Project ID: CRG 488

Competitive Research Grant

Sub-Project Completion Report

on

Development of Artificial Breeding Technique for *Cirrhinus reba* Collected from Different Regions of Bangladesh

Project Duration

July 2017 to September 2018

Department of Fisheries Biology and Genetics
Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur

Submitted to

Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215
Bangladesh

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Project Implementation Unit
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Acknowledgement

The execution of CRG sub-project has successfully been completed by the Department of Fisheries Biology and Genetics of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thank to the World Bank for arranging the grant fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who extended their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to generate technology as well as increase production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

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Acronyms

Abbreviated Form		Detailed Form
ANOVA	:	Analysis of Variance
BLAST	:	Basic Local Alignment Search Tools
cm	:	Centimeter
Co-PI	:	Co-Principal Investigator
CRG	:	Competitive Research Grants
Cytb	:	Cytochrome B gene
DMRT	:	Duncan Multiple Range Test
DNA	:	Deoxyribonucleic Acid
FBG	:	Fisheries Biology and Genetics
gm	:	Gram
hr	:	Hour
HSTU	:	Hajee Mohammad Danesh Science and Technology University
IMCs	:	Indian Major Carp
IUCN	:	International Union For Conservation of Nature
ML	:	Maximum Likelihood
mm	:	Millimeter
MP	:	Maximum Parsimony
NATP	:	National Agro-Technology Project
NCBI	:	National Center for Biotechnology Informatics
NT	:	Near Threatened
PG	:	Pituitary Glands
PI	:	Principal Investigator
SIS	:	Small Indigenous Fishes
VU	:	Vulnerable

Table of Contents

List of Tables

List of Figures

Executive Summary

Cirrihinus reba, has been reported native to Ganges-Brahmaputra basin including Bangladesh. The potential of this species as the candidate of polyculture system has been described because of it's delicious taste and market value. Now a days, this species has become endemic in selected regions of Bangladesh, hence it's wild populations are at risk of losing genetic diversity and variability. For sustainable aquaculture and revival of the threatened fish species, the genetic diversity of the wild stocks needed to be assessed for quality broodstock development. Thereafter sustainable spawn production and larval rearing techniques are needed to be established. This sub-project aimed to study the genetic variation of *C. reba* of different region of Bangladesh, as well as to develop the sustainable induced breeding and crossbreeding techniques. Brood fish were collected from four natural water bodies and followed by rearing, revealing of genetic variation among *b*roodstocks using cytochrome b gene analysis, induced breeding using several hormones and larval rearing. Finally crossbreeding between different stocks was conducted to improve the local *C. reba* stocks. The data were tested using ANOVA and the significant results (p<0.05) were further tested by DMRT using SPSS. About 5000 live brood fish were collected from three rivers of Dinajpur, 2000 brood from Bogura and about 500 from Mymensingh region were collected for the study. A few *C. reba* samples were collected from Jassore regions in frozen condition. Based on genetic assessment, *C. reba* population of Mymensingh and Dinajpur were found better in terms of genetic distance (0.805) than those of Jassore and Bogura regions. The Bogura population was found deteriorated compared to three other stocks which could be may be due to lower gene flow (0.00). Among three hormones tested, administration of pituitary gland (PG) extract showed the best performance in terms of ovulation, fertilization and hatching over Ovatide and Flash. After been proven PG as the best inducing agent for breeding of *C. reba*, 4 trails with variable doses such as: T_1 - Female 1.5, 5.5; Male 2.0 mg/kg, T_2 - Female 1.5, 6.5; Male 2.0 mg/Kg; T_3 - Female 1.5, 7.5; Male 2.0 mg/kg and T_4 - Female 1.5, 8.0; Male 2.0 mg/kg body weight were carried out to choose the best effective dose for breeding. Finally, the dose of pituitary gland (PG) with T_2 @ Female 1.5 mg/kg for 1^{St} injection, 6.5 mg/kg for 2^{nd} injection; Male 2.0 mg/kg for inducing C. reba breeders demonstrated the highest breeding performance (Ovulation: 86% Fertilization: 80% Hatching: 72%) compared to the other doses. Among the cross breeding trails, mating between females of Dinajpur and males of Mymensingh showed better performance in terms of ovulation (86.66%), fertilization (88%) and hatching rate (79%) compared to those of other trials. For sustainable seed production of this species; broodfish needed to be collected from at least 3 sources. In case of feeding, Factory made nursery feed (32% protein) along with plankton @10 spawn/liter of water showed higher specific growth rate (3.3%) and survival rate (90%) for fingerling production. As a result, nursery operators are recommended to supply artificial feed as well as apply organic fertilizer in their nursery ponds to grow plankton.

CRG Sub-Project Completion Report (PCR)-

A. Sub-project Description

1. **Title of the CRG sub-project: Development of Artificial Breeding Technique for *Cirrhinus reba* Collected from Different Regions of Bangladesh**
2. **Implementing organization:** Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh
3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

Principal Investigator (Position, full address with phone no; as applicable) :Dr. Imran Parvez, Professor, Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Cell Phone no. +8801521316996 and +8801914503031, Email: imran_bau2007@yahoo.com

Co-Principal Investigator(s)-(Proponent Institute/Collaborating Institute-Position, full address with phone no) : Mousumi Sarker Chhanda, Lecturer, Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Cell no. 01719471799, E-mail: chh.sarker@yahoo.com

4. **Sub-project budget (Tk):**
   4.1 Total: 3573048.00 (Thirty five lacs seventy three thousand fourty eight BD TK only)
   4.2 Revised (if any): N/A
5. **Duration of the sub-project:** One year two month 18th Days
   5.1 Start date (based on LoA signed): 13th July, 2017
   5.2 End date: 30 September 2018

6. **Justification of undertaking the sub-project:**

The demand of fish is increasing with the rising population in Bangladesh. Due to increased fishing pressure availability of some fish species in open water bodies had reduced drastically. As a result, the trends of dependence only on capturing natural fish have changed and attempts have been made to enhancing the production of fish from impounded waters through aquaculture practices since 1983-84. The interest of culturing Indian Major Carps (IMCs) and few exotic fish species quickly removed the small indigenous species (SIS) of fish during pond preparation prior to stocking for aquaculture. Due to habitat degradation and overfishing of SIS fish species leads them in threatened situation. IUCN-Bangladesh (2000) recognized about 54 freshwater fish species of Bangladesh are in threats of different levels of extinction. Among the threatened fish species the Bhagna, *Cirrihinus reba* is identified as vulnerable (IUCN-Bangladesh, 2000).

C. reba, has been reported native to Bangladesh, Ganges-Brahmaputra basin of India, Indus plain, hilly areas of Pakistan and Nepal. The potentialities of this species as the candidate for polyculture in ponds have also been described because of its faster growth, attractive appearance and higher consumer acceptance (Sarkar *et al.* 2004). Now a days this species has become endemic in some selected region of Bangladesh such as in Dinajpur, Bogra, Jessore, Mymensingh and Sylhet region of the country. Among the endemic region, the rivers of Dinajpur district provide the most important breeding ground for this species. The district is criss-crossed by several rivers such as the Atrai, the Punarbhaba and the Depha and some of their tributaries namely the Kakra, the Kanchan, and the Choto Jamuna etc. From last few years, over fishing of this species is greatly increased as water content of these small rivers are decreasing and

becomes about to dry during winter season, thus people catches fish at the extreme level by drying out water bodies (Personal observation).

The wild population of *C. reba* are at risk of loss of genetic diversity and variability due to extinction of genetically distinct wild stocks. Knowledge of genetic diversity data could have a vital role in scientific planning of the breeding programmes for genetic improvement and effective management of the wild genetic resources. Identification of polymorphic molecular markers is a critical requirement in the investigation to determine genetic variation and divergence (Ferguson *et al.,* 1995). Mitochondrial DNA analysis is widely used in studying population structure of animal species. MtDNA has fast evolution rate and its maternal mode of inheritance made it a very potential genetic marker system, alone or in combination with other nuclear markers such as microsatellites, for analyzing population structure and phylogenetic studies (Chaturvedi *et al.,* 2011; Mandal *et al.,* 2011; He A *et al.,* 2011). Variation in mitochondrial cyto b region has been used for population studies in fishes across taxonomic orders such as, Acipenceriformes (Fontana *et al.,*2007); Squaliformes (Murray *et al.,*2008); Salmoniformes (Bouza *et al.,*2008). The polymorphic cyto b region has been used in analyzing and determining genetic variability and diversity in cyprinid fish species (Li *et al.,* 2009; Watanabe *et al.,* 2009).

Though *C. reba* is a highly demanding and threatened fish in south-east Asia, published reports on this species are limited, most importantly on seed production and conservation of the gene pool as like some other threatened fish in the world including Bangladesh. The available literature on this species and also literatures available on induced breeding, selective breeding, larval rearing of some other fish species which are found pertinent to the present study are briefly described here. Rao *et al.,* (1972) described the breeding biology and spawning behavior *C. reba* from the Cauvery and Bhavani rivers. The Internal rhythm of sexual cycle in a carp *Cirrhinus reba* (Hamilton) under artificial conditions of darkness have been reported by Verghese (1975). The fecundity of *Cirrhinus reba* (Hamilton-Buchanan) from Baigul reservoir in Uttar Pradesh also investigated (Khan 1986). The phenotypic plasticity of *C. reba* from the riverine waters of Dinajpur district have been described very recently (Kibria *et al.* 2013). Captive breeding of *C. reba* with ovaprim for conservation of its wild populations has been described in India where the result showed higher breeding values in terms of ovulation, fertilization, spawning, hatching rate etc. (Sarkar 2004) For mass seed production, the development of induced breeding and larval rearing have already been developed by several other endangered fish species such as local sarpunti, *Puntius sarana* (Akhteruzzaman *et al.,*1992; Chakraborty *et al*.,2002, Parvez and Khan, 2005); baim, *Mastacembelus armatus* (Mollah 2005); jatpunti, *Puntius sophore* (Hossain and Shah, 2006); bata, *Labeo bata* (Rahman *et al.,* 2006) and shing, *Heteropneustes fossilis* (Gheyas *et al.,* 2000). All the above mentioned artificial seed production techniques were carried out by considering for the conservation of threatened fish species from being extinction and for the inclusion of these species in aquaculture to increase the aquaculture production of Bangladesh.

For sustainable aquaculture and revival of the threatened fish species from being extinction, the genetic diversity of the wild stocks needed to be assessed for quality broodstock development. Special emphasis on the development of effective seed production techniques and culture management are very essential. The seed production through hypophysation, cost effective larval rearing and grow out techniques, as well as stock assessment through molecular markers would be capable to fulfill the farmers demand for fish seed to increase their production and as well as to conserve the gene pool of this threaten fish species of Bangladesh.

7. Sub-project goal: Sustainable aquaculture and revival of threatened indigenous fish species *C. reba* through mass seed production using artificial propagation technique.
8. Sub-project objective (s):
   The specific objectives to achieve the goals are stated in below
   I. To assess the genetic variation of *C. reba* collected from different region of Bangladesh for quality Broodstock management
   II. To develop artificial breeding technique for *C. reba*
   III. Crossbreeding of *C. reba* for the genetic improvement of the stocks of Dinajpur region

9. Implementing location (s): Department of Fisheries Biology and Genetics, HSTU, Dinajpur, Fisheries Field Research Complex, Caritas, Dinajpur, two selected fish hatcheries.

10. Methodology in brief:

Approach: The proposed sub-project was consisted of both laboratory and field work.
The laboratory experiments and some parts of field trials were conducted in the Department of Fisheries Biology and Genetics of HSTU. The activities of Broodstock rearing, induced breeding, larval rearing etc. were conducted in the field research complex of Caritas Bangladesh, Dinajpur. Before submitting the concept note, several meeting and cross-visits were held between these two organizations and finally Caritas Bangladesh, Dinajpur agreed to provide the facilities of their fisheries field complex in this study. The hatchery was not in production for last 4/5 years, so renovation and set up of water pump were required (Fig. 10.1: A-D).

Figure 10.1: Caritas fish hatchery, Setabgonj, Dinajpur; A) The hatchery look before renovation, B) The hatchery after renovation, C) The set up of motor pump to ensure the water supply D) the identification of project sites by hanging the name board

Methodology: The research activities of this sub-project were divided into 3 experiments that briefly described in below:

Broodstock collection: The brood of *C. reba* were collected from the rivers of Dinajpur, Bogura, Mymensingh and Jashore regions of the country (Figure 10.2). A stock of the *C. reba* broods of Dinajpur region was transferred from the ponds of HSTU to the ponds of Caritas Hatchery. For each source about 2000 mature *C. reba* males and females were collected. The collected wild *C. reba* were transported to the research station by using both traditional and modern oxygenated packaging systems. (Figure 10.3)

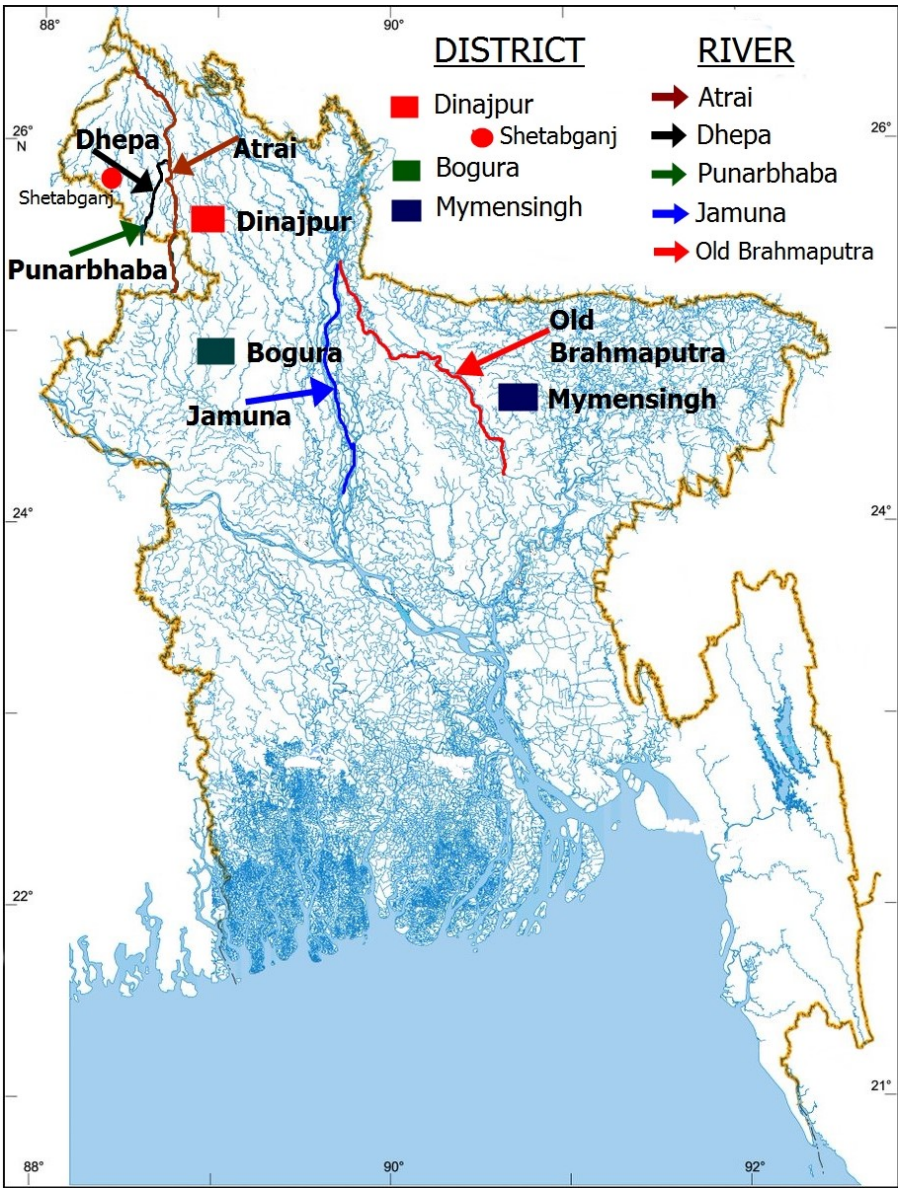


Fig.10.2: Map showing the collection of brood fish from the rivers of different district

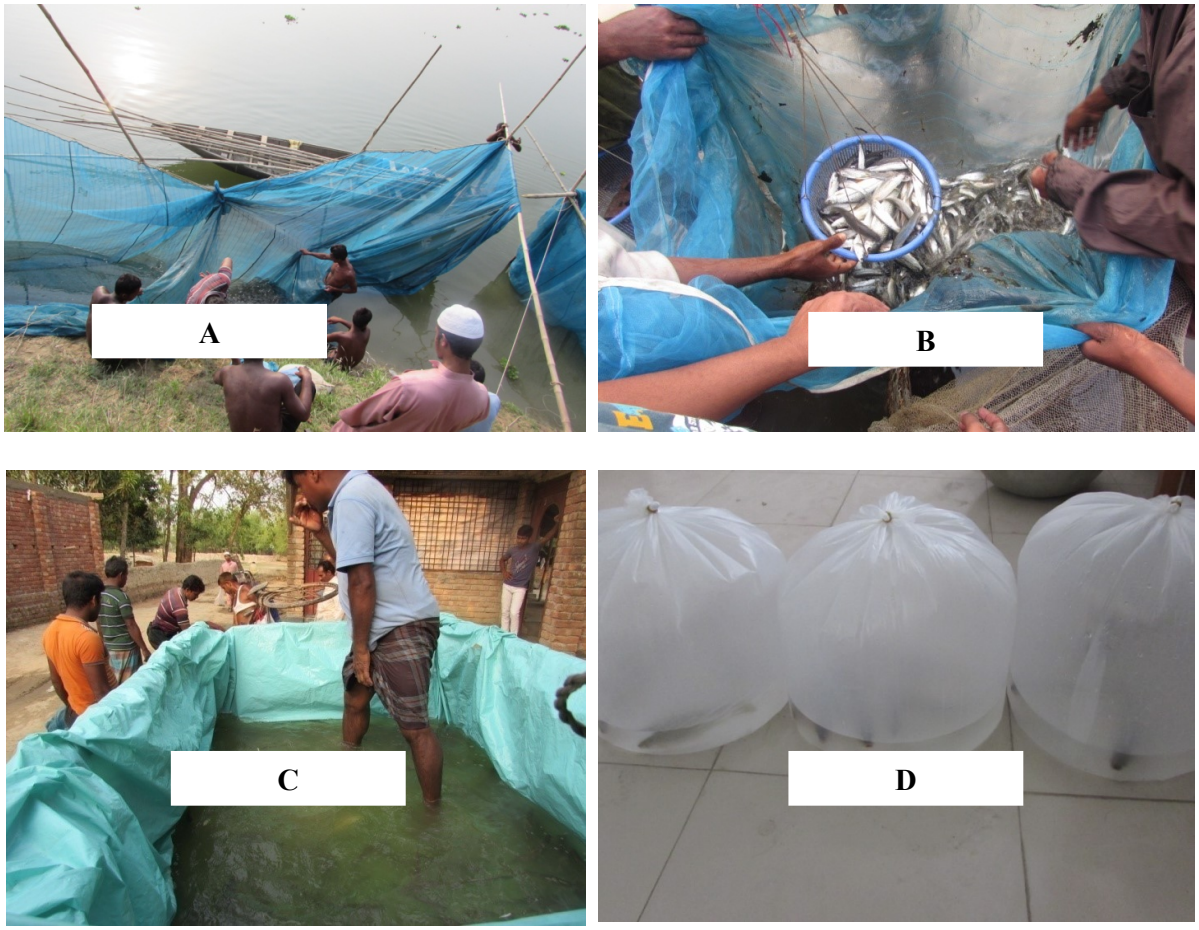


Figure 10.3: Brood fish collection and transportation; A) The fishermen harvesting *C. reba* from the river, B) Purchasing the harvested fish from the fishermen, C) Transportation of live collected fish through traditional system D) Transportation of live collected fish through modern oxygenated packaging systems

Conditioning of the brood before stocking

After collecting the broods at breeding stations, conditioning were done to give times to the breeders for adjusting with the new environment. For their adjustment with the new stocking condition 5-6 hours were kept for conditioning in circular tank. Broodstock were kept into separate rectangular tank for conditioning approximately for 6 hours. Adequate water flow was ensured for sufficient oxygen. In fasting condition breeding success might be achieved. So any food materials were not provided to the breeds. (Figure 10.4)

Figure 10.4: The conditioning of brood fish in cistern

Brood stock rearing in earthen ponds: The collected wild broodstocks were reared in separate ponds of experimental sites (Figure 10.5). The fish were stocked with a few other species which included Silver Carp, Bighead carp to control the phytoplankton bloom. Fish were stocked @ 50 C. reba, 5 Silver and Bighead carp per decimal of water area. In all the ponds a common broodstock rearing technique was practiced. Regular manuring with cowdung was done in 30 days interval @ 5 kg/decimal and further fertilization were done using Urea and TSP @ 200g/decimal and @ 100 g/decimal respectively. At the same time artificial feed having 25% protein were provided in every pond at the rate of 5-6% body weight. Apart from this management, feed enriched with protein and vitamin E was provided in early March and continued up to September to enhance the gonadal maturation in fishes. Feed were provided to the fish at two times per day at 9.00 am and 5.00 pm respectively (Figure 10.5). The water quality parameter such as pH, DO, temperature etc. were recorded during the experimentation. During broodstock rearing in earthen ponds, regular fortnightly samplings were conducted.

Growth and Specific Growth Rate were measured according to the following formula:

Growth= $\frac{W_2 - W_1}{W_1} \times 100$

Where, W_1=Initial weight in g and

W_2= Final weight in g

SGR= $\frac{LnW_2 - LnW_1}{T_2 - T_1} \times 100$

Where, W_1= The weight of fish at time T_1 and

W_2=the weight of fish at time T_2

Figure 10.5: Brood rearing activities in earthen ponds.

10.1. Experiment 1: Assessment of genetic variation of *C. reba* for quality broodstock management

10.1.1 Sample collection for brood stock genetic quality assessment

To know the genetic status of wild *C. reba* brood stock in Bangladesh, wild stocks were collected from four geographically isolated areas of Bangladesh (Figure 10.6). The areas were selected based on the previous information of their endemic situation in Dinajpur, Bogura, Jashore, Mymensingh. A total of 120 individuals of *C. reba* were collected from four regions, where 30 individuals from each location were collected. The details of sample collection for genetic assessment are given in Table (Table 10.1).

Table -10.1: The sample collection and location for genetic variation study.

Name of the Regions	Name of the river	Number of sample	Tissue Used for DNA extraction
Dinajpur	Punorvoba	30	Muscle
Bogura	Jamuna	30	Muscle
Jashore	Bergobindopurbaor	30	Muscle
Mymensingh	Bhramaputra	30	Muscle

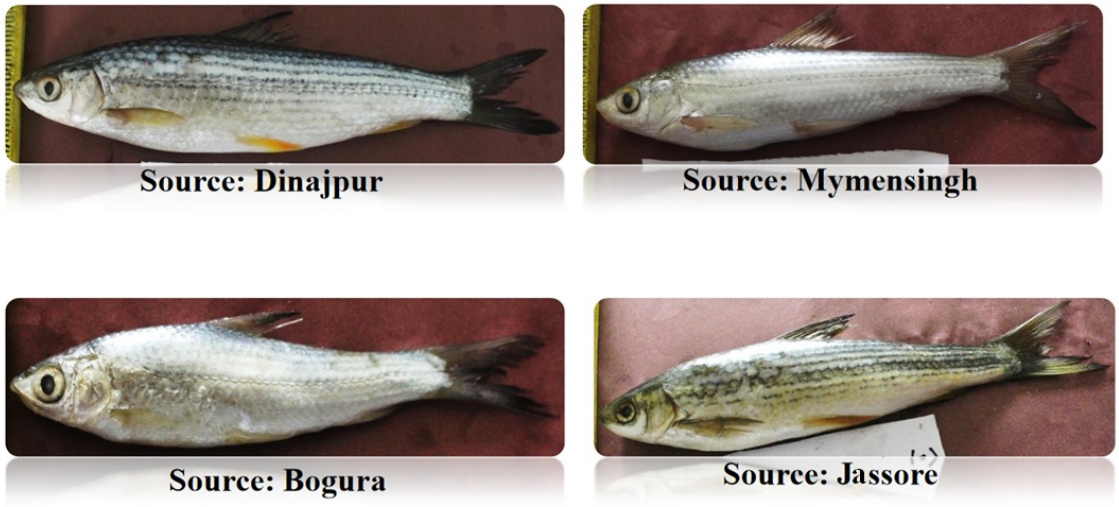


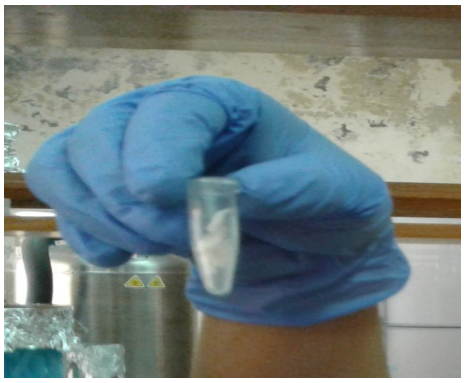
Figure-10.6: Representative fish of collected from four Districts of Bangladesh.

10.1.2 Fish Tissue Collection and Preservation

Approximately 15g of muscle tissue was dissected from each individual of collected *C. reba. F*rom each source of wild stock, muscle samples of 10 individual were collected. Prior to collecting tissue samples the instruments used were sterilized. The collected muscle tissues were preserved in 95% ethanol and kept at 4°C until the extraction of genomic DNA.

10.1.3 DNA extraction

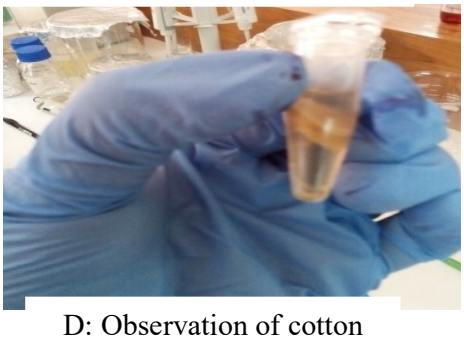
Genomic DNA was extracted for each muscle tissue of collected samples by using Phenol-Chloroform-Isoamyl alcohol and proteinase K digestion methods. To prevent contamination the required materials for extraction technique were previously sterilized and rinsed with 100% ethanol. Proteinase k helps to digest the protein percentage and isolate DNA sample and it was stored at -20°c as dissolved condition. At the end the cotton like DNA structure was observed after centrifuge at 10,000 rpm for 10 min to find clear DNA pellet. DNA pellet was observed very clearly at the bottom of tube after twice centrifugation. The DNA pellet dried in the safety cabinet and 100 µl TE buffer was added and finally preserved in the refrigerator at -20 degree centigrade until used for the next steps. (Figure 10.7: A-D)



A: Tissue sample collection

B: Tissue sample in water bath

C: Tissue samples in centrifuge



D: Observation of cotton like DNA structure

Figure 10.7 Activities of DNA extraction process; A) Tissue sample collection, B) Tissue samples in the water bath, C) Tissue samples in the Centrifuge and D) Observed the cotton like DNA pellet.

10.1.4. Purification of the extracted DNA

During the preparation of DNA, their presence of proteins, lipids, polysaccharides etc. can interfere with DNA content that reduces the quality of DNA. For this reason extracted DNA in the previous section (10.1.3) were purified by using 70% alcohol (900µl) and 7.5m ammonium acetate (20 µl). The mixtures were freezed at -20°C for 30 min and centrifuged at 12,000 rpm at 4°C temperature for 20 min. After that, the liquid of all eppendorf tubes were kept out and purified DNA pellet were observed inside. All tubes were dried carefully, added 100 µl TE buffer (10mM Tris CL and 1mM EDTA. These DNAs were carefully preserved to use as template DNA in PCR reaction for several times.

10.1.5. Quantification of the purified DNA

For ensuring complete solubility of DNA, the purity factor (A260/A280 nm) was measured by UV spectrophotometer (Cole Parmer Ins. Company, US) and its integrity was checked by loading 10 µl DNA preparation (2 µl extracted DNA, 2 µl dye and 6 µl sterile water) on 0.1 percent agarose gel and stained with ethidium bromide. After quantification the extracted DNA samples were stored at -20°C till their further uses.

10.1.6: Primer selection for amplification of Cytochrome-b gene (Cytb)

To amplify the Cytb gene from the purified DNA, following primer pair according to Linacre and Lee (2016) was used. (Table 10.2)

Table 10.2: The nucleotide base of Primer pairs

Name of the Primer	**Forward sequence (5´-3´)**	**Reverse sequence (5´-3´)**	**Reference**
Cytochrome-b	**CGAAGCTTGATATGAAAAACCATCGTTG**	**AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA**	Linacre & Lee (2016).

10.1.7: Primer dilution

The intact primer was powder form in the tube that was diluted with the nucleus free water according to the amount of primer label. Then the primer solution was vortexed for 30 sec. For this PCR, the primer solution had five fold dilutions.

10.1.8: Preparation of PCR reactions

About 25µL volumes of each PCR reaction mixture were prepared for PCR amplification of Cytb gene given in the (Table 10.3). The PCR master mix, template DNA and Primer were mixed and followed by centrifugation and homogenous mixing with vortex. The works were done carefully in the safety cabinet to prevent any contamination.

Table -10.3: Components of PCR reaction
Total amount=40x25=1000 µl

Name of the reagent	Per sample amount	Number of sample	Total amount (µl)
Master mix (DNA Tag Polymerase, dNTPs, $MgCl_2$)	12.5 µl	40x12.5	500
Forward primer	1 µl	40x1	40
Reverse primer	1 µl	40x1	40
Water	9.5 µl	40x9.5	380
Template	1 µl	40x1	40

10.1.9: Thermal condition of PCR

After that samples were taken in PCR machine which was set at certain time and temperature required for amplification of cytochrome b. The thermal profile was set up as required and run the PCR machine for amplification that shown in Table (10.4). The PCR tubes containing the reactions were kept on to the well of thermal cycler (Figure 10.1.8: A-B).

Table -10.4: PCR optimization

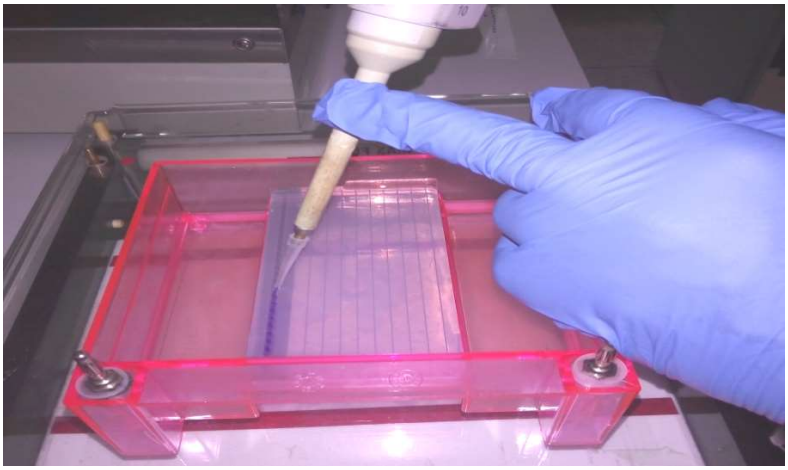
Name of the gene	PCR reaction	Temperature	Time	Cycle
Cytochrome b	Preheat temperature	105 ºC	2 min	
	Initial denaturation	94 ºC	4 min	1 cycle
	Denaturation	94ºC	40 sec	35 cycle
	Annealing temperature	48 ºC	1 min	
	Extension	72 ºC	1 min	
	Final extension	72 ºC	7 min	1 cycle

A B

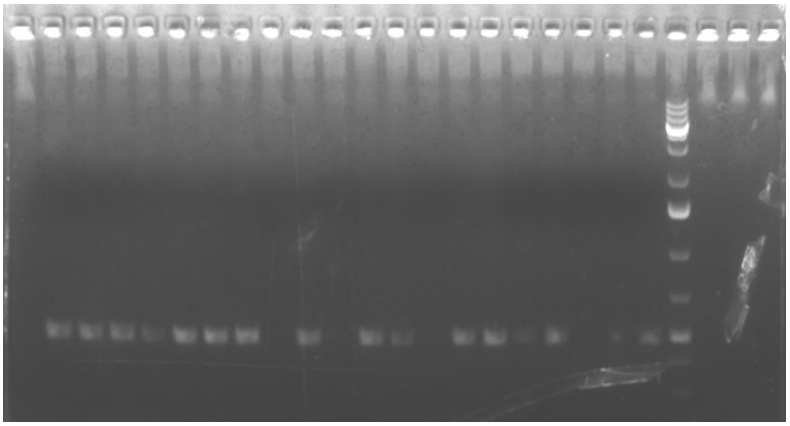
Figure 10.8: The PCR reactions: A) Placing of PCR tube into the wells of thermal cycler and B) the display of the PCR profile on the thermal cycler

10.1.10: Detection of amplified PCR products using agarose gel electrophoresis

Agarose gel (2%) which contains ethidium bromide run at 100V with 1x TBE solution was used to detect the amplified Cytb gene. The products were visualized by laser detection of fluorescence emitted by different emission spectra of fluorescent labeled terminators and observed under UV device (Figure 10.9. A & B).



A



B

Figure 10.9: Activition of Electrophoresis

10.1.11 Purification of the PCR products

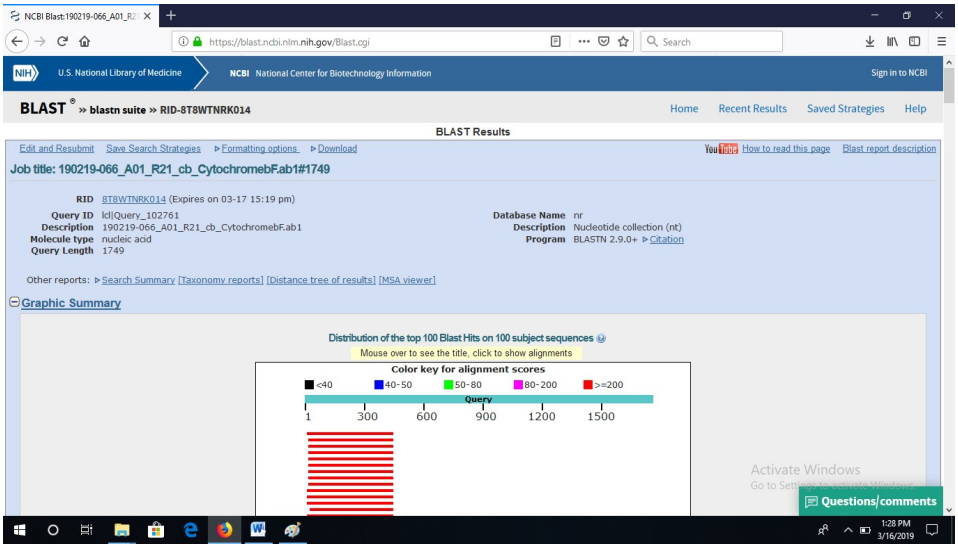
Purified PCR products of Cytochrome b genes were purified by using SV Minicolumn kit. Incubation and repeated centrifugation (@ 16000 RPM) were conducted according to the manufacturer's instructions. Then SV Minicolumns were carefully transferred to a clean 1.5ml micro centrifuge tube and apply 50 µL of nuclease-free water directly to the center of the column without touching the membrane with the pipette tip, then incubated at room temperature for 1 minute. Finally after centrifugation for 1 minute at 16,000 × g (14,000 rpm) the SV Minicolumn was discarded and eluted DNA was stored at 4°C or -20°C.

10.1.12: Sequencing of purified PCR products of Cytb gene nucleotides

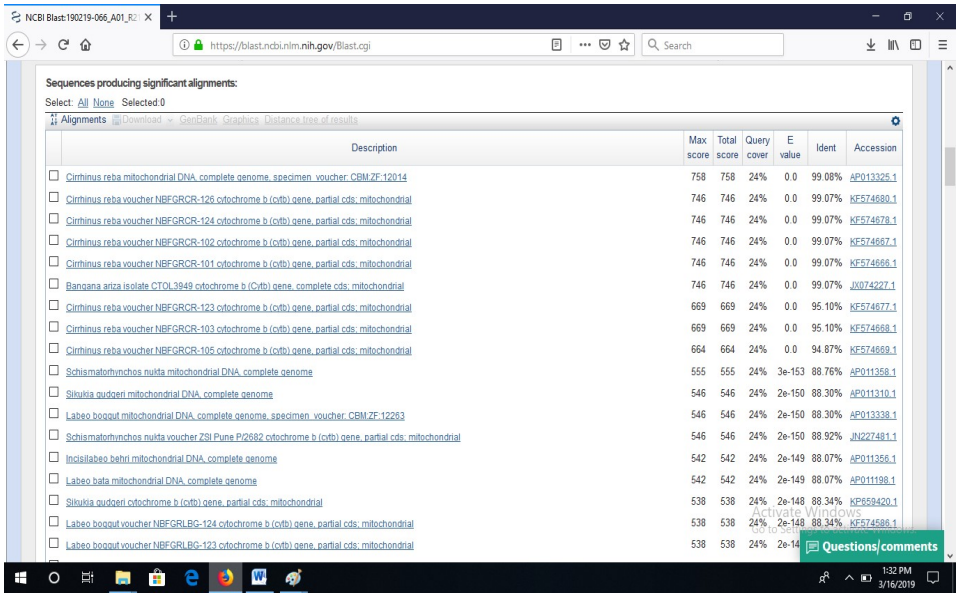
The purified PCR products were subjected to sequencing for revealing the genetic variation of *C. reba* wild populations. Five µl of PCR products, 0.7µl of Exonuclease I 10x Buffer (New England Biolabs, MA, USA), 0.5 µl of Exonuclease I and, 5.3 µl of distilled sterile water were incubated at 37°C for 30 min before being denatured at 80°C for 20 min. Then the purified products were labeled using the Big Dye terminator v.3.1 cycle sequencing kit (Sanger sequencer) in a total reaction mixture of 10 µl containing 4.94 µl of distilled sterile water, 1.94 µl of 5x Big Dye Buffer (400 mMTris–HCl pH 9.0 and 10 mM $MgCl_2$), 2 µl of 10 p mol of M13F or M13R, 0.12 µl of Big Dye terminator, and 1 µlExo SAP products. Sequence-PCR products were cleaned up using the ethanol precipitation method and sequenced bi-directionally on an ABI 3130 x l.DNA fragments were cleaned before sending to the Sequencing facility.

10.1.13: Identification of sequence similarities using BLAST at NCBI genebank database

Sequence of each specimen of this study was blasted at NCBI genebank database using Basic Local Alignment Search Tool (http://ncbi.nih.gov/BLAST/). The sequenced Cytb was found the similar with the stored Cytb nucleotide sequences of NCBI nucleotide depositories. The similarity of the identity were ranged from 88 to 96% (Figure 10.10: A-B).



(A): The snap shot of blasting sequences at NCBI nucleotide database



(B): The snap shot of sequenceproducing significant alignment with the representative query sequences of Cytb from *C. reba*

Figure10.10: The Identification of sequenced gene at NCBI Gene bank database by using BLAST

10.1.14: Genetic data analysis of C. reba wild populations

Consensus sequences were generated by aligning edited forward and reverse sequences. Contig assembly were performed as well Basic Local Alignment Search Tool (BLAST) database searches were performed to ensure the amplification of correct target sequences. Analyses were conducted using Molecular evolutionary genetics analysis (MEGA) 7.01 version software with the Maximum Likelihood model (Tamura *et al* 2013). The nucleotide composition, estimates of net base composition bias, nucleotide divergence (genetic distance), transition-transversion bias, disparity between sequences as disparity index per site and phylogenetic relationship were analyzed. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter models. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed according to (Felsenstin 1985).

Experiment 2 (A): Seed production of *C. reba* through artificial propagation

10.2.1. Brood selection for induced breeding

From the total stock few broods were caught for breeding. Only ready to spawn breeders were selected for breeding program. Male broods were easily selected by their rub pectoral fin. Female were selected by their swollen abdomen and pectoral fin. Female anal region was bold and pink color. The female broods are comparatively longer than male brood (Figure: 10.11)

Plate 10.11: Male and female broods of *Cirrhinus reba*

10.2.2. Conditioning of the brood before stocking

When broods were reached at the breeding stations then it needed to be conditioned. For their adjustment with the new stocking condition it was needed to provide 5-6 hours conditioning time in circular tank. Broodstocks were kept into separate rectangular tank for conditioning approximately for 6 hours. Adequate water flow was ensured for sufficient oxygen. In fasting condition breeding success may achieve. So any food materials were not provided to the breeds. (Figure 10.12)


Plate10.12: Conditioning of broods before breeding

Inducing C. reba with different hormones to optimize the doses of hormones:

10.2.3: Age and weight of brood fishes

For induced breeding of any fishes species, it is important aspect to maintain age and weight of brood stock. If brood fish is not fully matured, eggs quality and quantity could be deteriorated. Age and weight of broods vary from species to species. In this study, the average weight of the females and males were 135.5±10 g and 71±10 g respectively.

10.2.4: Breeding plan

For induced breeding of *C. reba* male and female broods were collected from the brood rearing ponds of the research station at 2:1 ratio of male and female. In the initial breeding program, the doses of inducing agents were optimized, for this purposes three hormones namely Pituitary Gland Extracts (PG), Synthetic hormone FLASH and OVATIDE at different doses were used. The details of hormone doses of each hormone are given in the (Table 10.5).

Table 10.5: The details of breeding experiment

Name of Hormones	Treatments	Replication	Hormones and Doses		
			Female		Male
PG extracts	T_1	R_1	1.5 mg/kg	5.5 mg/kg	2.0 mg/kg
		R_2	1.5	5.5	2.0
		R_3	1.5	5.5	2.0
	T_2	R_1	1.5	6.5	2.0
		R_2	1.5	6.5	2.0
		R_3	1.5	6.5	2.0
	T_3	R_1	1.5	7.5	2.0
		R_2	1.5	7.5	2.0
		R_3	1.5	7.5	2.0
Ovatide	T_1	R_1	-	0.5ml/kg	0.3 ml/kg
		R_2	-	0.5	0.3
		R_3	-	0.5	0.3
	T_2	R_1	-	0.6	0.3
		R_2	-	0.6	0.3
		R_3	-	0.6	0.3
		R_1	-	0.7	0.3
		R_2	-	0.7	0.3

	T_3	R_3	-	0.7	0.3
FLASH	T_1	R_1	-	0.4 ml/kg	0.2 ml/kg
		R_2	-	0.4	0.2
		R_3	-	0.4	0.2
	T_2	R_1	-	0.5	0.2
		R_2	-	0.5	0.2
		R_3	-	0.5	0.2
	T_3	R_1	-	0.6	0.2
		R_2	-	0.6	0.2
		R_3	-	0.6	0.2

10.2.5 Preparation of PG (Pituitary gland), Flash and Ovatide Solution

Locally available dry pituitary glands (PG) as well as Flash and Ovatide were collected from market in preserved condition in air tight vials. At first, the pituitary glands were gently removed from the vial with a pair of forceps and dried by using the filter paper for 2-3 minutes and then weighed by a sensitive analytical electronic balance The amount to be weighed out was calculated based on the total body weight of all the fish using the following formula :

$$\text{Weight of PG (mg)} = \frac{W_t \times P_t}{1000}$$

Where, Wt represents total body weight (g) of all the fish to be injected and Pt represents the rate in mg PG to be injected/kg body weight under a particular treatment.

The weighed PG was transferred to a tissue homogenizer for thoroughly crushed. The crushed PG was then diluted in distilled water to dissolve it and was centrifuged with a hand centrifuged for 10 minutes at 2000 rpm. The freshly prepared PG supernatant of hormone was then taken slowly in a 1 ml hypodermic syringe for injection.

Then, the required amount of Flash and Ovatide was taken in a small bowl by syringe which has a capacity of 1ml. Then sufficient amount of water added to the synthetic hormone.

10.2.6 Hormone administration with PG, Flash and Ovatide

After preparation of PG extract, Flash and Ovatide solution brood fish were caught carefully by using net, and kept in sponge and injected. They were covered by soft cloth; then injected near the pectoral fin base. The amount of hormone for each fish was determined before according to the body weight of the broods. Single dose of hormone was administered to the male and female then wait for the response releasing egg and milt of female and male respectively. After ovulation, female and male were selected for stripping from each treatment to optimize the dosage, ovulation and fertilization rate were determined.

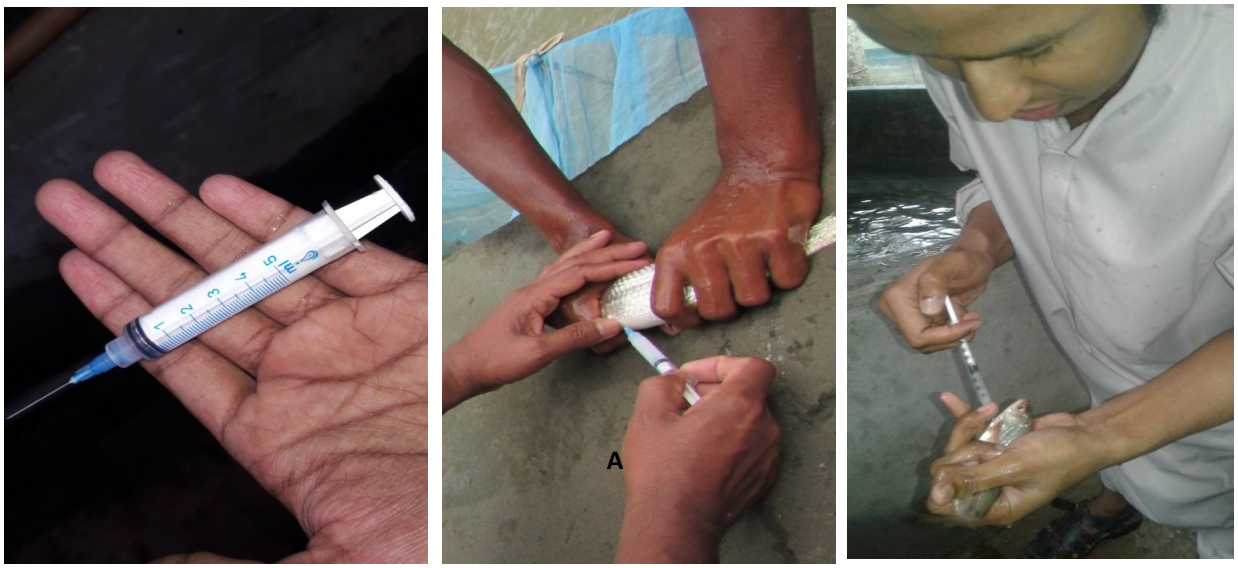


Plate 10.13: Induce Breeding activities: (A) injecting the brood fish, (B) observation of spawning

10.2.7. Collection of fertilized eggs and transferring to hatching tank

After 9 hrs of injection at 10:15am the fishes in maximum cases were found to ovulate. Then the fishes were removed from the rectangular tank by small net into other tanks. The eggs were transferred to the circular tank for hatching. The eggs were kept in plastic bowls to examine the rate of fertilization. During fertilization, approximately 100 eggs were placed in bowls with three replications after 10 minutes regular interval. At first the number of fertilized and unfertilized eggs of each bowl was counted with naked eyes.(Figure 10.14) After approximately 18-23h of fertilization when hatching almost completed, the number of hatchlings in each bowl were counted. The breeding parameters were studied by following formula:

$$\text{Ovulation rate (\%)} = \frac{\text{Number of fish ovulated}}{\text{Total number. of fish injected}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs (fertilized + unfertilized)}} \times 100$$

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

The water quality parameters such as pH, DO, temperature etc. will be recorded during ovulation, incubation and hatching.

Figure 10.14: Observation of Fertilization and counting

Figure 10.15: Incubation of fertilized eggs for hatching out

10.2.8. Data analysis

The collected data were summarized and processed for analysis. These data were verified to eliminate all possible errors and inconsistence. For the calculation of ovulation, fertilization and hatching rate simple statistical tools were used. These calculated data were input in SPSS sheet. Mean, standard deviations were collected from ANOVA table. Further level of significant of the trials was test by DMRT. Post hoc test was use to checking the similarity and dissimilarity of the trials. Then all numerical data were tabulated into MS Excel to take graphical representation of the trials.

10.2.2. Experiment 2(B): Larval rearing of *C. reba* for fingerling developments

10.2.2.1. Optimization of larval feed

The hatch out larvae were fed boiled chicken egg as first feed after 48 hours. The larvae were reared until 5/7 days in the hatchery unit than transferred to the ponds. From our previous studies, we have identified that *C. reba* is an omnivore fish. By considering its food habit the first larval rearing experiment were conducted in the glass aquarium to optimize its feed. Three treatments were conducted for verifying feeds and named as (T_1, T_2 and T_3) which are outlined in Table 10.6. Plankton was grown in a nearby pond by applying regular fertilization and harvested using plankton net.

Table 10.6: The experimental design to optimize the feeding regime and stocking density

Trial - 1		
Treatments	Feed	Stocking Density (Larvae/L)
T_1	Plankton	10
T_2	Commercial fish feed (32 % protein)	
T_3	Commercial fish feed (32 % protein) + Plankton	

10.2.2.2. Optimization of stocking density

After optimization of larval feed at glass aquaria, the stocking density was optimized in nylon net made hapa. Three treatments namely T_1, T_2 and T_3 were designed by considering the stocking density @ 600/hapa, 750/hapa and 900/hapa respectively (Table 10.7). Three replications of each were used and as feed plankton and commercial factory feed having 32% proteins were provided for a period of 8 weeks. Plankton was grown in the experimental hapa applying regular fertilization. Regular water quality monitoring was done during the experimental period.

Table 10.7: The experimental design to optimize the feeding regime and stocking density

Trial-2		
Treatments	Stocking Density (number/happa)	Feed
T_1	600	Plankton+ commercial fish feed (32% protein)
T_2	750	
T_3	900	

10.2.2.3. Production of fingerling in earthen ponds

Based on the findings of feed and stocking density optimization, finally initiatives were taken to produce fingerlings at large scale in earthen ponds (Figure 10.15). The study could not fulfill the scientific requirement due to limited number of experimental ponds. Only the growth and survival of larvae were observed using a single pond.

Figure 10.16: Larval rearing and nursing activities: A) in glass aquaria, B) in nylon net made hapa C) in Earthen pond

Statistical Analysis

Different growth parameter such as length gain, weight gain, total weight gain and specific growth rate (SGR) and survival rate were tested using one way analysis of variance (ANOVA). Significant results (p<0.05) further were further tested by Duncan's New Multiple Range Test (DMRT) to identify significant differences among means. These statistical analyses were performed with the aid of the computer software program MS Excel (Windows 10) and SPSS (Ver. 22).

10.3 Experiment 3: Crossbreeding of *C. reba* for the genetic improvement of Dinajpur stocks

10.3.1. Breeding plan and Procedure

Seven crossbreeding trials (line-1, line-2, line-3, kube-4, line-5, line-6 and line 7) were performed for the identification of the best line in terms of breeding performances (Table 10.8). Each line having three replications was conducted by using 6 females and 12 males for each source. For each lines, the ovulation, fertilization and hatching rate were recorded to identify the best performing line. From the experiment 2, the suitable hormone and optimized does were identified and used to induce the breeders in this experiment. PG hormone was used, where male were injected once and female twice. The first injection for the female was 1.5mg/kg body weight. After 6 hours of first injection of the female, the 2nd injection was administered @6.5 mg/kg body weight. During the 2nd injection of the female, the male breeders were injected @ 2mg/kg/body weight.

Table 10.8: The breeding plan for identifying the best line

Lines	Source of female breeders		Source of male breeders
Line 1 (DD)	Dinajpur (♀)	×	Dinajpur (♂)
Line 2 (MM)	Mymensingh (♀)	×	Mymensingh (♂)
Line 3 (BB)	Bogura (♀)	×	Bogura (♂)
Line 4 (DM)	Dinajpur (♀)	×	Mymensingh (♂)
Line 5 (MD)	Mymensingh(♀)	×	Dinajpur(♂)
Line 6 (DB)	Dinajpur(♀)	×	Bogura (♂)
Line 7 (BD)	Bogura (♀)	×	Dinajpur (♂)

The breeding performances were identified by determining the ovulation success, fertilization rate and hatching rate of the each breeding trails.

10.3.2 Statistical Analyses

Different breeding parameters; ovulation rate, fertilization rate and hatching rate were tested using one way analysis of variance (ANOVA). Significant results (p<0.05) further were further tested by Duncan's New Multiple Range Test (DMRT) to identify significant differences among means. These statistical analyses were performed with the aid of the computer software program MS Excel (Windows 10) and SPSS (Ver. 22).

11. Results and discussion:

11.1 Experiment I: Assessment of genetic variation of *C. reba* for quality broodstock management

The genetic differentiation of four stocks of *C. reba* were determined by analyzing the mitochondrial cytochrome b gene. After isolation of genomic DNA from each wild stock, the Cytb gene were amplified and sequenced. The sequenced nucleotides of the Cytb gene of studied population were confirmed by BLAST and found the identity and similarity about 88-100%. Then the sequences were analyzed which included the determination of the percentage of overall conserved and vaiable sites among the Cytb gene of studied population, nucletide composition, AT and GC content, Genetic Distance (Nucleotide divergence), Cluster formation of the populations through phylogenetic analysis were conducted. The resuts in brief are presented in below.

11.1.1 Sequence analysis

In the multiple sequence alignment of the sequenced mitochondrial gene of COI, a total of 855 sites were determined of which the overall conserved sites and variable sites were 39 (11.23%) and 759 (88.77%) respectively (Table 11.1.2). Among the studied sites about 533 nitrogen bases were parsimony informative. In the nucleotide sequences where the variable sites were higher (759) than the conserve sites (39) that may be due to the closely related species of the population. Wang *et al.,* 2007 studied on molecular phylogeny of *icefish Salangidae* based on cytochrome b sequences and found from the DNA sequence alignment, conserved sites 692 and variable sites 449 and of which 426 were parsimony informative. Molecular Phylogeny of Catfishes (Teleostei: Siluriformes) in the Philippines using the mitochondrial genes CO1, Cytb were done by (Yu *et al.,* 2014) and concatenated sequences yielded 1526 (36%) variable characters and 1385 (33%) parsimony-informative characters out of 4216 characters. Genetic variation and structure of *Clarias batrachus* from Southeast Asia using a mitochondrial DNA marker by (Lee *et al.,*2015) and results showed that alignment of 27 sequences of the cytochrome b gene resulted in 395 sites, of 260 sites were conservative (65.9%), 74 were variable (18.7%) and 61 were parsimony informative (15.4%). The percent of sequence analysis result of this study was more or less same as above the reference result.

Table 11.1: Sites of nucleotide in multiple sequence alignment

Sites of nucleotide	Value (s)	Percent (%)
Conserved sites	39	4.56
Variable sites	759	88.77
Parsimony sites	533	62.34

11.1.2. Nucleotide composition and GC content

The nucleotide composition of Cyto b gene for 20 sequences were estimated which showed that the average nucleotide composition of thymine (T), cytosine (C), adenine (A) and guanine (G) were 21.1, 31.4, 16.1 and 31.3 respectively. The cytosine content was highest 31.4% where the guanine content was 32.3%. In cases of different species and genus level the nucleotide composition were not highly varied. There was no larger difference in individual amount of nucleotide composition. The percent of thiamin and adenine were found more or less same throughout the Mymensingh population but the percent of adenine and thiamine were found different in the sequence of others three populations (Table 11.2). The average AT content was lower (37.3%) than the GC content (62.7%). Sarmah & Sarmah (2016) studied the average nucleotide composition for all the haplotype

sequences where 30.75% Adenine, 27.45% Thymine, 13.41% Guanine and 28.39% Cytosine were described. A high concentration of Cytosine (28.5%) was displayed in CAJOR5, CADHE2 and CRJJA1 while in CWBNA2 the lowest Guanine concentration (13.6%) was recorded. The average frequencies of four nucleotides for all the 23 samples of *C. marulius* are A: 25.49%; T: 28.16%; G: 15.79%; C: 30.55% were also reported (Habib, *et al.,* 2011).

Table 11.1.2: Nucleotide composition of Cytochrome B sequences of *C. reba* populations

Species *C. reba*	T(U)	C	A	G	Total
C. reba 21FCytb Din 348	27.6	22.4	20.7	29.3	348.0
C. reba 21Rcytb Din 351	7.7	39.0	30.5	22.8	351.0
C. reba 22Fcytb Din 261	30.3	20.7	19.9	29.1	261.0
C. reba 23Fcyt Din 156	17.9	26.9	19.9	35.3	156.0
C. reba 24Rcytb Din 123	30.9	22.0	25.2	22.0	123.0
C. reba 25Rcytb Din 390	26.4	23.3	22.3	27.9	390.0
C. reba 10Rcytb Bog 243	13.1	29.7	20.7	36.5	222.0
C. reba 26Rcytb Bog 435	18.2	31.7	5.1	45.1	435.0
C. reba 27Rcytb Bog 705	21.4	31.1	17.3	30.2	705.0
C. reba 29Rcytb Bog 363	25.9	24.8	22.0	27.3	363.0
C. reba 12Rcytb Myn 429	27.3	23.6	21.9	27.3	411.0
C. reba 31Fcytb Myn 276	30.8	20.7	19.2	29.3	276.0
C. reba 32Rcytb Myn 798	24.1	38.0	10.2	27.8	798.0
C. reba 34Rcytb Myn 660	25.9	25.5	17.3	31.3	642.0
C. reba 40Rcytb Myn 192	30.2	20.8	25.0	24.0	192.0
C. reba 16Rcytb Jes 387	27.4	23.5	20.7	28.4	387.0
C. reba 18Rcytb Jes 351	26.8	24.2	22.2	26.8	351.0
C. reba 35Rcytb Jes 678	1.5	64.0	1.9	32.6	678.0
C. reba 37Rcytb Jes 855	14.6	35.7	5.3	44.4	855.0
C. reba 39Rcytb Jes 390	23.1	26.2	23.6	27.2	390.0
Avg.	21.1	31.4	16.1	31.3	416.7

11.1.3. Genetic distance among the populations of C. reba based on Cytb sequences

To estimate the genetic distance of 20 species of four populations in terms evolutionary divergence between sequences were estimated to determine the genetic distances among them by using Molecular evolutionary genetics analysis 7.01version with the Maximum Likelihood model (Tamura *et al.,* 2013). The number of base differences per site from between sequences is shown in the Table (11.3). Standard error estimate(s) are shown below the diagonal. The analysis involved 20 nucleotide sequences. Analyses were conducted to assess the genetic distance among the polulations ranged from 0.000 to 0.805 shown in the Table (11.3). The highest genetic distance was found between Dinajpur and Mymensingh (*C. reba* _Din_384 and C. reba_Myn_*660*) population in Bangladesh where the lowest genetic distance (0.000) was found between several individual of populations such as *C. reba* _22Fcytb_Din*, C. reba* _31Fcytb_Myn*, C. reba* _18Rcytb_Jes.etc (Table11.3). Habib et al*.,* (2011) studied *Channa marulius* with partial Cytochrome b gene sequence of mtDNA for determining the genetic variation in wild populations. Results revealed the presence of 5 haplotypes with haplotype diversity value of 0.763 and nucleotide diversity value of 0.012. Single population specific

haplotype was observed in Mahanadi and Yamuna samples and 3 haplotypes in Teesta samples. The analysis of data demonstrated the suitability of partial Cytochrome b sequence in determining the genetic diversity in *C. marulius* population. The evolutionary divergence showed that they were evolved and accumulated in differences and ultimate changes in the body shape, size or any other function. The genetic distance was lowest between *A. baerii*-like *A. gueldenstaedtii* (1.63%) and highest between *A. stellatus*- *H. huso*(27.9%). The intraspecies sequence divergence ranged from 0.42% (*A. gueldenstaedtii*) to 0.17% (*H. huso*). Twelve species of *Puntius* were investigated using 60 partial sequences of the mitochondrial cytochrome b (Cyt b, 1096 bps) gene to estimate genetic divergence and to establish the phylogenetic relationship by (Goswami*et al.,* 2012). Briolay*et al.,* 1998 found the highest genetic distance 1.3 in a study of fish phylogeny. Mitochondrial Cytochrome b sequence variation in three sturgeon species *A. stellatus, A. gueldenstaedtii, H. huso* from the Black Sea Coasts of Turkey by Yılmaz*et al.,* 2013. Yang *et al.,* 2012 showed that the pair-wise genetic divergence ranges from 0.4 to 23.8% observed. Similar statement of pairwise sequence divergence between taxa that ranged from 0.3 to 26% was described by Zardoya *et al.,* (2010). Three described species (*Atule mate, Selarcrumenophthalmus* and *Seriolinanigrofasciata*) exhibited conspecific divergences up to ten times greater (4.32-4.82%) than mean estimates (0.24-0.39%) indicating a discrepancy with assigned morphological taxonomic identification, and the existence of cryptic species within the IMA keskin, (2012) showed that Two-parameter genetic distances such as 0.209 for interspecific distance and 0.009 for intraspecific variation. They clearly demonstrate the effectiveness of the DNA approach both for identifications at species level and revealing intraspecific variation among populations.

Table 11.3: Estimates of Evolutionary Divergence between Sequences (Genetic distance)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *C. reba* _Din_348		0.040	0.000	0.042	0.038	0.038	0.041	0.040	0.041	0.041	0.039	0.000	0.037	0.034	0.019	0.041	0.041	0.036	0.043	0.042
2. C. reba _Din_351	0.732		0.040	0.040	0.040	0.042	0.037	0.041	0.044	0.041	0.041	0.040	0.042	0.039	0.039	0.040	0.041	0.044	0.044	0.041
3. C. reba _Din_261	0.000	0.732		0.042	0.038	0.038	0.041	0.040	0.041	0.041	0.039	0.000	0.037	0.034	0.019	0.041	0.041	0.036	0.043	0.042
4. C. reba _Din_156	0.699	0.707	0.699		0.041	0.041	0.039	0.044	0.040	0.042	0.041	0.042	0.040	0.040	0.042	0.042	0.042	0.041	0.038	0.041
5. C. reba _Din_123	0.740	0.715	0.740	0.707		0.039	0.040	0.038	0.039	0.040	0.040	0.038	0.040	0.041	0.039	0.041	0.040	0.037	0.039	0.041
6. C. reba __Din_390	0.732	0.659	0.732	0.659	0.748		0.038	0.037	0.040	0.042	0.041	0.038	0.038	0.040	0.040	0.042	0.042	0.043	0.040	0.041
7. C. reba _Bog_243	0.724	0.748	0.724	0.724	0.732	0.764		0.039	0.039	0.043	0.043	0.041	0.044	0.042	0.042	0.043	0.043	0.039	0.042	0.043
8. C. reba _Bog_435	0.715	0.683	0.715	0.675	0.780	0.772	0.724		0.041	0.044	0.044	0.040	0.043	0.044	0.041	0.044	0.044	0.043	0.042	0.043
9. C. reba _Bog_705	0.667	0.626	0.667	0.699	0.740	0.667	0.748	0.675		0.039	0.040	0.041	0.040	0.038	0.040	0.039	0.039	0.043	0.039	0.040
10. C. reba _Bog_363	0.732	0.659	0.732	0.683	0.715	0.667	0.650	0.659	0.707		0.011	0.041	0.042	0.039	0.043	0.011	0.000	0.041	0.044	0.019
11. *C. reba* _Myn_429	0.748	0.642	0.748	0.691	0.715	0.675	0.642	0.642	0.699	0.016		0.039	0.042	0.040	0.041	0.015	0.011	0.041	0.044	0.022
12. C. reba _Myn_276	0.000	0.732	0.000	0.699	0.740	0.732	0.724	0.715	0.667	0.732	0.748		0.037	0.034	0.019	0.041	0.041	0.036	0.043	0.042
13. C. reba _Myn_798	0.780	0.650	0.780	0.715	0.732	0.740	0.577	0.610	0.724	0.667	0.650	0.780		0.044	0.038	0.042	0.042	0.044	0.044	0.042
14. C. reba __Myn_660	0.805	0.715	0.805	0.707	0.724	0.732	0.659	0.593	0.748	0.715	0.707	0.805	0.650		0.034	0.039	0.039	0.041	0.042	0.038
15. C. reba _Myn_192	0.049	0.764	0.049	0.683	0.715	0.715	0.699	0.699	0.683	0.707	0.724	0.049	0.772	0.805		0.043	0.043	0.037	0.041	0.044
16.C. reba _Jes_387	0.732	0.675	0.732	0.683	0.715	0.675	0.659	0.659	0.724	0.016	0.033	0.732	0.675	0.715	0.707		0.011	0.041	0.043	0.021
17. C. reba _Jes_351	0.732	0.659	0.732	0.683	0.715	0.667	0.650	0.659	0.707	0.000	0.016	0.732	0.667	0.715	0.707	0.016		0.041	0.044	0.019
18. C. reba _Jes_678	0.789	0.659	0.789	0.650	0.789	0.642	0.707	0.602	0.585	0.659	0.642	0.789	0.423	0.683	0.780	0.675	0.659		0.043	0.041
19. C. reba _Jes_855	0.667	0.593	0.667	0.748	0.715	0.691	0.650	0.691	0.715	0.634	0.626	0.667	0.675	0.691	0.691	0.650	0.634	0.634		0.044
20. C. reba _Jes_390	0.691	0.675	0.691	0.691	0.691	0.691	0.650	0.667	0.699	0.049	0.065	0.691	0.667	0.724	0.667	0.065	0.049	0.675	0.593	

11.1.4 Phylogenetic tree construction for observe cluster formation (if any)

The sequenced gene was further used for the study of evolutionary relationship among the population through the use of maximum likelihood method (ML) and the maximum parsimony method (MP).The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter models. The tree with the highest log likelihood (2197.4402) is shown. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the species analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Figure 11.1). Evolutionary analyses were conducted in MEGA 7.01 version (Tamura, *et al.,* 2013). The phylogenetic tree in maximum likelihood method divided the taxa in three clades. Clade1 and clade2 consisted of three population that included Bogura, Jashore and Mymensingh but Dinajpur population did not form sister with studied population of clade-1 and Clade 2. Dinajpur population form sister poorly with the Mymensingh and Bogura population in the clade 3. Overall the Dinajpur population did not sister with the Jashore population that shown in (Figure 11.1).

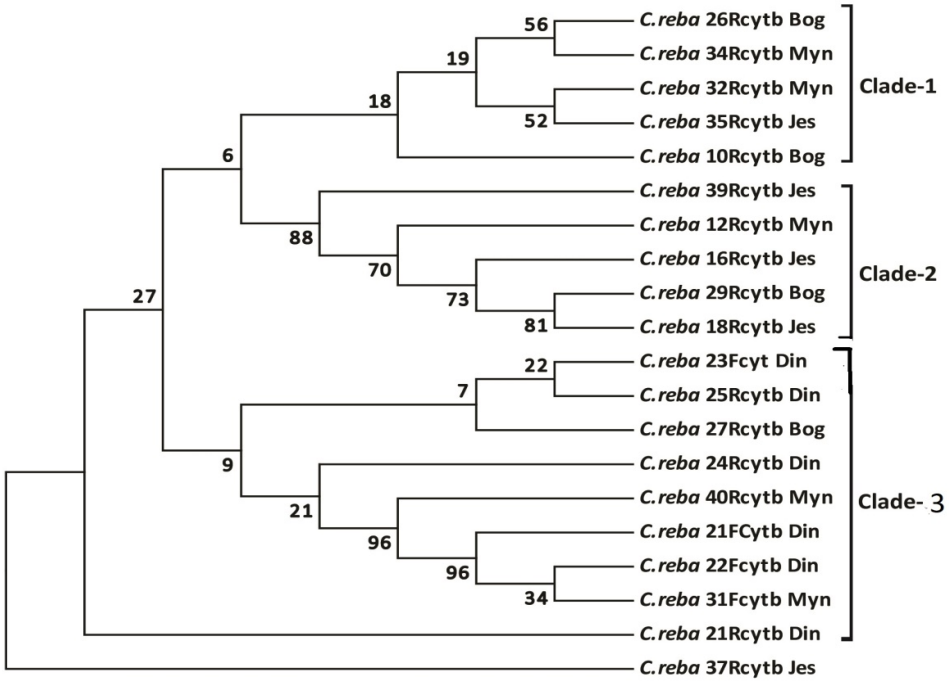


Figure 11.1: Molecular Phylogenetic analysis by Maximum Likelihood method

11.1.5 Bootstrap consensus tree analyzed with maximum parsimony method

The evolutionary history was inferred using the Maximum Parsimony method. Tree 1 out of 2 most parsimonious trees (length =710) is shown. The consistency index, the retention index, and the composite index were 0.579805, 0.263770 and 0.263770 respectively. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed according to Felsenstein, (1985). The analysis involved 20 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 123 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7.01 version according to (Tamura*, et al.,* 2013). Based on the Cytb nucleotide sequences the similar pattern of cluster formation in the MP

tree was found shown the (Figure 11.2). The three population that included Mymensingh, Jassore and Bogura were formed the sister in clade1 but the Dinajpur population did not form the sister clade 1. In the clade 3, poor sister formation of Dinajpur population with other populations were observed.

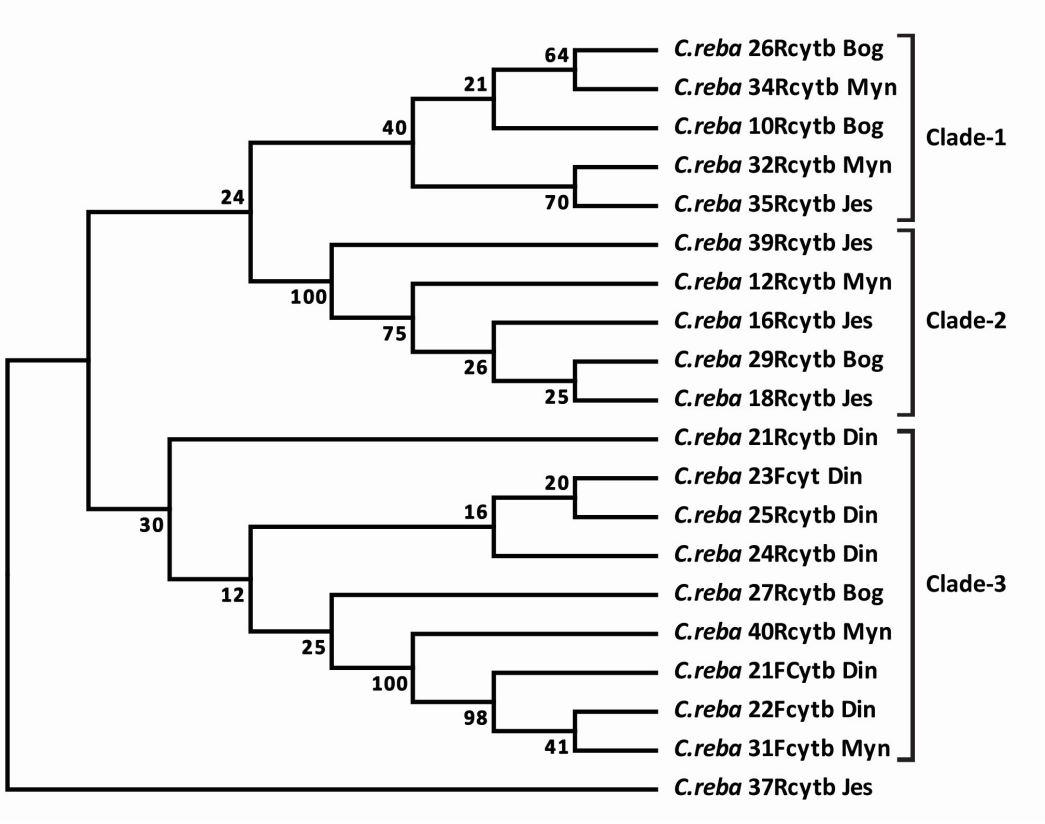


Figure 11.2: Evolutionary tree based on Maximum Parsimony analysis of four populations

A phylogenetic tree was constructed by using the Kimura 2-parameter distances for identification of Gadidae species and resulted in a well-differentiated clade, with the exception of *Gadusogac* and *Gadusmacrocephalus*. (Goswami *et al.,* 2012) observed the average intraspecies diversity by ML method was estimated as 0.002, whereas the average interspecies diversity was estimated as 0.177. The sequence analysis of the *Cyt b* gene revealed four distinct groups, which are genetically distinct species and exhibited identical phylogenetic relationship. In the Maximum Parsimony method Tree 1 out of 2 most parsimonious trees (length = 710) is shown. Based on the Cytb nucleotide sequences the similar pattern of cluster formation in the MP tree was found. The three population such as Mymensingh, Jassore and Bogura were formed the sister in clade1 and the clade 2 but the Dinajpur population did not form the sister clade 1 and clade 2. In the clade 3, Dinajpur population formed sister of other populationpoorly. (Tsipas *et al.,*2017) observed the Phylogenetic relationship by MP method of cyprinid species from the middle east representative partial D-loop sequences 573 bp, in length, sequences from European, Asian and Japanese domestic and wild common carp strains (n=45) have been analyzed. Phylogenetic analysis indicates that the Yangtze River wild common carp (YWC1) is the ancestor for the Greek. the Ozeros Lake *common carp* (OZE2a) and Volga River wild *common carp* (VWC1) strains consist of a closely and a distinct ancestor for European *Cyprinus carpio* populations.

11.2. Experiment II: Seed production of *C. reba* through artificial propagation

This experiment was initially done for selecting the best hormone to induce the *C. reba* breeders for artificial breeding. After selecting the hormone, more trials were conducted to standardize the doses. After spawning success, experiments were conducted for larval rearing. The results of these trials are described briefly in below.

11.2.1. Selection of inducing agents to induce C. reba breeders

Three hormones namely the natural dehydrated pituitary gland extracts (PG), two synthetic inducing agents OVATIDE and FLASH available in the local markets of Bangladesh were used in the breeding trials. Three doses of each hormone stated in the material method section (Table 11.4;) considered as Treatment 1 (T_1), Treatment 2 (T_2) and Treatment 3 (T_3) having three replications of each (R_1, R_2, R_3) were conducted. The results of hormone treatment with different doses interferes the ovulation rate, latency period, fertilization rate, hatching rate and hatching percentage as are described in Table. The highest ovulation, fertilization, and hatching rate were observed in Treatment 1 (PG) where the females were injected twice: 1st injection was administered @ 1.5 mg/kg body weight of the breeders and 2nd injection administered @ 6.5 mg/kg body weight of the breeders. The male breeders were injected once during the 2nd injection of the female. The ovulation, fertilization, hatching were 86%, 80% and 72% respectively in Treatment 2 which were significantly different from two other treatments (Figure 11.3)

Table 11.4: Optimization of inducing agents to ovulate the ready to spawn breeders of *C. reba* collected from different regions of Bangladesh

Treatments	Ovulation (%)	Ovulation period (hr)	Fertilization (%)	Hatching (%)	Hatching Period (hr)
T_1 PG: Female 1.5, 6.5; Male 2.0mg/kg	86.67±3.34	11.34±0.34	80.67±1.76	72.67±1.34	16.34±0.34
T_2 Ovatide: Female 0.6; Male 0.3ml/Kg	56.67±3.34	14.00±0.58	53.34±3.34	42.34±1.45	19.00±0.58
T_3 FLASH: Female 0.5; Male 0.2 ml/kg	46.67±6.67	13.17±0.17	44.34±2.34	37.34±1.76	18.34±0.34

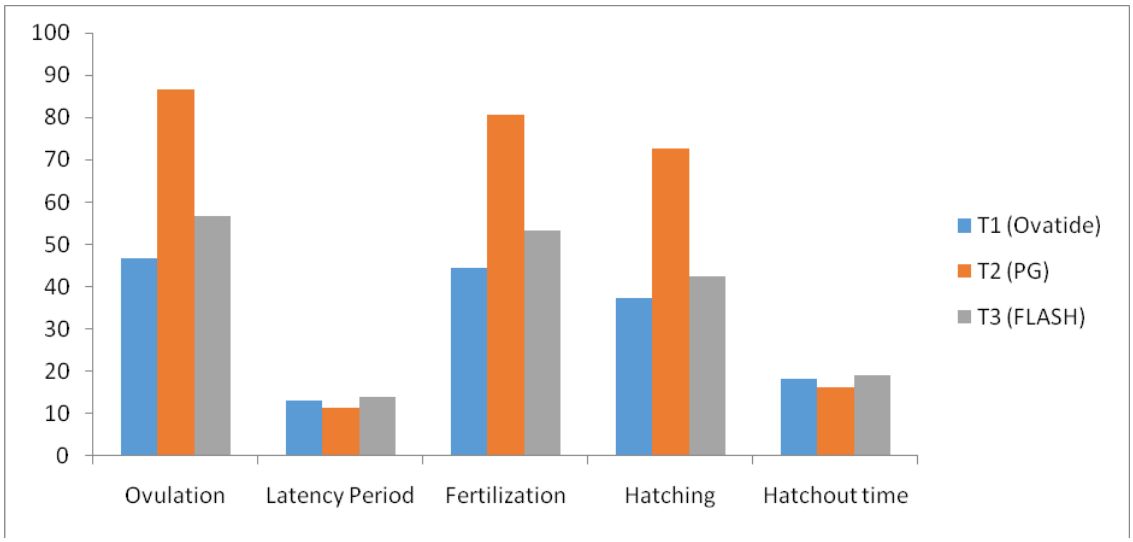


Figure 11.3: Induce breeding performances of *C. reba* induced by using different inducing agents

After selection of PG as the best inducing agents, trails with several does of PG were performed to select the best does to ovulate the *C. reba* breeders. The ovulation rate, ovulation period, fertilization rate, hatching rate, hatching period varied from treatment to treatment as shown in Table 11.5. The highest ovulation, fertilization, hatching rate were observed at treatment 2 where the female breeders were injected with the two doses, in the first doses of PG @ 1.5 were injected to the female and after 6 hrs again the female breeders were injected with PG extract @ 6.5 mg/kg body weight. The male breeders were only injected once @ 2 mg/kg body weight during the 2nd injection of the female. (Figure 11.4)

Table 11.5: Optimization of doses of PG extracts to ovulate the ready to spawn breeders of *C. reba* collected from different regions of Bangladesh

PG for 1st doses to female	Treatments PG for 2nd doses to female	PG doses for male	Ovulation (%)	Ovulation period (hr)	Fertilization (%)	Hatching (%)	Hatching Period (hr)
1.5mg/kg	T_1 (5.5mg/kg)	1.5mg/kg	36.00±3.34	12.83±0.44	66.67±3.33	54.33±2.33	16.67±0.33
	T_2 (6.5mg/kg)		88.00±1.67	11.33±0.33	81.33±1.33	77.67±1.45	16.67±0.67
	T_3 (7.5mg/kg)		50.00±0.00	10.67±0.33	62.00±2.00	58.33±1.66	17.33±0.33
	T_4 (8.0mg/kg)		20.00±0.00	10.0±0.33	24.50±1.47	48. 3±1.86	16.33±0.33

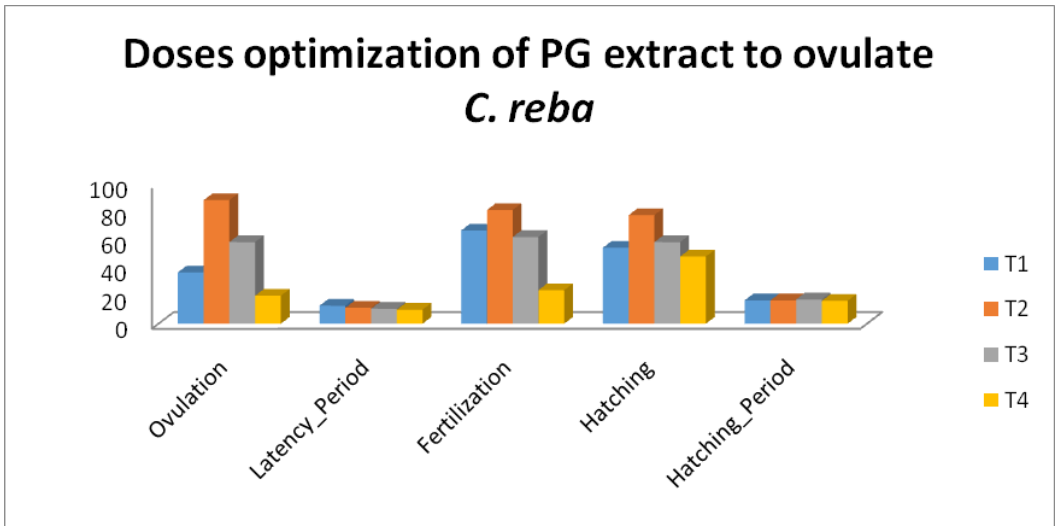


Figure 11.4: Breeding performance of *C. reba* with different doses

In inducing breeding, success of breeding mainly depends on an appropriate administration of hormone, condition of brood and surrounding environment. In induced breeding trials of this study the brood fish were treated with PG. The male fish were found to be responded by a single dose of 2 mg/kg body weight. On the other hand, females were treated twice, where in the first PG was injected at the rate of 1.5 mg/kg body weight and at the rate 6.5 mg/kg body weight in the 2nd dose. Rahman et al. (2007) studied the induced breeding of *C. reba* by using PG hormone, where they injected 0.5, 1.0 and 1.5 mg PG kg-1 body weight followed by a second dose of 4.0, 4.5 and 5.0 mg PG kg-1 body weight. A single dose for males *C. reba* @ 1.5 mg PG kg-1 body weight. The highest average fertilization and hatching rate of *C. reba* were 87.88% and 81.47% respectively. Recently Tausif (2016) induced *C. reba* by using PG and observed ovulation, fertilization, hatching rate 78%, 34.22%, 45.12% respectively. His ovulation rate was similar to the present study, but fertilization and hatching rate were comparatively lower than the present study.

11.2.2 Larval rearing of C. reba spwan to develop fingerlings

The produced spwan in different trails were subjected to utilize for larval rearing study. The larval rearing initially conducted at Glass aquaria to optimize the feeding regime and then in nylon net made hapa to optimize its stocking density and finally in earthen ponds by using the results of previous two systems for large scale production. The same feeding strategies and water quality monitoring were maintained in the entire experimental unit. During experimental research in nylon net made hapa and earthen ponds, regular water quality monitoring including plankton abundance were determined.

11.2.3 Larval rearing of C. reba for fingerling developments

11.2.3.1 Optimization of larval feed

The highest growth and survival were observed in treatment 3 where both plankton and factory made commercial nursery feed having 32% protein were provided. The highest length gain, weight gain, SGR and survival rate were observed in Treatment 3 and followed by Treatment 2 and Treatment 1 (Figure 11.5).

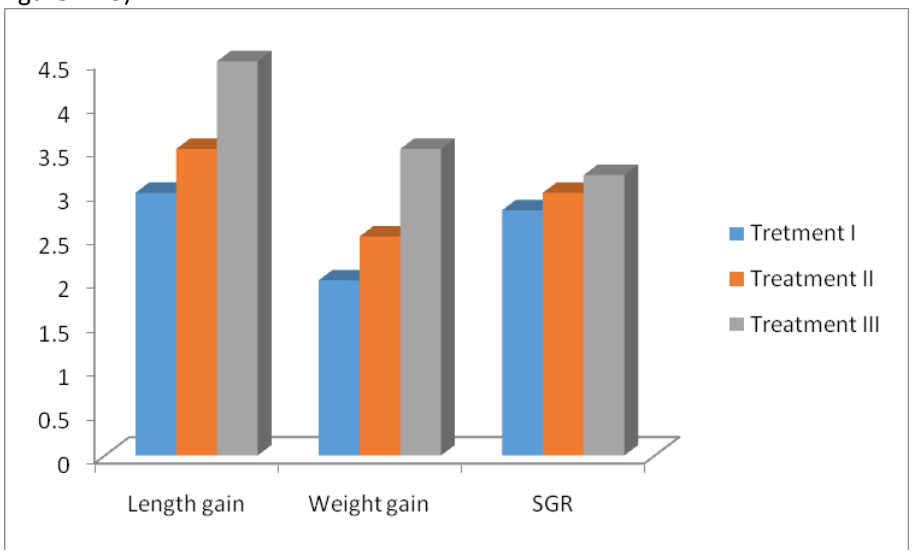


Figure 11.5: The growth performance for feed optimization for *C. reba* larvae.

11.2.3.2 Optimization of stocking density

The highest growth and survival were observed in treatment 1 and followed by treatment 2 and 3 where the stocking density were 600, 750 and 900 fry/hapa respectively. The highest weight gain, SGR and survival rate were observed in Treatment 1 and followed Treatment 2 and 3 (Figure 11.6). By considering the survival rate and total weight gain the farmers are suggested to stock spawn in their earthen ponds by following experiment Treatment 2 rather than Treatment 1 to maximize the utilization of pond water.

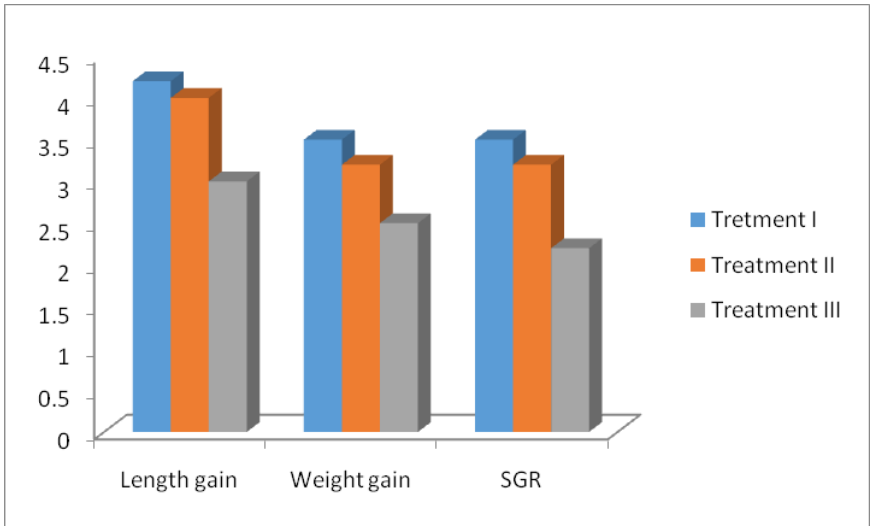


Figure 11.6: The growth performance of larval rearing at different stocking density

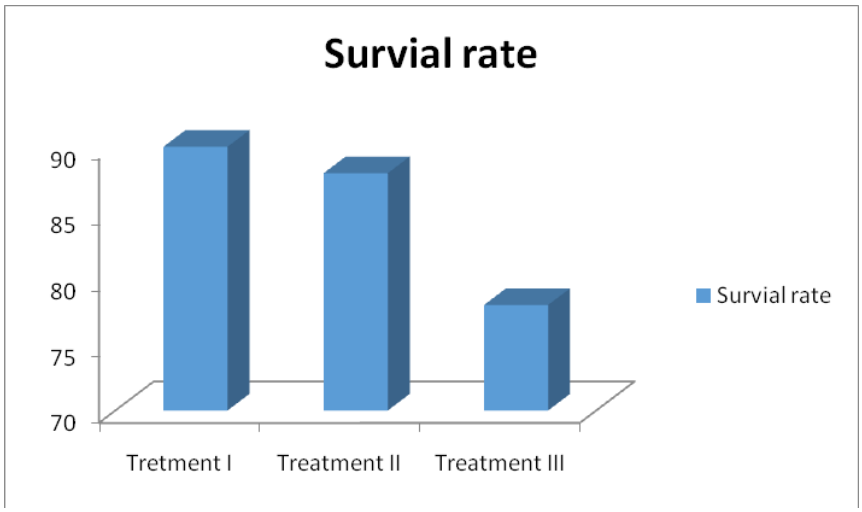


Figure 11.6: The survival rate of *C. reba* larvae at different stocking density

11.2.4 Production of fingerling in earthen ponds

Based on the findings of feed and stocking density optimization, finally initiatives were taken to produce fingerlings at large scale in earthen ponds. The study was not done with a proper scientific design due to lack of sufficient number of experimental ponds. Only the growth and survival of larvae were shown using a single pond. After 2 months nursing the spawns developed into fingerlings weighing 3.5±0.8 gm with a survival rate of 90% (Figure 11.7)

Figure 11.7: The produced larvae in earthen ponds

11.2. Experiment III: Crossbreeding of *C. reba* for the genetic improvement of Dinajpur stocks.
From the previous experiment (II.A) the hormone and doses of PG were optimized which were used to induce breeders of this experiment. Seven breeding trails were conducted using PG @ 1.5mg/kg body weight as first does to the females and @ 6.5 mg/kg body as the 2nd doses to the females. The males were injected once @ 2 mg/kg body during the 2nd injection of female.

11.3.1 Performance of different doses of PG on induce breeding of C. reba

The ovulation, fertilization and hatching rates of the seven breeding trials were shown in Table 4.1. Among 7 breeding lines, the highest ovulation (86.66±5.77%), fertilization (95.33±1.53 ^{c}) and

hatching rate (79.00±1.73[c]) were found in Line-4 (Dinajpur♀× Mymensingh♂), Line-2 (Mymensingh♀× Mymensingh♂) and Line-4 (Dinajpur♀× Mymensingh♂) respectively. And the lowest ovulation (71.66±2.88[a]%) fertilization (55.33±5.03[a]%) and hatching rate (46.66±5.77[a]%) were found in Line-7 (Dinajpur♀×Bogura♂). The ANOVA indicated that there was significant differences among seven trials at 0.5 level (Table 4.2). Further, the significant results were tested using Duncan Multiple Rang eTest (DMRT) to know the level of significance (Table 11.6).

Table 11.6 Performance of different breeding lines of *C. reba* using PG

Line	Treatments	Ovulation Rate (%)	Fertilization rate (%)	Hatching rate (%)
Line-1	Dinajpur♂× Dinajpur♀	84.00±6.92 [bc]	93.60±2.08 [c]	77.66±2.52 [c]
Line-2	Mymensingh♂× Mymensingh♀	85.00±5.0 [c]	95.33±1.53 [c]	75.00±5.00 [c]
Line-3	Bogura♂× Bogura♀	83.33±5.7[bc]	92.33±2.51 [c]	75.33±5.03 [c]
Line-4	Dinajpur ♀× Mymensingh♂	75.00±5.00 [ab]	58.66±5.50 [ab]	47.66±2.52 [a]
Line-5	Mymensingh♀×Dinajpur♂	86.66±5.77 [c]	88.00±7.0 [c]	79.00±1.73 [c]
Line-6	Dinajpur♀× Bogura♂	73.33±2.88 [a]	63.66±3.21 [b]	56.66±2.88 [b]
Line-7	Dinajpur♂× Bogura♀	71.66±2.88 [a]	55.33±5.03 [a]	46.66±5.77 [a]

The average ovulation rates of 7 breeding line showed in Figure. Among the breeding lines, the level of various occurred were significant (<0.05), the highest ovulation rate was 86.66 which was found in the line 4 where females of Dinajpur were mated with the male of Mymensingh and followed by Line 2 (Mymensingh x Mymensingh), Line 1 (Dinajpur x Dinajpur), Line 3 (BoguraxBogua) etc. (Figure 11.3.8)

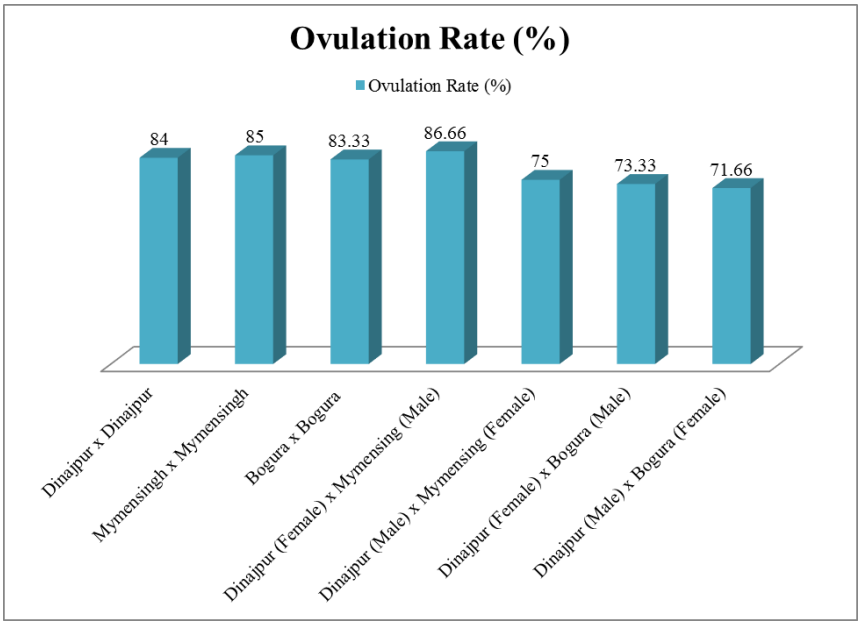


Fig. 11.8: Ovulation rate of 7 breeding trails of *C. reba*

Fertilization rate of the trials

The average fertilization rates of 7 breeding line showed in Figure. Fertilization rate was found to ranged from 55.33 to 95.33%. Among the breeding lines, the level of fertilization rate occurred were significant (<0.05), the highest fertilization rate was 95.33, which was found in the line 2 (Mymensingh x Mymensingh), and followed by Line 1 (Dinajpur x Dinajpur) etc (Figure 11.9).

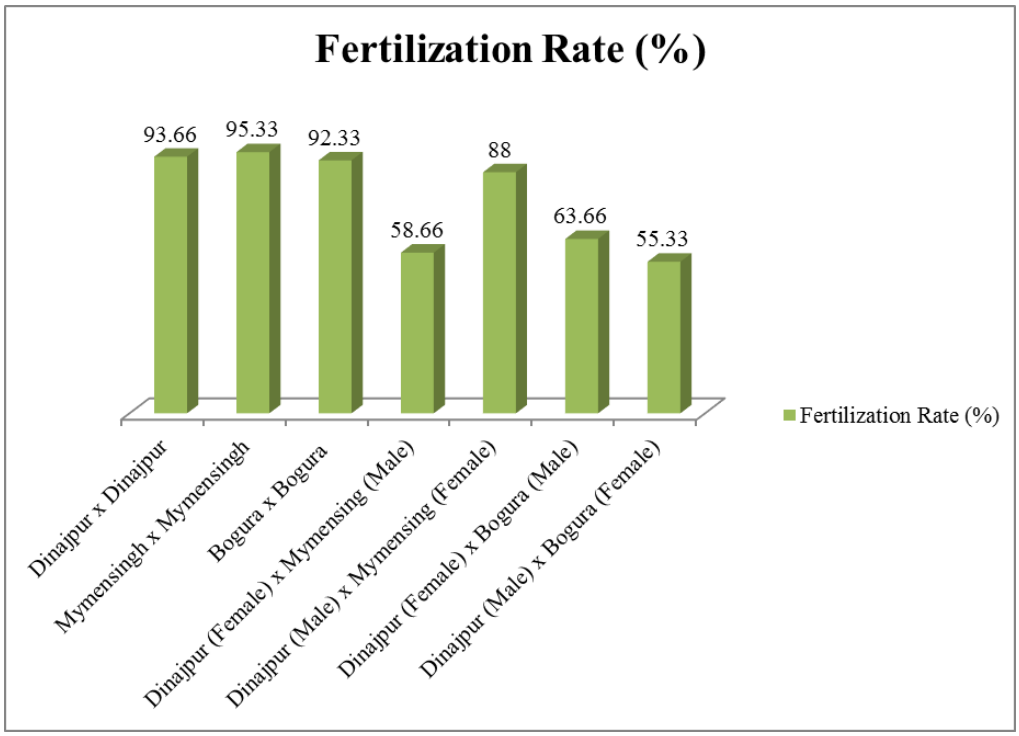


Fig. 11.9: Fertilization rate of 7 cross breeding trials

Hatching rate of the trials

The average hatching rates of 7 breeding line showed in (Figure 11.10). Hatching rate was found to be ranged from 46.66 79%. Among the breeding lines, the level of various hathcing rate occurred were significant (<0.05), the highest hatching rate was 79%, which was found in the line 4where females of Dinajpur were mated with males of Mymensingh). The hatch out time varied from 12-16 hours after fertilization.

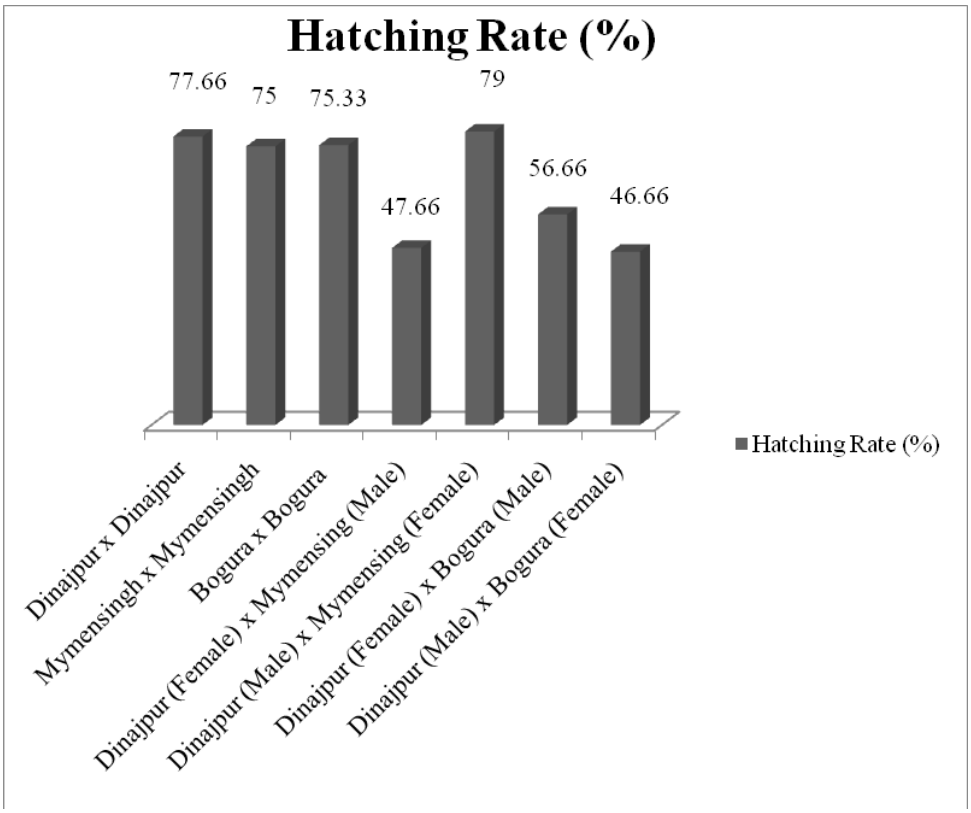


Fig. 11. 10: Hatching rate of fertilized eggs of the crossbreeding trials

12. A. Research highlight/findings (Bullet point – max 10 nos.):

I. The genetic variabilities of four wild stocks of *C. reba* were determined which could be the guidelines for the hatchery owner for the broodstock development of this species. The current study suggested the *C. reba* broodstock of Mymensingh and Dinajpur could be used for induced breeding purpose.

II. The hormone and hormone doses for the successful induced breeding of *C. reba* were established. The highest ovulation, fertilization, hatching rate were observed where the female breeders were injected with two doses, the first doses was PG @1.5 mg/kg body weight to the female and after 6 hrs 2nd doses with PG extract @6.5 mg/kg body weight. The male breeders were only injected once during the 2nd injection of the female @2.5 mg/kg body weight.

III. The larval feed for the production of fry of *C. reba* was established, commercial larvae feed (with 32% protein content) and plankton showed highest growth and survival.

IV. The stocking density to grow fingerling of *C. reba* were developed.

V. The better cross breeding trails which was between females of Dinajpur and Males of Mymensingh was identified.

VI. The laboratory of the Fisheries Biology and Genetics was enriched with a few sophisticated tools for molecular works

VII. The hatchery of the study site started to produce the seed of *C. reba* and providing to the local fish farmers

VIII. *C. reba* brood fish had been delivered to another private hatchery to start the propagation of its seed by using the developed technology

IX. Three theses at Master of Science have approved by the department of fisheries biology and genetics.

X. At least three article in the SCI index journal is expected to be published

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Computer and Accessories (Pakage-GD3)	139500.0	Computer and Accessories (Pakage-GD3)	139500.0	Figure 12.1. The office equipment purchased
(b) Lab &field equipment	Capital equipment (Thermal cycler /PCR Machine) (Package-GD4)	424500.0	Capital equipment (Thermal cycler /PCR Machine) (Package-GD4)	424500.0	Figure 12.2. The laboratory equipment purchased
(c) Other capital items	Small equipments and apparatus(Pakage-GD5)	415500.0	Small equipments and apparatus (Pakage-GD5)	415500.0	Figure 12.2. The laboratory equipment purchased
(D) Chemicals and Reagents	Chemicals (Pakage-GD6)	304200.0	Chemicals (Pakage-GD6)	304200.0	-

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
Laboratory workspace (Desk), Water Supply and Electric wiring	79,000/-	79,000/-	-	-	**Figure 12.3:** Part of the renovated laboratory
Hatchery infra-structure and pond netting			49,000/-	49,000/-	

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	417770.0	417770.0	417770.0	0.00	100%	Travelling were required between PI office and Hatchery sites
B. Field research/lab expenses and supplies	1583076.0	1576601.0	1558986.0	17615.0		
C. Operating expenses	293253.0	269515.0	269448.0	67.0		
D. Vehicle hire and fuel, oil & maintenance	210000.0	200000.0	210000.0	-10000.0		
E. Training/workshop/seminar etc.	0.00	0.00	0.00	0.00		
F. Publications and printing	115610.0	20724.0	25000.0	-4276.0		
G. Miscellaneous	50000.0	26754.0	28794.0	-2040.0		
H. Capital expenses	903339.0	880000.0	877200.0	2800.0		
Total	3573048.0	3391364.0	3387198.0	4166.0	-	Keep for Bank Charge

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 assess the genetic variation of *C. reba* collected from different region of Bangladesh for quality Broodstock management	✓ Brood fish collected from 4 geographically isolated regions: Dinajpur, Bogura, Jashore and Mymensingh ✓ Representative samples of each regions were taken for the isolation of cytochrome b genes and analyzed to reveal the genetic variation among them	✓ Genetic identification of *C. reba* stocks of Bangladesh fro 4 regions have identified	The fish breeders in Bangladesh could select the sources of broodstocks for their hatchery propagation

To develop artificial breeding technique for *C. reba*	✓ Live brood fish were collected from 3 geographically isolated regions: Dinajpur, Bogura, and Mymensingh ✓ Reared in separate earthen ponds in the experimental sites ✓ Hormone and hormone doses were optimized. ✓ Larval rearing were optimized in terms of feeding and stocking density	✓ Pituitary gland extracts @ 1.5 mg and 6.5mg/kg body weight to the female breeders as 1st and 2nd dose respectively; and 2.0 mg/kg/body weight to the male breeders are suggested to the fish breeders ✓ Utilization of larval factory made nursery feed having 32% proteins and production of plankton with fertilization is suggested to rear this fish.	The induced breeding and larval rearing to produce fingerlings of *C. reba* have been established
Crossbreeding of *C. reba* for the genetic improvement of the stocks of Dinajpur region	✓ Seven breeding trials within and between 3 geographically different stocks of *C. reba* were conducted to improve the genetic diversity ✓ Cross breeding trails between *C. reba* females of Dinajpur and males of Mymensingh showed better results interms of ovulation, fertilization and hatching rate ✓ Due to time limitation, growth and genetic studies of the produced crossbreed were not possible to identify.	✓ Seven breeding lines of *C. reba* were studied ✓ Within and between crossbreeding of Dinajpur and Mymensing stocks of *C. reba* may use for its seed production	Genetic improvement of *C. reba* by crossbreeding between geographically isolated broodstock may increase the genetic diversity, hence the sustainability of its seed production may ensure

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.	**1**	**-**	The *C. reba* fish seed production manual through induced breeding technology
Journal publication	3	1 conference proceeding is available	Seed production of *Cirrhinus reba*through hypophysation for sustainable aquaculture and conservation of threatened fishes in Bangladesh

			(Published and Presented in the 10th International Scientific Conference Organized by CIVASU, 2018
Information development	**Media coverage (Both Printed and Online)**		Figure 12.4: Media coverage (Both Printed and Online)
Other publications, if any	**3 thesis**	**1 thesis**	**1.** Study of genetic variation of wild *Cirrhinus reba* (Hamilton 1822) of Bangladesh using mitochondrial gene Cytochrome b gene **2.** Induced breeding within and between different wild stocks of *Cirrhinus reba* (Hamilton, 1822) to select the future breeders. **3.** Length-Weight Relationship and Geometric Morphometric Analyses of Wild *Cirrhinus reba* (Hamilton 1822) From Different Regions of Bangladesh

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

i. Generation of technology (Commodity & Non-commodity)

Induced breeding and larval rearing technology aquaculture potential threatened indigenous fish species *Cirrhinus reba*

ii. **Generation of new knowledge that help in developing more technology in future**

Broodstock's genetic variabilities determination and crossbreeding of endemic species *C. reba* may use for the genetic improvement of other endemic species.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Though for the demonstration of seed production technology in the form of structured training were not included in the project proposal, two local hatchery technician were informally demonstrated the seed production technology. Even, brood fish has given to one hatchery owner to breed in his hatchery.

iv. Policy Support

The research findings of this project will support the government policy to increase the fish production by creating the scope of production of new indigenous fish in the country's aquaculture system.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):

Desk monitoring was conducted by Institute of Research and Training (IRT), authorized authority of HSTU, and also by PIU-BARC, NATP-2.

ii) Field Monitoring (time& No. of visit, Team visit and output):

Desk monitoring was conducted by the university authority, the supreme authority the Vice-Chancellor, Treasurer and Registrar of the university. Besides, the internal monitoring team (Formed by the University) and teachers of the department of FBG visited the project sites. Moreover, Caritas officials also visited the sites to observe the project activities (Figure 12.5).

H. Lesson Learned/Challenges (if any)

I. Broodstock domestication in captive condition
II. Induced breeding for seed production
III. Larval rearing to produce fingerlings of *C. reba*
IV. Broodstock genetic assessment
V. Crossbreeding between different stocks to improve genetic quality

I. Challenges (if any)

I. Short project duration was the most crucial challenges to complete the works
II. Collection and transportation of broodstocks from nature was challenging
III. Sudden fluctuation in temperature caused larval death during larval rearing
IV. Flooding of ponds in August caused loss of some broodfish

Signature of the Principal Investigator
Date ……
Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal

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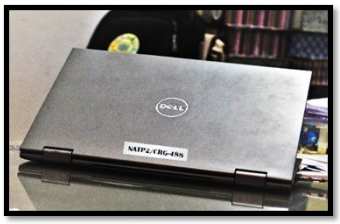
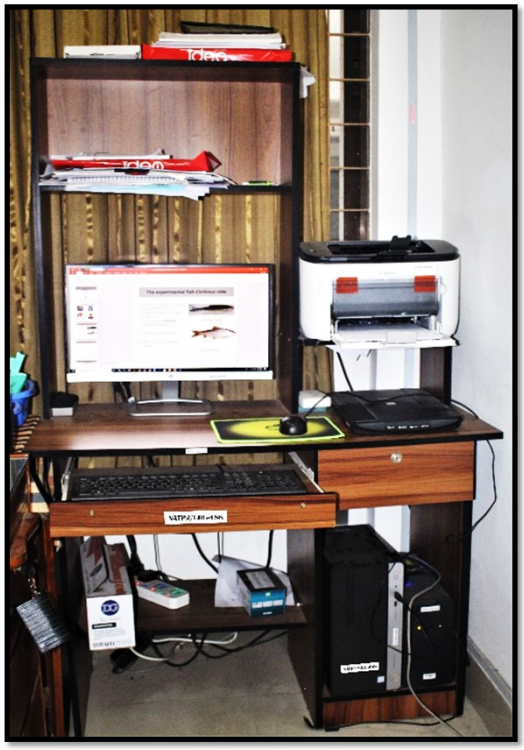
Appendices

Appendix 1 – Photographs of office equipment



Laptop

Almira



Desktop Computer with Accessories

Appendix 2 – Laboratory equipments purchased for Fisheries Biology and Genetics Laboratory-HTSU

Microwave Oven

Thermal Cycler

Centrifuge Machine

Fisheries Biology and Genetics Laboratory, HSTU

Appendix 3 – Newspaper reporting

Figure 12.4: The news coverage of project success in different dailies (A-D)

কৃষি সংবাদ

বিলুপ্তপ্রায় খরকি মাছ এখন পুকুরে চাষ

রিয়াজুল ইসলাম, দিনাজপুর

নানাবিধ প্রাকৃতিক ও মানবসৃষ্ট দুর্যোগসহ বিভিন্ন কারণে বিলুপ্তপ্রায় নদীর মিষ্টি ভাগ্না বা খরকি মাছ এখন পুকুরে চাষ হচ্ছে। পুকুরে কৃত্রিম প্রজননের মাধ্যমে ভাগ্না বা খরকি মাছের পোনা ও জাত উন্নয়নে সাফল্য পেয়েছেন দিনাজপুর হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়ের ফিশারিজ বায়োলজি ও জেনেটিক্স (এফবিজি) বিভাগের সহযোগী অধ্যাপক ড. ইমরান পারভেজের নেতৃত্বে থাকা একদল গবেষক। কৃত্রিম উপায়ে যদি মাছটির পোনা উৎপাদন এরপর পৃষ্ঠা ২ কলাম ৬

বিলুপ্তপ্রায়

[পেছনের পৃষ্ঠার পর] ও জাতের উন্নয়ন ঘটানো যায় তাহলে দেশের টেকসই মৎস্য উৎপাদন ও আমিষের চাহিদা পূরণে এক গুরুত্বপূর্ণ ভূমিকা পালন করতে পারবে। চাহিদা মিটানোর পাশাপাশি মৎস্যচাষিরা এই প্রজাতির মাছ চাষ করে আরও লাভবান হতে পারবেন। বাটা ও সরপুঁটি মাছের চেয়ে এ মাছ অনেক বেশি সুস্বাদু এবং চাহিদাসম্পন্ন। দেশের উত্তরাঞ্চলের যে জায়গাগুলোতে ছয় মাসের বেশি পানি থাকে না, সে জায়গাতেও এ মাছ চাষ করা যাবে। এর পরেও চলমান গবেষণা সম্পন্ন হলে ঘরের ভিতর রেস সিস্টেমেও চাষ করা যেতে পারে। এ মাছকে ছড়িয়ে দিতে কাহারোলে হাই হ্যাচারিতে ব্রুড ডেভেলপমেন্টের কাজ চলছে। প্রশিক্ষণের মাধ্যমে দেশের ১০টি মৎস্য হ্যাচারিতে পৌঁছে দেওয়ার পরিকল্পনা করছেন ড. ইমরান পারভেজ। আগামী বছর থেকে বাণিজ্যিকভাবে এ মাছের পোনা চাষিরা পাবেন। হাবিপ্রবির ড. ইমরান পারভেজ বলেন, ২০১৫ সাল থেকে বিলুপ্ত প্রায় প্রজাতির এই মাছের কৃত্রিম উপায়ে পোনা উৎপাদন ও জাত উন্নয়নের জন্য কাজ করে সফলতা পাওয়া গেছে। বাজারে এ প্রজাতির মাছের চাহিদাও বেশি এবং বাজার মূল্যও বেশি। তিনি বলেন, মাছের জাত উন্নয়ন ও পোনা উৎপাদনের জন্য আমি দিনাজপুর, বগুড়া, ময়মনসিংহ ও যশোর এই চারটি অঞ্চল থেকে মা-বাবা মাছ সংগ্রহ করে মাছের খাদ্যাভাস, প্রজনন বায়োলজি, সময় নির্ণয় করা হয়। পোনা উৎপাদনের জন্য পিটুইটারি গ্রন্থির নির্যাস, এইচসিজি, কৃত্রিম হরমোন ওভারপ্রিম ইত্যাদি প্রয়োগের মাধ্যমে মাছকে প্রজননের জন্য প্রণোদিত করা হয়েছিল। এরপর বিভিন্ন অঞ্চলের মাছের মধ্যে ক্রস করে ইন্টার ও ইন্ট্রা ব্রিডিং ঘটানো হয়। এতে পিটুইটারি গ্রন্থির নির্যাসের মাধ্যমে প্রজননে দিনাজপুর-দিনাজপুর, দিনাজপুর-ময়মনসিংহ ও ময়মনসিংহ-ময়মনসিংহ মাছে বেশি সফলতা পাওয়া যায়।

A. News paper reporting

উন্নয়নের মূলধারায়

আলোকিত বাংলাদেশ

জন্মদিনে মাশরাফিকে শুভেচ্ছা
আইসিসির : পৃষ্ঠা ৬

ওয়াশিংটনে কাভানার বিরুদ্ধে
বিক্ষোভ : গ্রেপ্তার ৩০২ : পৃষ্ঠা ৮

কলকাতার চলচ্চিত্রে আইরিন
তানি : পৃষ্ঠা ৫

াবর ২০১৮ রেজি নং-ডিএ ৬১৯৮ | বর্ষ ৯ সংখ্যা ৩৫ | ২১ আশ্বিন ১৪২৫ | ২৫ মহররম ১৪৪০ হিজরি www.alokitobangladesh.com thealokitobangladesh ১২ পৃষ্ঠা ৫ টাকা

সুসংবাদ প্রতিদিন alokitobd7@gmail.com

খরকি মাছের জাত উন্নয়নে সাফল্য

● কামরুল হুদা হেলাল, *দিনাজপুর*

হাজী মোহাম্মদ দানেশ বিজ্ঞান-এ প্রযুক্তি বিশ্ববিদ্যালয়ের (হাবিপ্রবি) ফিশারিজ বায়োলজি অ্যান্ড জেনেটিকস বিভাগের শিক্ষকদের গবেষণায় কৃত্রিম প্রজননের মাধ্যমে পুকুরেই খরকি মাছের পোনা উৎপাদন ও ক্রস-ব্রিডিংয়ের মাধ্যমে জাত উন্নয়নের কার্যক্রমে সাফল্য অর্জিত হয়েছে। বৈজ্ঞানিক এ সফলতার মাধ্যমে সুস্বাদু ও জনপ্রিয় খরকি মাছের চাষ ও উৎপাদন বেড়ে যাওয়ার সম্ভাবনা সৃষ্টি হয়েছে।

দিনাজপুরের হাবিপ্রবির ফিশারিজ বায়োলজি অ্যান্ড জেনেটিকস বিভাগের সহযোগী অধ্যাপক ড. ইমরান পারভেজের নেতৃত্বে একদল গবেষক প্রায় তিন বছর গবেষণা করে দিনাজপুরসহ ঠাকুরগাঁও, পঞ্চগড় ও নীলফামারীতে পুকুরেই খরকি মাছ চাষ করে কৃত্রিম প্রজননের মাধ্যমে পোনা উৎপাদন ও ক্রস-ব্রিডিং করে জাত উন্নয়নে সফলতা অর্জন করেছেন।

ড. ইমরান পারভেজ বলেন, খরকি মাছের বৈজ্ঞানিক নাম Cirrhinus reba (Hamilton 1822) এবং দেশের বিভিন্ন অঞ্চলে এ মাছ বিভিন্ন নামে পরিচিত। ময়মনসিংহ অঞ্চলে ভাগনা ও যশোর অঞ্চলে টাটকিনি নামে পরিচিত এ মাছ। বাংলাদেশসহ দক্ষিণ এশিয়ার ভারত, নেপাল, ভুটান, পাকিস্তানসহ

এরপর পৃষ্ঠা ১১ কলাম ১

খরকি মাছের জাত

● শেষ পৃষ্ঠার পর

অন্যান্য দেশে এ মাছ এক সময় প্রচুর পরিমাণে পাওয়া যেত। প্রাকৃতিক ও মনুষ্যসৃষ্ট কারণে খরকি মাছ হুমকির সম্মুখীন হওয়ায় এ প্রজাতির মাছের উৎপাদন ব্যাপকভাবে কমে যায়। হুমকির সম্মুখীন এ মাছকে ফিরিয়ে আনতে পুকুরে কৃত্রিম উপায়ে পোনা উৎপাদন ও পোনার লালনপালনের লক্ষ্যে ২০১৫ সালে গবেষণা কার্যক্রম শুরু হয়। কৃত্রিম উপায়ে পোনা উৎপাদন করা গেলে নদীতে পুনঃমজুতের মাধ্যমে একদিকে যেমন এর অস্তিত্ব রক্ষা হবে; পাশাপাশি দেশের টেকসই মৎস্য উৎপাদনে গুরুত্বপূর্ণ ভূমিকা রাখবে। বিশেষ করে দেশের যেসব অঞ্চলে সারা বছর পানি থাকে না এমন জেলা দিনাজপুর, ঠাকুরগাঁও, পঞ্চগড় ও নীলফামারীতে ৬ মাসের মধ্যে এ প্রজাতির মাছ চাষ করে বাজারজাত করা সম্ভব। খরকি মাছের পোনা উৎপাদনের লক্ষ্যে হাবিপ্রবির ফিশারিজ বায়োলজি অ্যান্ড জেনেটিক্স বিভাগ গবেষণা প্রকল্প হাতে নেয়। প্রকল্পটিতে ড. ইমরান পারভেজকে সহযোগী গবেষক হিসেবে সহযোগিতা করেন প্রভাষক মৌসুমী সরকার ছন্দা, ড. মাহবুবুল হাসান ও অনুষদীয় শিক্ষকরা।

প্রকল্পটির গবেষণাগারভিত্তিক কার্যক্রম বিশ্ববিদ্যালয়ের ফিশারিজ বিভাগের গবেষণাগারে করা হলেও মাঠ পর্যায়ের গবেষণা কাজগুলো শুরুর দিকে বাংলাদেশ মৎস্য গবেষণা প্রতিষ্ঠানের সৈয়দপুরের স্বাদু পানি উপকেন্দ্রে করা হয়েছিল। দিনাজপুরের এনজিও কারিতাস বাংলাদেশের মৎস্য হ্যাচারিতে গবেষণা কাজ পরিচালনা করা হয়।

খরকি মাছের কৃত্রিম প্রজননের জন্য এর প্রজননগত বায়োলজি যেমন ডিমের সংখ্যা, ডিমের পরিপক্বতার সময়, গোনাডো সোমাটিক সূচক ইত্যাদি নির্ণয় করা হয়। এ মাছের ডিম পরিপক্ব হয় মে থেকে এবং তা শেষ হয় আগস্টে। বয়স ও শারীরিক ওজন ভেদে একটি স্ত্রী মাছের ডিম ধারণক্ষমতা ৫০ হাজার থেকে ১ লাখ ২০ হাজার পর্যন্ত হয়ে থাকে।

প্রজননগত বায়োলজি নির্ণয়ের পর বিভিন্ন প্রণোদনা জাতীয় উপাদান যেমন- মাছের পিটুইটারি গ্রন্থির নির্যাস, এইচসিজি, কৃত্রিম হরমোন ওভাপ্রিম ইত্যাদি দিয়ে কৃত্রিমভাবে খরকি মাছকে প্রণোদিত করার চেষ্টা করা হয়। মাছের ডিম ছাড়ার হার, ডিম নিষিক্তকরণের হার, নিষিক্ত ডিম থেকে পোনা উৎপাদনের হার, বাঁচার হার ইত্যাদি বিষয়ের দিক থেকে পিটুইটারি গ্রন্থির নির্যাসের ব্যবহারই সবচেয়ে ভালো হিসেবে গ্রহণ করা হয়।

দিনাজপুর, বগুড়া, ময়মনসিংহ ও যশোর থেকে জীবিত খরকি মাছের মা-বাবা মাছ সংগ্রহ করা হয় এবং এদের মধ্যে মরফোলজিক্যাল পার্থক্য ও জেনেটিক পার্থক্য নিরূপণ করা হয়। ওই গবেষণা থেকে দেখা গেছে, এরা একই প্রজাতির মাছ হওয়া সত্ত্বেও নিজেদের মধ্যে পার্থক্য বজায় রাখছে। চারটি স্টকের মধ্যে জেনেটিক বিচিত্রতার দিক থেকে দিনাজপুর ও ময়মনসিংহের পপুলেশন অন্যতম। গবেষণায় দেখা যায়, একই প্রজাতির মাছ হওয়া সত্ত্বেও নিজেদের মধ্যে এরা পার্থক্য বজায় রাখে, তাই বিভিন্ন এলাকার মাছের মধ্যে ইন্টার ও ইন্ট্রা ব্রিডিং করানো হয়।

ড. ইমরান বলেন, নিষিক্ত ডিম ফুটে রেণু পোনা বের হওয়ার ৪৮ ঘণ্টা পর প্রথম খাবার হিসেবে সিদ্ধ ডিমের কুসুম পানিতে দ্রবণ তৈরি করে প্রদান করা হয়। এ অবস্থায় সাত দিন ধরে লালনপালন করা হয়। সাত সপ্তাহ তিন ধরনের খাবার প্রদান করে সবচেয়ে উপযুক্ত খাবার নির্বাচন করা হয়।

প্রকল্পটির সফলতার জন্য বর্তমানে কিছু বিশেষ গবেষণা কার্যক্রম যেমন- কৃষিকাজে ব্যবহৃত কীটনাশক মাছের জাইগোটের বৃদ্ধিকে কীভাবে ব্যাহত করে, লবণাক্ততা বৃদ্ধি পেলে এ মাছের স্পার্মাটোজোয়া কীভাবে এর গতি হারিয়ে ফেলে, তাপমাত্রা বৃদ্ধি পেলে এর বৃদ্ধির কী অবস্থা হয় তার ওপর কাজ চলছে।

দেশে বর্তমানে কার্প মাছের মিশ্র চাষে দেশীয় বাটা ও থাই সরপুঁটি চাষ করা হয়ে থাকে। বাটা মাছের বিকল্প হিসেবে মিশ্র চাষে খরকি মাছ চাষের ব্যাপক সম্ভাবনা রয়েছে। অন্যদিকে এ মাছ বাটা ও সরপুঁটি মাছের চেয়ে সুস্বাদু হওয়ায় বাজার মূল্যও বেশি।

হ্যাচারিতে পোনা উৎপাদনের প্রযুক্তিটি বিভিন্ন সরকারি-বেসরকারি প্রতিষ্ঠানে ছড়িয়ে দেওয়ার জন্য এরই মধ্যে উদ্যোগ নেওয়া হয়েছে। মোট ১০টি হ্যাচারিতে প্রশিক্ষণের মাধ্যমে এ প্রযুক্তিটি ছড়িয়ে দেওয়ার পরিকল্পনা রয়েছে। পাশাপাশি মিশ্রচাষের গবেষণা চাষির খামার পর্যায়ে করার পরিকল্পনা রয়েছে।

B: News of project success in Alokito Bangladesh

মোঃ শাহাদৎ হোসেন শাহ্
দিনাজপুর জেলা প্রতিনিধি
দৈনিক বাংলাদেশের খবর
মোবাইল ঃ ০১৭১৮৮৮০১৭৮
দিনাজপুর প্রেস ক্লাব, নিমতলা, দিনাজপুর।

১ বছর ধরে আলোকাগার কোমল যত্নে বসুন্ধরা টিস্যু

বন্ধুর প্রকাশিত দৈনিক

দেশের কথা দশের কথা

বাংলাদেশের খবর

বুধবার • ১৭ অক্টোবর ২০১৮ | ২ কার্তিক ১৪২৫। ৬ সফর ১৪৪০। রেজি. নং-ডিএ ১৪-১৫৮। বর্ষ ৪। সংখ্যা ৩২

ফিরে আসছে বিলুপ্তপ্রায় খরকি মাছ

কৃত্রিম প্রজননে পোনা উৎপাদন ও ক্রস-ব্রিডিংয়ের সাফল্য

মো. শাহাদৎ হোসেন শাহ, দিনাজপুর

হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়ের ফিশারিজ বায়োলজি অ্যান্ড জেনেটিকস বিভাগের শিক্ষকদের গবেষণায় কৃত্রিম প্রজননের মাধ্যমে পুকুরেই জনপ্রিয় খরকি মাছের পোনা উৎপাদন ও ক্রস-ব্রিডিংয়ের মাধ্যমে জাত উন্নয়নের কার্যক্রমে সাফল্য অর্জিত হয়েছে। বৈজ্ঞানিক এই সফলতার মাধ্যমে সুস্বাদু ও জনপ্রিয় খরকি মাছের চাষ ও উৎপাদন বাড়ার সম্ভাবনা সৃষ্টি হয়েছে। দিনাজপুরের হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়ের ফিশারিজ বায়োলজি অ্যান্ড জেনেটিকস বিভাগের সহযোগী অধ্যাপক ড. ইমরান পারভেজের নেতৃত্বে একদল গবেষক প্রায় তিন বছর গবেষণা করে দিনাজপুরসহ ঠাকুরগাঁও, পঞ্চগড় ও নীলফামারীর জনপ্রিয় মাছ খরকি পুকুরেই চাষ করে কৃত্রিম প্রজননের মাধ্যমে পোনা উৎপাদন ও ক্রস-ব্রিডিং করে জাত উন্নয়নে সাফল্য অর্জন করেছেন। গবেষণা দলের প্রধান ড. ইমরান পারভেজ বলেন, খরকি মাছের বৈজ্ঞানিক নাম. ঈরৎৎযরহঁং ৎবনধ (ঐধসরহঃড়হ ১৮২২) এবং দেশের বিভিন্ন অঞ্চলে বিভিন্ন নামে খরকি মাছ পরিচিত। ময়মনসিংহ অঞ্চলে ভাগ্না এবং যশোর অঞ্চলে টাটকিনি নামে পরিচিত। বাংলাদেশসহ দক্ষিণ এশিয়ার ভারত, নেপাল, ভুটান, পাকিস্তানসহ অন্যান্য দেশে খরকি মাছ একসময় প্রচুর পাওয়া যেত। প্রাকৃতিক ও মনুষ্যসৃষ্ট কারণে খরকি মাছ আজ হুমকির সম্মুখীন হওয়ায় এ প্রজাতির মাছের উৎপাদন ব্যাপকভাবে কমে যায়। হুমকির সম্মুখীন এই মাছকে ফিরিয়ে আনতে পুকুরে কৃত্রিম উপায়ে পোনা উৎপাদন ও পোনার লালন-পালনের লক্ষ্যে ২০১৫ সালে গবেষণা কার্যক্রম শুরু হয়। প্রকল্পটিতে প্রধান গবেষক ড. ইমরান পারভেজকে সহযোগী গবেষক হিসেবে সহযোগিতা করেন প্রভাষক মৌসুমী সরকার ছন্দা, ড. মাহবুবুল হাসান ও

অনুষদীয় শিক্ষকরা।

দিনাজপুর, বগুড়া, ময়মনসিংহ ও যশোর থেকে জীবিত খরকি মাঝের মা-বাবা মাছ সংগ্রহ করা হয় এবং এবং এদের মধ্যে মরফোলজিক্যাল ও জেনেটিক পার্থক্য নিরূপণ করা হয়। ওই গবেষণা থেকে দেখা যায়, এরা একই প্রজাতির মাছ হওয়া সত্ত্বেও নিজেদের মধ্যে পার্থক্য বজায় রাখছে। চারটি স্টকের মধ্যে জেনেটিক বৈচিত্র্যতার দিক থেকে দিনাজপুর ও ময়মনসিংহের পপুলেশন অন্যতম। গবেষণায় দেখা যায়, একই প্রজাতির মাছ হওয়া সত্ত্বেও নিজেদের মধ্যে এরা পার্থক্য বজায় রাখে, তাই বিভিন্ন এলাকার মাছের মধ্যে ইন্টার ও ইন্ট্রা ব্রিডিং করানো হয়। গবেষণায় দেখা যায় দিনাজপুরের মহিলা মাছ ও ময়মনসিংহের পুরুষ মাছের মধ্যে প্রজনন ঘটালে তারা সবচেয়ে ভালো ফল দেয়।

প্রধান গবেষক ড. ইমরান বলেন, নিষিক্ত ডিম ফুটে রেণু পোনা বের হওয়ার ৪৮ ঘণ্টা পর প্রথম খাবার হিসেবে সিদ্ধ ডিমের কুসুম পানিতে দ্রবণ তৈরি করে প্রদান করা হয়েছে। এ অবস্থায় ৭ দিন লালন-পালন করা হয়। সাত সপ্তাহ তিন ধরনের খাবার প্রদান করে সবচেয়ে উপযুক্ত খাবার নির্বাচন করা হয়। গবেষণা শেষে দেখা যায়, শতকরা ৩২ ভাগ প্রোটিনযুক্ত খাবার নিয়মিতভাবে প্রদান করার মাধ্যমে খুব দ্রুত পোনা উৎপাদন করা সম্ভব। অপর এক গবেষণায় চারা পোনা উৎপাদনের জন্য মাছের মজুত ঘনত্ব নির্ণয় করা হয়। দেখা যায়, প্রতি হেক্টর পুকুরে আড়াই লাখ ৭-১০ দিন বয়সী রেণু পোনা মজুত করলে সবচেয়ে বেশি বর্ধন ও বাঁচার হার পাওয়া যায়।

প্রকল্পটির সফলতার জন্য বর্তমানে কিছু বিশেষ গবেষণা কার্যক্রম, যেমন— কৃষিকাজে ব্যবহৃত কীটনাশক মাছের জাইগোটের বৃদ্ধিকে কীভাবে ব্যাহত করে, লবণাক্ততা বৃদ্ধি পেলে এই মাছের স্পার্মাটোজোয়া কীভাবে এর গতি হারিয়ে ফেলে, তাপমাত্রা বৃদ্ধি পেলে এর বৃদ্ধির কী অবস্থা হয় তার ওপর কাজ চলছে।

C: News of project success in Bangladesher Khobor

Appendix 4 – TV reporting

জাতীয় রাজনীতি রাজধানী দেশজুড়ে আন্তর্জাতিক অর্থ-বাণিজ্য খেলাধুলা বিনোদন লাইফস

কৃত্রিম প্রজননের মাধ্যমে খরকি মাছের পোনা বিস্তার (ভিডিও)

একুশে টেলিভিশন

প্রকাশিত : ২২:৪৯ ২৯ সেপ্টেম্বর ২০১৮ | আপডেট: ১৬:৩৭ ২৯ সেপ্টেম্বর ২০১৮

কৃত্রিম প্রজননের মাধ্যমে ভাগ্না বা খরকি মাছের পোনা ও জাত উন্নয়নে সফল হয়েছেন দিনাজপুর হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়ের ফিশারিজ বায়োলজি ও জেনেটিক্স(এফবিজি) বিভাগের সহযোগী অধ্যাপক ড. ইমরান পারভেজের নেতৃত্বে থাকা একদল গবেষক।

কৃত্রিম প্রজননের মাধ্যমে উৎপাদিত ভাগ্না বা খরকি মাছের পোনা দেখতে ২২ সেপ্টেম্বর (শনিবার) দিনাজপুর কারিতাস মৎস্য হ্যাচারীতে যান বিশ্ববিদ্যালয়ের উপাচার্য প্রফেসর ড. মু. আবুল কাসেম, কোষাধ্যক্ষ প্রফেসর ড. বিধান চন্দ্র হালদার, রেজিস্ট্রার প্রফেসর ড. মো. নজিউল আলমসহ বেশ কয়েকজন শিক্ষক। সেখানে তারা ড. ইমরান পারভেজ এর প্রকল্পের আওতাধীন পুকুরগুলো ঘুরে দেখেন এবং কৃত্রিম প্রজননের মাধ্যমে উৎপাদিত পোনা ও জাতের বিভিন্ন বিষয়ে খোঁজ খবর নেন।

এ সময় উপাচার্য প্রফেসর ড. মু. আবুল কাসেম বলেন, বিলুপ্ত প্রজাতির এই মাছগুলোকে কৃত্রিম প্রজননের মাধ্যমে পোনা উৎপাদন ও জাত উন্নয়নে তিনি যে সফলতা দেখিয়েছেন তা প্রশংসনীয়। আশা করি, এই মাছ চাষের মাধ্যমে মৎস্য চাষীরা সফলতা লাভ করবে। এবং দেশের টেকসই মৎস্য উৎপাদনে এটি গুরুত্বপূর্ণ ভূমিকা পালন করবে। আমি তার এই কাজের উত্তরোত্তর সফলতা কামনা করছি।

https://www.ekushey-tv.com/%E0%A6%95%E0%A7%83%E0%A6%A4%E0%A7%8D%E0%A6%B0%E0%A6%BF%E0%A6%AE-%E0%A6%AA%E0%A7%8D%E0%A6%B%E0%A6%9C%E0%A6%A8%E0%A6%A8%E0%A7%87%E0%A6%B0-

আর্থিক সহযোগিতায় ২০১৫ সাল থেকে বিলুপ্ত (প্রায়) প্রজাতির এই মাছের কৃত্রিম উপায়ে পোনা উৎপাদন ও জাত উন্নয়নের জন্য কাজ করে আসছি। এক সময়ে এ মাছটি বাংলাদেশসহ দক্ষিণ এশিয়ার ভারত, নেপাল, ভুটান,পাকিস্তান ইত্যাদি দেশে পাওয়া যেতো। কিন্তু নানাবিধ প্রাকৃতিক ও মানবসৃষ্ট দুর্যোগের কারণে মাছটি আজ বিলীন প্রায়। ঝুকির সম্মুখীন এই মাছটিকে ফিরিয়ে আনতে আমি কাজ শুরু করি। আমি চিন্তা করেছি কৃত্রিম উপায়ে যদি মাছটির পোনা উৎপাদন ও জাতের উন্নয়ন ঘটানো যায় তাহলে দেশের টেকসই মৎস্য উৎপাদন ও আমিষের চাহিদা পূরণে এক গুরুত্বপূর্ণ ভূমিকা পালন করতে পারবে। পাশাপাশি মৎস্যচাষীরা এই প্রজাতির মাছ চাষ করে লাভবান হতে পারবে। বাজারে এ প্রজাতি মাছের বেশ চাহিদা এবং বাজার মূল্যও অনেক বেশি। কেজি প্রতি ২৫০-৩৫০টাকা।

মাছের জাত উন্নয়ন ও পোনা উৎপাদনের জন্য আমি দেশের দিনাজপুর, বগুড়া, ময়মনসিংহ ও যশোর এই চারটি অঞ্চল থেকে মা-বাবা মাছ সংগ্রহ করে মাছের খাদ্যাভ্যাস, প্রজনন বায়োলজী, সময় নির্ণয় করা হয়। এরা একই প্রজাতি হওয়া সত্ত্বেও গবেষণায় দেখা যায় এরা নিজেদের মধ্যে একটা পার্থক্য বজায় রাখে। পোনা উৎপাদনের জন্য পিটুইটারী গ্রন্থির নির্যাস, এইচসিজি, কৃত্রিম হরমোন ওভাপ্রিম ইত্যাদি প্রয়োগের মাধ্যমে মাছকে প্রজননের জন্য প্রণোদিত করা হয়েছিল। এরপর বিভিন্ন অঞ্চলের মাছের মধ্যে ক্রস করে ইন্টার ও ইন্ট্রা ব্রিডিং ঘটানো হয়। এতে পিটুইটারী গ্রন্থির নির্যাসের মাধ্যমে প্রজননে দিনাজপুর-দিনাজপুর' দিনাজপুর-ময়মনসিংহ ওময়মনসিংহ-ময়মনসিংহ মাছে বেশি সফলতা পাওয়া যায়।

তিনি আরও বলেন, বাটা মাছের বিকল্প হিসেবে বিশেষভাবে এই খরকি মাছ চাষের বেশ সম্ভাবনা রয়েছে। বাটা ও সরপুটি মাছের চেয়ে এ মাছ অনেক বেশি সুস্বাদু এবং চাহিদা সম্পন্ন। দেশের উত্তরাঞ্চলের যে জায়গাগুলোতে ছয় মাসের বেশি পানি থাকে না সে জায়গাতেও এ মাছ চাষ করা যাবে।

কেআই/ এসএইচ/

D: News of project success in Online Ekushey News Portal.

Appendix 5 – Field Monitoring

Figure 12.5: Honroable Vice-Chancellor Prof. Dr. M. Abul Kashem, Treasurer, Registrar and Teachers of the Department of Fisheries Biology and Genetics visited the hatchery sites and monitored the project activities.