doses of ovulin hormone respectively. The highest hatching rate was found 71.07 % in T_2 and lowest hatching rate found 31.50% in T_4.

There was a significant difference found among the four doses of ovulin hormone whereas T_2 was significantly (p<0.001) higher than that of T_1, T_2 and T_3 (Figure 3 and Table 7).

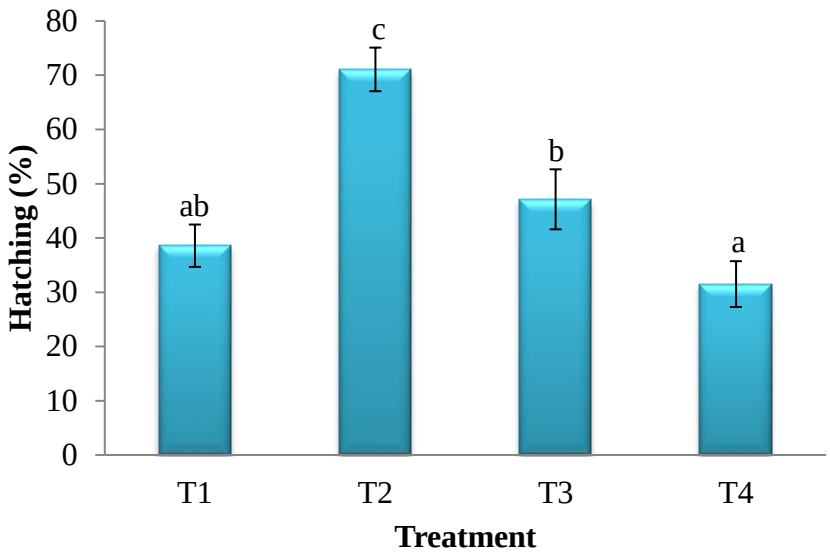

Figure 3: Comparison of hatching rate (%) of *C. auratus* during induced breeding with the administration of different doses of synthetic ovulin hormone. Values are presented as mean ± SEM and are statistically significant at p< 0.001.

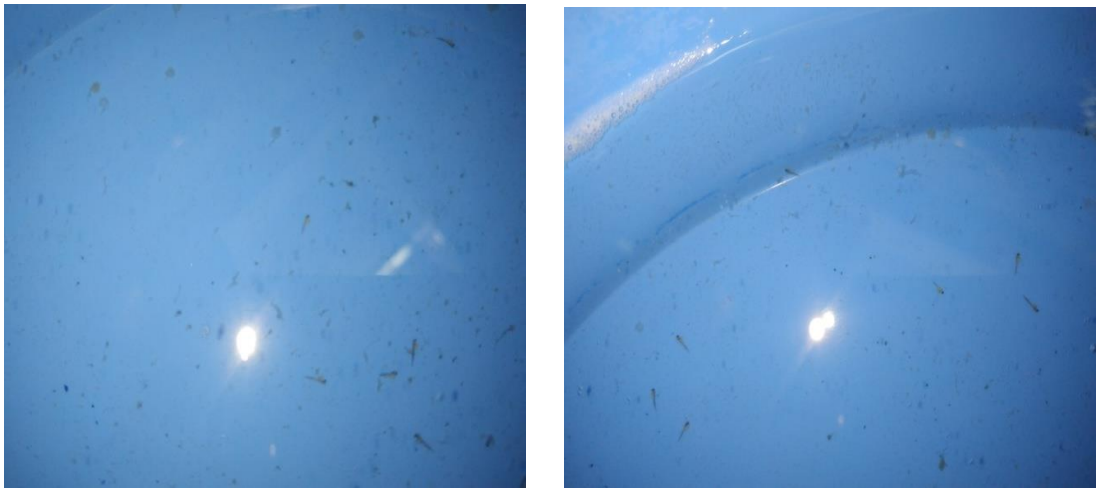

Plate 12: Observation of hatching

4.2.6 Survival rate:

Survival rate was recorded 31.92, 69.64, 51.74 and 27.43% in the treatments of T_1, T_2, T_3 and T_4 respectively. There was a significant difference among the four doses of ovulin hormone whereas T_2 significantly (p<0.001) higher than the other treatments.

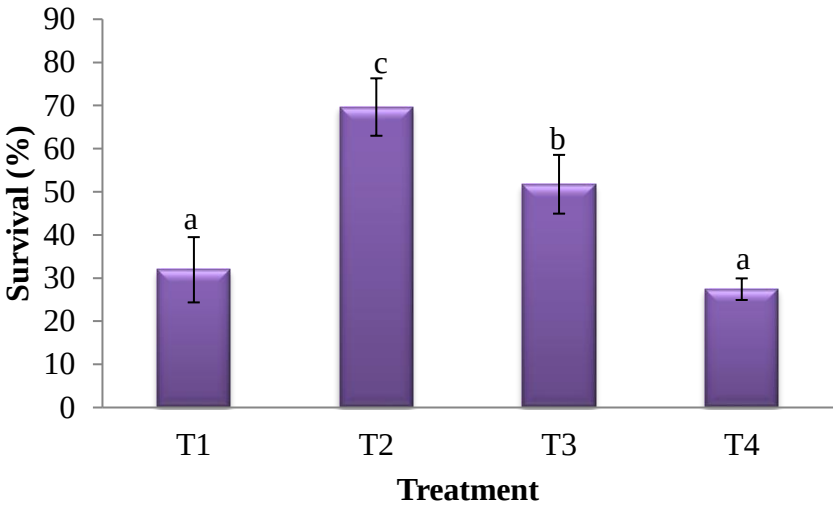

Figure 4: Comparison of survival rate (%) of *C. auratus* during induced breeding with the administration of different doses of synthetic ovulin hormone. Values are presented as mean ± SEM and are statistically significant at p< 0.001.

Plate 13: Larvae counting

Plate 14: Survived larvae

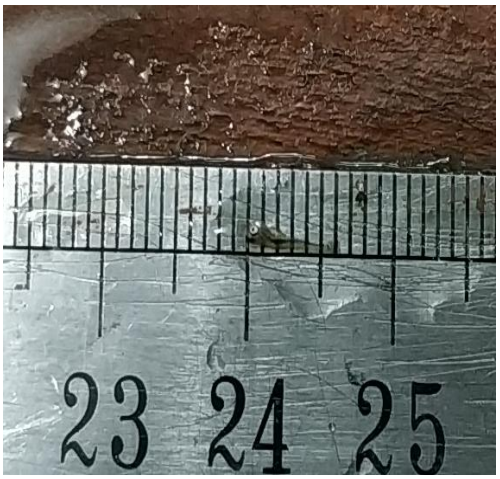

Plate 15: 7 day's old goldfish (6mm)

Plate 16: 14 day's old larvae (9mm)

Plate 17: 21 day's old goldfish (1.2 cm)

Plate 18: 30 day's old goldfish (1.6 cm)

4.2.7 Larval development:

a. Newly-hatched larva:

Newly hatched larvae (1-12 hours) freed itself by frequent movements of the tail which made the break of the egg shell. Total length larva varied with the range of 1.1-1.3mm. Pigmentation was not found. The major portion of yolk scattered melanophores more especially in anterior position. Movements were confined due to the volume of yolk contents. The larva found attached in the wall of hatching bowl. Notochord was clearly

visible. Heart differentiation into chambers was started in vertical position at the foremost part of yolk sac.

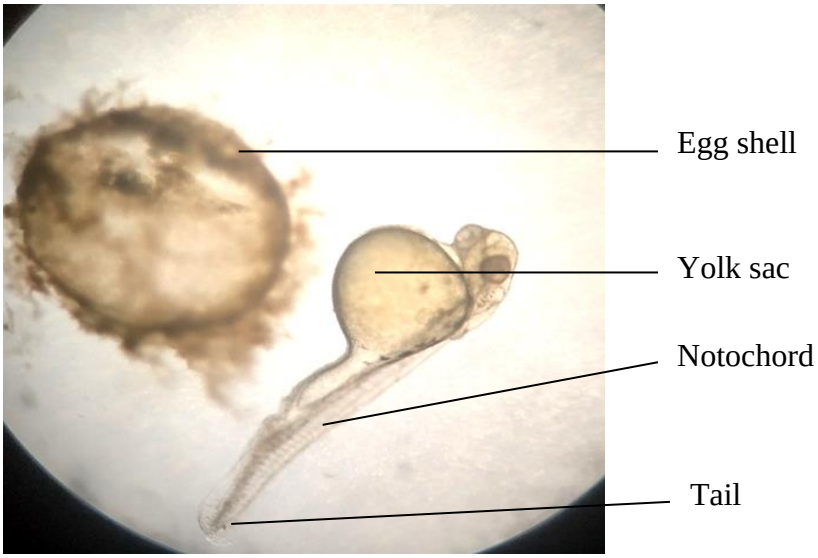

Plate 19: Early hatched larvae

b. 13- 20 hours old larva:

After 13-20 hours of length increased and the fin folds were seen continuously around the tail area. The gill and vent rudiments were apparently formed. Gut was straight to slightly curve in anterior portion. Pigmentation was denser throughout the head and body. Movement was very little as the free movement was not achieved yet.

Plate 20: 15 hours Larvae

c. 2-7 day's old larva:

The total length of the larvae was 2.5- 4.4 mm. Yolk sac totally reduced between these periods. A certain reduction in the in the size of the yolk sac was found in tubular form due to its greater absorption anteriorly. Yellow pigmentation was increased highly than before and the density of pigment spot (xanthophores) found excessively in head, dorsal musculature and lateral line area. Movements of larvae were frequent. The mouth was opened but not potrusible at this stage. Opercular membrane was not fully developed. The air bladder was partially inflated and growing antero-posteriorly and divided into two branches. The gut was appeared as tube like structure extended behind the air bladder.

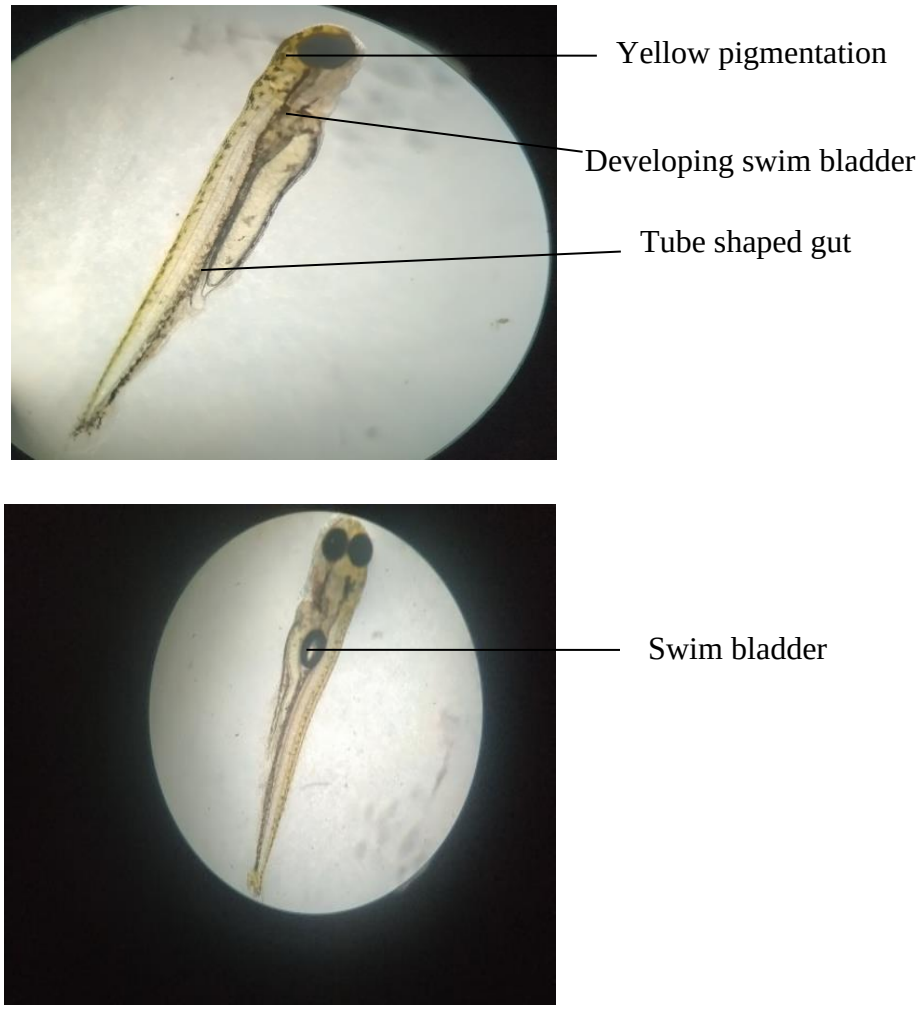

Plate 21: Development of swim bladder

d. 8-15 day's old larva:

Total length of the larvae was 6- 9 mm at this stage. Lower jaw movements were rhythmic with enlarged mouth opening. The posterior end of the notochord had become bent upward slightly and caudal fin rays were evident in the fin fold among the melanophores below the curved notochord. Dense yellow pigmentation was distributed all over the surface of the body. Distinct lateral line was found and the alimentary canal was look like a straight tube which enlarged anteriorly. The liver appeared as a triangular mass on the ventral surface immediately posterior to the heart.

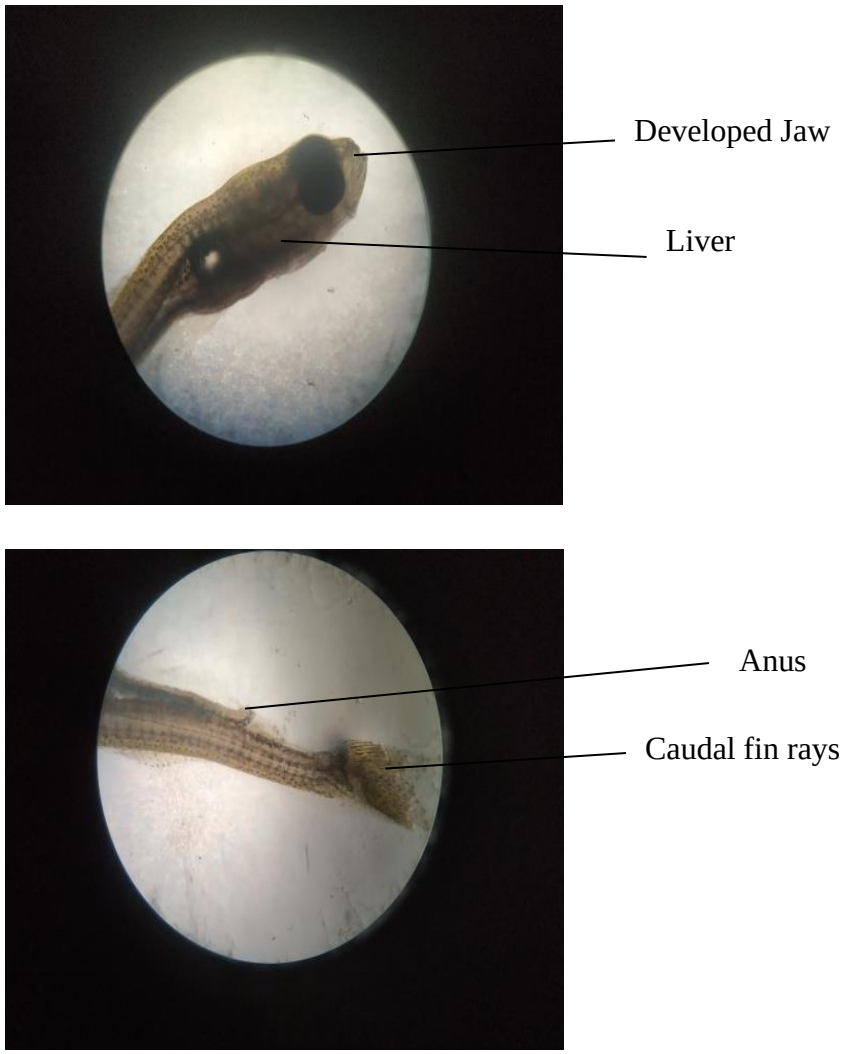

Plate 22: Jaw development and evidence of caudal fin rays

e. 16-21 day's old goldfish:

Total length became 8.7- 11.5 mm and the larva apparently resembles with their parents but actual goldfish coloration was not achieved. Operculum and branchiostegal rays were clearly visible. The caudal fin had forked and tri lobed and supported by un-branched fin rays. The air bladder completely spread in the abdominal cavity. The yellow pigmentation is heavy and the general body surface is taking on a transparent appearance. Dorsal fin and anal fin ray was visible but true fins rays were still on growing stage.

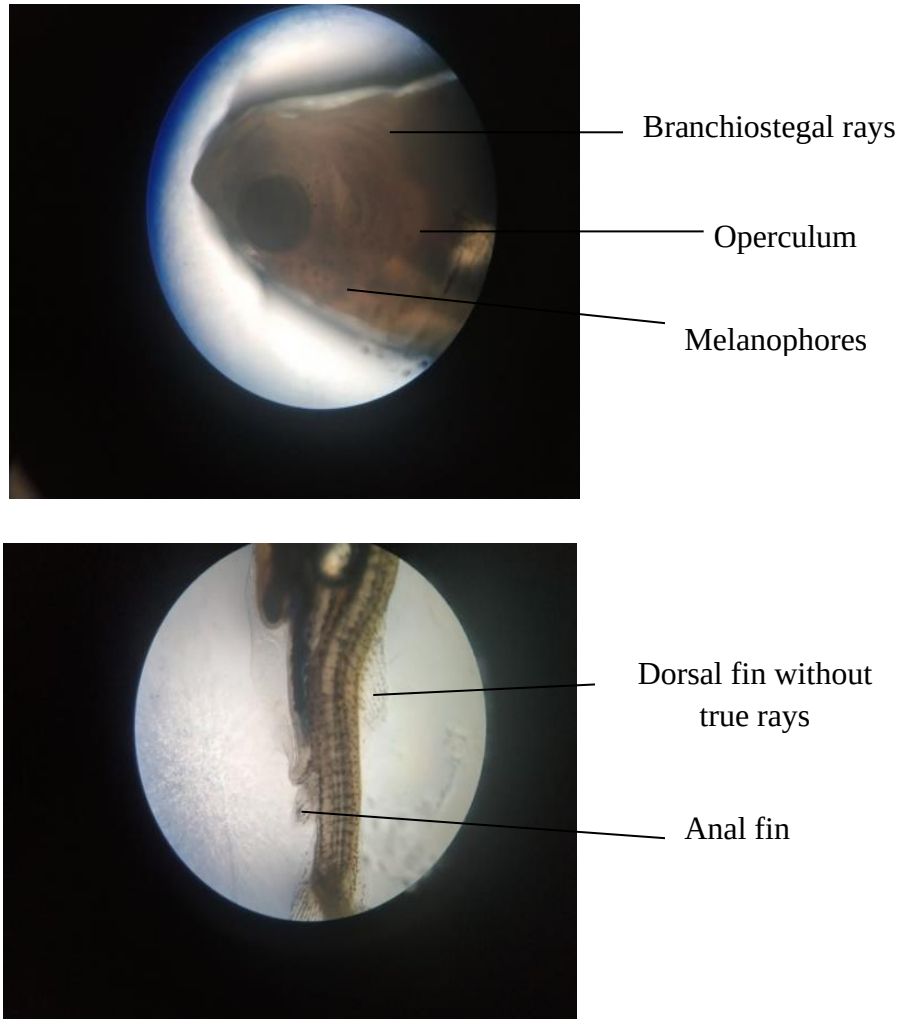

Plate 23: Various dimension of three weeks old oranda

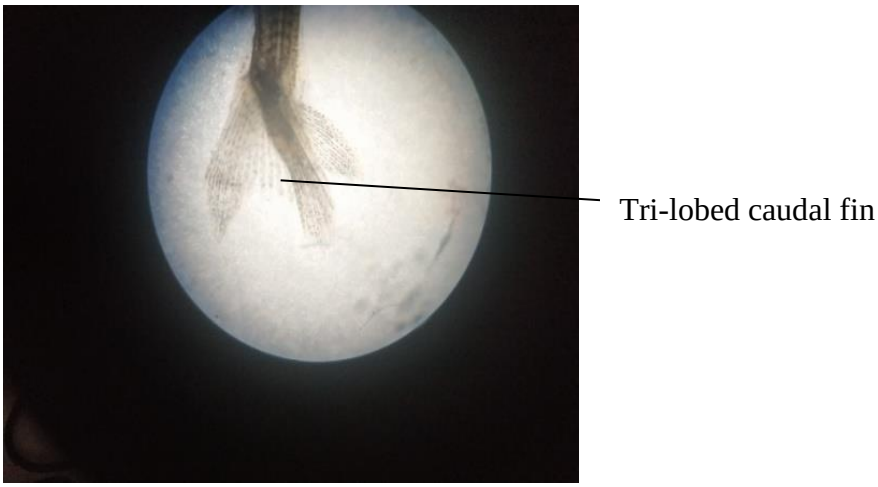

Plate 24: Developed caudal fin with true rays

f. One month old age:

This stage fry resembles with the adults. Body color also resembles with the adult. Wen was visible clearly. The caudal fin length advancement over the 3.5-4.2 mm and the dorsal fin consists of 8-12 true rays. The anal and pectoral fins had developed distinct rays. The pelvic fins had appeared as minute lateral fleshy protuberances.

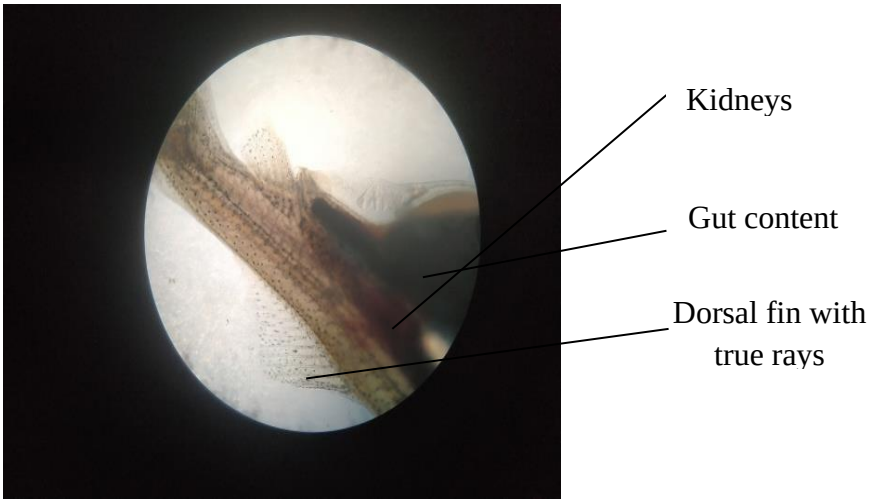

Plate 25: Various dimension of one month old oranda

Plate 26: Goldfish fry-parents resemblance

4.3 Cost-benefit analysis of the assay:

At the end of the experimental study cost benefit analysis was done based on dose 0.4ml and 0.6 ml/kg body weight. After the analysis it was concluded that induced breeding by the use of 0.4ml ovulin hormone is profitable which found almost three times higher than that of 0.6m/kg bodyweight of ovulin hormone (Table 8 and Table 9).

Table 8: Cost benefit analysis at the dose of 0.6 ml ovulin /kg body weight:

Capital cost	Amount	Unit price (BDT)	Total price (BDT)
Plastic bowls	15	25	375
Hormone(0.6ml)	28.8	45	2160
Injection	5	5	25
Goldfish	36	150	5400
Aerator	6	250	1500
Culture cost			
Feed(g) for one year	2000	5	10000
Electricity(unit)	60	5	300
		Total cost	= 19760 taka
Production Monthly production of 510fry Yearly production of 2040fry			
Sale			
2040 fry		12 taka/fry	24480 taka
Annual profit: **Monthly profit**		Sale-Total cost	24480-19760=4760 taka 396.66 taka

Table 9: Cost benefit analysis at the dose of 0.4 ml ovulin /kg body weight:

Capital cost	Amount	Unit price (BDT)	Total price (BDT)
Plastic bowls	15	25	375
Hormone(0.4ml)	19.2	45	1440
Injection	5	5	25
Goldfish	36	150	5400
Aerator	6	250	1500
Culture cost			
Feed(g) for one year	1200	5	10000
Electricity(unit)	60	5	300
			Total cost = 19040 taka
Production Monthly production of 690fry Yearly production of 2760fry			
		Sale	
2760 fry		12 taka/fry	2760×12= 33120 taka
Annual profit: **Monthly profit**		Sale-Total cost	33120-19040=14080 taka 1173.33 taka

CHAPTER-5

DISCUSSION

Ovulin hormone was used only in catfish species and no record was found in goldfish before this study. Different doses of ovulin hormone administrated in undiluted form. According to Olumuji and Mustapha (2002), the synthetic hormone is used undiluted form unlike natural hormone in fish induced breeding. Therefore, not much, if any, has been reported on the use of ovulin diluted with other substances on induced breeding of fish (Madu, 1989). In the experimental trial, the ovulin hormone at the doses of (0.3 ml, 0.5 ml, 0.7 ml and 0.9 ml/kg body weight) administrated at the temperature range of 28.5-30.3^{o}C in oranda goldfish broods was not succeeded on induced breeding and mortality of the broods found after 1-2 hours of hormone administration. One of the major constraints of that result was the use of low weight broods (20-25g). Low weight goldfish broods were also used on induced breeding by administrating Wova-FH and Tirapost hormone (Mahadevi et al., 2018; Jagtab et al., 2000) and they concluded with successive results. Low weight oranda goldfish were purchased due to unavailability of higher weighted fish in the local market at that period. A survey of local breeders ensured that low (20-25g) weighted broods breed by using hormone injection. Cost effectiveness was also under consideration during the procurement. But ovulin hormone did not show the complete response in that weight range of oranda goldfish. Two female's response was positive in terms of ovulation but the stripped eggs were not fertilized. The unsuccessful results of experimental trail reveal that, ovulin hormone is not effective in low weight oranda goldfish as an inducing agent though this finding is not sufficient to make a conclusive decision. For these circumstances, further study was designed and brood selection was carried out properly to overcome the results of experimental trial one.

At the present study, intra-peritoneal administration of ovulin hormone at the doses of (0.2, 0.4, 0.6 and 0.8ml/kg body weight) were done to establish the induced breeding technique of oranda goldfish weighted (30-44g). The doses were selected on the basis of previous reports where ovulin were used as inducing agent (Ibrahim et al., 2019; Uruku et

al., 2018; Maradun et al., 2018; Ukwe and Abu 2016; Ayoola et al., 2012) and the reports of other synthetic hormones used in goldfish artificial breeding (Nirmal et al., 2016; Cejko and Kucharczyk , 2015; Mahmud et al., 2012; Paulo and Antonio ,2005; Jagtab et al., 2000; Lam et al., 1976; Sokolowaska et al., 1934).

During the experimental period water quality was maintained properly. The physico-chemical parameters of a body of water are very crucial factor for growth and survival of the aquatic organisms (Adebisi, 1981; Owhonda et al., 2007). The study was lined with the statements of Gurung et al., (2018), as they mentioned that oranda are hardy species and tolerate a wide range of temperature and the pH tolerance level ranged between 7.0 and 8.0. Common goldfish ovulation is not occurred at 13-14^{o}C; raising at 20°C ovulation occurs (Yamamoto et al., 1966; Yamamoto and Yamazaki, 1967) but present study reveals that ovulation of oranda goldfish occurred at 25-27^{o}C. There is a variation in terms of temperature of ovulation found in different varieties of goldfish.

The temperature of the present study observed between 25-27^{o}C which is said to be line with Bichi et al., (2014) and (Ayinla 1988), who reported that, the time of interval between the start of fertilization and hatching changes with the increase in temperature. Fertilization was found after 1-2 hour at 25-27^{o}C in the study. Afzal et al., (2007) recommended a temperature range of between 25^{o}C to 32°C for good performance of fishes. The potentiality of low hatching period and increase hatching rate and fry survival rate depends on increase rate of temperature. Blaxter (1992) stated that, temperature is known to be the main environmental factor governing fish egg development. Low hatching and larval survival were also found in present study which agrees with the statement of Blaxter (1992).

The pH range in present study is in agreement with the world health organization international standard for the fresh water. It also corresponds with works of Huet (1972). Yang et al., (2011) reported that, hatching took place at 32 hours high pH 10 and it needed 64 hours to hatch when the pH was lowered. They stated that, the lower the pH the higher the hatching period. Hatching period of the current study was ranged 36-50 hours at pH range of 7.4-7.8.

The DO value measured during the experimental period ranges between 4.8-5.8mg/L. Bichi et al., (2014) reported the DO value of his study was 5.0mg/l and these agreed with those of Ufodike and Garba, (1992) who reported that, a minimum constant value of 4.0mg/l DO is sufficient for survival of most species and their developmental stages. Brain (2006) and Ita et al., (1995) reported that, increased DO level is essential for reproduction. Saplkhale et al., (2011) reported that, hatching rate of eggs and incubation period decreased with increase in temperatures from 26 to 30°C and pH levels from 5.5 to 8.5. Fertilization rate, hatching rate survival rate of the present study found lower due to this similar kind of pH range and temperature range.

Present study revealed that, relative fecundity found highest at the dose 0.4ml/kg weight of ovuline. Relative fecundity of crucian carp ranged between 13-17% at which also agree with the present study while they used 0.5ml/kg body weight of natural carp pituitary hormone (Targon´ska et al., 2012). Relative fecundity of oranda goldfish is also collaborated with the findings of the study of common goldfish (Vahid et al., 2012).

Stripped eggs were counted 360-717 in the present study. Low numbers of eggs were found due to the low body weight of the broods. Mahadevi et al., (2018) administrated different doses of WOVA-FH hormone at low weight telescopic eyed goldfish (*Carassisus auratus auratus*) while highest numbers of eggs estimated as 1189 eggs. Higher numbers of eggs estimated in the induce breeding of (*Carassius* sp.) in the previous studies (Jagtab et al., 2011; Mamun and Basudev, 2016). The significant result of the stripped eggs of the present study was found higher than that of crucian carps (Targon'ska et al., 2012).

The egg diameter of the oranda goldfish estimated as a ranged of 0.6-0.9 mm. Egg diameter of the present study agrees with the range found by Ruhul et al., (2013), but lower than the most of the previous studies (Arindam et al., 2018; Mahadevi et al., 2018; Mahmud et al., 2012; Vahid et al., 2012; Jagtap et al., 2011; Helen and Battle, 1940) due to use of low weight broods. Sargent et al., (1987) reported that egg size may also affected by age at maturity in fish and the size of the broods (De-Martini, 1991; L'Abee et al., 1990). At the present study, oranda broods were lower weighted in comparison with

the other studies. The present study also reveals that, low diameter of eggs can produce viable offspring.

Ovulation period of the oranda goldfish observed after 8-13 hours at the temperature range of 25-27^{o}C of the hormone injection and no significance differences found among the doses. Similar ovulation period was observed by Stacey et al., (1979) and Jagtab et al., (2011). Early ovulation of goldfish in induced breeding observed by Mahmud et al, (2012) and late ovulation of goldfish was observed by Targon´ska et al., (2012). Mahadevi et al., (2018) reported that, the increase of hormone doses reduce the time of ovulation period in goldfish (*Carassius auaratus*). Ovulation in other fishes while applying ovulin hormone observed 13 hours (Ukwe and Abu, 2016), 14 hours (Ayoola et. al., 2012), 10 hours (Uruku et al., 2018) in *Clarias gariepinus* induced breeding. Targon´ska et al., (2012) found 14-16 hours of ovulation on induced breeding of crucian carp *(Carassius carassius)*. Ovulin hormones at the present study of oranda goldfish performed similarly in terms of ovulation that of *Clarias gariepinus* induced breeding. Though ovulation of present study occurred in late summer, according to (Kobayashi et al. 1986a, 1986b) female goldfishes start vitellogenesis during winter and ovulate single to several times over an extended spring-summer spawning season. Most importantly, 100% ovulation was occurred among the hormone administrated fish. These findings strongly agree with the study of Khan et al., (2013), while they used ovatide hormones in major carps. Ovulation rate of the present study is also collaborative to that of major carps where 95-100% ovulation was achieved Basavaraj et al., (1999). The findings of ovulation rate of Oranda goldfish found higher than the studies other goldfish (Cejko and Kucharczyk, 2015; Lam et al., 1976; Sokolowaska et al., 1934). No ovulation was found in the non-administrated groups of the present study. But telescopic eyed goldfish ovulated without administrating hormone doses (Mahadevi et al., 2018). The present study made a conclusive point that, ovulation will be found in any doses of ovulin hormone.

Highest rate of fertilization (73%) occurred in oranda goldfish eggs at the doses of 0.4ml/kg doses of ovulin hormone. Fertilization rate were found lower than present study recorded as a rate of 81% (Ukwe and Abu, 2019), a rate of 88.12% (Maradun et al.,

2018) found higher than present study as a rate of 67% (Ayoola et al., 2012) and a rate of 15.27% (Uruku et al., 2018) while ovulin hormone used as inducing agent in *Clarias gariepinus.* This finding ensures that, ovulin hormone performed differently in catfish and goldfish with a clear variation of fertilization of eggs. Fertilization rate of the present study is also comparable to the study of Mahmud et al., (2012), who administrated different doses of ovaprim hormone on comet goldfish where highest rate of fertilization found 51.47% at the dose of 0.7ml/kg dose. In the present study fertilization rate was found higher with the low dose of ovulin in oranda goldfish compared to the study of Mahmud et al., (2012) conducted in comet goldfish. These variations might be occurred due to temperature, hormone and species variation. Higher rate of fertilization was observed by Jagtab et al., (2011) at the administration of cloprostenol and tirapose hormone in common goldfish which agrees with the present study and also makes line with the study of Khan et al., (2012) where synthetic hormones administrated in carps. Crucian carp eggs fertility rate were observed by Ali et al., (2015) where the higher fertility rate achieved at 1.0mg of PG/kg body weight. This report reveals that, natural hormone also performed better than synthetic hormone at the induce breeding of crucian carp.

Hatching period of the present study occurred at of 36-50 hours of fertilization at the range of 25-27^{o}C. This also agrees with the hatching period of telescopic eyed goldfish Mahadevi et al., (2018). Comet goldfish eggs needs 76 hours of fertilization at the temperature range of 15-20^{o}C (Mahmud et al., 2012). Crucian carp eggs hatched after 72 hours at 22^{o}C.This comparative study shows that, goldfish hatching period is increased with the decrease of the temperature. Hatching rates of the comet goldfish (Mahmud et al., 2012) found lower than the present study. Higher rate of hatching was occurred in telescopic eyed goldfish (Mahadevi et al., 2018) while comparing with the present study. Vahid et al., (2012) found 96% of hatching rate in goldfish. High rate of hatching rate observed in their study might be due to the seasonal variation. They conducted their experiment in spring season where present study was conducted in late summer season.

Higher rate of survival of oranda larvae was found in T_2 and T_3 of the present study. Lower survival rate observed in application of ovulin hormone in *Clarias gariepinus*

(Ukwe and Abu, 2016; Uruku et al., 2018 and Ibrahim et al., 2019). Maradu et al., (2018) found highest rate of survival (88.12%), while applying ovulin hormone in *Clarias gariepinus.* Survival rate of goldfish (Paulo and Anonio, 2005 and Arindam et al., 2018) and crucian carp (Targon'ska et al., 2012) was found higher than the present study. Cost benefit analysis showed that the dose of 0.4ml kg body weight is profitable in oranda goldfish induced breeding.

The larval development of the study is collaborated with the study of (Mahmud et al, 2012) though they conducted the experiment in lower temperature 15-20^{o}C. Length increment of oranda larvae was found lower than that of the common goldfish (Helen and Battle, 1940). Changes in the pattern of the entire structure of an organ in relation to the environment are decisive for evaluating the developmental patterns of species (Balon, 1999).

Though it is difficult to identify the variability of the results, above discussion concludes that ovulin hormone can be used as inducing agent for goldfish and the dose of 0.4 ml and 6 ml ovulin/kg body weight of may be recommended doses of induced breeding technique of breeding approaches.

CHAPTER-6

CONCLUSIONS

To breed oranda goldfish the doses of 0.4ml and 0.6 ml ovulin/kg body weight can be used. Considering the cost effectiveness, the dose of 0.4 ml ovulin/kg body weight is applicable to perform the breeding program of this elegant fish in commercial purpose. The demand of fish as pet is becoming popular in our country day by day. At past it was considered that, aquarium keeping is very costly and limited in elegant citizens but at present aquariums are seen almost in every urban household. Though the local suppliers managed to fulfill the local demands still they have to rely on import because hatchery and hatchery produced seed of ornamental fishes is very low and only few species are successfully propagated in our country by local breeders. Millions of foreign currencies spent to import ornamental fishes every year. It is a matter of regret that, ornamental fishery as recreational fisheries is an underrated and neglected sector due to attention in capture and culture fishery where our neighboring countries earn millions of foreign earnings by exporting their hatchery produced ornamental fish seeds and other products. It is high time we should concentrate on this sector because our country has great resource potentials with the environmental suitability for oranda goldfish as well as other ornamental fish's artificial propagation. Initiative should be taken to improve the market trade of ornamental fish as well as international collaboration is needed to be effective to export ornamental fishery products. Ornamental fish breeding and farming can be an alternative income source for many unemployed people of our country. The breeding of oranda goldfish required little space and less initial investment than most other forms of aquaculture practices, so it can be practiced even in urban areas with little alteration of backyard or even the roof of a dwelling by understanding the biology of the fishes.

CHAPTER-7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

The ambition of this study was to establish the induced breeding of oranda goldfish (*C. auratus*) by using synthetic ovulin hormone. Although a qualitative approach was followed to explore the objectives of the research, there are some limitations of the study which can be minimized by the following recommendations:

- Higher weight of goldfish should be used considering cost effectiveness, may obtain better outcome.
- The duration of the brood stock management should be increased if the facilities are available.
- This assay of induced breeding can be conducted in aquaria rather than plastic bowls to get better results.
- Further experimental trial can be conducted to confirm the results and experimental error.

Future perspectives of the present study may include:

- Induced breeding of goldfish varieties (telescope, bubble eye, comet, ryukin etc.) and other ornamental egg layer fishes can be conducted using ovulin hormone.
- Cross breeding program may be possible between the goldfish varieties by applying synthetic ovulin hormone.
- Comparison of the performance of ovulin hormone with other synthetic hormones (ovaprim, ovatide, ovopel etc.) in breeding program of goldfish and other ornamental fish species.

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APPENDICES

Appendix 1. Data of breeding performances of oranda goldfish

Treatment	OP (hr)	SE	RF %	FR %	HP hr	HR %	SR %
T_1R_1	8	309	8.38	31	36	41.94	30.77
T_1R_2	10	401	13.17	38	36	39.48	40
T_1R_3	12	372	11.48	35	48	34.29	25
T_2R_1	12	723	23.94	78	38	75.65	62.72
T_2R_2	10	628	17.49	72	45	69.45	76
T_2R_3	9	802	23.79	69	48	68.12	70.22
T_3R_1	12	533	14.77	55	45	52.73	58.62
T_3R_2	12	428	11.67	66	36	46.97	51.62
T_3R_3	11	322	10.55	48	39	41.67	45
T_4R_1	13	123	3.83	29	48	34.49	30
T_4R_2	9	692	15.79	30	48	33.34	27.28
T_4R_3	12	325	10.63	33	50	26.67	25

*OP= Ovulation Period, SE= Stripped Eggs, RF=Relative Fecundity, FR=Fertilization Rate, HP=Hatching Period, HR=Hatching Rate, SR=Survival rate

Appendix 2. Multiple comparisons of the parameters of breeding performance of oranda goldfish:

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Ovulation Period	Between Groups	5.667	3	1.889	0.687	0.585
	Within Groups	22	8	2.750		
	Total	27.667	11			
Stripped eggs	Between Groups	249527	3	83175.667	3.195	0.084
	Within Groups	208284	8	26035.500		
	Total	457811	11			
Relative Fecundity	Between Groups	260.406	3	86.802	5.766	0.021
	Within Groups	120.435	8	15.054		
	Total	380.841	11			
Fertilization rate	Between Groups	3512.667	3	1170.889	39.030	0.000
	Within Groups	240.000	8	30.000		
	Total	3752.667	11			
Hatching Period	Between Groups	145.667	3	48.556	2.088	0.180
	Within Groups	186.000	8	23.250		
	Total	331.667	11			
Hatching rate	Between Groups	2672.513	3	890.838	44.637	0.000
	Within Groups	159.658	8	19.957		
	Total	2832.171	11			
Survival rate	Between Groups	3397.976	3	1132.659	29.374	0.000
	Within Groups	308.476	8	38.560		
	Total	3706.452	11			

Post Hoc Tests

Homogeneous Subsets

Ovulation Period

Tukey B[a]

Treatment	N	Subset for alpha = 0.05
		1
1.0	3	10.000
2.0	3	10.333
4.0	3	11.333
3.0	3	11.667

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Stripped eggs

Tukey B[a]

Treatment	N	Subset for alpha = 0.05
		1
1.0	3	360.667
4.0	3	380.000
3.0	3	427.667
2.0	3	717.667

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Relative Fecundity

Tukey B[a]

Treatment	N	Subset for alpha = 0.05	
		1	2
4.0	3	10.0833	
1.0	3	11.0100	
3.0	3	12.3300	
2.0	3		21.7400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Fertilization rate

Tukey B[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
4.0	3	30.667		
1.0	3	34.667		
3.0	3		56.333	
2.0	3			73.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Hatching Period

Tukey B[a]

Treatment	N	Subset for alpha = 0.05
		1
3.0	3	40.000
1.0	3	40.333
2.0	3	43.667
4.0	3	48.667

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Hatching rate

Tukey B[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
4.0	3	31.5000		
1.0	3	38.5700	38.5700	
3.0	3		47.1233	
2.0	3			71.0733

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Survival rate

Tukey B[a]

Treatment	N	Subset for alpha = 0.05		
		1	2	3
4.0	3	27.4267		
1.0	3	31.9233		
3.0	3		51.7467	
2.0	3			69.6467

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.