

Project ID 553

Competitive Research Grant

Sub-Project Completion Report

on

Induction of breeding and larval rearing of *Botia dario* and *Lepidocephalichthys guntea* for aquaculture and recreational uses in Bangladesh

Project Duration

May 2017 to September 2018

Department of Fisheries and Marine Science
Noakhali Science and Technology University

Submitted to

Project Implementation Unit-BARC, NATP - 2
Bangladesh Agricultural Research Council
Farmgate. Dhaka-1215



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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
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Acronyms

DoF	Department of Forestry
BFRI	Bangladesh Fisheries Research Institute
NATP	National Agricultural Technology Program
CRG	Competitive Research Grant
PI	Principal Investigator
Co-PI	Co-Principal Investigator
IUCN	International Union for Conservation of Nature
GPS	Global Position System
BW	Body Weight
GSI	Gonado-Somatic-Index
DO	Dissolves Oxygen
TDS	Total Dissolved Solids
HCG	Human Cronic Gonadotropin
CPG	Carp Pituitary Glands
ADAC	Association of Official Analytical Chemicals
SIS	Small Indegenous Species
IU	International Units
ml	milliliter
mg	milligram

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Executive Summary

This study was conducted to find out the best induced breeding protocols along with larvae rearing techniques of two loaches, *Botia dario* and *Lepidocephalichthys guntea*. Artificial breeding was attempted in two types of incubators: glass jar aquarium (G1 to G6) and aluminum cistern (L1 to L3) incubator. Broods of both species were injected hormone under three treatments (CPG, HCG and Ovaprium) with 9 replications. Fish were injected once to prevent injury and stress. Females of both the species were administered carp pituitary gland (CPG) @ 5 to 20 mg/kg (G1, G2 & L1), HCG @ 300 to 2000 IU/kg (G3, G4 & L2) and Ovaprium @ 0.5 to 2.0 ml/kg (G5, G6 & L3) where males were administered @ half of the doses for females. Fry rearing was done in two systems: Hapa A (lower part of hapa fixed 20 cm above the pond bottom) and Hapa B (lower part of net inserted into pond bottom). During investigating breeding performance of *B. dario* and *L. guntea*, the lowest average GSI values of the species were recorded $0.2 \pm 0.032\%$ and $0.75 \pm 0.041\%$ in August whereas the highest average GSI values were observed $10.94 \pm 1.17\%$ and $11.38 \pm 1.23\%$ in June respectively. The average range of fecundity was 170 ± 27.34 to 12521 ± 232.57 for female of *B. dario* having the average range of body weight 6.45 ± 1.23 g to 13.8 ± 1.32 g respectively. The average range of fecundity was 698 ± 45.27 to 9284 ± 213.46 for female of *L. guntea* with the average body weight ranged 5.29 ± 1.2 g to 13.5 ± 1.3 g respectively. The highest oocyte diameters were recorded 0.53 ± 0.05 and 0.61 ± 0.07 mm in *L. guntea* and *B. dario* in June. Among the two species, *L. guntea* responded to induced breeding with different stimulating hormones whereas the *B. dario* didn't respond. The average spawning rate was found about 80% when injected with Ovaprium (1-1.5 ml/kg) and 68% with CPG hormone (8-10 mg/kg) and 34% with HCG hormone (1200-1500 IU/kg). Spawning rate was higher (78%) in the aluminum cistern compared to glass jar aquarium (62%). Average latency period of *L. guntea* varied from 3.17 to 4.38 hrs. among the treatments under two types of incubators. The highest fertilization (92-94%) occurred when induced @ 1.0 ml/kg Ovaprium (G6) & 0.8 ml/kg Ovaprium (L3) in May and 1.2 ml/kg Ovaprium (L3) in June and the lowest fertilization (41-42%) occurred @ 2.0 ml/kg Ovaprium (G6) in June and 2.5 ml/kg Ovaprium (G5) in July. The highest hatching period (15.52 hrs.) was observed when injected @ 900 IU/kg HCG (G3) and 1200 IU/kg HCG (G4) in June and the lowest (12.22 hrs.) was @ 10 mg/kg CPG (G2) in May and 1.5 ml/kg Ovaprium (G5) in June. During the experimental period, about 81-85% hatching occurred when induced @ CPG (10-12 mg/kg) and 78-87% @ Ovaprium (0.8-1.5 ml/kg) and was 62-71% @ (1400-1500 IU/kg). The hatching rate was 0% in some of replications, injected @ 5 mg/kg CPG (G1), 300 IU/kg HCG (G3), 600 IU/kg HCG (G4) in May, and 2000 IU/kg HCG (G4) and 3.0 ml/kg Ovaprium (G6) in July. Average hatching rate of *L. guntea* was higher in aluminum cistern ($67.88\% \pm 5.18$) compared to glass aquarium ($54.85\% \pm 4.72$). Among the treatments, the most remarkable survival rate (about 79-86%) of *L. guntea* was obtained with the treatment of Ovaprium @ (0.8 and 2.0 ml/kg) and @ CPG (8-12 mg/kg). In case of larvae rearing, the survival rate of larvae was calculated in the month of May, June and July that were 21%, 18% and 15% in Hapa A and 31%, 36% and 26% in Hapa B. From the study it was observed that the higher survival rate of Gutum larvae was found in Hapa B compared to Hapa A. From the study of breeding biology, the GSI value and fecundity were higher for the both species in the month of June and therefore concluded that the peak breeding season of both the species were in June. The hatching period was found lower with the treatments of CPG and Ovaprium hormone compared to that of HCG. Higher hatching and survival rates were found with the treatment of CPG compared to Ovaprium and HCG treatment. For successful induced breeding of *L. guntea*, the effective doses for female and male were determined as: CPG @ 10-12 mg/Kg BW and 5-6 mg PG/kg BW (G2 & L1); Ovaprim @ 0.8- 1.0 ml/kg BW and 0.4-0.5 ml/kg BW (G6 & L3). While surveying the status of Rani and Gutum as ornamental fish, found to have a pretty potentiality but yet to be commercialized as many other exotic ornamental fish species. Among the ornamental fish traders interviewed; *L. annandalei*, *L. guntea*, *B. dario* and *B. lohachata* were stocked by 1%, 3%, 2% and 16% shopkeepers predominantly in Katabon, Dhaka and Kumilla.

Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project: Induction of breeding and larval rearing of *Botia dario* and *Lepidocephalichthys guntea* for aquaculture and recreational uses in Bangladesh**
2. **Implementing organization:**Noakhali Science and Technology University, Sonapur, Noakhali-3814.
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

3.1 Dr. Shyamal Kumar Paul,

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3.2 Bhakta Supratim Sarkar, Assistant Professor and Co-PI, Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali. Cell: 01911712791. Email: vktt79@yahoo.com

4. **Sub-project budget (Tk):** 2918240 BDT
Total: 2918240 BDT (Twenty nine lac eighteen thousand two hundred forty taka only).
Revised: 2918240 BDT

5. Duration of the sub-project:

Start date (based on LoA signed) : 27 July 2017
End date : 30 September 2018

6. Justification of undertaking the sub-project

Indigenous fishes traditionally occupy an unenviable position and an inseparable link in the life, livelihood, health and the general well-being of the rural mass, especially the poor. It has been reported that some species such as molla (*A. molla*), dhela (*O. cotiocotio*), darkina (*E. danricus*) and kaski (*C. soborna*) contain a high amount of vitamin A and other micronutrients and minerals. Many of the "Small Indigenous Species"(SIS) are self-breeders and need proper management practices to facilitate natural breeding mostly in monsoon.

In the past, these small indigenous fishes were abundantly available in rivers, beels, jheels, canals, haors, baors, ditches and floodplains of Bangladesh and had a low market value (Shafi and Quddus, 1982; Ahmed, 1984 and Jhingram and Talwar, 1991). Some of these species have been disappearing gradually from the systems which in turn severely affecting the biodiversity of our ecosystem; *Botia dario* and *Lepidocephalichthys guntea* considered as endangered species.

Botio dario and *Lepidocephalichthys guntea* considered as small indigenous fish species and are listed to endangered species (IUCN 2015). *B. dario* is known as rani fish locally in Bangladesh. This fish is found in the beel and river especially northern part of Bangladesh and Mymensingh and the haor area of Sylhet. The abundances of the rani fishes are decreasing day by day due to various natural and man-

made causes in our country. The ecological importance of rani fish is to control the snail production and as an aquarium fish in our country.

Lepidocephalichthys guntea is considered as of much concern species and locally known as Gutum or puiya (IUCN 2015). This species found in freshwater and brackish water all over Bangladesh. Gutum is a scavenger and cleans up organic debris from the ecosystem. Currently the species is not as common as it was during the mid-1950s (Flora and fauna, Encyclopedia). Siltation and drying of habitats and indiscriminate fishing of ponds, beels and ditches have reduced the stock. This species is found small quantities with others species in the market. Sufficient number of fry and fingerlings of those fishes are, however, quite difficult to obtain from natural waters for stocking in the ponds. Proper techniques of induced breeding and mass production of fry in commercial scale seem to be the most crucial factors in expanding culture practice for those species. Nutrition in the diet of brood fish is known to have a profound effect on gonad development, fecundity, quality of eggs and larvae. Although precise information on the nutritional requirements of brood stock for gonad maturation is scanty, it has been known quantity and quality of feed as well as the feeding regime is important for maintenance of egg quality and successful spawning. Both of these fishes can be a good sources for the aquarium and culture fish through induce breeding.

7. Sub-project goal:

The main goal of this project was to produce fry of endangered *Botia dario* and *Lepidocephalichthys guntea* available for commercial as well as ornamental culture in Bangladesh through induced breeding and larval rearing technique. It will create new avenue for income generation for the hatchery operators and ornamental fish traders. Availability of fish fries of those species will create employment opportunity for rural poor people through nursery management and culture. In near future, the enhanced production of those species could have provided low price protein sources for the resource-poor of the country. In long run, sustainability in terms of production and biodiversity would be established.

8. Sub-project objective (s): The specific objectives of the sub-project are to:

- develop the breeding technique of *Botia dario* and *Lepidocephalichthys guntea* through optimizing hormones for successful induced breeding
- develop nursery management techniques
- assess their feasibility for commercial and recreational aquaculture

9. Implementing location (s):

- Bismillah Fish Seed Production Center and Farm, Kumilla
- Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali.

10. Methodology:

10.1 Regional Distribution of Study Area

This research work was conducted in a private hatchery called Bismillah Fish Seed Production Centre (Fig.1) and Farm situated at Kumilla district (GPS Location: Lat.- 23.16542° and long.- 91.20135°) and the Fish Biology Laboratory in the Department of Fisheries and Marine Science, Noakhali Science and Technology University. The hatchery was one of the leading fish seed producers of Bangladesh. Study area could be divided into two parts, i.e. Hatchery complex and Pond complex. Pond complex was 200 meters away from Hatchery complex.

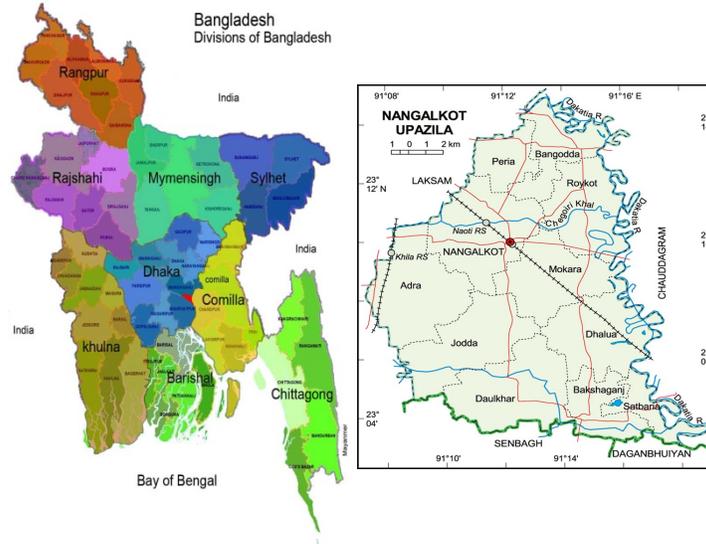


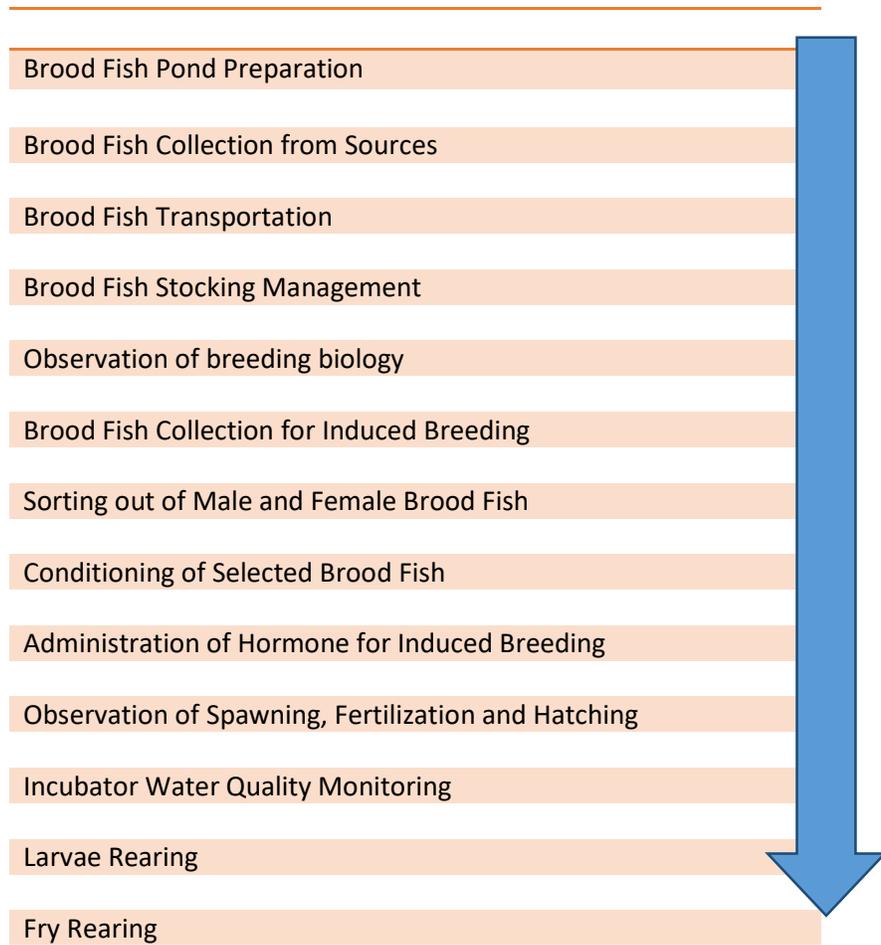
Fig.1: Geographical Location of Study Area

10.2 Study Period

This research work took more than 1 year to complete all the relative activities properly. The activities started at July, 2017 with preparation of brood stock pond and completed at September, 2018 with completion of fry rearing.

10.3 Experimental Design

An appropriate experimental design or methodology is like a heart for conducting a scientific research. In a scientific research the acceptability of the results depends to a great extent on the appropriate methodology. The results may be erroneous for the use of imperfect methodology. In this study a scientific and logical methodology had been taken by the researcher. This research work was done by using the following methodology:



10.3.1 Preparation of Brood Pond

days of liming. Inorganic fertilizers such as Urea and TSP were also used at the rate of 100g and 50g per decimal respectively. Seven days after fertilization, the brood ponds were ready for brood fish stocking. Collected broods of *Botia dario* and *Lepidocephalichthys guntea* were managed in two brood ponds separately with area of 20 decimal tagged as Pond-1 and Pond-2 and maintained the water depth between 0.9-1.2 meters.



Fig. 02: Google Earth Image of Brood Ponds



Fig. 03: Brood Ponds Preparation

10.3.2 Collection of Brood Fish

Brood fish of *L. guntea* and *B. dario* were collected from various regions of Bangladesh depend on regional availability.

Brood Fish of *L. guntea*: Gutum fish are abundant throughout Bangladesh. In this study, brood fishes were collected from Kewatkhali bank of the Brhamaputra river of Mymensingh sadar, floodplains and canals of Subarnachar of Noakhali, Floodplains and canals of Feni sadar and floodplains and canals of Nangolkot of Kumilla.

Brood Fish of *B. dario*: Bou Rani fish are an endangered species (IUCN 2016). Their availability are confined to few areas. Matured broods were collected from Pagla ghat of Dekhar Haor of Sunamganj Sadar, Jabar khal of Balaganj of Sylhet and Kataban Ornamental Fish Market of Dhaka.

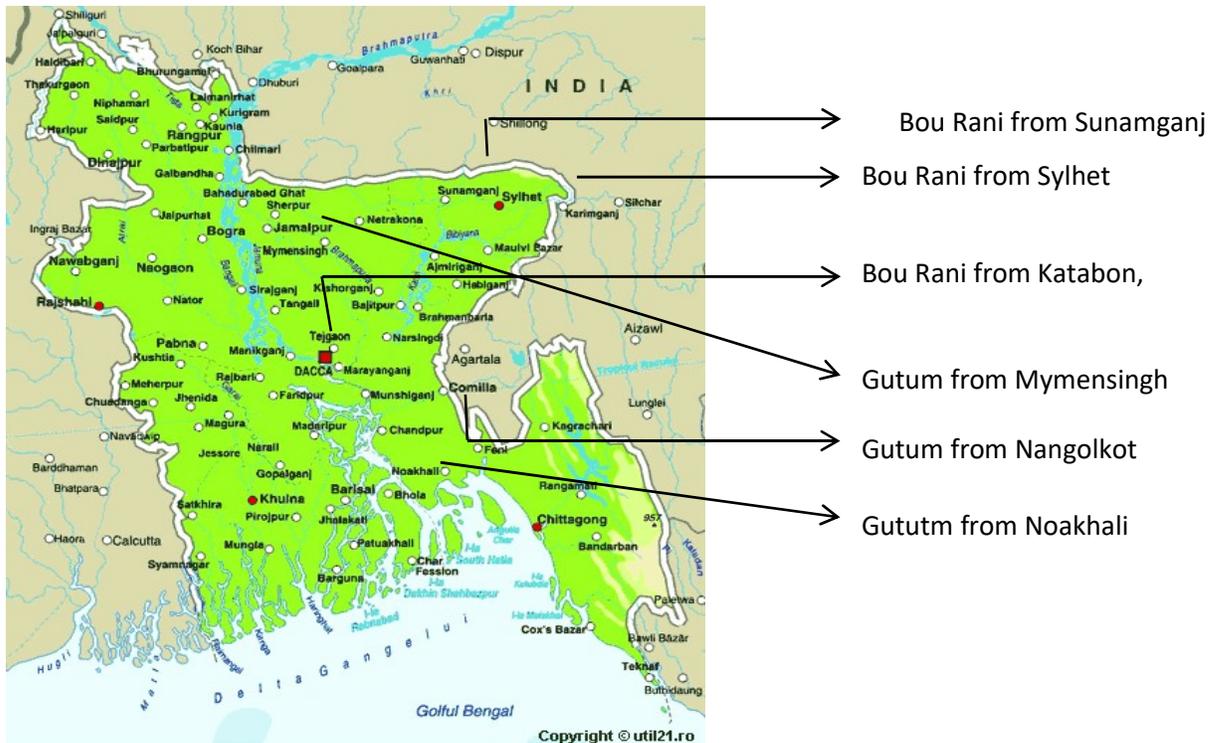


Fig. 04: Geographical Location of Brood Fish Sources

10.3.3 Transportation of Brood Fish

The researcher used following two methods to transport the brood fish from the sources region to study area:



- by oxygenated poly bag
- by plastic drum with continuous aeration

Fig. 05: Transport of broods by Oxygenated Polybag and Aerated Plastic Drums.

10.3.4 Brood Fish Stock Management

10.3.4.1 Brood Fish Stock

Two species of brood fish were reared in two ponds separately. *B. dario* and *L. guntea* were stocked at the rate of 80 Ind. /decimal and 100 Ind. /decimal respectively.



Fig. 6: Brood Fish Stocking

10.3.4.2 Feeding Management of Brood Fish

Brood fishes were fed at the rate of 4% of their body weight with supplementary feed (fish meal 30%, meat and bone meal 10%, mustered oil cake 15%, rice bran 20%, soybean oil cake 20%, wheat flour 4%, vitamin and minerals 1%). Feeding frequency were planned with twice per day at morning (9 am) and afternoon (4pm). Ensured the 32% protein (min.) with 5% lipid and 10% moisture (max.) in the supplied feed.

10.3.4.3 Measurement the breeding parameters

In this experiment, parameters of breeding biology (fecundity and gonadosomatic index) were being measured of brood fishes at every fortnight. In every sampling, 15 individuals of broods were being taken and measured their weight, length, fecundity and gonadosomatic index (GSI) respectively. Feeding rate were adjust at every fifteen days interval. Harvesting of broods were done by seine net. Biological parameters of fishes (weight, length, fecundity and GSI) were done with weight machine, measurement tape, cutting blade, magnifying glass and high resolution camera.

Measurement of Fecundity: Fecundity of both species was estimated by 'Direct Counting Method'. At first, fish's ovary was cut and weighted. A sub-sample was taken and weighted from the anterior, middle and the posterior part of the ovary. This sub-sample took onslide and kept under magnifying glass and the eggs of whole slide counted one by one. Counted data entry on datasheet and fecundity was estimated by using following formula:

$$\text{Fecundity} = (\text{No. of eggs in sub-sample} \times \text{Gonad weight}) \div \text{Weight of sub-sample}$$

Measurement of GSI: The GSI was the percentage of gonad weight to the total weight of the fish. The value of GSI was being measured at every fifteen days interval by the following formula:

$$\text{GSI} = (\text{Weight of Ovary} \div \text{Weight of fish}) \times 100$$



Fig. 7: Measuring GSI and Fecundity

10.3.4.4 Pond Water Quality Monitoring

Water qualities were being measured in the brood ponds and larvae'srearing hapa at every fortnight. Temperature, pH, dissolved Oxygen and TDS were regularly measured and recorded and taken necessary actions if any needed.

Water Temperature: Water temperature was measured fortnightly in brood rearing ponds during the experimental period. Temperature was measured by Digital Temperature Meter.

pH: pH of the water was recorded fortnightly in brood rearing pond during the study period. The water pH was measured by using a pH meter (model:HANNA-HI96107).

Dissolved Oxygen (DO): During the study period, Dissolved Oxygen was recorded fortnightly in brood ponds. DO value was recorded by using a dissolved oxygen (DO) meter (EZDO-DO-O₂-Temp).

Total Dissolved Solids (TDS): TDS value of the brood ponds was recorded fortnightly during the study period by using a TDS meter (model: EZDO-TDS5031).



Fig. 8: Water Quality Measurement of Brood pond and rearing Hapa.

10.3.5 Collection of Brood Fish for breeding

Brood fishes were collected by two consecutive steps i.e. firstly, netting the fish after reducing the water of the pond and then collecting by using hand after dewatering the pond (Fig.9). Fish were collected from the ponds at very early morning to reduce the stress on fish as much as possible. Mature broods, capable of induced breeding, were sorted out from the collected stock and kept in plastic jar and provided aeration by portable aerator. The selected broods were carried away to the hatchery and kept in a rectangular tank for sex based sorting out.

10.3.6 Sorting out of Male and Female Brood Fish

Brood fish selection was one of the most important aspects for successful induced breeding. Good looking, healthy and sexually mature broods were selected for breeding. Mature male and female broods were sorted out on the basis of secondary sexual characteristics.



Fig. 9 Brood Fish Collection by Netting and Dewatering

10.3.6.1 Selection of mature Gutum

In mature males, the pectoral fins were enlarged with fused, thickened innermost (7th and 8th) rays forming a structure known as the lamina circularis. Adult females were typically heavier-bodied and have spotted patterning on the flanks as opposed to a dark stripe. Mature males were shorter and less weighed than the female ones. Mature female was easily recognized by their soft and swollen abdomen (Fig. 10a.).

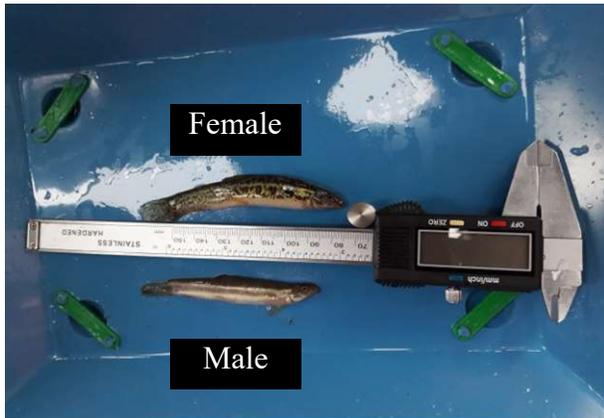


Fig. 10(a): Male and Female of *L. guntea*



Fig. 10(b): Male and Female of *B. dario*

10.3.6.2 Selection of Mature Bou Rani

Mature male fishes were shorter and less weighed than the females. The mature males were identified by their flat abdomens and long protruded genital papillae. On the other hand, the females were recognized by their soft and swollen abdomen and round and swollen urogenital papillae (Fig. 10b.).

10.3.7 Conditioning Selected Brood Fish

Selected male and female broods were conditioned in two separate rectangular tanks for 10 hours to make them ready for induced breeding. Continuous water flow was maintained through water shower to keep ambient temperature and suitable dissolved oxygen level. No feed were provided during the conditioning hours (Fig. 11.).



Fig. 11: Brood Fish Conditioning

10.3.8 Administration of Hormone for Induced Breeding

After conditioning of brood fishes, the broods were injected with hormone at definite doses. Hormones were injected on dorsal region near the tail of dorsal fin of brood fish at early night from 8 pm to 10 pm with using 1 ml syringe.

10.3.8.1 Used hormone for Induced Breeding

Carp Pituitary Gland (CPG), Human Chorionic Gonadotropin Hormone (HCG) and Ovaprium were used for induced breeding of *B. dario* and *L. guntea*.

Male fishes were administered of hormones (CPG, HCG and Ovaprium) at the half doses of hormone of female fishes. In the breeding protocol, hormone was administered at one dose for male and female fishes. Hormone administration of the broods fishes are shown in Fig. 12.



Fig. 12: Hormone Injecting in *L. guntea* and *B. dario*

10.3.8.2 Used Incubator for Induced Breeding

Two types of incubator were used for Induced breeding of *L. guntea* and *B. dario* (shown in Fig. 13a and Fig. 13b):



Fig. 13a: Glass Jar Aquarium Incubator (Panel A)



Fig. 13b: Aluminum Cistern Incubator (Panel B)

Glass Jar Aquarium (Panel A): Six glass jar aquarium incubator(G1 to G6) were used for induced breeding for both species. The areaof each aquarium was 70cm×70cm×70cm. Inlet and outlet water facilities was fixed with each glass jar aquarium. The rate of inlet and outlet of water was 500 ml water/minutes in this system.

Aluminum Cistern Incubator (Panel B):Aluminum cistern incubator (L1 to L3) had a diameter of 1 m and height of 1 m having running water facility with increasing and decreasing speed of water flow at 100 L/hr.

10.3.8.3 Hormone Protocol for Induced Breeding

This experiment was conducted with the two types of incubator namely asglass jar aquarium incubator (Panel A) and aluminum cistern incubator (Panel B). Broods of both species were injected hormone under three treatments (CPG, HCG and Ovaprium treatment) with 27 replications where each treatment was planned with 9replications. Male fishes were administered of hormones (CPG, HCG and Ovaprium) at the half doses of hormone of female fishes. Both sexes of fishes were planned with one dose of hormone protocol. Hormone protocols of the targeted two species of female are described into following Table 1:

Table 1: Hormone Protocol for *B. dario*and *L. guntea* (shown doses are for female, male to be induced @half of female)

Subject	sex ratio	Glass at Aquarium (Panel A)						Aluminum Cistern (B)		
		CPG hormone (mg/kg)		HCG hormone (IU/kg)		Ovaprium hormone (ml/kg)		CPG hormone (mg/kg)	HCG hormone (IU/kg)	Ovaprium hormone (ml/kg)
		G1/R1	G2/R2	G3/R3	G4/R4	G5/R5	G6/R6	L1/D1	L2/D12	L3/D3
Hormone dose in May	1:1	5	10	300	600	0.5	1.0	8	800	0.8
Hormone dose in June		8	12	900	1200	1.5	2.0	10	1000	1.2
Hormone dose in July		15	20	1500	2000	2.5	3.0	12	1400	1.7

Note: Replication G₁-G₆ for Gutum, Replication R₁-R₆ for Bou Rani, Replication L₁-L₃ for Gutum and D₁-D₃ for Bou Rani

10.3.9 Observation of Spawning

a) Calculation of Fertilization Rate

Total number of eggs and rate of fertilization were calculated one hour after natural spawning by direct counting method. For this three bowls were used with a capacity of 1.25 liter. Three replications were used for each sampling day. The average values of three bowls are used in formula. For determination of fertilization and hatching rates, approximately 100 eggs were placed in bowls. After two hours of fertilization, the transparent eggs were considered as fertilized eggs whereas, the opaque eggs were considered as dead eggs. The fertilization rate was determined by following formula:

$$\text{Fertilization rate (\%)} = (\text{No. of Fertilized Eggs} \div \text{Total No. of Eggs}) \times 100$$



Fig. 14: *Botia dario* and *L. guntealaying* Eggs

b) Calculation of Hatching Rate

The hatching rate was counted by direct counting method. The hatchlings were taken in white enamel bowl (1.25 liter), then the water with hatchlings were shifted to another bowl by siphoning. The average values of three bowls were used in formula. The hatching rate was determined using the following formula:

$$\text{Hatching rate (\%)} = (\text{No. of Hatchlings} \div \text{Total No. of Eggs}) \times 100$$

c) Calculation of Survival Rate

The survival rate was counted by direct counting method. At first the hatchling were taken in white enamel bowl which have a capacity of 1.25 liter. Hatchlings were collected from homogenously distributed water in three bowls. Hatchlings were counted by naked eyes. The average values of three bowls are used in formula. The survival rate was determined by following formula:

$$\text{Survival rate (\%)} = (\text{No. of Alive Hatchlings} \div \text{Total No. of Eggs}) \times 100$$

10.3.10 Monitoring of water quality parameters in Incubator

The water quality parameters have direct impact on induced breeding. The fecundity, fertilization rate, hatching rate and survival rate largely depends on the water quality parameters. Water quality parameters like dissolved oxygen (mg/l); water temperature (°C) and pH were monitored at every four hours in incubators.

10.3.11 Larvae Rearing

Larvae were kept in 2m×1m×0.3m sized rectangular cement tank for 2 and half days. This rectangular tank was designed with inlet and outlet water system and provided continuous shower of water. They fed on their boiled yolk sack for 2 and half days. Feeding frequency was setted four times per day. A one yolk sac was provided for 20 thousands larvae per day. Two days old larvae were transferred to hapa for further rearing.

10.3.12 Fry Rearing in Hapa

Fish larvae aged of two and half days were transferred into Hapa which was made of silky cotton. Only boiled yolk sac was provided for the first three days and then provided supplementary feed powder contained minimum 30% protein were given according to their 8% BW with three feeding frequency. Water qualities and natural plankton density of hapa were monitored twice in weekly and kept ambient by taking necessary steps if needed. For protection and shelter of larvae, water hyacinths were used in Hapa (Fig.15). Two systems of hapa were setted for fry rearing. One system was (Hapa A) that lower part of net was settled 20 cm above from the pond bottom and another system was (Hapa B) that lower part of net inert into pond bottom at 10 cm depth.



Fig. 15: Fry Rearing in Hapa

10.4. Status of ornamental fish market

The majority of the shops of aquarium fishes were located in Dhaka city. Katabon market, is the most common and popular market in Dhaka city for aquariums and other aquarium products such as aquarium fishes, aquarium foods, chemicals, toys, plants etc. In this experiment, Katabon ornamental fish market was selected as one of the study areas in assessing the status of *B. dario* and *L. guntea* as ornamental fish species. A total number of 25 ornamental fish sellers were selected randomly for the study.

10.5 Measurement of biochemical (proximate) analysis of those species

Fish sample of *B. dario* and *L. guntea* were collected from experimental ponds of Kumilla. A 300 gm of sample was brought from the target ponds, preserved in ice box and carried to the laboratory of Aquaculture Department, Bangladesh Agricultural University, Mymensingh. The conventional method of AOAC (Association of official analytical chemicals) (AOAC, 1995) was followed for the determination of ash, moisture, protein, lipid and carbohydrate on wet basis.

10.6 Materials and Equipment Used during Study Period

Various materials and equipment were used during the research activities. Different materials were needed at different works like fish transportation, fish health observation, water quality measurement, induced breeding and fry rearing etc. All equipment were free from error and proper calibration was done before every time they were used.

- Brood fish were transported from sources to study area by using oxygenated plastic bag and plastic drum aerated with portable aeration.

- The equipment such as scoop net, plastic mug, plastic bucket, tray, weight machine, scale, measuring ribbon, scissor and blades etc. were used for fish health observation.
- DO meter, TDS meter, pH meter, and Digital Probe Thermometer etc. were used to measure the water quality parameters.
- Aeration was provided by using both portable and electric aerators.
- During breeding activities equipment like mortar and pestle for hormone preparation, syringe (1 ml and 3 ml), bottled mineral water, foam sheet and gloves etc. were used.



Fig.16: Equipment Used for the Study (Dissection Box, Slides, Water Quality Measuring Meters)



Fig. 16 (a): Equipment Used for the Study: Weight Machine, Chemical Kit, Sample Container, Syringe)

Data Analysis

The obtained data were scrutinized and summarized carefully before the final tabulation. All data were calculated in international units and analyzed using Microsoft Excel-2010. The research paper is written in Microsoft Word-2010

11. Results and Discussion:

11.1 Observation of Water Quality Parameters of Brood Ponds (Pond 1&2)

Average monthly temperature ranged from $22.04 \pm 0.68^\circ\text{C}$ to $28.89 \pm 1.02^\circ\text{C}$ of Pond-1 respectively. The average lower temperature ($22.04 \pm 0.68^\circ\text{C}$) was observed in the month of January and February and the higher temperature ($29.24 \pm 0.25^\circ\text{C}$) was found in the month of April and May shown in Fig. 17. Average temperature of Pond-2 ranged from $21.62 \pm 0.54^\circ\text{C}$ to $28.43 \pm 0.85^\circ\text{C}$ during the study period shown in Fig. 18. The average lower temperature ($21.38 \pm 0.27^\circ\text{C}$) was observed in the month of January and February and the higher temperature ($28.33 \pm 0.19^\circ\text{C}$) was found in the month of August, April and May shown in Fig. 18. These ranges of temperature of Pond-1 and Pond -2 were suitable for fish growth supported by the works of Ali *et al.*, (1997), Jhingram and Pullin (1985), Aminul (1996) and Mumtazuddin and Khaleque (1981).

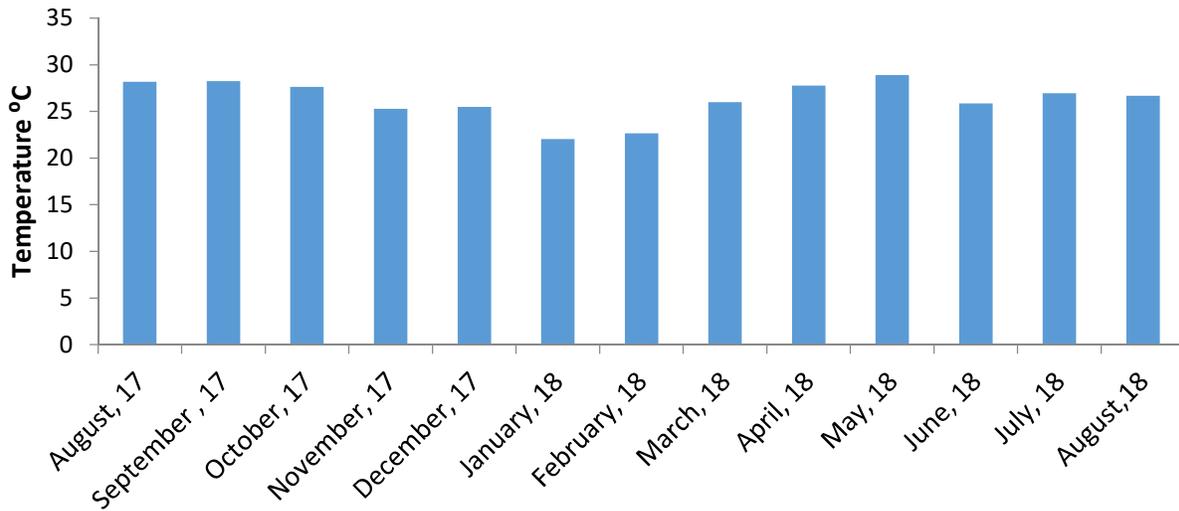


Fig.17: Average Temperature of Brood Pond-1

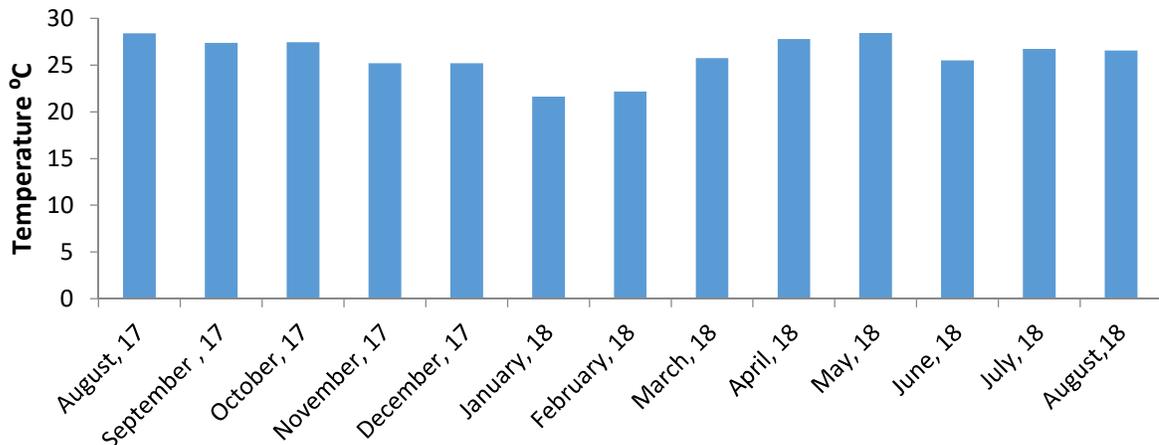


Fig. 18: Average Temperature of Brood Pond-2

Average dissolved oxygen (DO) of Pond-1 and Pond-2 were ranged from 6.23 ± 0.4 mg/L to 7.34 ± 0.28 mg/L and 6.06 ± 0.18 mg/L to 7.35 ± 0.46 mg/L respectively during the study period that shown in Fig. 19 and 20. The monthly average DO of Pond 1 and Pond 2 was lower in the month of April and May (Fig 19 & 20). According to the findings of DoF (1996), Alikunhi (1957), Banarjee (1967), Bhuiyan (1970) and Ali *et al.*,(1982) dissolved oxygen rate of Pond-1 and Pond-2 were suitable for fish growth.

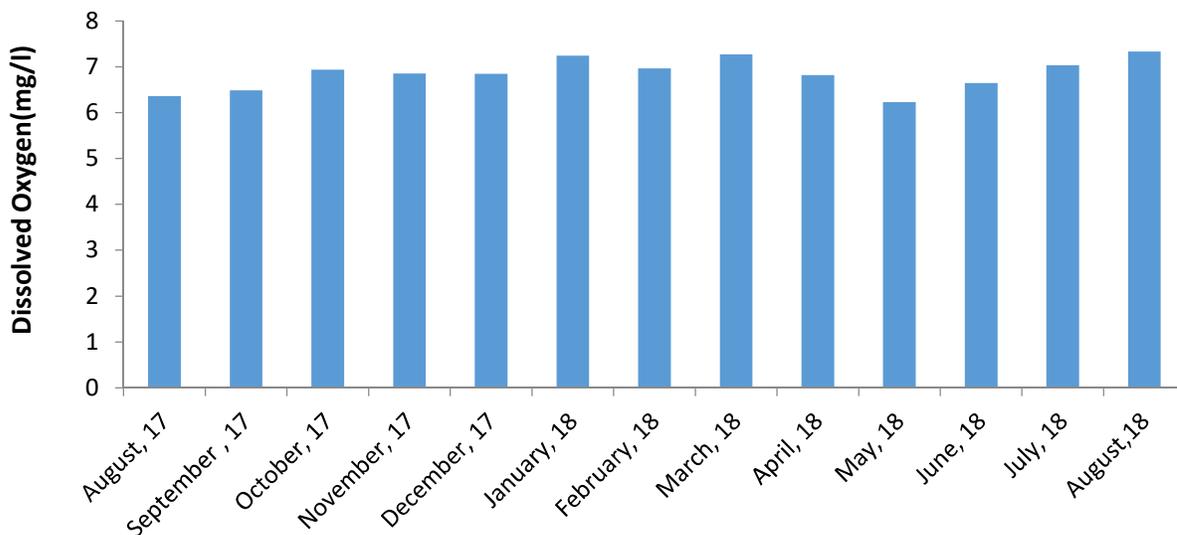


Fig.19: Average DO of Brood Pond-1

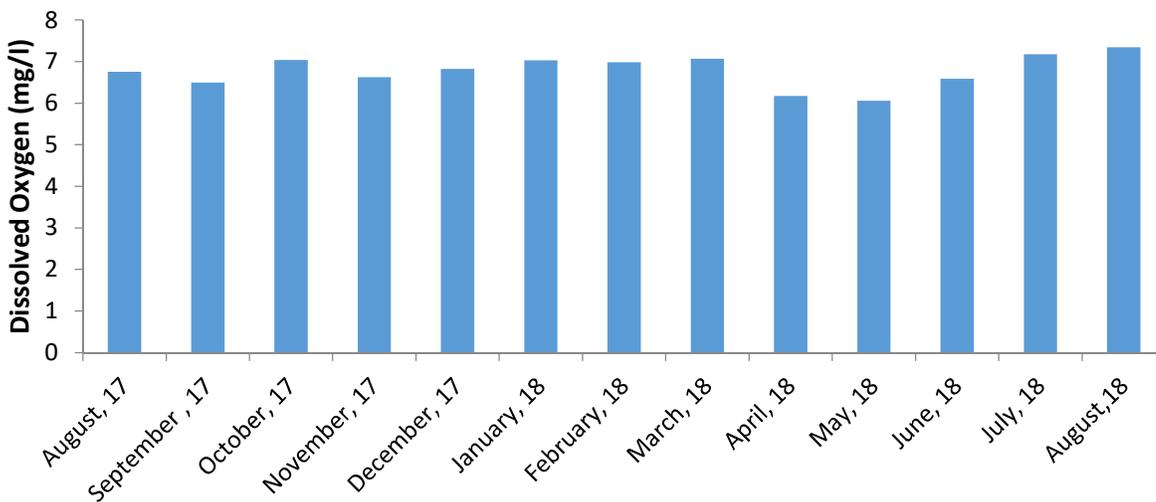


Fig. 20: Average DO of Brood Pond-2

Average pH of Pond-1 and Pond-2 were ranged from 7.45 ± 0.29 to 7.94 ± 0.67 and 7.52 ± 0.20 to 7.72 ± 0.12 during the study period that shown in the Fig. 21 and 22. In pond-1, the highest pH was found in the month of November (7.98 ± 0.17) and lowest was found in the month of January (7.31 ± 0.12) respectively. In pond-2, the highest pH was found in the month of November (7.71 ± 0.11) and lowest was found in the month of October (7.51 ± 0.27) and March (7.52 ± 0.22); this happen could be the heavy rainfall and short rainfall. Above mentioned the ranges of pH were suitable for the

growth of the brood fishes supported by the findings of Swingle (1957), Banarjee (1967), Bhuiyan (1970) and Ali *et al.*, (1982).

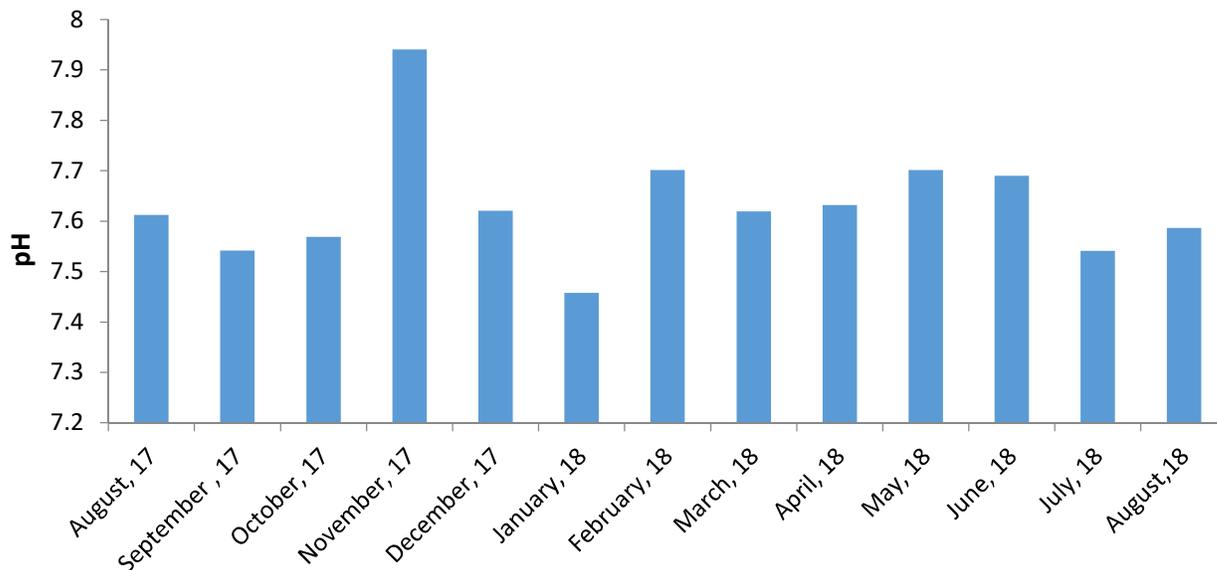


Fig. 21: Average pH of Brood Pond-1

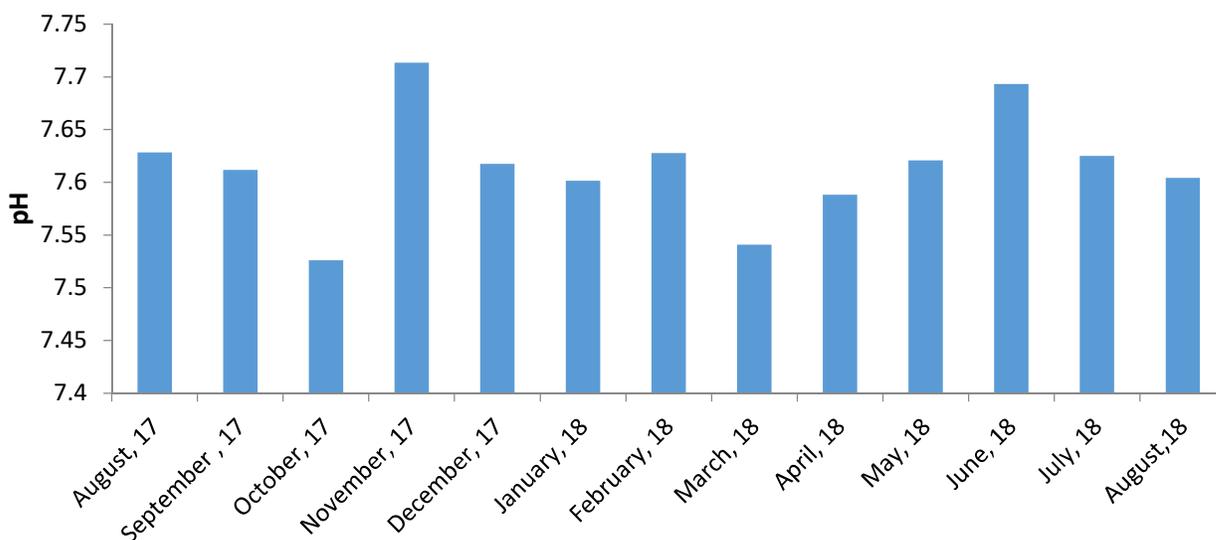


Fig. 22: Average pH of Brood Pond-2

Average monthly total dissolved solids (TDS) of Pond-1 and Pond-2 were varied from 283.58 ± 13.65 ppm to 382.16 ± 10.26 ppm and 258.42 ± 16.80 ppm to 343.34 ± 11.59 during the study period (Fig. 23 & 24). This level of TDS was below than the standard level of 500 mg/L set by the USA Environmental Protection Agency (Charkhabi and Sakizadeh, 2006) but far greater than the standard value of 0.13 mg/l recommended by Davis (1993).

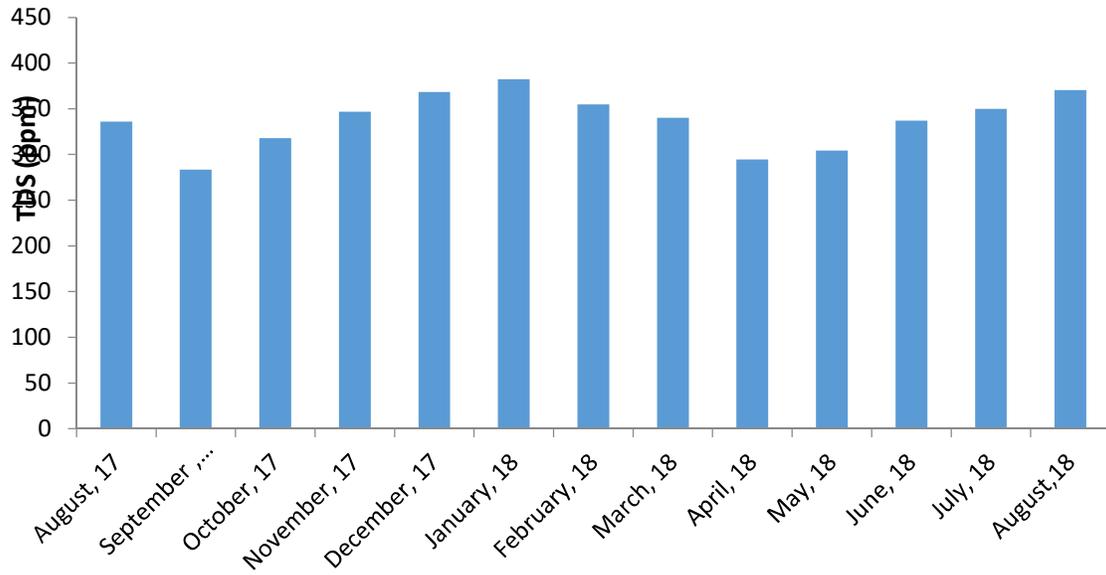


Fig. 23: Average TDS of Brood Pond-1

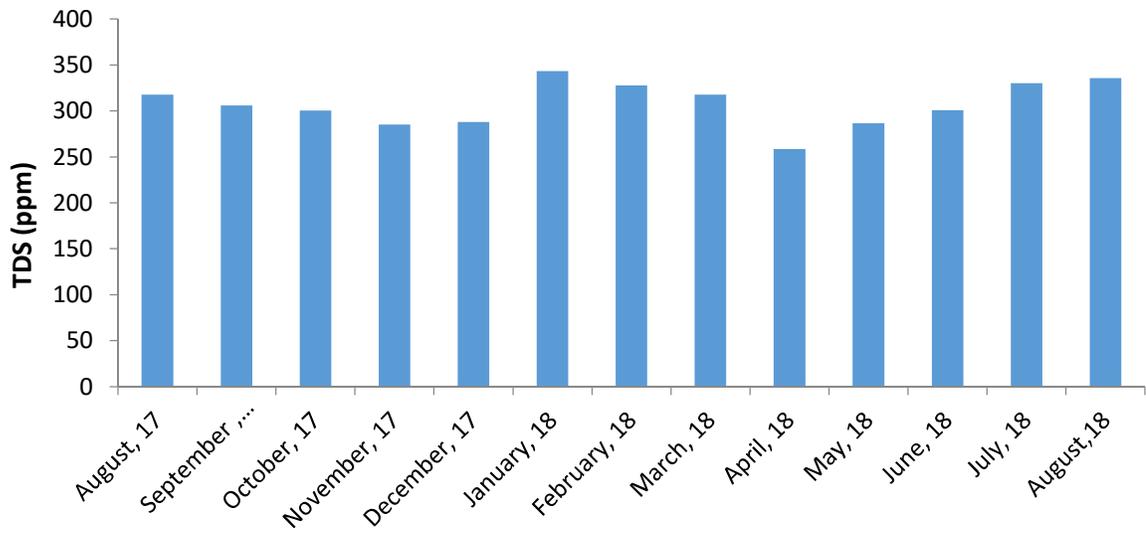


Fig. 24: Average TDS of Brood Pond-2

11.2 Observation of Growth of Brood Fishes

11.2.1 Growth performance of *L. guntea* and *B. dario*

Weight and length of fishes were measured fortnightly. Weight and length gain of those species were very slow in pond condition. Initial and final average weight of *L. guntea* were 2.64 ± 0.41 and 9.438 ± 1.77 gm respectively (Fig. 25) which supported the findings of Biswas *et al.*, (2018) and Gohain *et al.*, (2017). Initial and final average weight of *B. dario* were 3.57 ± 0.92 gm and 10.38 ± 1.789 gm respectively which were more or less similar as described by Hussain *et al.*, (2007). In the month of May June and July, the weight gain was much higher due to gonad development of those species (Fig. 25).

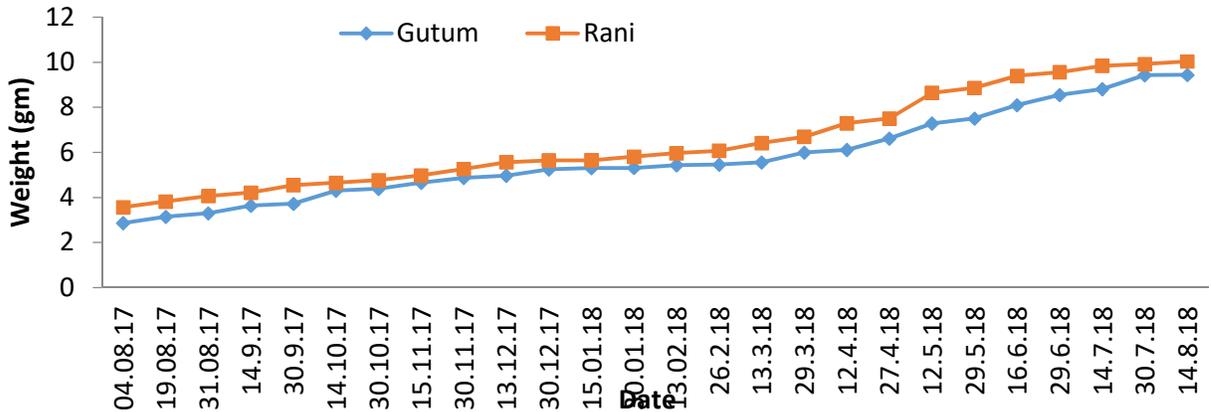


Fig. 25: Growth performance of *L. guntea* and *B. dario*

Initial and final average length of *L. guntea* was 5.52 ± 0.47 and 9.686 ± 0.87 cm respectively. According to Talwar and Jhingran (1991) and Rahman (2005), the average length of mature Gutumis 9-11 cm which supported the present findings. Initial and final average length of *B. dario* was 4.13 ± 0.17 and 9.369 ± 0.21 cm respectively (Fig. 26) which supported the findings of Hossen *et al.*, (2014). From the month of May to August, the average length gain was stagnant of those species (Fig. 26).

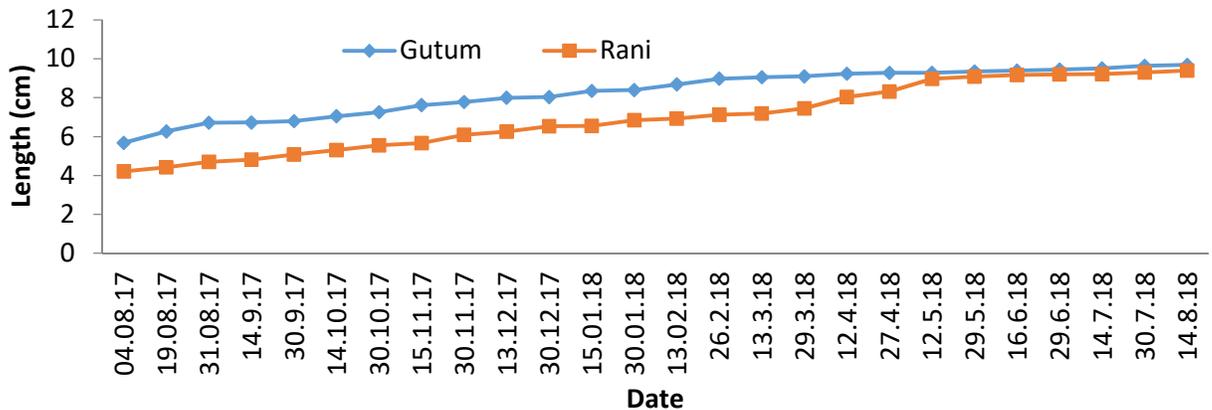


Fig. 26: Length performance of *L. guntea* and *B. dario*

11.3 Gonado- somatic index (GSI) of *L. guntea*

Breeding season can be determined on the basis of Gonado- somatic index (GSI) of fishes. The mean value of GSI of *L. guntea* was determined during the study period from August, 2017 to August, 2018. In the study period, didn't found the GSI value from the month of September to November (Fig. 27). The highest mean value of GSI value was calculated $11.38 \pm 1.23\%$ in June and lowest mean value of GSI value was $0.75 \pm 0.041\%$ in December as shown in Fig. 27. From the study, higher GSI value was found in the month of April, May and June compare to others months which had similarities with the Rahman *et al.*, (1997), Baishya *et al.*, (2010) and Dey *et al.*, (2016). GSI correspond to increased gonadal development, which is between March to July and followed by a gradual decrease in value after July which was similarities to Choudhury *et al.*, 2015. The GSI showed a single highest peak during the months of April to July, thereby indicating that a greater percentage of fish were maturing during this period. The sudden drop of GSI during the month of August clearly suggested the onset of spawning activity in this fish, indicating that the fish had a definite breeding season in a year. Similar results were reported for *Rasbora rasbora* by Agarwal (1982) and showed the highest value of GSI for the month of July and a sudden drop in the month of August.

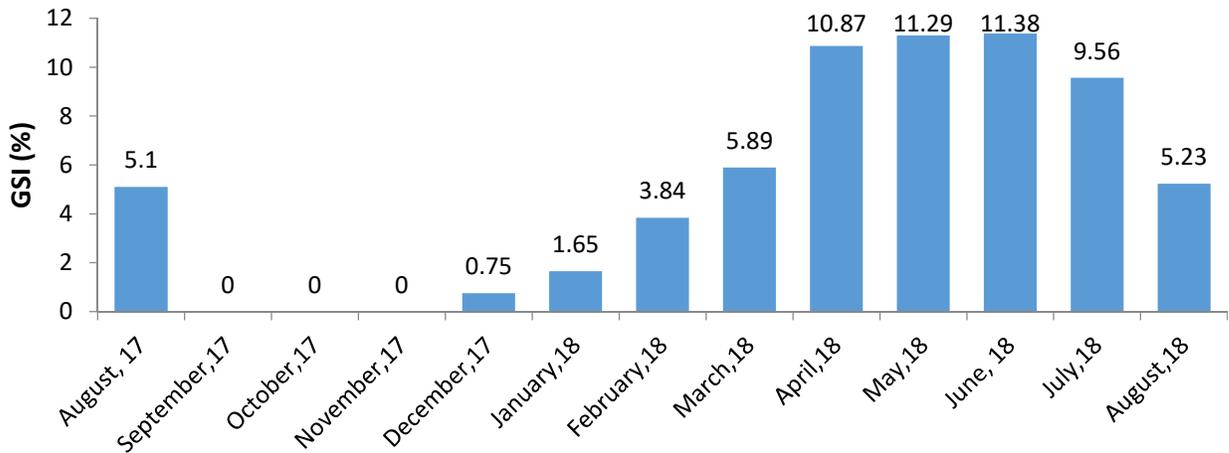


Fig. 27: Average Monthly GSI of *L. guntea*

11.4 Gonado- somatic index (GSI) of *B. dario*

The mean value of GSI was observed during the study period from August, 2017 to August, 2018. GSI value of *B. dario* was found from January to July. From the study, didn't found the GSI value from August to December. The highest mean value of GSI was determined $10.94 \pm 1.17\%$ in June and lowest mean GSI value was $0.2 \pm 0.032\%$ in August as shown in Fig. 28. From the result, concluded that the peak breeding season of the *B. dario* was May and June which showed similarities with the findings of Dey *et al.*, (2015), Hoque and Rahman (2008), Bithy *et al.*, (2012) and Dey *et al.*, (2016). GSI indicates gonadal development and maturity of fish which increases with the maturation of the fish and declines abruptly thereafter (Parameswarn *et al.*, 1974). Yeldan and Avsar (2000) also reported that GSI is widely used especially for the bony fishes in order to examine the spawning period because its value is directly related to the development of the gonad.

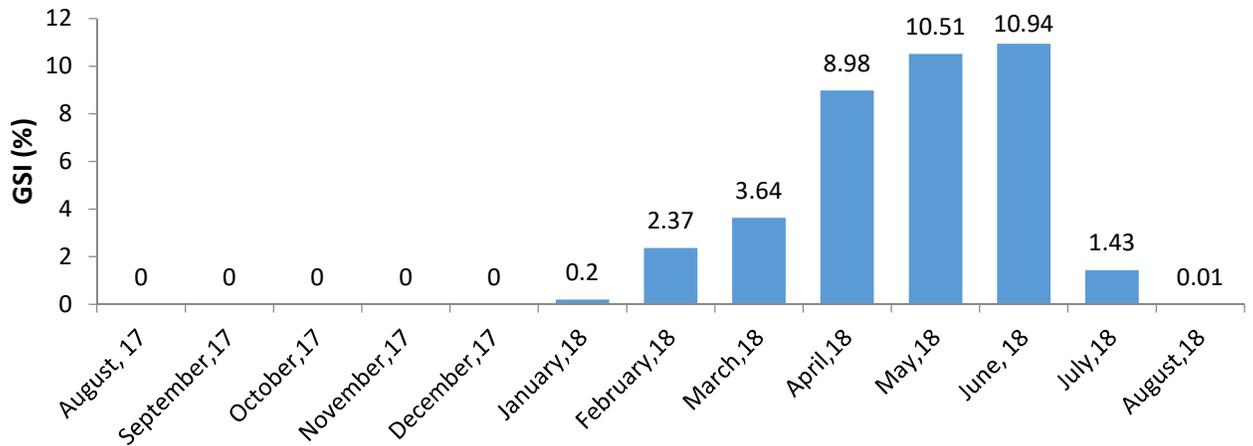


Fig. 28: Monthly Average GSI of *B. dario*

11.5 Fecundity of *B. dario* and *L. guntea*

Fecundity is the estimation of ova content in the ovary of a matured female specimen. Fecundity was estimated by sampling and direct counting method. During the study period, the mean fecundity ranged from 170 ± 27.34 to 12521 ± 232.57 for female of *B. dario* having average body weight of 6.45 ± 1.23 g to 13.8 ± 1.32 g. In this study, the mean fecundity of female was higher in the month of May (12452 ± 209.28) and June (12521 ± 232.57) compare to other months. The fecundity of this species was gradually decrease since the month of July (Fig. 29). This result was almost similar to the findings of other researchers like as the study of Dey *et al.*, (2015), Hussain *et al.*, (2007) and Dey *et al.*, (2016) and Roy and Hossain *et al.*, (2006). Bagenal (1967) reported that length and weight are reliable indicators of the capacity of egg production; hence the fecundity increases with the increase of the fish in size and weight. This condition was also found in the present work, in which the number of eggs increases with an increase of length and weight of fish.

The mean fecundity was ranged from 698 ± 45.27 to 9284 ± 213.46 for female of *L. guntea* having average body weight of 5.29 ± 1.2 g to 13.5 ± 1.3 g (Fig. 30). In this study, the mean fecundity of female was higher in the month of May (9143 ± 119.34) and June (9284 ± 164.52) compare to other months. During the study period, didn't found the fecundity from the month of August to December (Fig. 30). The fecundity of this species was gradually decreasesince the month of July (Fig. 30). From the study, the breeding season was the April to June for the species of *B. dario* and May to June for the species of *L. guntea* (Fig. 29 and 30). This result supported by the findings of *et al.*, (2016) and Roy and Hossain *et al.*, (2006). Rahman *et al.*, (1997), Baishya (2010), Dey

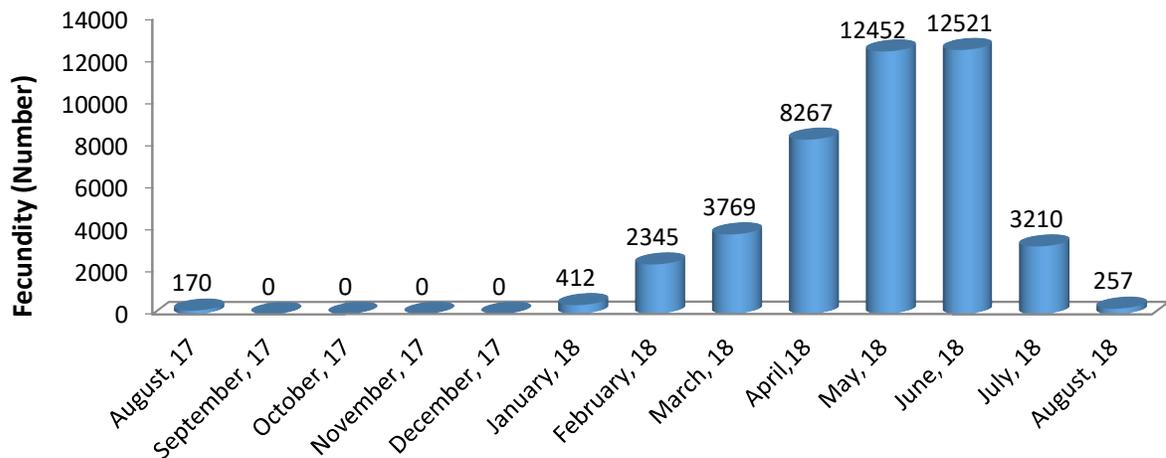


Fig.29: Monthly Average Fecundity of *B. dario*

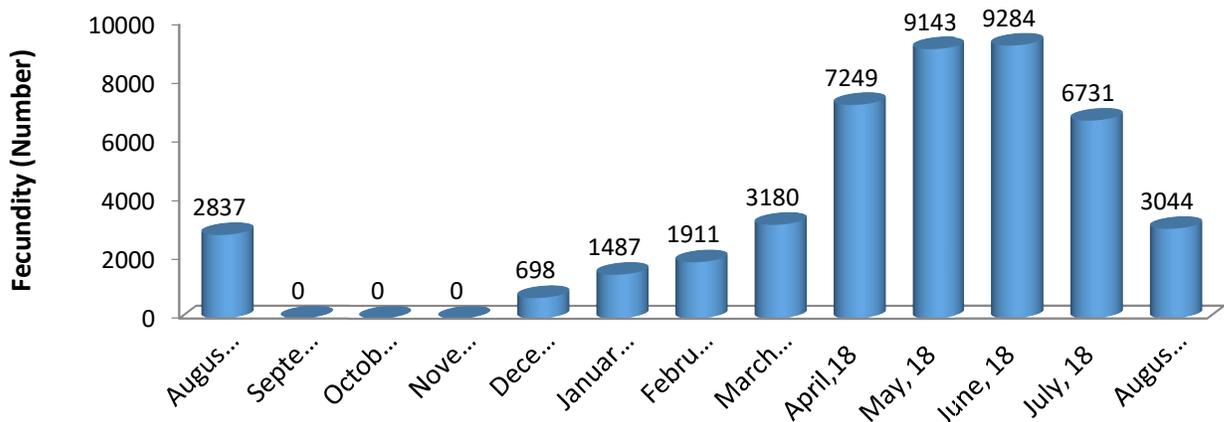


Fig. 30: Average Monthly Fecundity of *L. guntea*

11.6. Gonado-Somatic Index and Correlation between body weight, fecundity, gonad weight and GSI

Spawning period was confirmed by the Gonado-somatic index (GSI). GSI increased from April to July. Gonado-Somatic Index was higher in female than male. For establish the mathematical relationship between body weight, gonad weight and fecundity. Gonado-Somatic Index, Coefficient of Correlation (r) was done using the MS- Excel. In case of *B. dario*, the scatter diagram of female gonado-somatic index and fecundity (Fig. 31), fecundity and gonad weight (Fig. 32) showed a straight line relationship and is expressed as $Y = a + bx$, where, 'a' and 'b' are constants; X and Y are the variables. The coefficient of correlation (r) showed significance at $p \leq 0.01$.

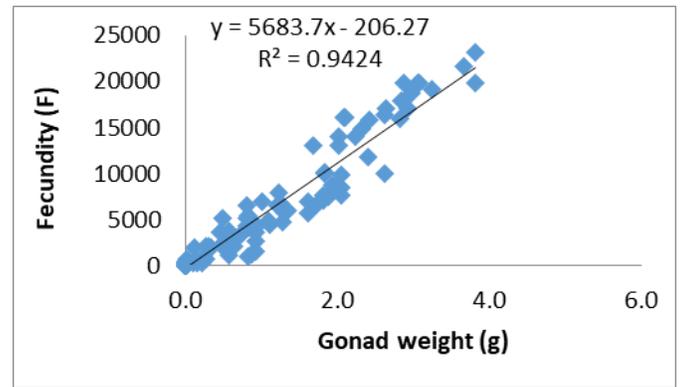
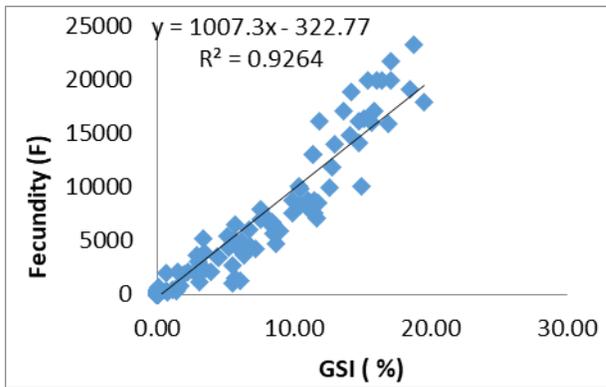


Fig.: 31 Relationship between fecundity and GSI of *B. dario* Fig. : 32 Relationship between fecundity and Gonad weight of *B. dario*

In case of *L. guntea*, the scatter diagram of female GSI and fecundity (Fig. 33), fecundity and gonad weight (Fig. 34), gonad weight and body weight (Fig. 35) showed a straight line relationship and is expressed as $Y = a + bx$, where, 'a' and 'b' are constants; X and Y are the variables. The coefficient of correlation (r) showed significance at $p \leq 0.01$. Linear relationships between fecundity, gonad weight, GSI and body weight were found to be highly significant (Fig. 33, 34 and 35).

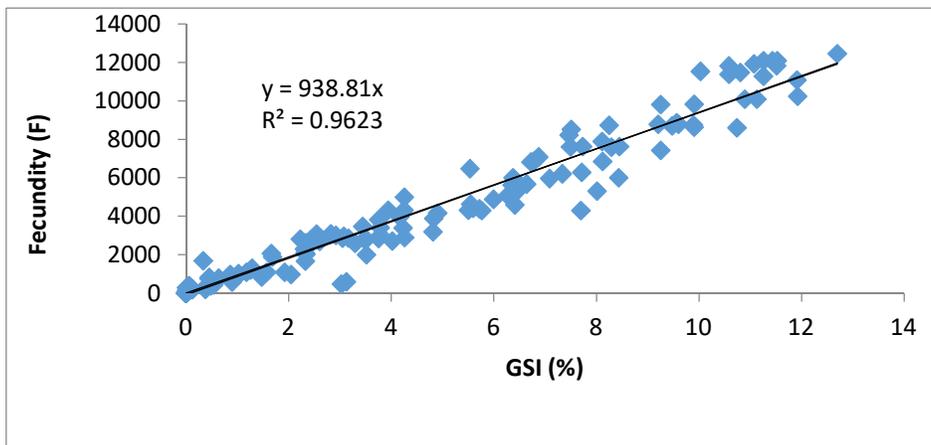


Fig. 33: Relationship between fecundity and GSI of *L. guntea*

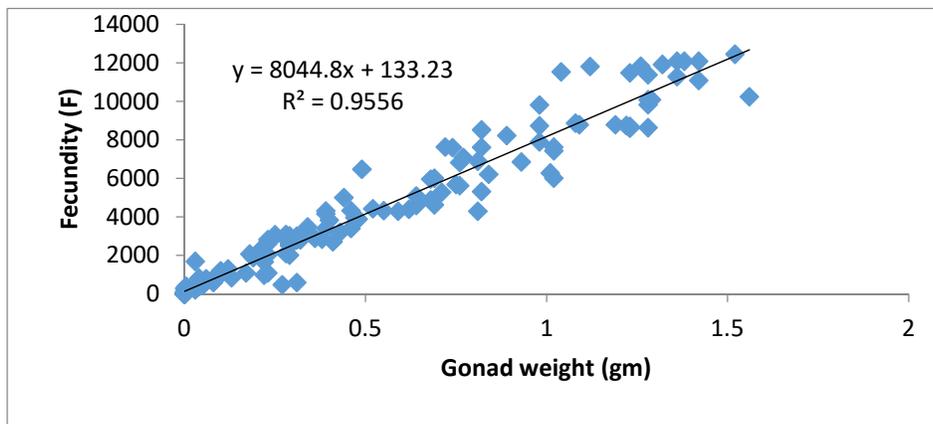


Fig. 34: Relationship between fecundity and gonad weight of *L. guntea*

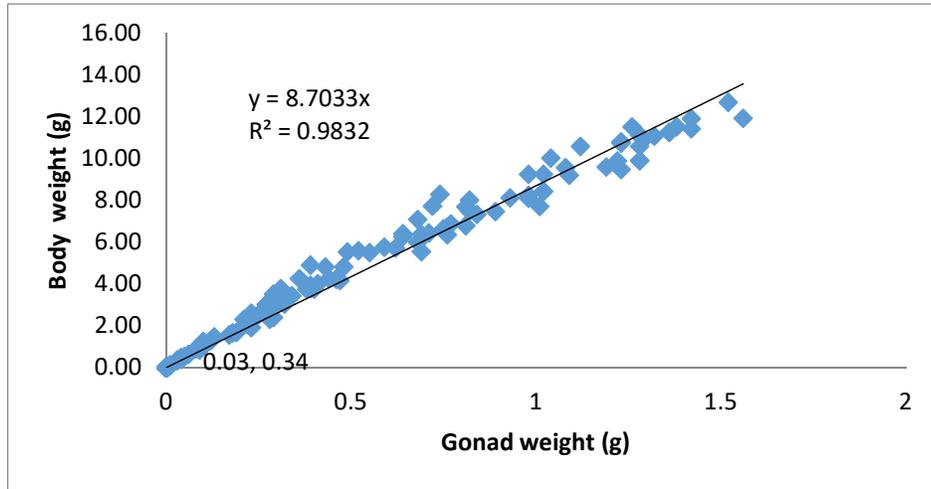


Fig. 35:
Relationship between body weight and gonad weight of *L. guntea*
11.7 Oocyte diameter of fish eggs

During the breeding season, measured the egg size of *B. dario* and *L. guntea*. Highest egg diameter was found for both species in the month of June, 2018 that shown in the Table 2. Samples of eggs were collected from the anterior part, middle part and the post anterior part of the gonad and measured the eggs diameter and presented the average value in the Table 2. Histopathological study of eggs can be more accurate result instead of egg diameter. But can draw the conclusion of egg maturity with the measure of diameter. From the result, it can be concluded that egg maturity was high in the month of May and June of both species.

The ovum was an almost oval shape in the mature stage. The mean oocyte diameter increased with the maturational stage of the ovary of *L. guntea* and *B. dario*. The oocyte diameter progressively increased from April to June, along with the progression of the maturity phase (Das *et. al.*, 1998). Chakraborty *et al.*, (2007) reported that the egg diameter of *Puntius sarana* was significantly higher in the month of June, where the diameter of the ova was decreased in the month of October, which indicates that the diameter of the ova was highest in the peak spawning season (Chakraborty *et al.*, 2000).

Table 2: Oocyte diameter of *L. guntea* and *B. dario* in different month.

Fish	April	May	June	July	August
<i>L. guntea</i>	0.22±0.04 mm	0.42 ± 0.07 mm	0.53±0.05mm	0.39±0.07mm	0.27±0.09mm
<i>B. dario</i>	0.44 ±0.08mm	0.56±0.09 mm	0.61±0.07mm	0.31±0.05mm	0.18±0.06mm

11.8 Water Quality Parameter of Incubators

Water quality parameters such as, temperature (°C), pH, dissolved oxygen (mg/l) and TDS (ppm) were monitored at every hour interval in those incubators during the experimental period. Water qualities (pH, DO, Temp. and TDS) were measured for *L. guntea* and *B. dario* in glass jar aquaria (Panel A) and aluminum cistern incubator (Panel B) are shown in Table 3 and Table 4. The average temperature of incubators (Panel A and Panel B) was ranged from 25°C to 31°C where minimum level was recorded at the treatments conducted on May and the maximum level of temperature was recorded at July. In May, measured the average temperature of nine replications for incubators ranged 25.32±0.74°C to 26.4±0.70°C. In June, measured the average temperature of nine replications for incubators ranged 25.48±0.21°C to 26.93±0.54°C. In July, measured the average temperature of nine replications for

incubators ranged $28.5\pm 0.87^{\circ}\text{C}$ to $30.5\pm 0.73^{\circ}\text{C}$. Average temperature of incubators in May and June of both types of incubator were suitable for fish breeding while the average temperature at July was little bit higher. Temperature is very important factor for the incubation of eggs. The development of embryo and the variability of hatching time in fertilized eggs and their viability of most of the fish generally are influenced by the temperature of water (Hoar and Randall, 1969; Jhingran, 1983 and Rahman, 1975). Temperature is inversely proportional to the time of hatching (Alikunhi et al., 1962; Mollah, 1983; Rana, 1990) and hatching success (Hoar and Randall, 1969).

Measured the average dissolved oxygen (DO) of the incubators in the month of May, June and July ranged 7.64 ± 0.24 mg/L to 7.87 ± 0.23 mg/L, 7.34 ± 0.21 to 8.23 ± 0.26 mg/L and 7.67 ± 0.25 mg/L to 8.23 ± 0.29 mg/L respectively. These levels of DO were appropriate for induced breeding of fishes conducted earlier by Islam, et al., (2015), Dey et al., (2015), Bashuda et al., (2017) and Mumtazuddin and Khaleque (1981).

In the month of May, June and July, measured the average value of pH of both Panel A and Panel B ranged 7.45 ± 0.18 to 7.52 ± 0.20 , 7.21 ± 0.18 to 7.69 ± 0.21 and 7.53 ± 0.49 to 7.82 ± 0.23 respectively (Table 3 & 4). These levels of pH was suitable for induced breeding and had showed similarities with the findings of Islam et al., (2015), Dey et al., (2015), Bashuda et al., (2017) and Mumtazuddin and Khaleque (1981). In the month of May, June and July of incubators, measured the average total dissolved solids (TDS) ranged 415.81 ± 91.25 ppm to 479.79 ± 40.89 ppm, 357.35 ± 66.7 ppm to 556.77 ± 91.2 ppm and 377.2 ± 41.3 ppm to 467.58 ± 79.80 ppm respectively (Table 3 & 4). These levels of TDS were far away greater than the recommendations of Bashuda et al., (2017), Mumtazuddin and Khaleque (1981) and Davis (1993).

For the induced breeding of *B. dario*, measured the temperature, DO, pH, and TDS were measured of incubators. In the month of May, June and July, measured the average value of temperature of incubators ranged 25.32 ± 0.74 to $26.29\pm 0.43^{\circ}\text{C}$, 25.48 ± 0.21 to $26.9\pm 0.37^{\circ}\text{C}$ and 28.81 ± 0.88 to $30.2\pm 0.97^{\circ}\text{C}$ (Table 5 and 6). Average temperature was higher in July and lower was in May of incubators. Temperature of water is the vital factor for hatching of eggs. The temperature of incubators was similar to the findings of Hoar and Randall (1969). In the month of May, June and July, measured the average value of DO of incubators ranged 7.64 ± 0.32 to 7.77 ± 0.2 mg/l, 7.34 ± 0.31 to 7.82 ± 0.23 mg/l and 7.43 ± 0.25 to 7.92 ± 0.17 mg/l (Table 5 and 6). The range of pH value of incubators was 7.43 ± 0.18 to 7.51 ± 0.13 in May and 7.43 ± 0.13 to 7.87 ± 0.21 in June and 7.44 ± 0.21 to 7.73 ± 0.67 in July. From the study, the average value of pH was lower in May and higher in May of incubators (Table 5 and 6). These levels of pH was suitable for induced breeding and had showed similarities with the findings of Islam et al., (2015), Dey et al., (2015), Bashuda et al., (2017) and Mumtazuddin and Khaleque (1981).

In the month of May, June and July of incubators, measured the average total dissolved solids (TDS) ranged 414.8 ± 91.25 to 508.75 ± 40.89 ppm, 401.98 ± 59.6 to 474.79 ± 74.2 ppm and 387.65 ± 48.23 to 453.63 ± 76.4 ppm respectively (Table 5 & 6). These levels of TDS were far away greater than the recommendations of Bashuda et al., (2017), Mumtazuddin and Khaleque (1981) and Davis (1993).

Table 3: Water Quality Parameters of Glass jar aquaria (Panel A) for *L. guntea*

Month	Parameters (Average)	Glass Jar Aquaria (Panel A)					
Hormone protocol		G1	G2	G3	G4	G5	G6
		CPG (5mg/kg)	CPG (10 mg/kg)	HCG (300 IU/kg)	HCG (600 IU/kg)	Ovaprium (0.5 ml/kg)	Ovaprium (1.0 ml/kg)
May	Temperature (°C)	25.6±0.88	26.4±0.70	26±0.51	25.7±0.89	26.2±0.57	25.8±0.97
	DO (mg/l)	7.75±0.35	7.87±0.23	7.76±0.29	7.78±0.24	7.71±0.18	7.64±0.30
	pH	7.44±0.14	7.47±0.19	7.47±0.14	7.47±0.15	7.52±0.13	7.45±0.21
	TDS (ppm)	456.9±79.8	435.9±93.2	420.34±89.1	464.7±71.6	421.1±90.1	447.4±74.4
Hormone protocol		CPG (8mg/kg)	CPG (12 mg/kg)	HCG (900 IU/kg)	HCG (1200 IU/kg)	Ovaprium (1.5 ml/kg)	Ovaprium (2.0 ml/kg)
June	Temperature (°C)	25.76±0.74	26.54±0.43	26.09±0.76	26.93±0.54	26.23±0.46	26.48±0.78
	DO (mg/l)	8.1±0.24	7.82±0.28	7.65±0.32	7.34±0.21	8.23±0.34	7.67±0.49
	pH	7.21±0.18	7.43±0.13	7.69±0.21	7.58±0.19	7.34±0.27	7.52±0.15
	TDS (ppm)	525.67±45.2	476.54±40.8	357.35±66.7	480.98±71.7	556.77±91.2	498.23±54.2
Hormone protocol		CPG (15mg/kg)	CPG (20 mg/kg)	HCG (1500 IU/kg)	HCG (2000 IU/kg)	Ovaprium (2.5 ml/kg)	Ovaprium (3.0 ml/kg)
July	Temperature (°C)	29.7±0.33	30.1±0.45	29.6±0.76	28.9±0.87	30.3±0.98	29.8±0.23
	DO (mg/l)	7.65±0.14	7.67±0.23	8.23±0.29	8.02±0.47	7.82±0.33	7.73±0.25
	pH	7.54±0.45	7.34±0.37	7.48±0.29	7.53±0.49	7.68±0.51	7.82±0.23
	TDS (ppm)	377.2±41.3	384.58±65.4	445.49±72.7	402.37±59.3	432.61±78.3	398.34±47.2

Table 4: Water Quality Parameters of Aluminum cistern (Panel B) for *L. guntea*

Month	Parameters (Average)	Aluminum Cistern Incubator (Panel B)		
Hormone protocol		L1	L2	L3
		CPG (8 mg/kg)	HCG (800 IU/kg)	Ovaprium (0.8 ml/kg)
May	Temperature (°C)	25.79±0.496	26.06±0.60	25.95±0.51
	DO (mg/l)	7.77±0.31	7.52±0.24	7.73±0.21
	pH	7.49±0.11	7.45±0.12	7.49±0.12
	TDS (ppm)	435±90.08	415.81±85.58	479.79±59.66
Hormone protocol		CPG (10 mg/kg)	HCG (1000 IU/kg)	Ovaprium (1.2 ml/kg)
June	Temperature (°C)	26.93±0.76	26.52±0.43	25.73±0.89
	DO (mg/l)	7.88±0.29	7.53±0.37	8.23±0.26
	pH	7.54±0.19	7.47±0.18	7.23±0.21
	TDS (ppm)	380.34±45.56	404.56±65.34	397.89±72.12

Month	Parameters (Average)	Aluminum Cistern Incubator (Panel B)		
Hormone protocol		CPG (12 mg/kg)	HCG (1400 IU/kg)	Ovaprium (1.7 ml/kg)
July	Temperature (°C)	28.5±0.87	30.5±0.73	29.7±0.58
	DO (mg/l)	7.65±0.42	7.88±0.28	8.02±0.57
	pH	7.39±0.17	7.67±0.14	7.27±0.23
	TDS (ppm)	401.08±87.76	467.58±79.80	453.39±89.1

Table 5: Water Quality Parameters of Glass jar incubator (Panel A) for *B. dario*

Month	Parameters (Average)	Glass Jar Aquaria (Panel A)					
		R1	R2	R3	R4	R5	R6
Hormone protocol		CPG (5mg/kg)	CPG (10 mg/kg)	HCG (300 IU/kg)	HCG (600 IU/kg)	Ovaprium (0.5 ml/kg)	Ovaprium (1.0 ml/kg)
May	Temperature (°C)	26.29±0.43	25.32±0.74	25.67±0.78	25.79±0.76	26.24±0.46	25.92±0.54
	DO (mg/l)	7.73±0.27	7.64±0.32	7.7±0.27	7.76±0.27	7.72±0.27	7.77±0.21
	pH	7.47±0.18	7.44±0.13	7.47±0.20	7.49±0.12	7.43±0.18	7.51±0.13
	TDS (ppm)	414.8±91.25	418.3±92.9	459.54±66	423.36±90.39	508.75±40.89	488.41±53.93
Hormone protocol		CPG (8mg/kg)	CPG (12 mg/kg)	HCG (900 IU/kg)	HCG (1200 IU/kg)	Ovaprium (1.5 ml/kg)	Ovaprium (2.0 ml/kg)
June	Temperature (°C)	26.9±0.37	26.77±0.43	26.33±0.67	25.84±0.39	26.09±0.57	26.59±0.88
	DO (mg/l)	7.65±0.14	7.58±0.35	7.82±0.23	7.59±0.25	7.34±0.31	7.43±0.29
	pH	7.43±0.13	7.65±0.19	7.54±0.15	7.47±0.12	7.87±0.21	7.77±0.21
	TDS (ppm)	421.32±89.1	443.47±74.4	408.59±85.6	436.78±66.1	401.98±59.6	474.79±74.2
Hormone protocol		CPG (15mg/kg)		HCG (1500 IU/kg)	HCG (2000 IU/kg)	Ovaprium (2.5 ml/kg)	Ovaprium (3.0 ml/kg)
July	Temperature (°C)	29.7±0.88	28.9±0.7	30.1±52	29.7±0.74	30.2±0.97	29.8±0.61
	DO (mg/l)	7.81±0.3	7.59±0.46	7.64±0.32	7.92±0.17	7.43±0.25	7.64±0.15
	pH	7.49±0.17	7.44±0.21	7.55±0.37	7.47±0.49	7.67±0.38	7.59±0.27
	TDS (ppm)	421.45±67.8	443.37±47.8	439.73±77.3	408.29±53.8	453.63±76.4	415.17±92.6

Table 6: Water Quality Parameters of Aluminum cistern incubator (Panel B) for *B. dario*

Month	Parameters (Average)	Aluminum Cistern Incubator (Panel B)		
		D1	D2	D3
Hormone protocol		CPG (8 mg/kg)	HCG (800 IU/kg)	Ovaprium (0.8 ml/kg)
May	Temperature (°C)	25.80±0.88	25.64±0.91	25.77±0.89
	DO (mg/l)	7.74±0.25	7.67±0.26	7.77±0.24
	pH	7.45±0.18	7.48±0.20	7.47±0.15
	TDS (ppm)	454.11±72.31	435.61±73.14	459.87±71.58
Hormone protocol		CPG (10 mg/kg)	HCG (1000 IU/kg)	Ovaprium (1.2 ml/kg)
June	Temperature (°C)	25.48±0.21	26.07±0.46	25.87±0.64
	DO (mg/l)	7.80±0.37	7.48±0.56	7.35±0.43
	pH	7.73±0.67	7.66±0.21	7.47±0.39
	TDS (ppm)	455±77.98	478±82.23	498±57.32
Hormone protocol		CPG (12 mg/kg)	HCG (1400 IU/kg)	Ovaprium (1.7 ml/kg)
July	Temperature (°C)	28.81±0.88	29.23±0.71	29.62±0.97
	DO (mg/l)	7.87±0.35	7.64±0.29	7.82±0.18
	pH	7.58±0.23	7.73±0.16	7.34±0.34
	TDS (ppm)	401.34±74.89	387.65±48.23	410.59±81.37

11.9 Spawning Rate of *L. guntea*

L. guntea broods were injected with inducing hormone at three different trials conducted in May, 18; June 18 and July 18. All trials were done with three treatments and twenty seven replications under two types of incubator. Three types of hormone viz. CPG, HCG and Ovaprium were used. Species of *L. guntea* responded of spawning differently with these hormones. The spawning rate of this species ranged 0 to 100% at different treatments with different replications. Monthly spawning rate of *L. guntea* at different hormonal treatment are shown in Table 7, Table 8 and Table 9.

Spawning Rate at May: In the treatment of Ovaprium, replication of 1.0 ml/kg (G6), 0.8 ml/kg (L3), 0.5 ml/kg (G5), were effective for spawning. In the treatment of CPG, replication of 10 mg/kg (G2), 8 mg/kg (L1) were effective for spawning and 800 IU/kg (L2) of treatment HCG showed spawning during the experimental period of May. Replication of 1.0 ml/kg (G6) and 0.8 ml/kg (L3) performed 100% and 80 % spawning among nine replications in May, 2018 as shown in the Table 7. From this experiment, occurred higher spawning with the treatment of Ovaprium hormone compare to other treatments of CPG and HCG. During the trial of this month, spawning didn't occurred with the replication 5 mg/kg (G1), 300 IU/kg (G3), 600 IU/kg (G4) of hormones (Table 7).

Spawning Rate at June: Species of *L. guntea* was spawning with the different types and doses of hormones in June. Among the nine replications, replication of 12 mg/kg (G2) and 1.5 ml/kg (G5) showed almost 100% spawning rate at the trial of June, 2018 (Table 8). In Other replication, occurred spawning (80% > 70% > 70% > 60% > 60% > 60% > 50%) with the replication of 1.2 ml/kg (L3) > 2.0 ml/kg (G6) > 10 mg/kg (L1) > 8 mg/kg (G1) > 1000 IU/kg (L2) > 1200 IU/kg (G4) > 900 IU/kg (G3). During the trial of the month, didn't occurred any death of broods among the nine replications (Table 8).

Spawning Rate at July: In the month of July, higher (almost 80%) spawning rate was occurred with the replication of 12 mg/kg CPG (L1) and 1.7 ml/kg Ovaprium (L3) compare to others replications. Spawning didn't occurred with the replication of 3.0 ml/kg (G6) and 2000 IU/kg (G4). During this experiment, occurred death of broods with the replication of 2000 IU/kg(G4), 2.5 ml/kg (G5), 3.0 ml/kg (G6) and 1.7 ml/kg (L3). In Other replication, observed spawning with the replication of 2.5 ml/kg (G5) > 1400 IU/kg (L2) > 15 mg/kg (G1) > 20 mg/kg (G2) > 1500 IU/kg (G3) at 60% > 60% > 50% > 50%. Dead fishes were found since 3 days after the injection time. This could be happened due to overdose of hormone and injured by niddle of syringe (Table 9).

Table 7: Spawning Rate of *L. guntea* In May

Month	Incubator	Replication	Hormone	Hormone dose	Spawning Rate (%)	Remarks
May	Glass jar Incubator (Panel A)	G1	CPG	5 mg/kg	0	Not found egg
		G2		10 mg/kg	60	Found egg
		G3	HCG	300IU/kg	0	Not found egg
		G4		600 IU/kg	0	Not found egg
		G5	Ovaprium	0.5 ml/kg	50	Found egg
		G6		1.0 ml/kg	100	Found egg
	Aluminum Cistern incubator (Panel B)	L1	CPG	8 mg/kg	40	Found egg
		L2	HCG	800 IU/kg	30	Found egg
		L3	Ovaprium	0.8 ml/kg	80	Found egg

Table 8: Spawning Rate of *L. guntea* In June

Month	Incubator	Replication	Hormone	Hormone dose	Spawning Rate (%)	Remarks
June	Glass jar Incubator (Panel A)	G1	CPG	8mg/kg	60	Found egg
		G2		12 mg/kg	100	Found egg
		G3	HCG	900 IU/kg	50	Found egg
		G4		1200 IU/kg	60	Found egg
		G5	Ovaprium	1.5 ml/kg	100	Found egg
		G6		2.0 ml/kg	70	Found egg
	Aluminum Cistern incubator (Panel B)	L1	CPG	10 mg/kg	70	Found egg
		L2	HCG	1000 IU/kg	60	Found egg
		L3	Ovaprium	1.2 ml/kg	80	Not found egg

Table 9: Spawning Rate of *L. guntea* In July

Month	Incubator	Replication	Hormone	Hormone dose	Spawning Rate (%)	Remarks
July	Glass jar Incubator (Panel A)	G1	CPG	15 mg/kg	50	Found egg
		G2		20 mg/kg	50	Found egg
		G3	HCG	1500 IU/kg	50	Found egg
		G4		2000 IU/kg	0	Fish dead
		G5	Ovaprium	2.5 ml/kg	60	Found egg and dead fish
		G6		3.0 ml/kg	0	Fish dead
	Aluminum Cistern incubator (Panel B)	L1	CPG	12 mg/kg	80	Found egg
		L2	HCG	1400 IU/kg	60	Found egg
		L3	Ovaprium	1.7 ml/kg	80	Found egg and dead fish

11.10 Spawning Rate of *B. dario*

Brood fishes of *B. dario* were injected with inducing hormone at three different trials conducted in May, 18; June, 18 and July, 18. Different doses of hormones were done with 27 replications under three treatments into two types of incubator. Three types of hormone viz. CPG, HCG and Ovaprium were used. But *B. dario* didn't responded to spawning with the administrated of different types and doses of hormones under different condition. Spawning rates were zero at every replication of treatments as shown in table 10.

Table 10: Spawning Rate of *B. dario*

Incubator	Replication	Hormone	Hormone dose			Hormone dose			Remarks
			May	June	July	May	June	July	
Flass jar Incubator (Panel A)	R1	CPG	5 mg/kg	8 mg/kg	15 mg/kg	0	0	0	Not found egg
	R2		10 mg/kg	12 mg/kg	20 mg/kg	0	0	0	Not found egg
	R3	HCG	300 IU/kg	900 IU/kg	1500 IU/kg	0	0	0	Not found egg
	R4		600 IU/KG	1200 IU/KG	2000 IU/KG	0	0	0	Not found egg
	R5	Ovaprium	0.5 ml/kg	1.5 ml/kg	2.5 ml/kg	0	0	0	Not found egg
	R6		1.0 ml/kg	2.0 ml/kg	3.0 ml/kg	0	0	0	Not found egg
Aluminum Cistern incubator (Panel B)	D1	CPG	8 mg/kg	10 mg/kg	12mg/kg	0	0	0	Not found egg
	D2	HCG	800 IU/kg	1000 IU/kg	1400 IU/kg	0	0	0	Not found egg
	D3	Ovaprium	8 ml/kg	1.2 ml/kg	1.7 ml/kg	0	0	0	Not found egg

11.11 Latency Period of *L. guntea*

Average spawning period of *L. guntea* were varied from 3.17 h to 4.38 h among the replications under two types of incubator. Average spawning period of *L. guntea* are shown in Fig. 36 and Fig. 37. The highest latency period (4.38 hrs.) was observed with 8mg/kg CPG (L1) in June and lowest period (3.11) with 1400 IU/kg HCG (L2) in July. Average latency period was lower with HCG treatments compare to other CPG and Ovaprium treatments. In addition that latency period of Aluminum Cistern incubator (Panel B) was little higher compare to Glass jar Incubator (Panel A) (Fig. 36& 37). In other replications, didn't show any latency period such as 5 mg/kg (G1) in May, 300 IU/kg (G3) in May and 600 IU/kg and 2000 IU/kg (G4) in May and July and 3.0 ml/kg (G6) in July due to occur spawning in those replications (Fig. 36).

The range of latency period of *L. guntea* was less than the other findings (vary 5 to 8 hours) of Bashuda et al., (2017), Purkayastha et al., (2012), Udit et al., (2014) and Sridhar et al., (1998) and almost similar to the opinion of Pal (2000) who stated that the courtship behavior of *N. nandus* started after 4 to 6 hrs. Ovaprim and high dose of HCG treatment lead to shorter latency time (40 h 40'), but ovulation percent, percentage of live embryos in the eyed stage and ovulation synchronization were lower than groups treated with Ovaprim singly or Ovaprim plus low dose of HCG (Abdolali et al., 2014). The latency period of *B. dero* was 7-10 hours. Udit et al. (2014) observed the latency period of *Puntius sarana* was also to be 8 to 9 hours. However, the latency period of Ompok pabda was found to be 6 to 8 hours when ovatide hormone used (Purukayastha et. al., 2012).

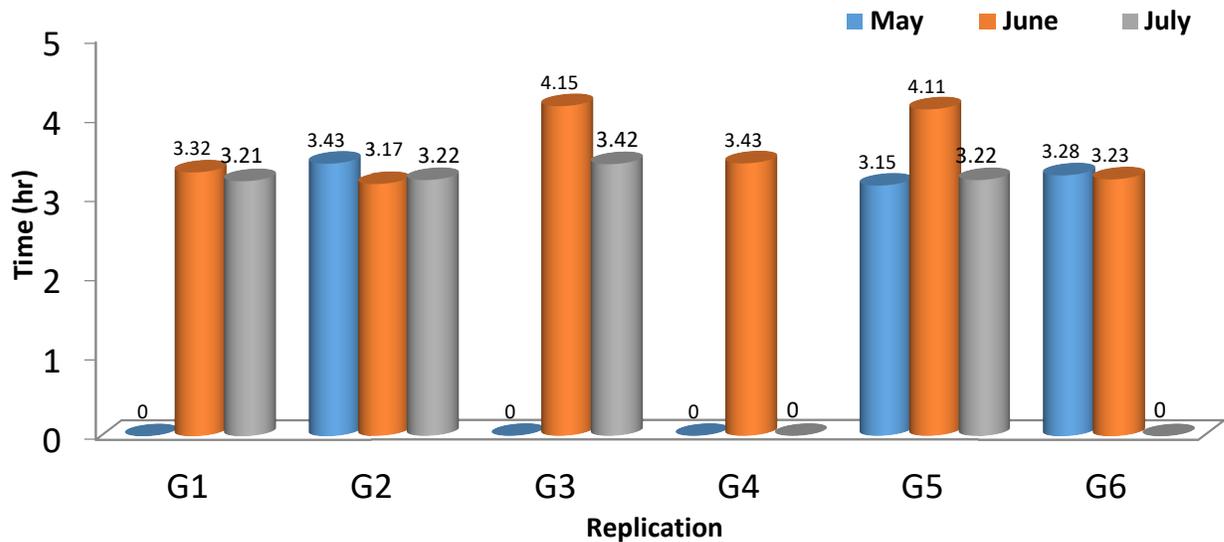


Fig.36: Latency period of *L. guntea* in Panel A

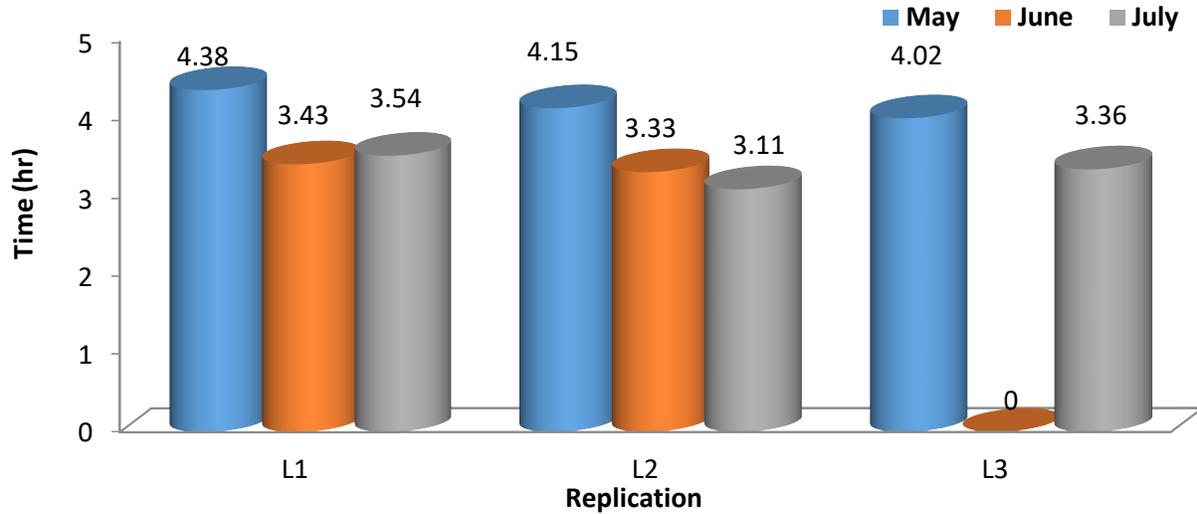


Fig. 37: Latency period of *L. guntea* at Panel B

11.12 Fertilization rate of *L. guntea*

Fertilization rate was dependent on the environmental condition, water quality, maturity, sex ratio and type and doses of hormone. After spawning, the brood fishes were removed from the incubators because they tend to consume their own eggs. Fertilization occurred in each replication with spawned eggs. Among the replications, the fertilization rate ranged $41 \pm 2.31\%$ to $94 \pm 4.63\%$ in the month of May to July 2018. The highest fertilization (92-94%) took place at replication of 1.0 ml /kg (G6)&0.8 ml/kg (L3) in May and 1.2 ml/kg (L3)in June and lowest (41-42%) occurred at 2.0 ml /kg (G6) in June and 2.5 ml/kg (G5)in July. In those replications, higher fertilization rate was observed with the CPG hormone compared to Ovaprium and HCG hormone. As per findings, replication of 10 mg/kg (G2), 1.0 ml /kg (G6)& 0.8 ml/kg (L3)in May; 12 mg/kg (G2), 1.5 ml/kg (G5)&10mg/kg(L1)in June and 12 mg/kg (L1)in July were the best combination for successful induced breeding. It concluded that the successful doses for female were 10-12 mg CPG/kg BW and 0.8-1.5 ml Ovaprium/kg BW where the male doses were the half doses of female doses. All of hormonal doses were applied in single injection dose due to avoid injuries and handling of this small indigenous species. In the present study, the fertilization rate was higher with the treatment of CPG hormones compare to the findings of Sayeed et al., (2009) whereas the average fertilization rates (%) of 60.12 ± 5.53 , 62.18 ± 2.22 , 75.87 ± 5.66 and 78.60 ± 3.21 in different doses. The fertilization was recorded 70-87% which was reported by Basudha et. al., 2016. Islam et al., 2011 reported that different hormone injection doses provided 57-80% fertilization rates of *Mystus vittatus*. Average fertilization rate of *L. guntea* shown in Fig. 38 and Fig.39.

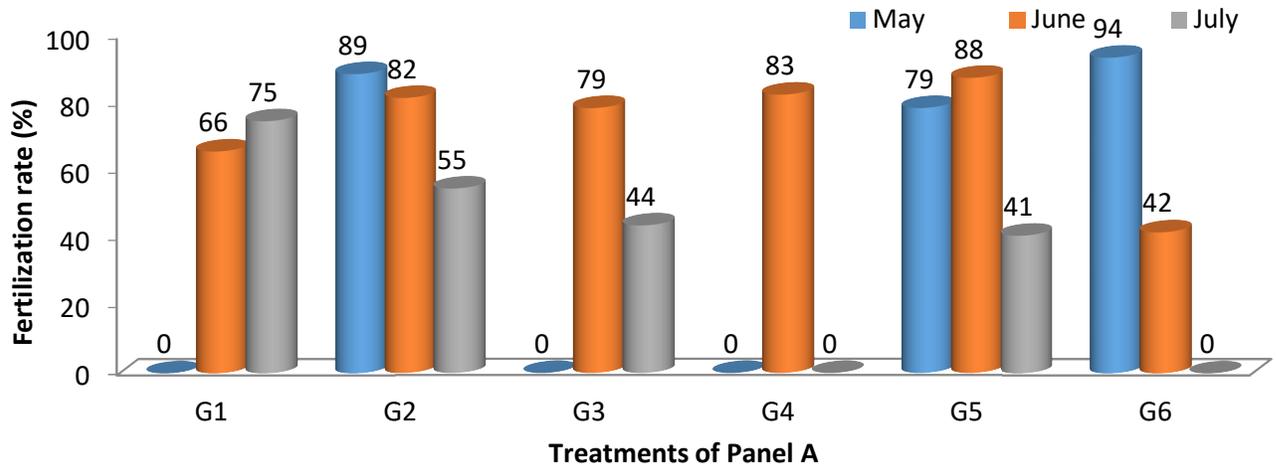


Fig. 38: Fertilization at Panel A of *L. guntea*

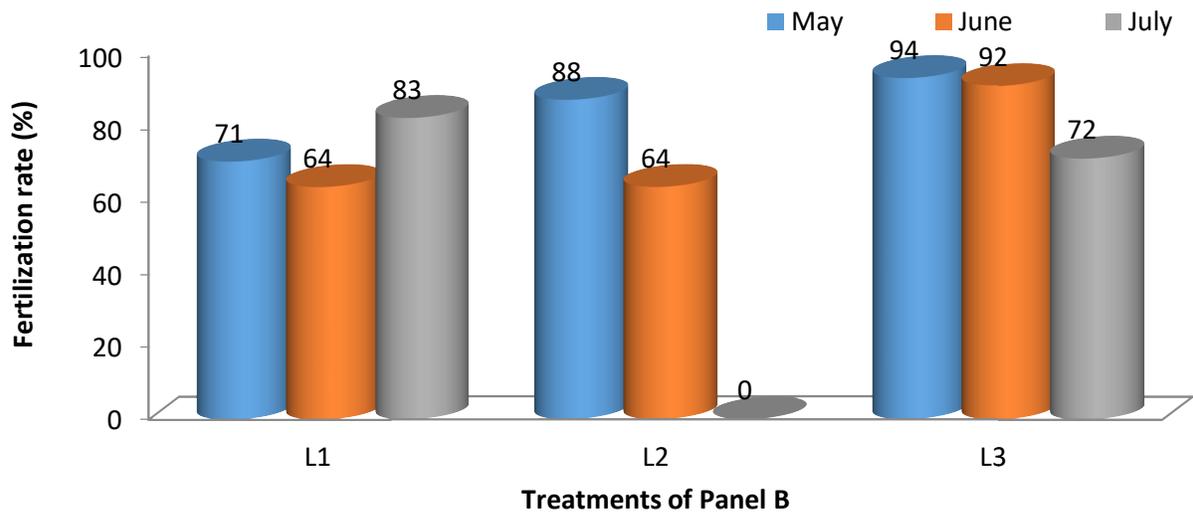


Fig. 39: Fertilization rate in Panel B of *L. guntea*

11.13 Fertilization Rate of *B. dario*

In the consequence of spawning, didn't occurred fertilization of *B. dario* during the experimental period. Fertilization rate of *B. dario* shown in Fig. 40 and Fig. 41. Dey et al., (2015) counted average fertilization rate about 82.09% when they injected WOVA-FH to *Botia* sp.

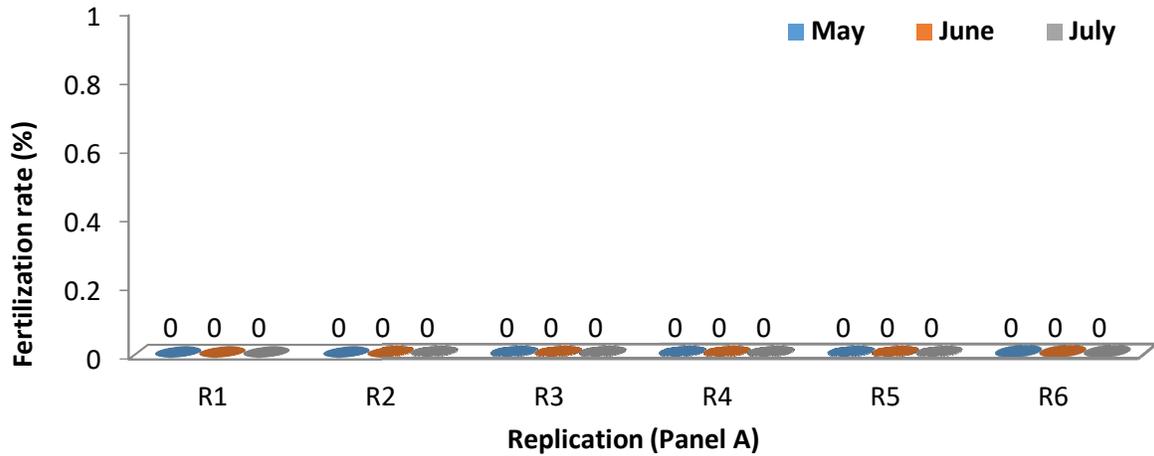


Fig. 40: Fertilization rate of *B. darioin* Panel A

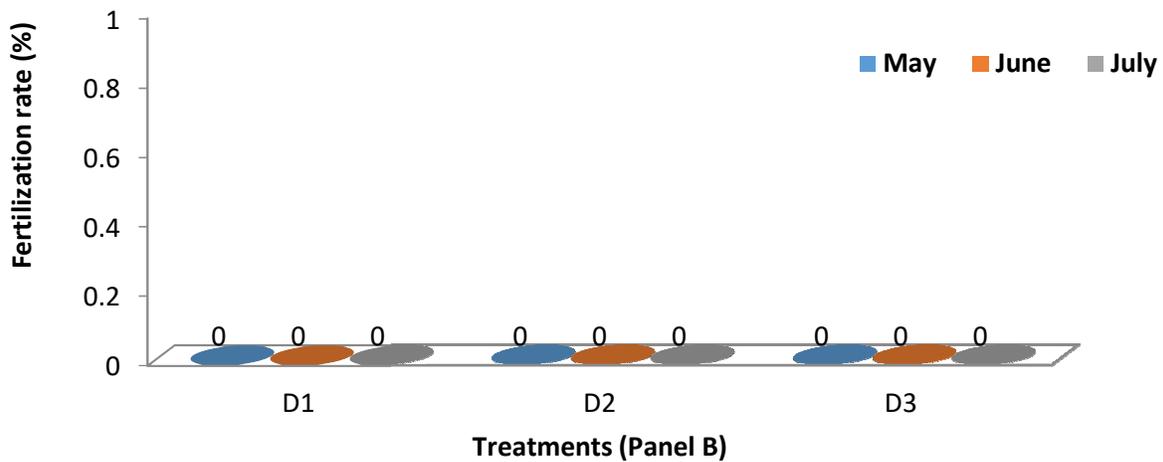


Fig. 41: Fertilization rate of *B. darioin* Panel B

11.14 Hatching Period of *L. guntea*

Average hatching period of *L. guntea* were varied from 12.32 to 15.52 hrs. among the replications. Average hatching period of *L. guntea* are shown in Fig.42 and Fig.43. Highest hatching period (15.52 hrs.) was found in the replication of 900 IU/kg (G3) and 1200 IU/kg (G4) in June and lowest was in 10 mg/kg (G2) in May and 1.5 ml/kg (G5) in June. From the result, concluded that the hatching period was lower with the treatment of CPG and Ovaprium hormone compare to HCG hormone treatment. The average hatching period was higher in the glass jar incubator (14.21 hrs.) compare to aluminum cistern incubator (13.16 hrs.). The values of hatching period were less than findings of Sayeed et al., (2009), Islam et al., (2018) and Sridhar et al., (1998) and showed similarities with Bashuda et al., (2017) and Udit et al., (2014).

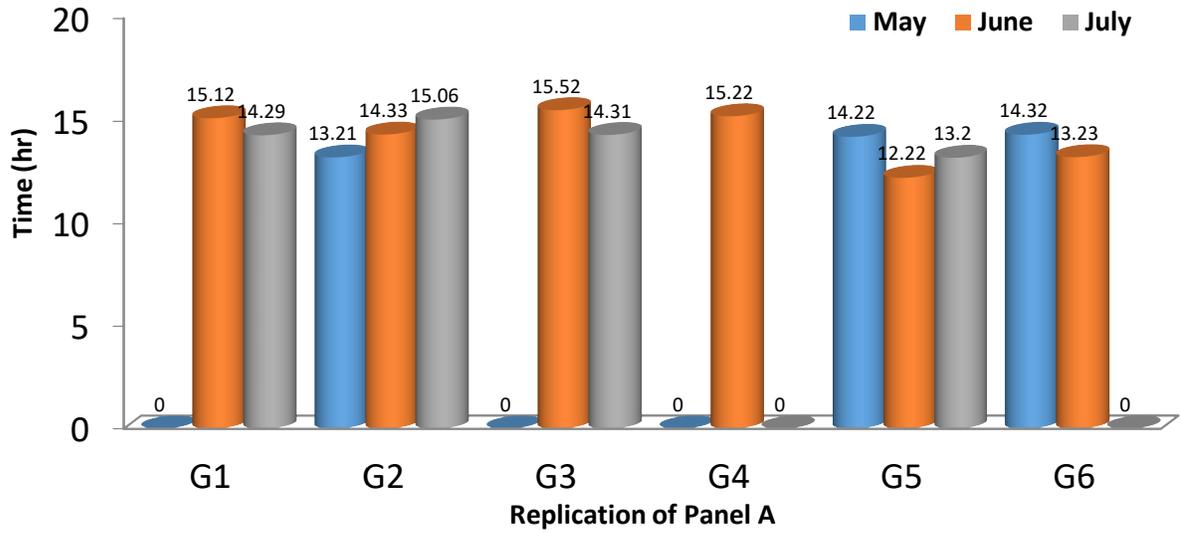


Fig. 42: Hatching Period of *L. gunteain* Glass jar Incubator (Panel A)

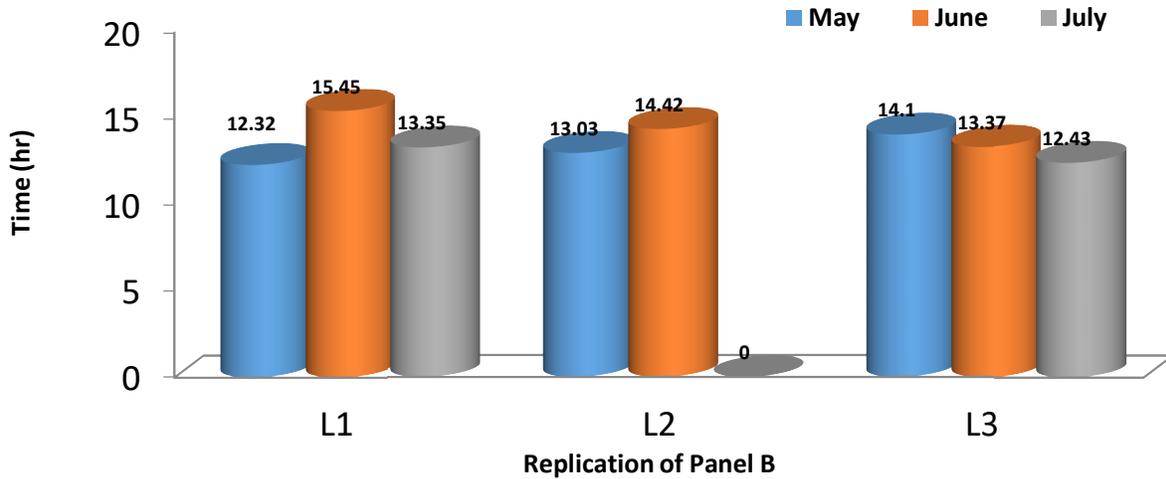


Fig.43: Hatching Period of *L. gunteain* Aluminum cistern incubator (Panel B)

11.15 Hatching Rate of *L. guntea*

In this experiment, found the average hatching rate of *L. guntea* ranged $21 \pm 2.08\%$ to $87 \pm 5.52\%$ with twenty seven replications of three treatments (CPG, HCG and Ovaprium) under glass jar incubator (Panel A) and aluminum cistern incubator (Panel B) shown in Fig.44 and Fig.45. During the experimental period, higher hatching rate (85%, 87% and 78%) was found with the replication of 10

mg/kg CPG(G2),1.0 ml/kg Ovaprium (G6) &0.8 ml/kg Ovaprium (L3) in May; hatching rate was 73% , 69% and 76% with the replication of 12 mg/kg CPG (G2), 1.5 ml/kg Ovaprium (G5) &10mg/kg CPG (L1) in June and the hatching rate was 87%, and 85% with the replication of 12 mg/kg CPG (L1) and1400 IU/kg HCG (L2)in July were the best combination for best successful hatching rateby different stimulating hormone. The hatching rate was 0% in some of replications of 5 mg/kg CPG (G1), 300 IU/kg HCG (G3), 600 IU/kg HCG (G4) in May, and 2000 IU/kg HCG (G4) and 3.0 ml /kg Ovaprium (G6) in July due to doesn't occurred of spawning with the hormone treatments that shown in Fig. of 44 and 45. It proposed that the successful doses for female were 10 mg/kg CPG (G2), 0.8 ml/kg Ovaprium (L1), 1.0 ml /kg Ovaprium (G6) in May and 12 mg/kg (G2) and 1.5 ml/kg Ovaprium (G5) in June. In this experiment, average fertilization rate was higher compare to other researcher that Islam et al., (2011) estimated 56% hatching rates of *Mystus vittatus* by using CPG hormone and 49 to 65% hatching rate of *L. guntea* was found (Sayeed et al., 2009). Udit et al., (2014) found the average fertilization and hatching rates of *Puntius sarana* 90.5% and 75.39 % respectively. Hatching rate (87.73%) were found in *Bangana dero* with hormone @ 0.5ml/kg female (Basudha et. al., 2016). Normally the SIS shows comparatively lower fertilization and hatching rates with regard to the larger carps (Wahab et al. 2003).

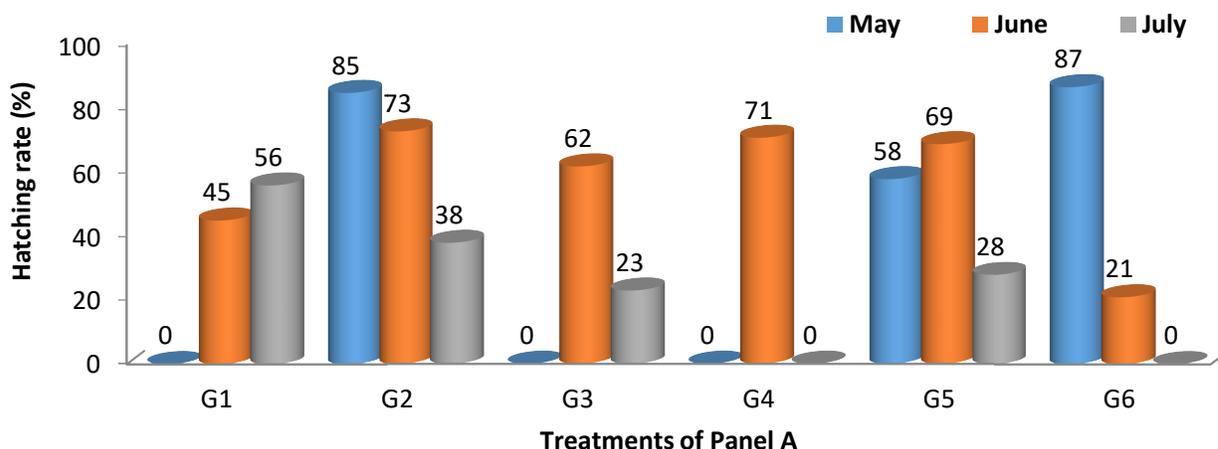


Fig.44: Hatching Rate of *L. guntea* at Panel A

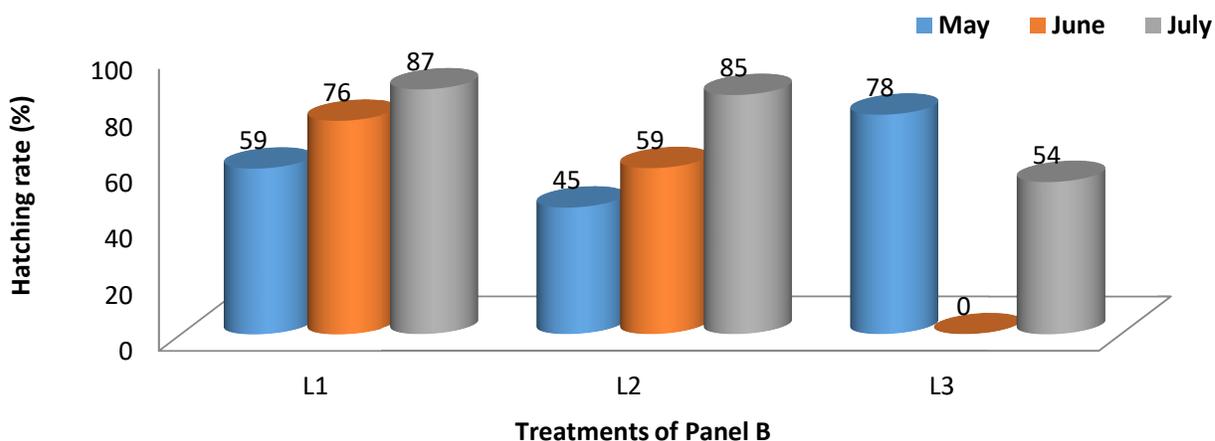


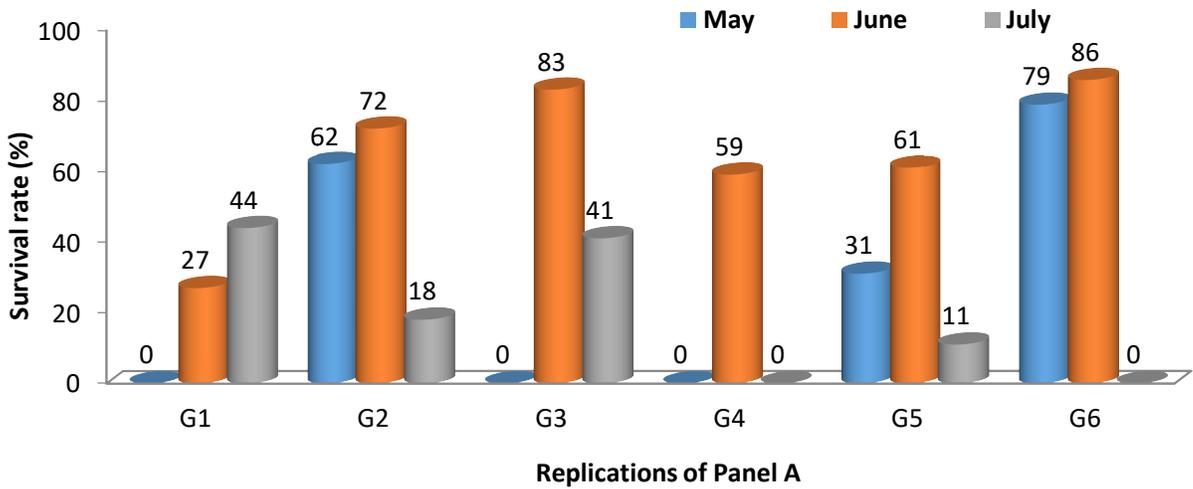
Fig.45: Hatching Rate of *L. guntea* at Panel B

11.16 Survival Rate of *L. guntea* Larvae in Incubator

From the result, found the average survival rate of *L. guntea* larvae which were varied 11±2.53% to 86±5.09 % with twenty seven replications of three treatments (CPG, HCG and Ovaprium) under glass jar incubator (Panel A) and aluminum cistern incubator (Panel B) shown in Fig. 46 and Fig. 47. Larvae were kept in incubator for two and half days. Every 4 hours interval, counted the survival rate of larvae in different replications. In those replications, found different survival rate of larvae in incubators. In this experiment, survival rate was decreased with the time lapsed. Survival rate of larvae depends on the physio-chemical properties of water.

In May, found the highest survival rate (83%) of larvae in the replication of 0.8 ml/kg (L3) and Lowest (31%) was in the replication of 0.5 ml/kg (G5). In June, found the highest survival rate (86%) of larvae in the replication of 2.0 ml/kg Ovaprium (G6) and Lowest (27%) was in the replication of 8mg/kg CPG (G1). In July, found the highest survival rate (82%) of larvae in the replication of 12 mg/kg CPG (L1) and Lowest (11%) was in the replication of 2.5 ml/kg Ovaprium (G5). From the result, concluded that higher survival rate was found with the treatment of Ovaprium compare to HCG and CPG treatment and beside that higher survival rate was found in the aluminum cistern incubator compare to glass jar incubator.

Fig. 46:



Survival Rate of *L. guntea* Larvae in Incubator of Panel A

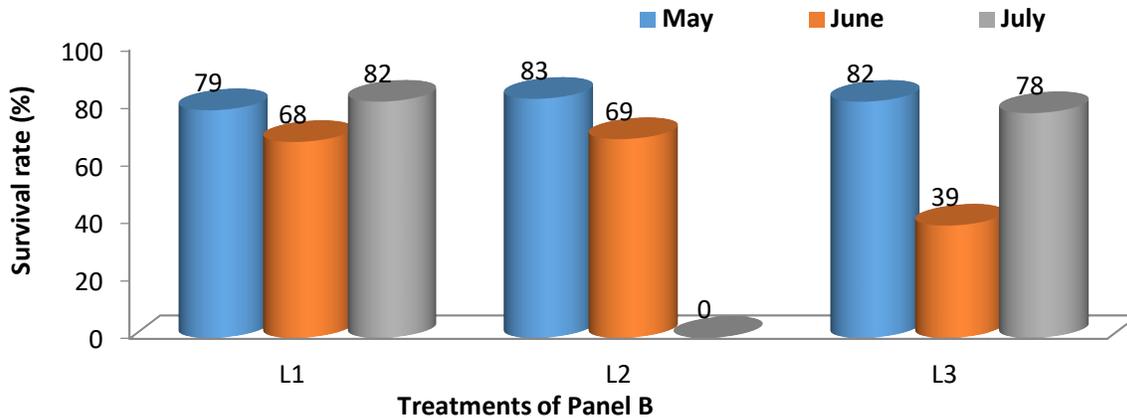


Fig.47: Survival Rate of *L. guntea* Larvae at Incubator of Panel B

11.17 Survival Rate of *L. gunteain* Rearing Stage

Fry rearing was done with the two systems; those were Hapa A (lower part of net was settled 20 cm above from the pond bottom) and Hapa B (lower part of net inert into pond bottom at 10 cm depth). Two and half days old larvae were transferred into different Hapa for rearing of larvae. For each batch production, *L. guntea* of larvae were rearing for a month. In the month of May 2018, survival rate of larvae was calculated that were 21% in the Hapa A and 31% in Hapa B (Fig. 48). The survival rate was 18% in Hapa A and 36% in Hapa B in the month of June 2018 (Fig. 49). The survival rate was 15% in Hapa A and 26% in Hapa B in the month of July 2018 (Fig.50). From the study that observed the higher survival rate of Gutum larvae in Hapa B compare to Hapa A. The highest survival rate of larvae was up to 36% after 30 days of rearing in Hapa B. Sayeed *et al.*, (2009) found highest 50% survival after they fed the Gutum’s larvae with egg yolk. The deterioration of water quality and tank cleanliness due to the use of formulated feeds also probably affected the growth and survival rate of larvae in their early stage as reported by Abi-Ayad and Kestemont (1994). Giriet *al.*, 2003, observed higher survival of *Wallago attu* larvae fed on live zooplanktons and under dry feed conditions as compared with live zooplanktons alone. However, it has been reported that some freshwater fish species were exclusively reared on artificial diets from the beginning of exogenous feeding (Charlonet *al.*, 1986; Appelbaum & Van Damme 1988; Legendre *et al.*, 1995; Carvalho, *et al.*, 2006).

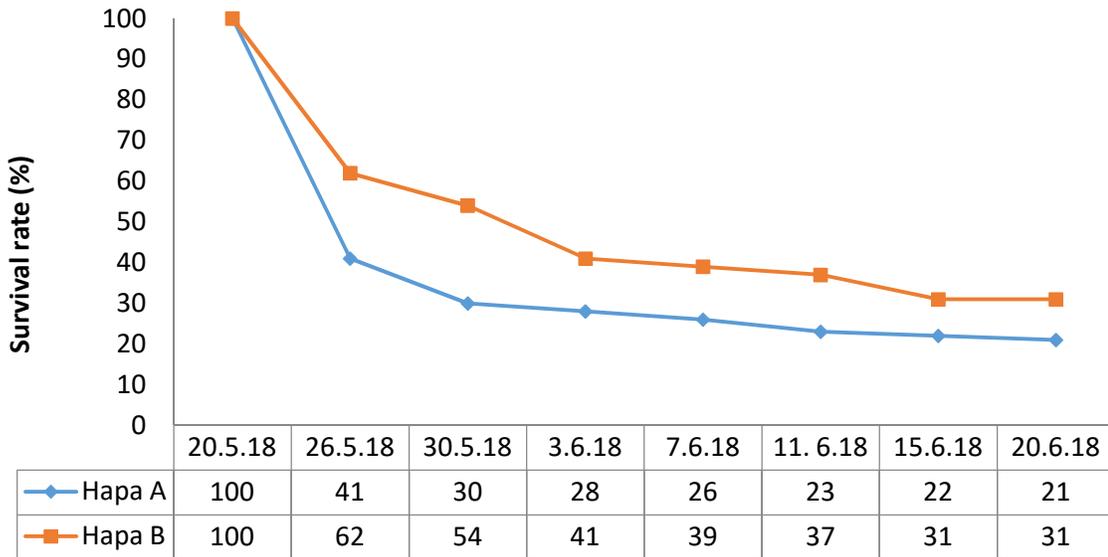


Fig.48: Survival Rate of *L. guntea* Fry in May, 2018

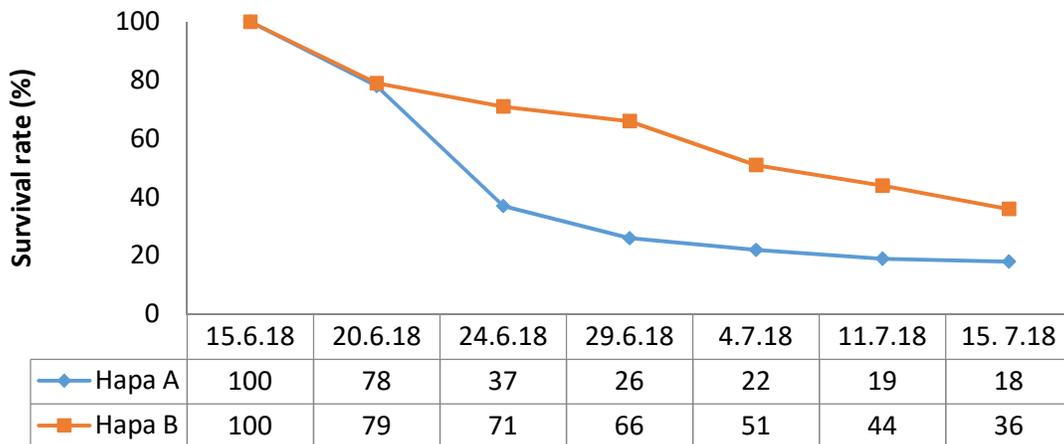


Fig. 49: Survival Rate of *L. guntea* Fry in June, 2018

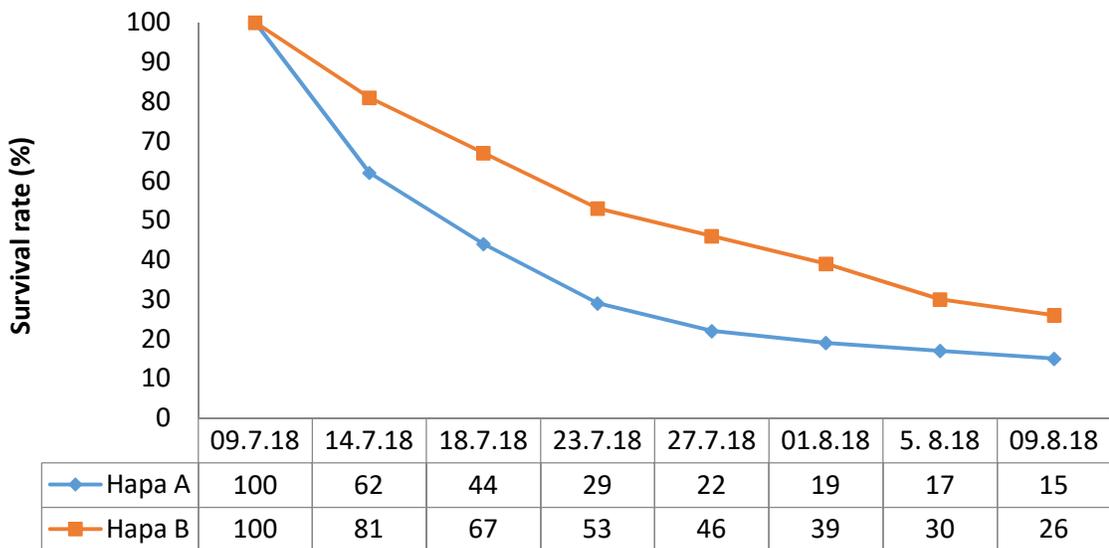


Fig. 50: Survival Rate of *L. guntea* Fry in July, 2018

11.18. Status of Gutum and Rani fish in aquarium fish market

11.18.1 Abundance of *Botia dario* and *L. Guntea* in different months

In this experiment, a survey was conducted to assess the availability of *L. guntea* and *B. dario* in the aquarium fish market. *Lepidocephalichthys guntea* are found especially in ditches, beel, haor, baor etc all over the Bangladesh. The availability of *L. guntea* was observed in the fish market from May to July especially in rainy season. *B. dario* was not available in all areas of Bangladesh excepting the areas such as Sunamgonj, Mymensingh, Sylhet and Kishoregonj and North Bengal area. This fish was most abundant during the month of September and October in the above mentioned areas.

11.18.2 Marketing channel of *Botia dario* and *L. Guntea*

The marketing channel of *Botia dario* and *L. guntea* in Bangladesh starts from the wild catchers to consumers and passes through a number of intermediaries such as fishermen, foria, aratdars, wholesalers and retailers.

Those target fishes were harvested by small fish catchers from the rivers or haors, bill and ditches. Almost all small fish catchers intentionally or unintentionally caught this species from the freshwater body. Harvesting was continued during the months of May to June and August to December.

In this study, three marketing channels were identified such as:

- Home consumption
- Food fish market
- Ornamental fish market

In the first marketing channel, fishermen consumed about 3.4% of their total catch (Fig.51). In the second channel, fishermen directly sold about 22.6% to the consumer and 19% to the retailers. But 50% of the catch was sold to the aratdar in presence of foria. Then the aratdars sold the total fish to the retailers by auctioning. Consumer bought these fishes from the retailers in the local fish market (Fig.46). The third marketing channel shows that fishermen sold about 5% of those fishes to the ornamental fish market on order basis from the wholesaler. Among these 5% wholesaler, 90% of *Botia*

dario wholesaler directly sold these fishes to the customer and the remaining 10% was sold to the retailers (Fig.46). Alam *et al.*, (2016) found *Botia dario* and *Botia lohachata* species in the ornamental fish market in Barisal division. Mostafa (2004) stated that SIS distribution chain starts from farmers or catchers to consumer through middlemen or foria, wholesaler or aratdar and retailers. He described two market channel, wholesale market and retail market, in his study. Ahmed *et al.*, (2005) reported that the market chain from producers to consumer passes through a number of intermediaries: local traders, agents/suppliers, wholesalers and retailers.

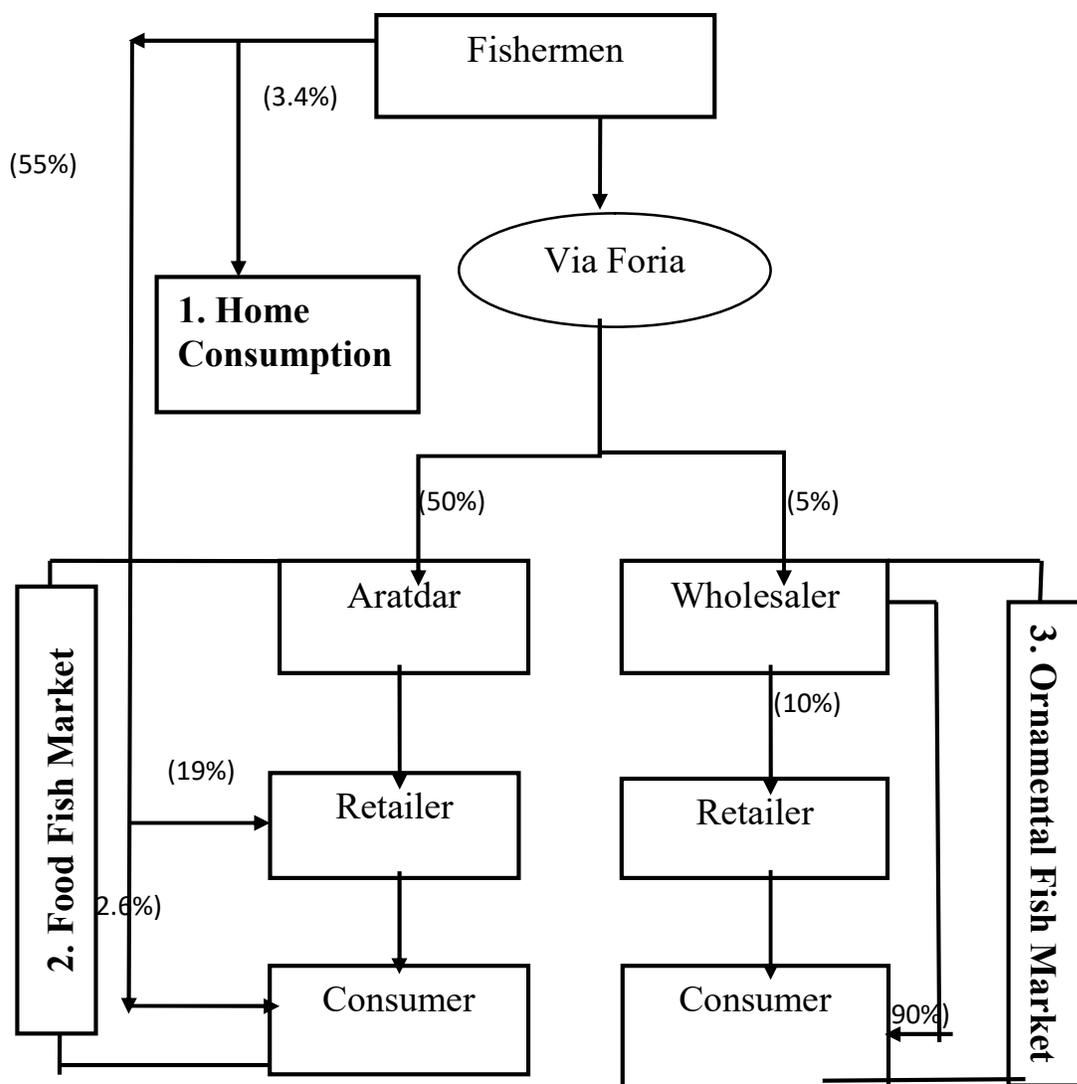


Fig. 51: Marketing channel of *B. dario* and *L. guntea*.

11.18.3 Status of *Botia dario* in ornamental fish market

In the ornamental fish market, two species were found under the genus *Botia*; *Botia dario* and *Botia lohachata*. According to ornamental fish seller, *Botia dario* and *Botia lohachata* were not seen abundantly all the year round due to low availability of spawners in the natural waterbody. These two

species were found in the ornamental fish market of Katabon since the month of September to November and not found in the other aquarium fish markets such as Kumilla and Noakhali. Among the aquarium fish traders, *B. dario* was stocked by 2% traders, 16% traders stocked *B. lohachata* and the remaining

82 %
not
Botia
fish
Dhaka
.

traders were
keeping the
genus in the
aquarium
market,
Katabon,
(Fig.52)

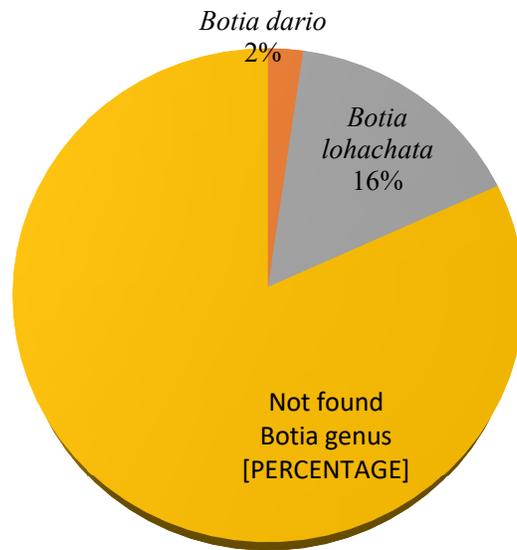


Fig. 52: Status of *Botia dario* in ornamental fish market

11.18.4 Status of *L. guntea* in ornamental fish market

Two genus of *Lepidocephalichthys* was found in the ornamental fish market of Katabon, Noakhali and Kumilla. The availability of those species (*L. guntea* and *L. annandalei*) was in the month of April to July in the aquarium fish markets. Among the aquarium fish traders, about 1% traders stocked the *L. annandalei* and 3% stocked *L. guntea* and remaining 96% did not stock the genus *Lepidocephalichthys* (Fig. 53).

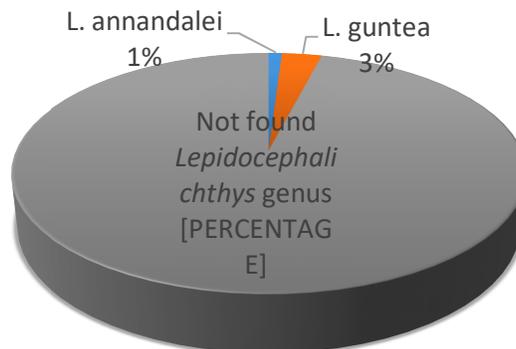


Fig. 53: Status of *L. guntea* in ornamental fish market

11.19. Proximate analysis of those species

11.19.1 Proximate analysis of Gutum fish (*Lepidocephalichthys guntea*) in dry matter basis

The composition of dry matter content of the experimented sample was as 12.11 % (Fig. 54). The ash content of *Lepidocephalichthys guntea* was 13.84 %. The crude protein content was 74.81 % by Micro-Kjeldhal method (Pearson, 1999). The quantity of crude protein generally remains higher than all other nutrient compositions in the fish (Ahmed *et al.*, 2012). The protein content of SIS were also similar to that found in major carp species (Rohu, *Labeo rohita*) with a value of 17.91% (Hossain *et al.*, 2015). The lipid content was 6.92 % with acetone in a Soxhlet Extractor for 4 hours. Begum and Minar (2012) analyzed the biochemical composition of small and large carp species of Bangladesh where they found lipid ranged around 3-4 %. With the above data, we found our observation of lipid content was higher than mentioned values. The carbohydrate content was 4.43 %. Muscle of the live fish generally contains between 0.1-1% glycogen (Balachandran, 2001). Carbohydrate and other nutrient contents were found very close to the values estimated by Foline *et al.*, 2011.

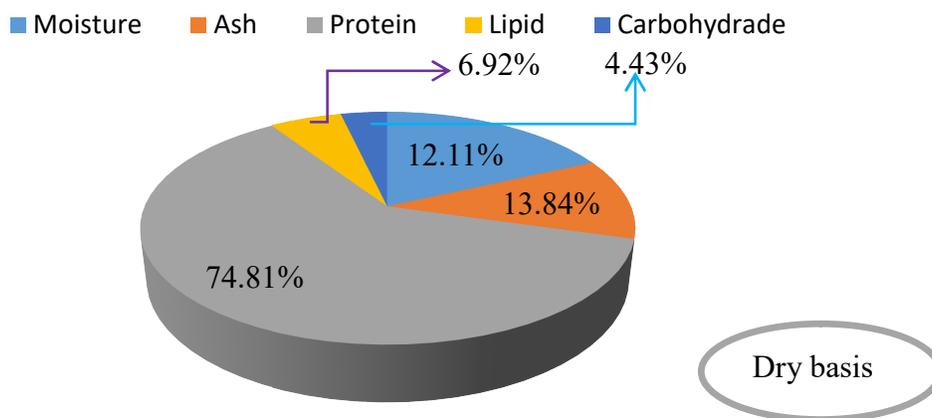


Fig.54: The nutritional composition of Gutum fish (*Lepidocephalichthys guntea*) in dry matter basis.

11.19.2 Proximate analysis of Gutum fish (*Lepidocephalichthys guntea*) in wet basis

The moisture content of Gutum was estimated 77.89 % as wet basis. Rana *et al.*, (2017), analyzed that the moisture content of fresh Tengra and found 76.06 %. The ash content, crude protein content, lipid content and carbohydrate contents of *Lepidocephalichthys guntea* were estimated as 3.09 %, 16.54 %, 1.53 % and 0.95 % respectively (Fig. 55). The results of this study showed that the major component of this studied fish was moisture.

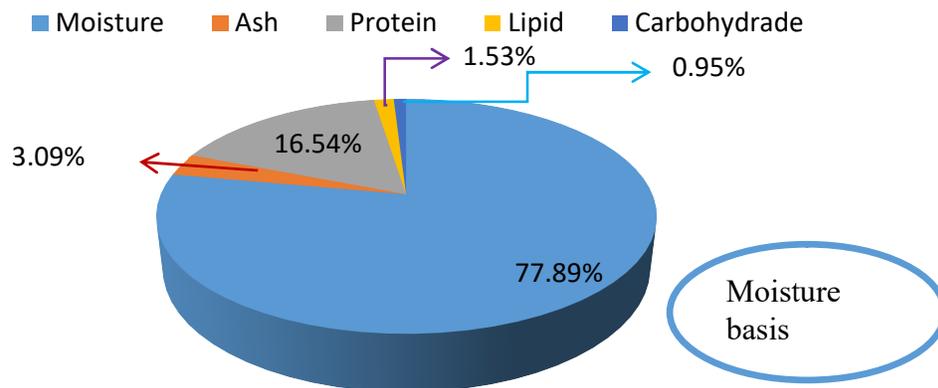


Fig.55: The nutritional composition of Gutum fish (*Lepidocephalichthys guntea*) in moisture basis.

11.20. Biochemical composition of *B. dario*

11.20.1 Proximate analysis of rani fish (*B. dario*) in moisture basis

Moisture is the principal constituent of fish. Moisture content in many fish varies between 60-80%. Moisture content is also found to vary considerably within the same species of fish depending on the age, fat content, feeding condition, spawning etc. Proteins are essential to the structure of cells, proper functioning of antibodies resisting infections, regulation of enzymes and hormones, and growth and repair of body tissues. Lipid serves as source of energy during starvation and fasting, Ash is a measure of the mineral content of food item. Ash is constituted by the minerals present in the flesh. A good source of instant energy that comes to the mind is carbohydrate. It also helps in the body's development and growth. The biochemical composition of the studied fishes in the experiments has been shown in the Fig.56 and 57. This study gives us an idea about biochemical suitability of the fish for inclusion in diet.

The moisture, ash, protein, lipid, carbohydrate content of *Botia dario* were 77.89%, 3.09%, 16.54%, 1.53% and 0.95% respectively as wet matter basis (Fig.55). Mazumder (2008) demonstrated the biochemical composition of four fish species such as *P. atherinoides*, *P. chola*, *G. chapra*, and *A. mola* where *P. atherinoides* contained 73.32% moisture while *P. chola* contained 74.43%, and *G. chapra* contained 75.06%, and *A. mola*, contained 76.38% respectively. The lipid content of selected catfish were recorded as 2% in *Clarias batrachus*, 1.15% in *Clarias gariepinus*, 2.97% in *Mystus tengara*, 1.99% in *Ompok pabda* and 2.83% in *Pangasius pangasius* by Dewangan (2012). About 15.62% protein was recorded in *Mystus vittatus* (Kamal *et al.*, 2007), and 18.17% crude protein was reported in *Pangasius pangasius* (Razzaque *et al.*, 2008). Thus a similar pattern of protein content of *B. Dario* (16.54%) was observed in the present investigation. Dewangan (2012) found that the carbohydrate content of selected catfish were recorded as 0.97% in *Clarias batrachus*, 0.95% in *Clarias gariepinus*, 0.72% in *Mystus tengara*, 0.64% in *Ompok pabda* and 0.66% in *Pangasius pangasius* respectively. In the present investigation carbohydrate content was 0.95% which was the lowest among carbohydrate contents of the species described.

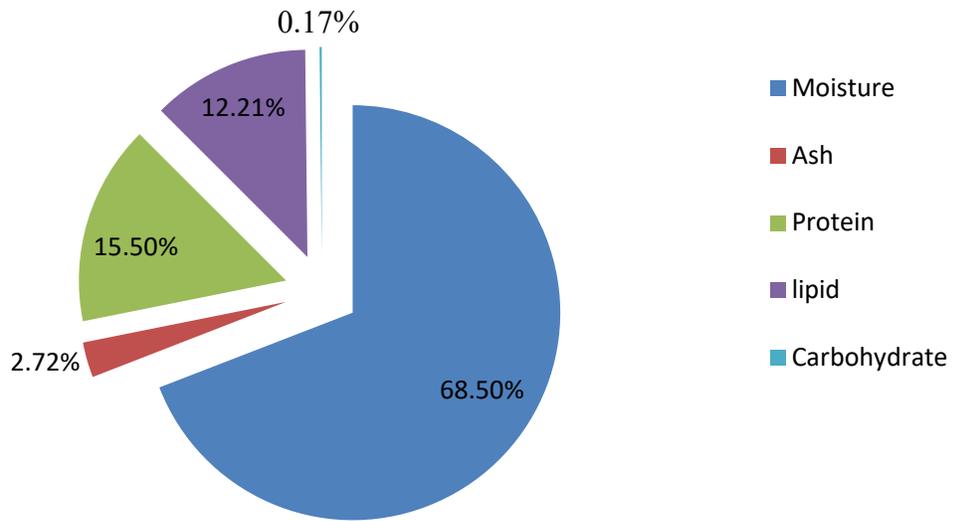


Fig. 56: Biochemical composition of rani fish in wet basis.

11.20.2 Nutritive analysis of rani fish (*B. dario*) in dry matter basis

Nutritive analysis was done in dry matter basis where the moisture, ash, protein, and lipid, carbohydrate contents were 13.5%, 8.68%, 52.51%, 38.7% and 0.11% respectively (Fig. 57)

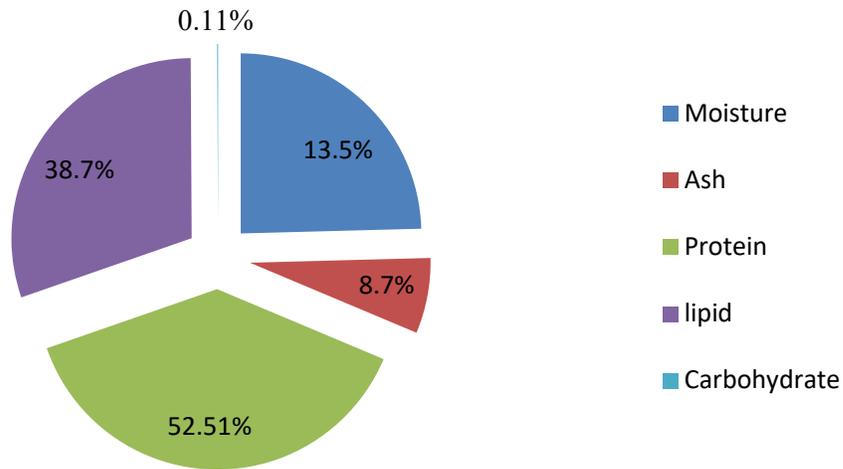


Fig. 57: Biochemical composition of rani fish in dry matter basis

L. guntea and *B. dario* are two loaches found in water bodies of Bangladesh which have both ornamental and nutritional value. Due to several natural and man-made reasons they are facing extinction. This present study tried to find out their breeding biology including GSI, fecundity and breeding season and attempted induced breeding using three hormones viz. CPG, HCG and Ovaprium. Each species was injected hormones under different treatments and replications in different months. *B. dario* didn't perform induce breeding by inducing hormone; where *L. guntea* showed different responses with different treatments. The fertilization rate, hatching rate and survival rate ranged 41 to

94%, 21 to 87% and 11 to 83% respectively. The best successful combination occurred at those treatments with 10-12 mg PG/Kg BW and 0.8-1.5 ml Ovaprium/Kg BW. In Bangladesh breeding of SIS is confined to a few species to a few hatcheries and they mostly rely on wild catches for broods. Emphasis must be given to expand the induced breeding activities of SIS at highest number of hatcheries. Induced breeding is an effective means to save a species from its extinction as the seed production of any species is completely dependent on brood stock development. To conserve those SIS species in the natural environment through induced breeding and a complete knowledge about their biology including breeding behavior, fecundity, fertilization and hatching was essential. Expert technicians and compact hatchery facilities are necessary for successful breeding. With further improvement of the breeding and hatching techniques and standardization of doses, it would be possible to meet a larger requirement of quality fish seed in the country. The technique is a simple one and can be disseminated to the farmers for mass production of fry and fingerlings.

12. Research highlight/findings:

- The month of June had been identified as the peak breeding season for Gutum and Rani mach based on higher GSI and fecundity values.
- Determined the effective doses of hormones for induced breeding of *L. guntea* were identified as: CPG@10-12mg/Kg BW of female and 5-6mg PG/kg BW of male (G2 & L1); Ovaprim @ 0.8-1.0 ml/kg BW of female and 0.4-0.5 ml/kg BW of male (G6 & L3).
- Produced viable fry of *L. guntia*
- Developed fry rearing technique of *L. guntia*
- Determined the nutritional value of the species
- Identified the status of those species in ornamental fish market

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Printer, Computer, Camera, Almirah (100%)	124000	Printer, Computer, Camera, Almirah (100%)	124000	All items were purchased as per procurement plan in due time
(b) Lab & field equipment	Incubator Pump Cistern Aerator DO meter pH meter Temperature meter TDS meter (100%)	276000	Incubator Pump Cistern Aerator DO meter pH meter Temperature meter TDS meter (100%)	276000	All items were purchased as per procurement plan in due time

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(c) Other capital items	Hormone & apparatus HCG Hormone PG Hormone Ovaprium Hormone Hapa Net (4X3X1m) Portable aerator Hand gloves Porcelain pestle & mortar Disposable syringe (22 size) Rubber foam mats (60*40*12.5 cm) J)Scoops nets 0.5 cm mesh, 1 m handle	262000	HCG Hormone PG Hormone Ovaprium Hormone Hapa Net (4X3X1m) Portable aerator Hand gloves Porcelain pestle & mortar Disposable syringe (22 size) Rubber foam mats (60*40*12.5 cm) J)Scoops nets 0.5 cm mesh, 1 m handle (100%)	262000	All items were purchased as per procurement plan in due time
(d)	Chemical and apparatus Cleaner brush Slide calipers Plastic bucket Feather Plastic bowl Ice box Tray Dissection box Weight machine (Digital) Punch machine xLime Oxy gold Bio pH	189250	Cleaner brush Slide calipers Plastic bucket Feather Plastic bowl Ice box Tray Dissection box Weight machine (Digital) Punch machine xLime Oxy gold Bio pH (100%)	189250	All of are purchased as procurement plan
(e) others	Malachite green Eco Solution Virex Charger gel Megavit Aqua	210750	Malachite green Eco Solution Virex Charger gel	210750	All items were purchased as per procurement plan in due time

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
	Amoxifish Matrix Ossi-C Geotox Aqua photo Biozyme Salt 2-phenoxy ethanol		Megavit Aqua Amoxifish Matrix Ossi-C Geotox Aqua photo Biozyme Salt 2-phenoxy ethanol		

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig. in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	331466	331466	331466	0	100	
B. Field research/lab expenses and supplies	1665559	1626147	1635139	-8992	100	Brood & fry rearing
C. Operating expenses	218500	216259	215855	404	100	
D. Vehicle hire and fuel, oil & maintenance	177715	177715	177715	0	100	
E. Training/workshop/seminar etc.	0	0	0	0	-	
F. Publications and printing	95000	0	0	0	0	
G. Miscellaneous	30000	27821	27821	0	100	
H. Capital expenses	400000	400000	387000	13000	100	GOB fund was not available

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
To develop appropriate protocol for breeding, rearing and nursing of <i>Botia dario</i> and <i>L.guntea</i>	Brood collection and reared, Measured breeding biology, Induced breeding with hormones, developed fry rearing technique	Standardized hormone dose , Produced viable fry of <i>L. guntea</i>	In near future, the enhanced production of those species might provide low price protein sources for the resource- poor and enhance fish biodiversity in nature
To measure the nutritional value of <i>Botia dario</i> and <i>L. guntea</i>	Proximate analysis such as protein, ash, carbohydrate, lipid and moisture from the fish flesh	On dry matter basis, Lipid content lower (6.92 %) in <i>L. guntea</i> compared to 38.7% in <i>B. dario</i> Crude protein content was 74.81 % in <i>L. guntea</i> and 49.2% in <i>B. dario</i>	More protein and low fat can be obtained from <i>L. guntea</i> and had high fat and low protein can be obtain from <i>B. dario</i> .
To know the status of those species in ornamental fish market	Survey was conducted in some ornamental fish market eg. katabon, dhaka	About 2% shop keeper stocked <i>B. dario</i> and 18% stocked <i>L. guntea</i> in the aquarium market	Could be good source for ornamental fish seller and might meet consumers's demand as food fish.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	01	Not completed	
Journal publication	02	Not completed	
Information development		Not completed	
Other publications, if any		Bangladesh protidin, Prothom alo, Kumillar kagoj, Amader kumilla, Jamuna television	

F. Technology/Knowledge generation/Policy Support (as applied):

Generation of technology (Commodity & Non-commodity)

Developed breeding technique of *Lepidocephalichthys guntea* through optimizing hormones for successful induced breeding

Generation of new knowledge that help in developing more technology in future

Mass production of *Lepidocephalichthys guntea* is now possible to supply as fry and that might help to develop the culture technique and conserve the fish from extinction

Technology transferred that help increased agricultural productivity and farmers' income

If invented induced breeding technique is transfer might create new avenue for income generation for the hatchery operators, fish farmers and ornamental fish traders

Policy Support

The new knowledge might be useful for updating national policy for ornamental fish business-especially with indigenous= species.

G. Information regarding Desk and Field Monitoring

- i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):
 - Monitoring Workshop organized by NATP-2, BARC on 12 January 2018, Presented Research Progress and found satisfactory.
- ii) Field Monitoring (time& No. of visit, Team visit and output):

Monitoring team	Date(s) of visit	Total visit till date (No.)	Output
PIU-BARC, NATP-2	10, January, 2018	01	Satisfactory
Presentation	11 April, 2018	01	Satisfactory
Internal Monitoring	22 May, 2018	01	Satisfactory
Others Visitors (Journalist)	30 May and 24 June , 2018	02	Project status

H. Lesson Learned/Challenges (if any)

- Identified the breeding season of *L. guntea* and *Botia dario*.
- Standardized the hormone dose for successful induce breeding of *L. guntea*.
- Learned the fry rearing technique of *L. guntea* in Hapa.
- Learned the biochemical composition of the two species.

I. Challenges (if any)

- Difficult to collect live quality brood from the nature.
- Difficult to catch or harvest *L. guntea* using various kinds of net from wild or pond.

Signature of the Principal Investigator

Date

Seal

Counter signature of the Head of the
organization/authorized representative

Date

Seal

J References

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