Project ID: 397

Competitive Research Grant

Sub-Project Completion Report

on

Seed Production of Bhagna, *Labeo ariza* (Hamilton, 1807) Through Line Breeding Trial in Bangladesh

Project Duration

May 2017 to September 2018

Department of Fisheries Biology & Genetics
Bangladesh Agricultural University, Mymensingh-2202

Submitted to

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

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

NATP	:	National Agricultural Technology Project
BARC	:	Bangladesh Agricultural Research Council
CRG	:	Competitive Research Grant
PIU	:	Project Implementation Unit
USAID	:	United States Agency for International Development
DoF	:	Department of Fisheries
GDP	:	Gross Domestic Product
FAO	:	Food and Agriculture Organization
et al.	:	Associates
cm	:	Centimeter
Kg	:	Kilogram
BAU	:	Bangladesh Agricultural University
^{0}C	:	Degree Celsius
PG		Pituitary gland
g		Gram
MS		Microsoft
µm		Micrometer
SPSS		Statistical Package for Social Science
GSI		Gonado-Somatic Index
SGR		Specific Growth Rate

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Executive Summary

The high demand, high flesh quality and high price of the Bhagna, *Labeo ariza* has generated much interest in aquaculture production. Moreover, their wild population is rapidly declining due to increased fishing pressure, siltation, aquatic pollution and various anthropogenic activities. As a result, Bhagna is recently listed as endangered species in Bangladesh. For their conservation and culture, information about quality seed production is necessary. In this regard, wild populations of Bhagna (*L. ariza*) were collected from three geographically isolated rivers of Bangladesh and domesticated for four months supplying commercial supplementary feed (Mega feed) enriched with protein and vitamin-E for early gonadal maturation. For GSI study, males and females of *L. ariza* were examined to determine the gonadal maturation and peak breeding season of *Labeo ariza*. Then line breeding trial was performed in accordance with the line breeding outline based on individual selection system. Six different lines (Three con-specific lines, line 1: Kangsha♀×Kangsha♂, line 2: Jamuna♀×Jamuna♂& line 3: Atrai♀×Atrai♂, and three heterospecific lines, line 4: Kangsha♀×Atrai♂, line 5: Kangsha♀×Jamuna♂& line 6: Atrai♀×Kangsha♂) were produced. Subsequently, offspring from each line were reared in glass aquaria using Thermostat and hapas setted in six different earthen ponds for a period of two months. Muscle cellularity analysis through histological observation was performed to identify the best line. After domestication period, the highest length and weight gain were observed in the Atrai population (16.78±0.68 cm and 95.92±2.24 g) followed by the Jamuna (13.30±3.05 cm, 89.65±2.46 g) and the Kangsha (14.36±1.05 cm, 90.36±1.88 g) populations. The highest survival rate was observed in Kangsha (91%) followed by Atrai (86%) and Jamuna (84%). During domestication, higher values of GSI were observed from July to September. It ranged between 3.60 to 4.95 and 9.80 to 11.5 for males and females, respectively indicating the breeding season extends from July to September. There was no significant variation existed in fertilization, hatching and survival rates of the six different lines. Line 4 (Kangsha♀×Atrai♂) showed the highest fertilization, hatching and survival rate (95±2.11%, 88±1.03% and 82±1.88%, respectively) among the six lines. The specific growth rate (SGR) was significantly higher in heterospecific groups (3.23±0.22% in line 4, 3.12±0.34% in line 5 and 3.07±0.37% in line 6) than the conspecific groups (3.07±0.16% in line 1, 3.00±0.29% in line 3 and 2.98±0.24% in line 2).The line 4 (Kangsha♀×Atrai♂) exhibited the highest relative percentage of hyperplastic fibers (new fibers) among the six lines. The relative percentage hyperplastic fibers were significantly higher in heterospecific groups (35.28% in line 4, 33.65%in line 5 and 32.38% in line 6) than the conspecific group (30.19% in line 3, 30.10% in line 1 and 28.15% in line 2). Present results indicated that the heterospecific group (especially line 4 Kangsha♀×Atrai♂) has higher breeding and growth performance than the conspecific groups.The knowledge obtained through the present experiments on domestication, GSI data, line breeding, growth performance and muscle fiber histology of Bhagna, *L. ariza,* may be used as a baseline for further research to save this species from possible threat of extinction.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project:** Seed Production of Bhagna, *Labeoariza* (Hamilton, 1807) Through Line Breeding Trial in Bangladesh

2. **Implementing organization:**
   National Agricultural Technology Program-Phase II Project (NATP-2)
   Bangladesh Agricultural Research Council (BARC)
   New Airport Road, Farmgate, Dhaka – 1215

3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

Principal Investigator:

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Office Address: Professor, Dept. of Fisheries Biology and Genetics, BAU, Mymensingh-2202, Bangladesh
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4. **Sub-project budget (Tk):**
   4.1 Total: **16,23,560/-**
   4.2 Revised (if any): Not Applicable
5. **Duration of the sub-project:**
   5.1 Start date (based on LoA signed): 08 May, 2017
   5.2 End date: 30 September 2018

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

Bangladesh is emerging as one of the leading fish producing country of the world and is blessed with huge open water resources with a wide range of aquatic diversity. Bangladesh is an agro-based riverine country with a huge delta of water resources. The country has vast and diversified water resources of 4.34 million ha (Mazid, 2002). Along with potential water resources, the country is also rich in the diversity of various fish species. This aquatic biodiversity ranked third in Asia, with approximately 293 indigenous freshwater species and 20 exotic species (DoF, 2016; Hossain *et al.,* 2012). The fisheries sector of Bangladesh is playing an increasingly significant role in the economy for the last few decades. It contributes 3.61% to national GDP and around one-fourth (24.41%) to the agricultural GDP (DoF, 2017). Bangladesh has a total fish production of 41.34 lakh MT in 2016-17, whereas inland open water (capture) contributes 28.14% (11.63 lakh MT), inland closed water (culture) contributes 56.44% (23.33 lakh MT) and marine fisheries production contributes 15.42% (6.37 lakh MT) to total production (DoF,

2017). According to FAO 2018, Bangladesh ranked third in global inland water capture production just after China and India and fifth in the inland aquaculture production.

The inland fisheries of Bangladesh mainly consist of carps, minnows and catfishes. Hossain *et al.* (2012) recorded 293 species of indigenous fin fish species in fresh water of Bangladesh. There are also about 40-50 indigenous fish species. These species are defined as small species which grow to a maximum length of about 25 cm (Felt *et al*., 1996). Prior to 1970, many different small indigenous fishes like koi, bheda, taki, kaski, rani, bata, bhagna, pabda, gulsha, mola, baim, puti, shing, magur etc. were abundant in almost all the fresh waters of Bangladesh. Among the small indigenous species, *Labeoariza* (Hamilton, 1807) is locally known as Bhagna, Bhagnabata, Raik or Tatkini and considered as one of the most important indigenous minor carp species in Bangladesh (Ahammad *et al.,* 2015). Taxonomically, it can be identified by thin stripes lying mainly dorsally of the lateral line; larger specimens often have a broad mid-lateral stripe. The colour of this species is variable, from dull dirty white or greyish to silvery or yellow. Bhagna can be easily recognizable by its medium sized silvery scales, deep bluish or darkish upper body part and the shiny and silvery lower body part. The lateral line has 38 or 39 scales, most of them appearing as a black mark elongated from the base margin of the operculum to the tail during the early stage of life (Rahman *et al.,* 2009) while in the adult stage, this black mark becomes shorter and remains between the operculum and the end of the belly (Talwar and Jhingran, 1991; Rahman, 2005). Also it can reach lengths of about 30 cm in natural waters. The males are smaller than females. Bhagna feeds on plankton and detritus and also an omnivore and column feeder in nature and grows to the largest size of 40 to 50 cm in length (Felts *et al*., 1996; Akhteruzzaman*et al*., 1998; Rahman *et al.,* 2009). Females and males attain sexual maturity within one year and customarily by the terminus of first year. This fish breeds in flooded shallow water from June to September (Roberts, 1997).The spawning season of Bhagna ranges from April to the August with a peak spawning time during the rainy time in flowing flood waters of these months (Hussain and Mazid, 2001; Akhteruzzaman *et al.,* 1998). It is also a prolific breeder, laying about 3 million ova per female (Roberts, 1997). Absolute fecundity of Bhagna was recorded between 2, 00,000 and 2, 50,000 per kg body weight of female (Hussain and Mazid, 2001). It has also high nutritional value with good amount of protein, calcium and low fatty acid content (Gupta, 1975). However protein, fat and carbohydrate calories of Bhagna (*L. ariza*) are relatively higher than those in the Indian major carps (Khawaja, 1966; Sharma and Simlot, 1971). It is distributed over Indo-pacific region (Jhingran, 1991; Walter, 1994; Rahman, 2005; Hogan, 2012). It inhabits clear rivers and tanks (Naser, 2015). In Bangladesh it is widely distributed in the Karnafuly River and adjacent basins in Chittagong Hill Tracts (Hogan, 2012; Roberts, 1997; Kohinoor *et al.,* 1998). Naturally this fish is also distributed in most of the small rivers, floodplains, creeks natural depressions of Bangladesh (Hussain and Mazid, 2001; Felts *et al.,* 1996). Recently, population of Bhagna has declined considerably due to increased fishing pressure, and various anthropogenic activities leading to siltation, aquatic pollution, and loss of natural habitat for spawning and growth (Akhteruzzaman *et al.*, 1998; Hussain and Mazid, 2001). These factors not only destroyed the breeding grounds but also caused stress to the availability of brood fish including fry and fingerlings (Hussain and Mazid, 2001). As a result, the fish is recently listed as one of the most important vulnerable and threatened species in Bangladesh (Naser, 2015; DoF, 2007; DoF, 2014). Although they are listed as threatened and vulnerable, a small number of the fish is still available in different rivers, beels, haors and baors (DoF, 2014). For these reasons, it is necessary to conserve and rehabilitable through breeding and culture practices.

Rahman *et al*. (2009) reported that *Labeo ariza* is slow growing pattern in culture pond. Therefore, line breeding can be used an effective tool to develop its base population and high quality seed production as well as increase fish muscle growth. As fish muscle growth take places in recruitment of new fibers (hyperplasia) and enlargement of existing fibers (hypertrophy) within a muscle mass in fish. So,

conduction of line breeding program based on individual selection system among the Bhagna populations might be an effective pathway to develop different lines of Bhagna. Genetic improvement can be accomplished through linebreeding and this approach offers an additional management tool for developing base population of Bhagna. However, if it is possible to collect wild Bhagna in *ex-situ* condition for their suitable breeding performance through line breeding, then this fish can become a permanent candidate of aquaculture. In this regard, good quality fish seed can be produced through outstanding line bred of Bhagna. Thus, a successful fish culture package of *L. ariza* will be established. Correspondingly, to get maximum economic returns, it is essential to develop base population of *L. ariza* stock which will provide improved fish seed and ultimately increase fish production. No work has yet been undertaken on seed production through line breeding technique under captive condition. However, the present work has therefore been undertaken to develop a practical and economically viable technique for mass seed production of *L. ariza* in captive condition.

7. Sub-project goal:

Seed production through line breeding technique and development of base population of *L. ariza*

8. Sub-project objective (s):

The specific objectives of this proposed research project are-

a) to domesticate the wild sources of *L. ariza* for gonadal maturation under captive condition;
b) to perform the induced breeding of *L. ariza* under different line breeding trial.
c) to identify the best line using muscle cellularity analysis through histological observation.

9. Implementing location (s):

The present study was conducted in the Fisheries Faculty Wet Laboratory Complex, Fish Genetics and Biotechnology Laboratory, Fish Physiology Laboratory and Experimental ponds belonging to the Department of Fisheries Biology and Genetics, BAU, Mymensingh.

10. Methodology in brief:

10.1. Study areas

The studies were conducted in the Fisheries Field Complex, Wet Laboratory, Fish Physiology Laboratory and Mini Hatchery Complex of the Department of Fisheries Biology and Genetics adjacent to the Faculty of Fisheries, BAU, Mymensingh.

10.2 Domestication of wild sources of Bhagna (*L. ariza*) for their gonadal maturation

10.2.1 Collection of wild sources of Bhagna (*L. ariza*):

Populations of Baghna were collected from three geographically distinct areas viz. Atrai River, Dinajpur District; Kangsha River, Mymensingh District; and Jamuna River, Sirajganj District in July, 2017 (Figure 1). At least 300 fries/fingerlings of each source were collected in live condition and transported to the Fisheries Faculty Field Laboratory Complex, BAU, Mymensingh. The fish were stocked in three different earthen ponds.

Figure 1: Collected Wild Bhagna populations from the Atrai River, Dinajpur (A, B); Kangsha River, Mymensingh (C, D); and Jamuna River, Sirajganj (E, F).

10.2.2 Improved nursery management:

Collected fingerlings were reared for 6 months in the previously prepared separate rectangular ponds (size 18×14 m^2 and average depth 1.3 m) (Figure 2). Supplementary feed (Mega feed, 35% protein) was provided during the nursing period. The feed were applied to fish at the rate of 5% of their body weight twice a day. Subsequently, length and weight were measured using a meter scale and a digital balance machine. After that shoot growth of adult Bhagna were selected for growout culture.

Figure 2: Bhagna populations collected from three different sources were reared in nursery ponds and observed their growth (A-I): A-C, Atrai river; D-F, Kangsha river; and G-I, Jamuna River.

j10.2.3 Rearing of adult Bhagna at growout ponds:

Adult Bhagna were reared for 4 months in growout ponds until their gonadal maturation (Figure 3). All facilities including water supply, inlet and outlet in the ponds were provided. A special feed enriched with protein (35%) and vitamin-E, were provided which enhances the gonadal maturation in fishes (Mollah *et al.,* 2009). Feed were provided to the fish at the rate of 5% of body weight.

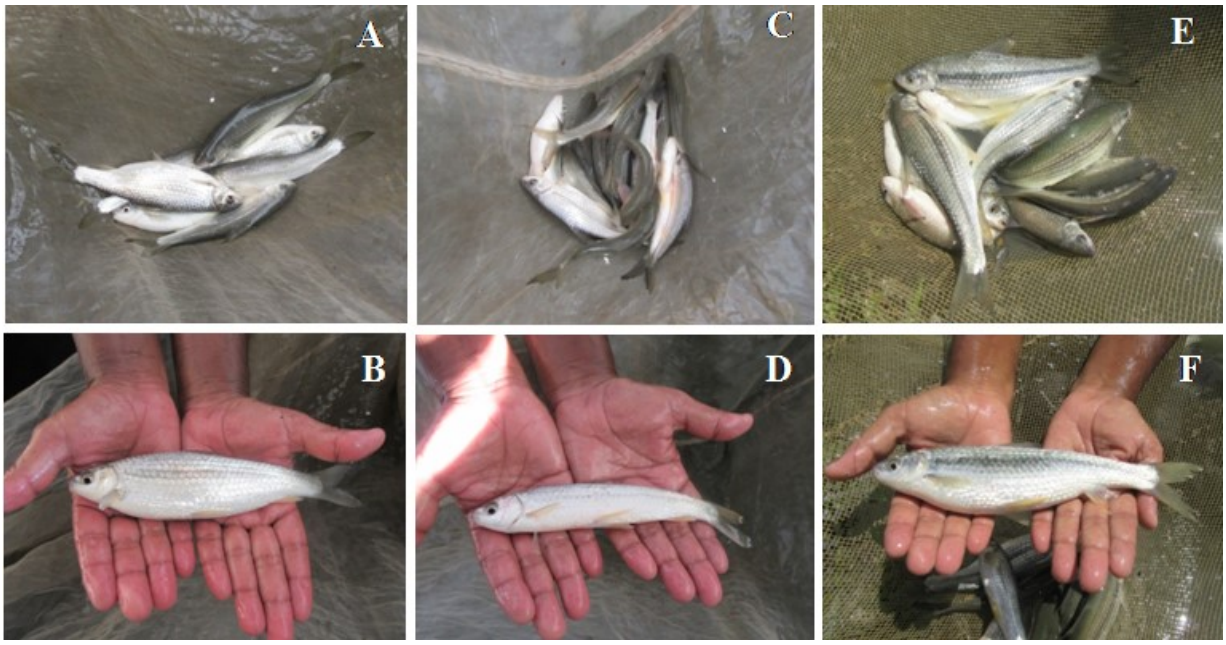

Figure 3: Gonadally matured Bhagna developed through feeding of vitamin premixes commercial feed (35% protein) during domestication (A-F). A-B, Atrai river population; C-D, Kangsha river population; and E-F, Jamuna River population.

10.2.4 Gonadosomatic index (GSI) of Bhagna (*L. ariza*):

The development of gonads as well as its size and weight increased along with its body mass increase. GSI was frequently applied to determine the reproductive cycle of a fish species over the year at monthly or less intervals as well as to know the peak spawning season. The value of GSI is the percentage of gonad weight to the total body weight of fish. Using the following formula the GSI of male and female *L. ariza* was determined during the period October, 2017 to September, 2018.

Gonadosomatic index (GSI) = (GW/BW)×100

Where, GW = Gonad weight of fish

BW = Body weight of fish

10.3 Performing Line breeding experiment for improved seed production

10.3.1 Brood fish selection

The main selection criteria to identify brood fish for a suitable breeder is not scientifically stated therefore followed the common sense. However, brood fish were selected based on some characteristics i.e. normal body shape and colour; absence of skeletal deformities; overall healthy status; normal behaviour; the largest size within its age group; and the best growth within its age group. In addition, mature female and male broods were identified by observing their secondary sexual

characters. Females were comparatively large in size with soft and swollen abdomen. Males were comparatively small in size and milts were available following stripping. Brood fish were selected based on individual selection system (Figure 4). Considering aforesaid criteria, the best 10% broods were selected from three different sources.

Figure 4: Selection of brood fish based on individual selection (A-D). A, Collected brood; B-D, Selected brood fish through individual selection.

10.3.2 Line breeding trials of three population of Bhagna:

Line breeding trials (line-1, line-2, line-3, line-4, line-5 & line-6) were performed using pituitary gland (PG) extract (Table 1; Figure 5 & 6). In this regard, 1:1 sex ratio of both free oozing male and female broods were selected for induced breeding. Females were treated with PG extracts at the doses of 4 mg kg^{-1} body weight (1^{st} dose) & 8 mg kg^{-1} body weight (2^{nd} dose) and males were treated with 4 mg kg-1 body weights during the second injection of female. Just prior to hypophysation, selected females and males were caught from the cistern. During administration of injection, the fish was wrapped by a soft cloth and kept lying on soaked foam. The PG solution was injected intramuscularly at the ventral side behind the pectoral fin. The broods were ovulated after 7-8 hours of injection. Then the fishes were subjected to strip and the fertilized eggs were transferred into the hatching tank with continuous water flow for proper aeration.

Table 1. Schematic representation of six breeding line of Bhagna, *L. ariza*

SL. No.	Group	Breeding line	Pattern
1	Conspecific group	Line-1	Kangsha♀×Kangsha♂
2		Line-2	Jamuna♀×Jamuna♂
3		Line-3	Atrai♀×Atrai♂
4	Heterospecific group	Line-4	Kangsha♀×Atrai♂
5		Line-5	Kangsha♀×Jamuna♂
6		Line-6	Atrai♀×Kangsha♂

Each of the lines was treated as a treatment in this experiment and the variation among the lines were observed and evaluated during the total experimental period.

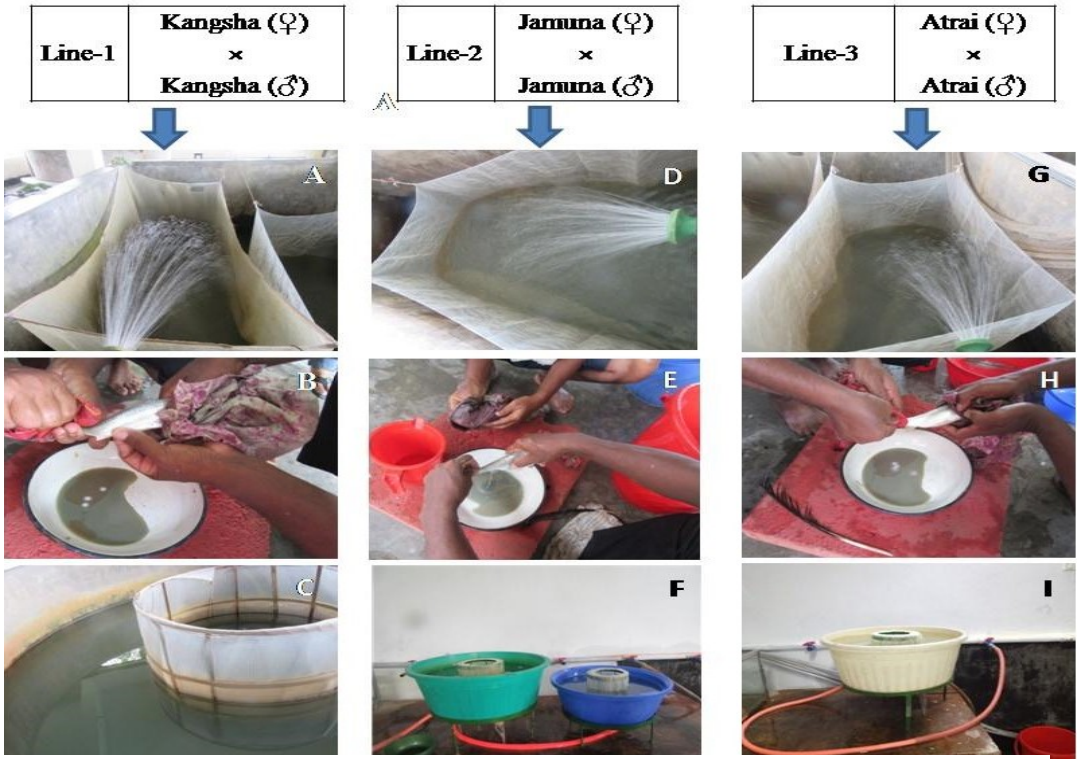

Figure 5: Line breeding trial of Bhagna of Conspecific group (Line 1 to Line 3).

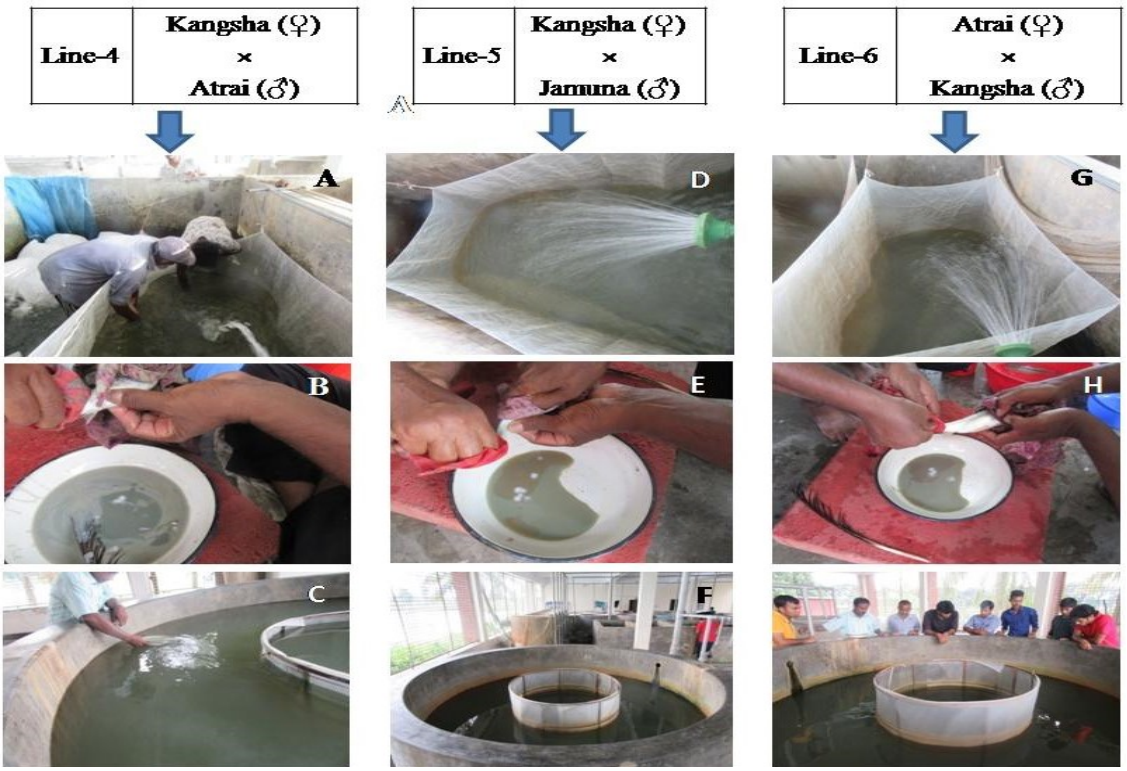

Figure 6: Line breeding trial of Bhagna of Heterospecific group (Line 4-Line 6).

10.3.3 Analysis of breeding performances

For determining fertilization rate a sample of eggs was taken in a petridish and the total number of eggs and the number of fertilized eggs were carefully counted. The fertilized eggs were found clear and transparent and the unfertilized eggs were opaque. Thus the percentage of fertilization of each replicate and the average of the percentages within each treatment were determined using the following formula:

- **Percentage of fertilization = (Number of fertilized eggs/Total number of eggs sample) × 100**

After 22±2 hours of fertilization the newly born hatchlings came out from egg shell. The percentage of hatching from each replicate and the average percentages within each treatment were calculated by counting the number of hatchlings coming out from the fertilized eggs, according to the following formula:

- **Percentage of hatching = (Number of hatchlings/Total number of fertilized eggs) × 100**
- **Survival rate = (Total no. of survived larvae/Total number of larvae) × 100**

10.4 Rearing of larvae of six different breeding line

10.4.1 Larval rearing using thermostat in glass aquaria and hapas:

Newly hatched larvae were reared in glass aquaria under different stocking densities with temperature controlled system (using Thermostat) for a period of 21 days (Figure 7). After that, larvae were released in six different hapas in earthen pond maintaining stocking densities for a period of two weeks (Figure 8). Here, regular aeration and siphoning were done. Feed were provided to the larvae at the rate of 5% of

body weight. At this stage, physico-chemical parameters viz. DO and pH were measured using pH and DO meter.

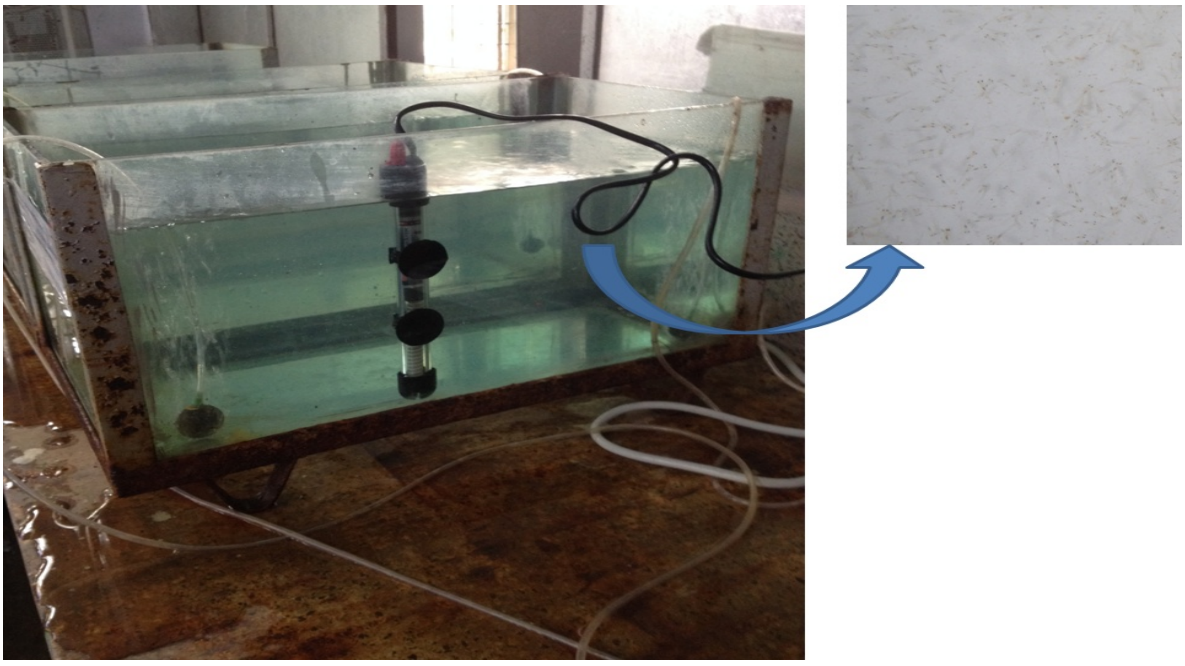

Figure 7: Larval rearing in glass aquaria using Thermostats.

Figure 8: Stocking of Larvae into hapa of six different nursery ponds.

10.4.2 Rearing of larvae in grow-out ponds:

Subsequently, larvae were released in previously prepared grow-out ponds in Field Laboratory Complex, Faculty of Fisheries, BAU and reared for a period of four weeks. Supplementary mega feed (35% protein) with probiotic (20 g/decimal; VC-7, TEAM Aqua Corporation) was supplied two times in a day at 5% body weight of the fish.

10.4.3 Regular sampling for length and weight data

Sampling was done in each pond after every 15 days interval for two months periods. Length and weight of 20 fishes were measured from each pond.

Figure 9: Periodic sampling for length and weight measurement (A-D), A- Netting in the ponds, B- fingerlings in the net, C- measurement of weight and D- measurement of length.

10.4.4 Analysis of growth parameters

Length gain, Weight gain, Specific Growth Rate (SGR) were calculated using the following formulae:

- Length gain (cm) = Final body length – Initial body length
- Weight gain (g) = Final body weight – Initial body weight
- Specific Growth Rate (SGR) % day^{-1} = SGR = $(\ln W_f - \ln W_i \times 100) / t$

  Where, $\ln W_f$ = the natural logarithm of the final weight

  $\ln W_i$ = the natural logarithm of the initial weight

  t = time (days) between $\ln W_f$ and $\ln W_i$

10.5 Cellular analysis using histological observation for new fiber detection:

Furthermore, among the possible six line of fingerling stage, improved seed condition were detected in the earlier presence of new muscle fiber through myotomal cross-sections on the basis of histochemistry techniques using cellular approaches (Jhonston, 1993; Ahammad *et al.,* 2015). In this regard, the preserved trunk muscle (80 dpf stage fry) was taken in a perforated plastic holder covered by perforated steel plates. Cleaning, infiltration and dehydration process were carried out in an automatic tissue processor unit using a series of alcohol of increasing concentrations, two times changes of xylene and finally through molten wax (three series) (Table 2). Paraffin embedded blocks were cut by microtome knife at 4-5 µm size and left the sections into a water bath at a temperature of 37°C. The sections were placed on a glass slide and kept overnight on a slide drier hot plate at a temperature of 20°C. Then the sections were stained routinely with haematoxyline and eosin (Humason, 1972) (Table 3). Moreover, the sample containing slides were covered by cover slip and mounted using Canada balsam. After that, some photographs were taken under compound microscope (Olympus, CX41).

Table 2. Automatic tissue processor system

SL. No.	Steps of Process	Times (Hours)	Process
1	50%Methylated spirit	1	Dehydration
2	80%Methylated spirit	2	
3	100%Methylated spirit	2	
4	100%Methylated spirit	2	
5	100%Methylated spirit	2	
6	100%alcohol	2	
7	100%alcohol	2	
8	Xylene	2	Cleaning
9	Xylene	1	
10	Molten wax	1	Infiltration
11	Molten wax	2	
12	Molten wax	2	
Total		21	

Table 3. Staining procedure for haematoxylin and eosin stain

Process	Solution	Times
Cleaning	Xylene	3 min
	Xylene	3 min
	Xylene	3 min
Rehydration	100% alcohol	2 min
	100% alcohol	2 min
	95% alcohol	1 min
	80% alcohol	1 min
Running up water		5 min
Staining	**Hematoxylin**	1 dip
Running tap water		25 min
Staining	**Eosin**	30 sec

Dehydration	95% alcohol	15 sec
	100% alcohol	30 sec
	100% alcohol	1 min
	100% alcohol	3 min
Cleaning	Xylene	3 min
	Xylene	3 min

10.6 Observation and measurement of the muscle fiber:

The section slide was observed under a high magnification microscope and the fiber was counted. A new fiber arising through hyperplasia is relatively small in size and through hypertrophic growth it increases in size. Several researchers have considered fibers less than 20 mm in diameter to represent fibers recruited by hyperplasia and those exceeding this diameter to represent fibers which had subsequently grown by hypertrophy (Rowlerson *et al.,* 1995). Hyperplastic and hypertrophic growth in the Bhagna (*L. ariza*) was similarly assessed using this established 20 mm diameter criterion in order to make comparisons with other fish species. The conversion of cross-sectional areas to equivalent diameter values was made according to Weatherley and Gill (1984) using the assumption that individual fiber cross-sections are circular. Under this assumption the cross-sectional area of a fiber is considered to be equal to that of a circle (πr^2). Therefore, fibers exhibiting areas under 314 mm^2 can be attributed to hyperplasia whereas those exceeding 314 mm^2 are considered a result of hypertrophic growth. Three size ranges of muscle fiber (small<20 µm, medium 21 to 30 µm, and large >30 µm) were observed according to Johnston *et al.* (2009).Then the fiber size was measured using Sigma Scan Pro Software (V. 5.0). Fifteen photos from each replication were considered for the analysis.

10.7 Statistical analysis

MS Excel 2016 and SPSS-V.21 software were used to analyze and interpret experimental data. Different bar diagrams were generated using MS excel 2016 and the different mean values of the growth parameters and mean number of muscle fibers were compared using ANOVA followed by Duncan's multiple range test at 95% level of confidence. Muscle fiber morphometry analysis was done using Sigma Scan Pro software (V. 5.0).

11. Results and discussion:

11.1 Growth response and survival rate of different populations during domestication period:

The initial and final average length, weight and survival rate of the Atrai, Jamuna and Kangsha populations during domestication period are presented in Table 4. The results showed that there was significant difference in growth variation and survival rate in each population. During stocking, the initial average length of the Atrai, Jamuna and Kangsha populations were 5.89±0.85, 5.85±0.92 and 5.80±0.73cm and initial weight were 7.08±0.94, 7.01±0.86 and 7.06±0.91 g respectively. After ten months of domestication in earthen ponds, there were significant differences ($P<0.05$) among the populations fed with same commercial supplementary feed (Mega feed) enriched with protein and vitamin-E. The highest length and weight gain (16.78±0.68 cm and 95.92±2.24 g) was observed in the Atrai population compared to the Jamuna (13.30±3.05 cm, 89.65±2.46 g) and the Kangsha (14.36±1.05 cm, 90.36±1.88 g) populations. Survival rate of fish was significantly higher in Kangsha (91%) than Atrai (86%) and Jamuna (84%). The observed growth response and survival of three river populations of *L. ariza* shown in the Table 4.

Table 4. Growth response and survival rate of three different populations during domestication period

Populations	Initial size		Final size		Survival rate (%)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	
Atrai	5.89±0.85[a]	7.08±0.94[a]	16.78±0.68[a]	95.92±2.24[a]	86[a]
Kangsha	5.80±0.73[a]	7.06±0.91[a]	14.36±1.05[b]	90.36±1.88[b]	91[b]
Jamuna	5.85±0.92[a]	7.01±0.86[a]	13.30±3.05[b]	89.65±2.46[b]	84[a]

Different superscript in the same column indicates significant difference among the lines

11.2 Gonadosomatic index (GSI) of *L. ariza*

For GSI study males and females of *L. ariza* were examined to determine the gonadal maturation and peak breeding season of *Labeoariza*. The gonadosomatic index is an indicator of the state of gonadal development. GSI values ranged from 0.55±0.26 to 4.95±0.35 in male and 1.15±0.21 to 11.5±0.37 in female and showed only one peak in August for both male and female (Figure 10). During the present study the higher values of GSI were observed from July to September. It ranged between 3.60 to 4.95 and 9.80 to 11.5 for males and females, respectively indicating the breeding season extends from July to September. Sudden decrease in gonad weight from October to January as indicated by the decline of GSI after spawning to minimum indices during November and December 0.55±0.26 and 0.76±0.32 in male and 1.15±0.21 and 1.31±0.26 in female, respectively (Figure 10).

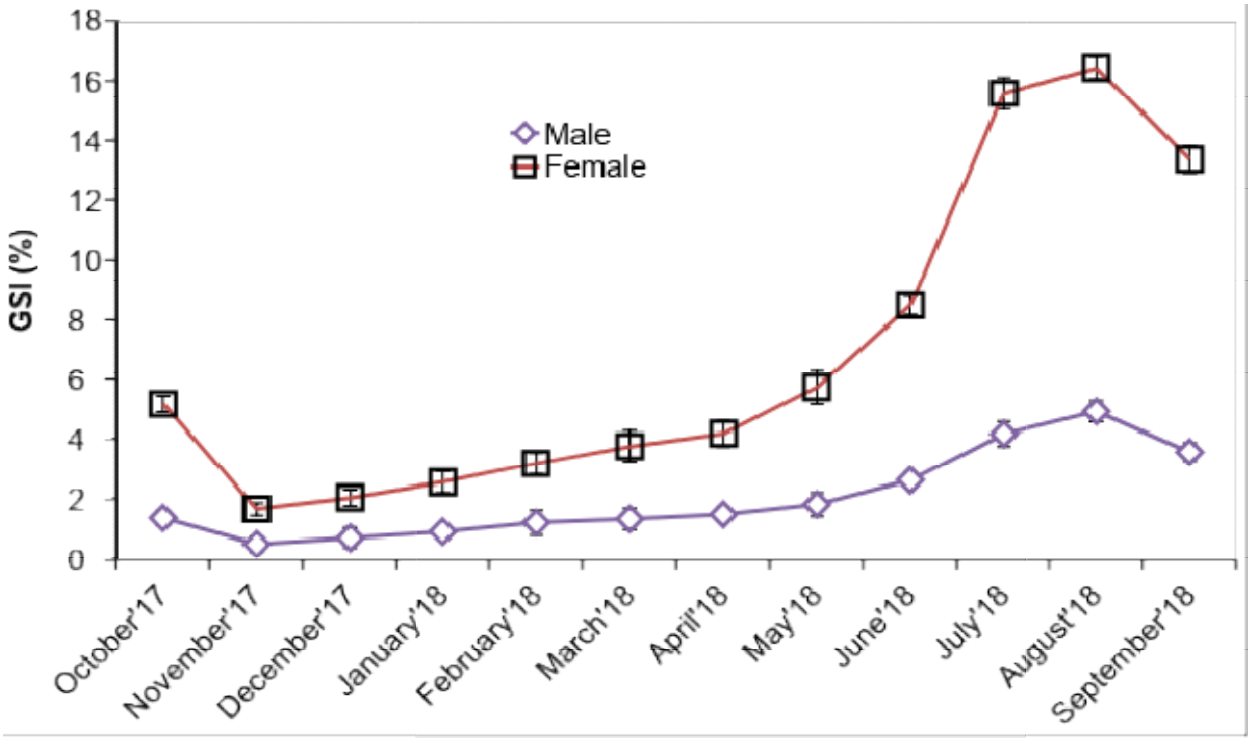

Figure 10: Gonadosomatic index (GSI) of male and female *L. ariza*

11.3 Fertilization, Hatching and Survival rate

The response of PG dose on female broods was excellent. After a certain period (18±2 hr.) of first dose of PG injection all the treated female broods were ovulated. The fertilization, hatching and survival rate of six different line of Bhagna (*L. ariza*) during seed production is shown in Table-5. There were significant variations (P<0.05) in fertilization, hatching and survival rate among the six different lines.

The highest fertilization, hatching and survival rate (95±2.11%, 88±1.03% and 82±1.88% respectively) was found in the line 4 during seed production. The fertilization rates from line 1 to line 6 were found as 92±2.01%, 94±1.09%, 93±1.11%, 95±2.11%, 91±3.03%, 90±1.99% respectively. The highest fertilization rate was observed at line 4 (95±2.11%) while the lowest was found at line 6 (90±1.99%). The differences among the fertilization rate of lines were found to be insignificant (P>0.05) (Table 5).

Hatching rate among the six lines (Line 1 to Line 6) was observed 87±1.2%, 84±3.08%, 85±2.08%, 88±1.03%, 86±2.58%, 81±4.01% respectively (Table 5) where the difference among the hatching rate was apparent but no significant difference among them existed (P>0.05).However, the survival rate of the six different lines from line 1 to line 6 was observed as 79±1.03%, 76±1.04%, 81±3.44%,82±1.88%,80±1.66% and 81±3.77% respectively. In this case, the highest survival rate was found in line 4 (82±1.88%) and the lowest survival rate was found in line 2 (76±1.04%).

Table 5. Fertilization, hatching and survival rate of six different lines of Bhagna during seed production

Parameters	Line 1	Line 2	Line 3	Line 4	Line 5	Line 6
Fertilization rate (%)	92±2.01	94±1.09	93±1.11	95±2.11	91±3.03	90±1.99
Hatching rate (%)	87±1.21	84±3.08	85±2.08	88±1.03	86±2.58	81±4.01
Survival rate (%)	79±1.03	76±1.04	81±3.44	82±1.88	80±1.66	81±3.77

11.4 Growth performance of *L. ariza* larvae of six different lines

The results of growth and production of *L. ariza* in terms of gain in weight in six different lines are presented in Figure 11 and 12. The results showed that there was significant difference in growth variation existed in each line during the experiment period.

The average initial length of six different lines were 3.81±0.25 cm in line 1, 3.84±0.35 cm in line 2, 3.95±0.16 cm in line 3, 4.08±0.67 cm in line 4, 3.85±0.85 cm in line 5 and 3.9±0.52 cm inline 6 respectively (Figure 11). The final length after two months (60 days) rearing in six different ponds showed that the length of fingerlings in heterospecific groups was comparatively higher than the conspecific groups. The final length were recorded the highest in line 4 (6.65±0.50 cm) followed by line 6 (6.53±0.34 cm); line 5 (6.42±0.62 cm); line 3 (6.35±0.53 cm); line 1(6.30±0.34 cm) and the lowest in line 2 (6.28±0.54 cm) (Figure 10). The length gain was the highest in line 4 (2.67±0.42 cm) followed by line 6 (2.63±0.23 cm), line 5 (2.57±0.27 cm), line 1 (2.49±0.31 cm), line 2 (2.44±0.25 cm) and line 3 (2.40±0.39 cm).

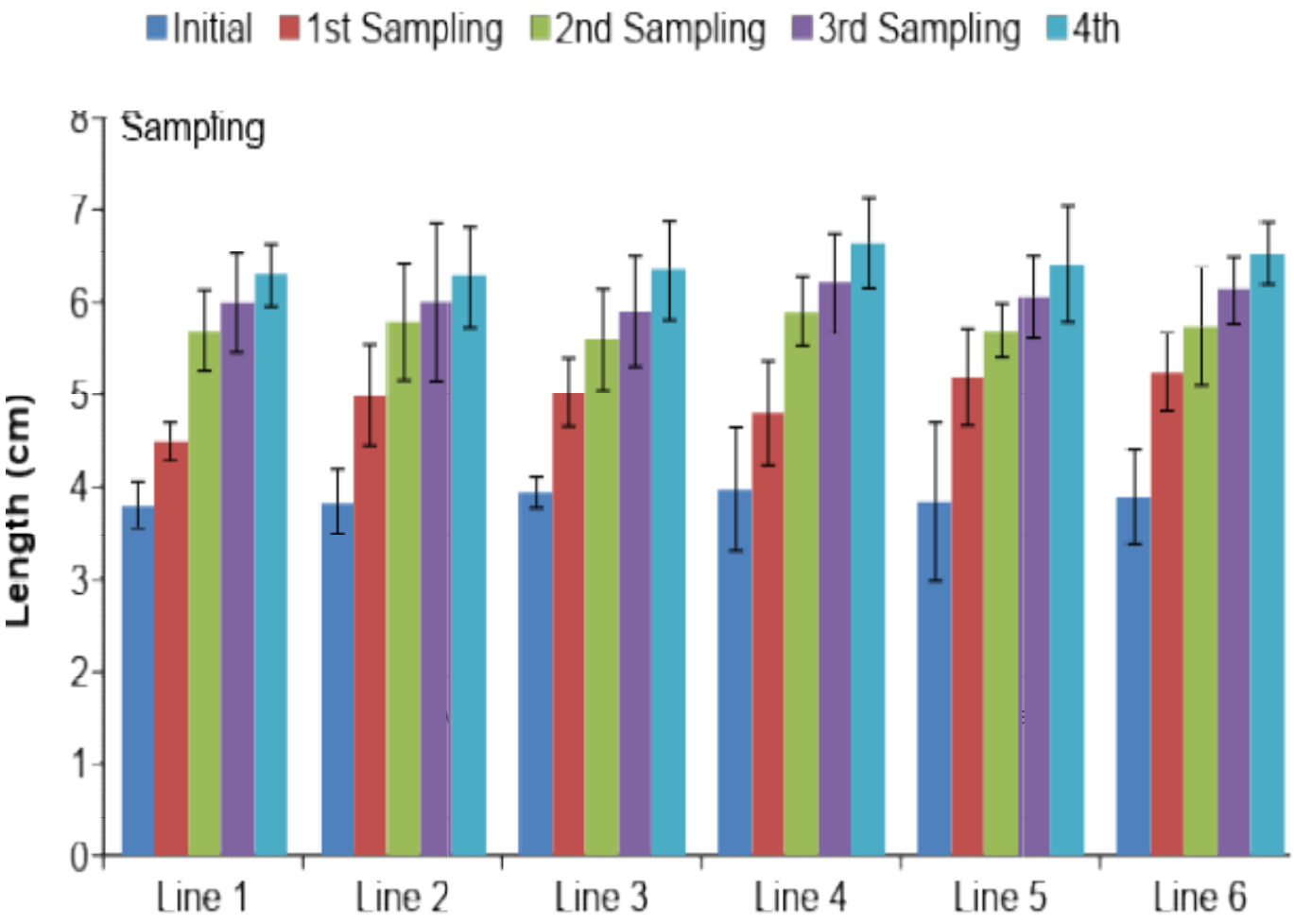

Fig. 11: Result of growth variation (Length) in six different lines of Bhagna *L. ariza*

The average initial weight of six different lines were 0.59±0.23 g in line 1, 0.61±0.36 g in line 2, 0.62±0.26 g in line 3, 0.67±0.37 g in line 4, 0.59±0.25 g in line 5 and 0.60±0.12 g in line 6. The final weight after two months of rearing in six different ponds showed that the weight of fingerlings in heterospecific groups was comparatively higher than the conspecific groups. The final weight was recorded the highest in line 4 (3.96±0.55 g) followed by line 5 (3.83±0.49 g); line 6 (3.79±0.34 g); line 3 (3.76±0.38 g); line 1(3.72±0.43 g) and the lowest in line 2 (3.65±0.54 g) (Figure 12). Significant difference existed ($p< 0.05$) among the final weight of the larvae of six different lines. The weight gain was found the highest in line 4 (3.39±0.26 g) and the lowest in line 2 (3.04±0.26 g) (Table 6).

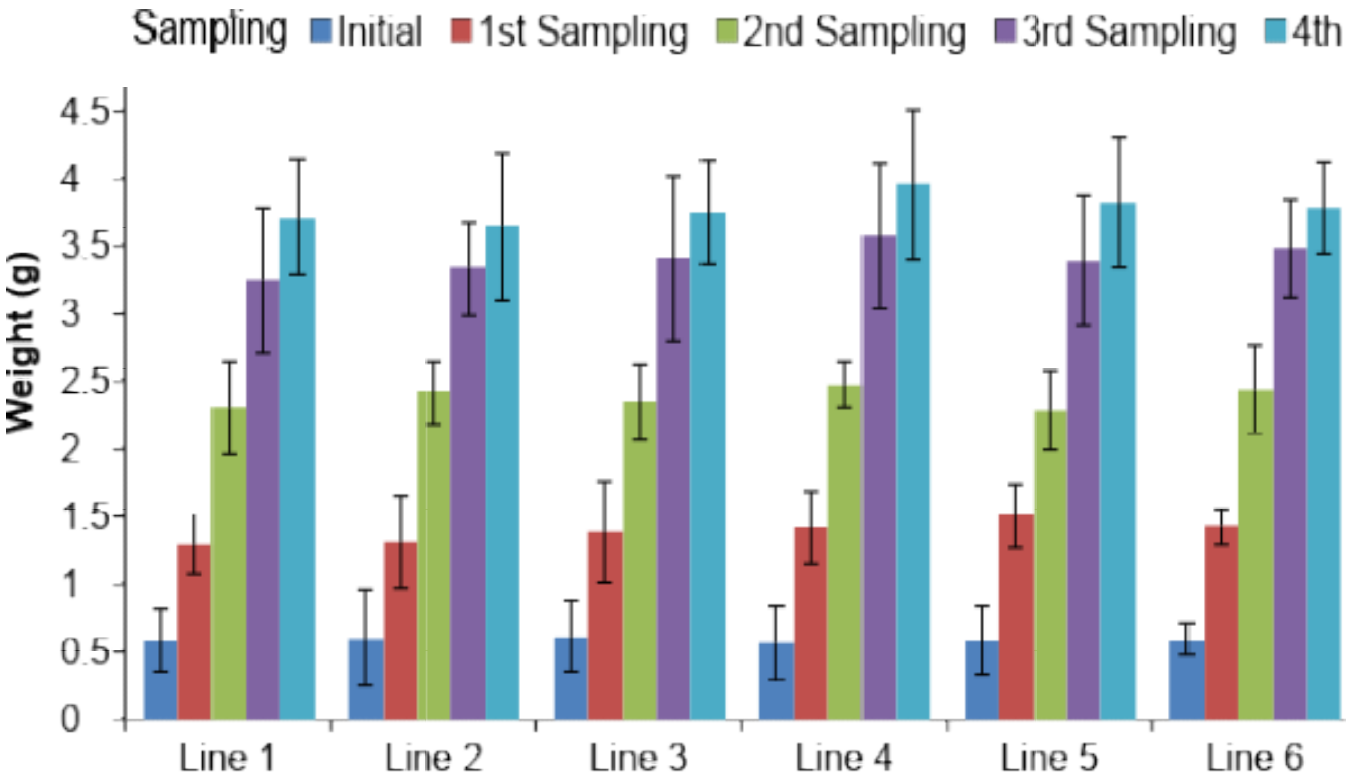

Figure 12: Result of growth variation (Weight) in six different lines of Bhagna*L. ariza*

The results of growth and production of *L. ariza* in terms of gain in length and weight in the six different lines are presented in Table 6. The results showed that significant difference existed in growth variation in each line and continued throughout the experimental period. The specific growth rate (SGR) of the larvae in different lines showed significant difference ($p<0.05$). The specific growth rate (SGR) was found is significantly higher in heterospecific groups (3.23±0.22% in line 4, 3.12±0.34% in line 5 and 3.07±0.37% in line 6) than the conspecific groups (3.07±0.16% in line 1, 3.00±0.29% in line 3 and 2.98±0.24% in line 2). The specific growth rate (SGR) value in Table 6 showed that significant variation between line 4 (highest) and line 2 (lowest).

Table 6. Growth performance of six different lines of Bhagna, *Labeo ariza*

Line	Initial Length (cm.)	Final length (cm.)	Initial Weight (g)	Final Weight (g)	Length gain (cm.)	Weight gain (g)	SGR (% day^{-1})
Line 1 (Kangsha♀×Kangsha♂	3.81±0.25^{a}	6.3±0.34^{a}	0.59±0.23^{a}	3.72±0.43^{a}	2.49±0.31^{a}	3.13±0.21^{a}	3.07±0.1^{a}
Line 2 (Jamuna♀×Jamuna♂	3.84±0.35^{a}	6.28±0.54^{a}	0.61±0.36^{a}	3.65±0.54^{a}	2.44±0.25^{a}	3.04±0.26^{b}	2.98±0.2^{b}
Line 3 (Atrai♀×Atrai♂	3.95±0.16^{a}	6.35±0.53^{a}	0.62±0.26^{a}	3.76±0.38^{a}	2.40±0.39^{a}	3.14±0.32^{a}	3.00±0.2^{b}
Line 4 (Kangsha♀×Atrai♂	3.98±0.67^{a}	6.65±0.50^{b}	0.57±0.27^{a}	3.96±0.55^{b}	2.67±0.42^{b}	3.39±0.26^{c}	3.23±0.2^{c}
Line 5 (Kangsha♀×Jamuna♂	3.85±0.85^{a}	6.42±0.62^{a}	0.59±0.25^{a}	3.83±0.49^{c}	2.57±0.27^{c}	3.24±0.18^{d}	3.12±0.3^{a}
Line 6 (Atrai♀×Kangsha♂	3.90±0.52^{a}	6.53±0.34^{c}	0.60±0.12^{a}	3.79±0.34^{c}	2.63±0.23^{b}	3.19±0.32^{a}	3.07±0.3^{a}

Different superscript in the same column indicates significant differences among the lines

11.5 Muscle fiber morphomerty of different lines

Horizontal sections of fast skeletal muscle of six different lines of *L. ariza* fingerlings are shown in Figure 12. Histological data revealed that the muscle fibers were dispersed in a mosaic pattern which was categorized by fibers of different diameters. All fiber diameter classes were found significantly different among the different lines. Muscle morphometric analysis showed that the percent hyperplastic small diameters fibers in heterospecific groups were comparatively higher than the conspecific groups, whereas in line 4, the highest number of small diameter fibers (new fiber) were documented (Figure 13 &14). The percent hyperplastic small diameter fibers in six different lines from Line 1 to Line 6 were observed as 30.10%, 28.15%, 30.19%, 35.28%, 33.65% and 32.38%, respectively (Figure 15). Muscle fiber morphometric analysis confirmed that increased growth rate of *L. ariza* in Line 4 was mostly governed by the hyperplastic muscle growth rather than hypertrophic muscle growth.

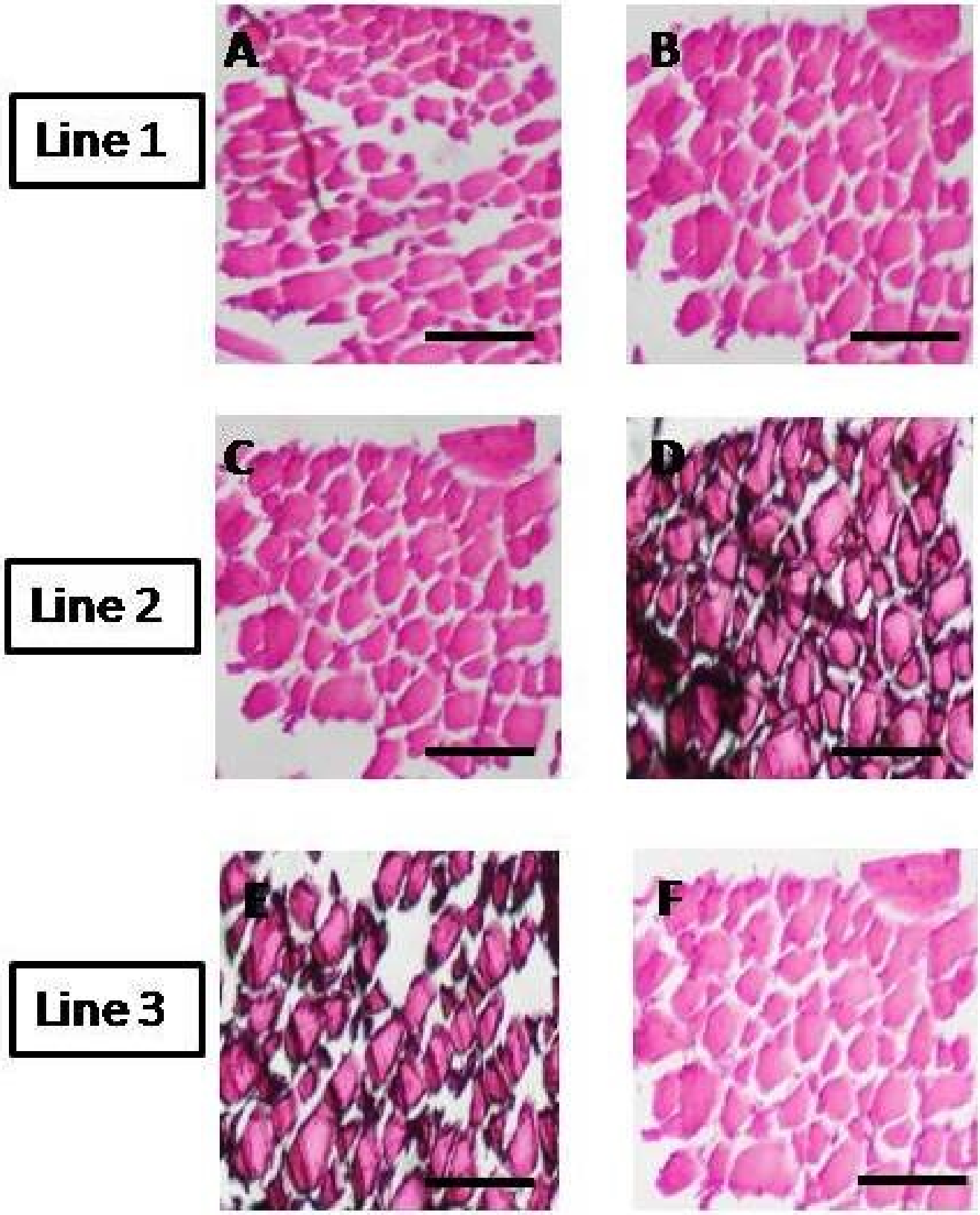

Figure 13: Histological observation of different sized muscle fibers in three different lines (Conspecific group) of Bhagna *L. ariza* (A-F represets line 1-3)

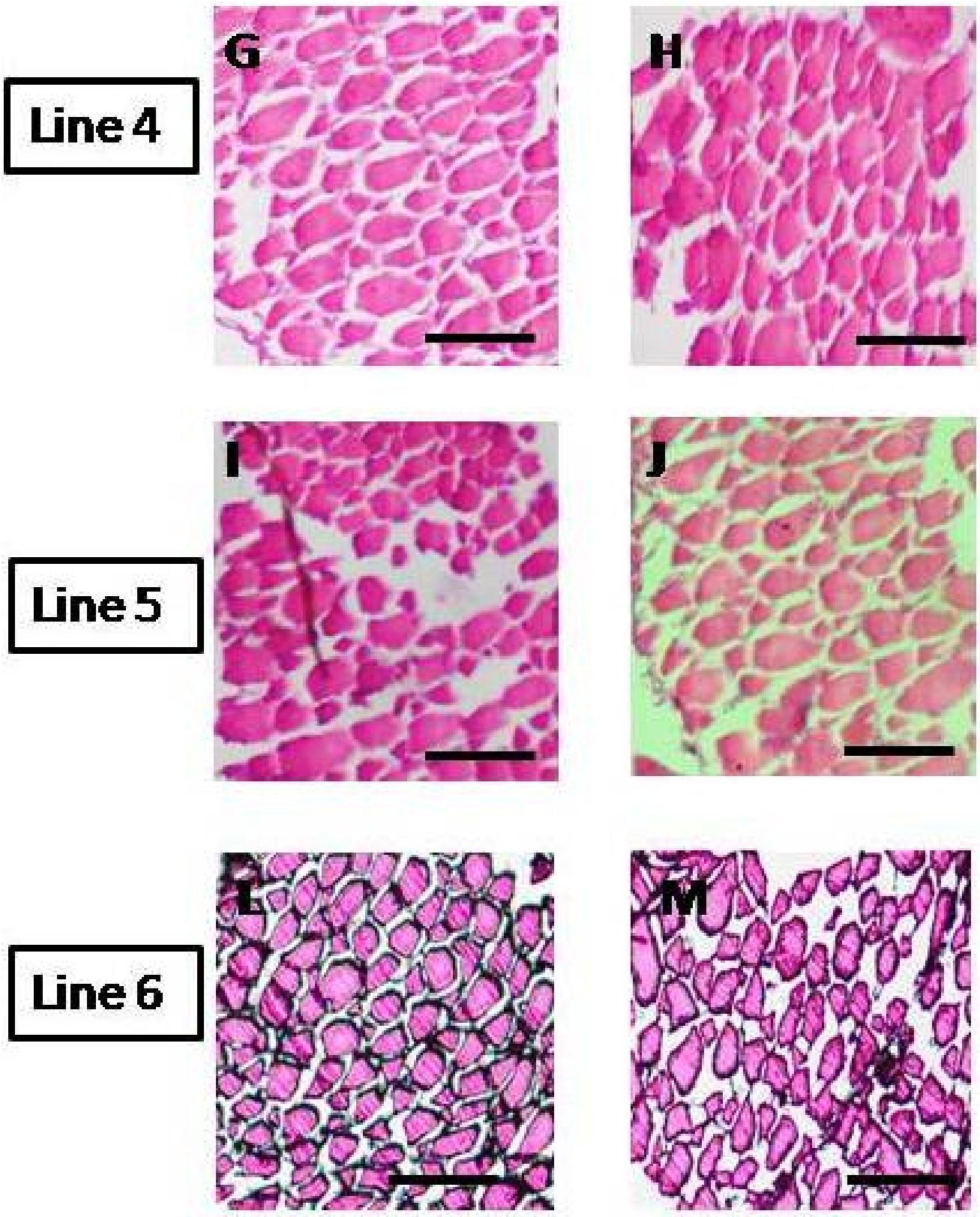

Figure 14: Histological observation of different sized muscle fibers in three different lines (hetero-specific group)

of Bhagna *L. ariza* (G-M represents line 4-6)

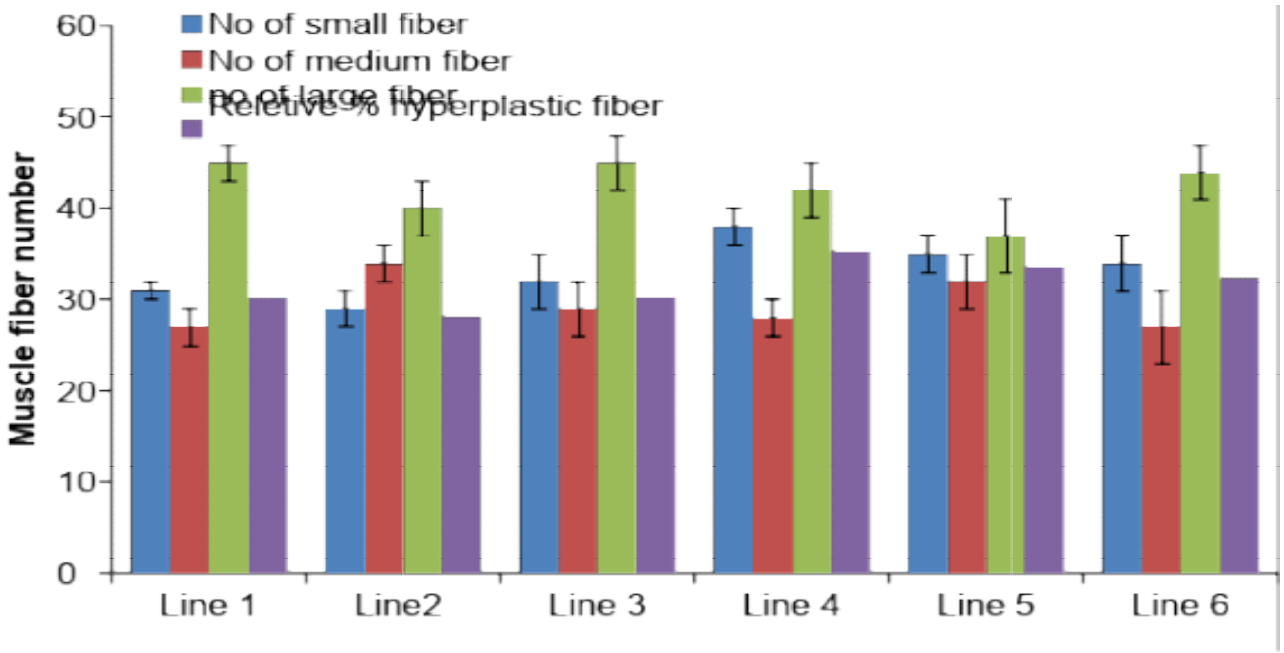

Figure 15: Number variation of different diameter (small, medium and large) fibers and percentage hyperplastic fibers in different lines of Bhagna*L. ariza*.

Published information on the biology, pertinently reproduction, breeding and muscle fiber composition of Bhagna (*L. ariza*) are very poor in Bangladesh. At the backdrop of rapid decline in abundance of this species from the natural waters of Bangladesh, necessities of adequate technology for bringing this species under culture system, the present attempt on the growth and muscle fiber development of Bhagna can be demanding.

In this study, collection of all the samples was done at the similar time but final size had shown to be highest in Atrai population followed by Kangsha and Jamuna populations. The Jamuna River is geographically nearer to the Kangsha River and Atrai River is geographically far from the Jamuna and the Kangsha rivers. In the present study, the Atrai population was collected from the Chirrirbandar Upazilla (Dinajpur District), where there is less chance of migration with the Jamuna (Sirajganj District) and the Kanghsa populations (Mymensingh District). Similar result of length and weight was found by Ahammad *et al. (*2015) for morphological analysis of Bhagna (*L. ariza*). Ahammad *et al.* (2018) found that the phenotypic differences among three populations (Atrai, Jamuna and Kangsha) might occur due to their separate geographical locations, different feeding habit, existing environmental variation of their three habitats or may be originated from different ancestors as well as genetic variation. In natural conditions, the growth of a particular species could be influenced by different environmental factors which in turn, reflect the reproductive activity of that species (Mookerjee and Majumder, 2006). After ten months domestication, the Atrai population showed higher length and weight but the Kangsha population showed higher survival rate (91%) among the three populations (Atrai, Jamuna andKangsha). The survival rate might be slowdown due to transportation of the fingerling from different geographical distances to the experimental site.

Morphological characters of fish can show high flexibility in response to changes in environmental conditions, such as food abundance and temperature (Swain *et al.,* 1991). Generally, fish show greater variances in morphological characters both within and between populations than any other vertebrates and are more vulnerable to environmentally induced morphological variations (Wimberger, 1992). The study of the genetic variation is a key step in any selection program, for the reason that, the genetic improvement depends mainly on maintaining the genetic variation between the mixed populations (Ponzoni *et al.*, 2005).

Nutrition is very important for sound growth and maturation of gonads of a fish and for the domestication of the fish as well. The quality of eggs depends on the quality of feed provided. Nahar *et al*. (2000) and Mollah *et al.* (2009) found the best results using 58% protein containing feed. They mentioned that with the increment of protein concentration in feed the growth of fish also increased simultaneously. In the present experiment, a special feed enriched with protein and vitamin-E (Mega feed) was supplied to the fish and the better growth performance in respect to length and weight was observed in Atrai population. Higher survival rate was found in the Kangsha population(91%). It could be due to better genetic combinations and adaptability of the Kangsha population in different natural environment.

The gonadosomatic index study of Bhagna, *L. ariza* from three different populations was recorded from October 2017 to September 2018 (see Figure 10). It was observed that Bhagna, *L. ariza* have only one breeding season of short duration, running from July to September, with a peak in August. Report of Rahman *et al.* (2007) supports the findings of the present study as he reported that the breeding season of Rebafrom June to October. Almost similar observations have been made by Lashari *et al*. (2007) in Reba carp, *C. reba* from Jacobabad District (Sindh) Pakistan. The GSI during the present investigations showed one peak during summer in the month of August (4.95±0.35 and 11.5±0.37 for males and females. Qazi (2001) and Lashari *et al.* (2007) reported the breeding season of *C. reba* from June to August, with a peak in July from India, Bangladesh and Pakistan waters which supports the present findings.

Maintaining a large genetic variance, the subsequent selection between groups (families) results in considerable progress while maintaining a large genetic variance. Moav & Wohlfarth, (1976) reported that in case of common carp (*Cyprinus carpio*) when two lines were crossed, the F_1, showed a high degree of heterosis. It supports our result also. The analysis of variance for fertilization, hatching and survival rate of six different line of Bhagna (*Labeo ariza*) showed significantly higher fertilization, hatching and survival rate in the line-4 (Kangsha♀× Atrai♂ compared to other lines. This may be attributed to the improved genetic variations of their parents in case of these phenotypic traits. Chattopadhyay *et al.* (2013) found the fertilization rate of 86.4% and hatching rate of 91.6% during seed production of *Cirrhinus reba* by using Ovaprim and maintaining physico-chemical parameters at temperature: 26.5-27.8ºC pH: 7.2-7.4, D.O.: 5.2-6.4 mgL^{-1} . The survival rate of the fry of line 4 was 82±1.88% where the survival rate of parental line (Kangsha and Atrai population) was 91% and 86%, respectively.

The growth (length and weight gain) was superior in the heterospecific groups than the conspecific groups. This may be due to the heterozygosity present in the heterospecific groups due to the crossing between two different populations or due to heterosis. According to Ahammad*et al.* (2018) there are phenotypic differences among three populations (Atrai, Jamuna and Kangsha) may as well as genetic variation due to their separate geographical location. Thus the heterospecific groups attain a high level of heterozygous alleles compared to the conspecific groups. Again in line 4 (Atrai♂×Kangsha♀) showed the highest Specific Growth Rate (SGR), length and weight gain among the six lines. According to Wohlfarth (1993)heterosis was found commonly in young of the year carp during their first

summer. According to the findings of Ali *et al*. (2017) better growth rate can be obtained by selective breeding between different parent populations with genetic differences due to geographical differences as happened in case of Nile tilapia. The results of Bentsen *et al.* (2017) about the response to selection for increased body weight at harvest in *Oreochromis niloticus* also supports the present findings.

Analysis of muscle fiber morphometry in different lines showed many small-diameter fibers surrounded by larger fibers. This pattern is observed in most fish species during muscle growth and is characteristic of hypertrophy and hyperplasia (Johnston, 1999; Rowlerson and Veggetti, 2001; Aguiar *et al.,* 2005; Almeida *et al.,* 2008). In the majority of fish species, these mechanisms are employed throughout their life cycle and have been well documented in a variety of species, particularly in species with a large potential for aquaculture (Johnston *et al.,* 2000; Rowlerson and Veggetti, 2001; Aguiar *et al.*, 2005). In the present work, it has been observed that larval stage *L. ariza* comprised of many immature fibers, demonstrating that hyperplasia is probably the main muscle growth mechanism at that stage. This mechanism is responsible for the new fibers formation in fish species, once proliferative satellite cells aggregate to the mature fibers surface and generate new myotubes (Johnston *et al*., 2000; Dal Pai *et. al.,* 2003). The hyperplasia was identified by the occurrence of mosaic groups of fibers.

In the present study, the percent hyperplastic small diameters fibers (new fibers) in line 4 were the highest (35.28%) among the six lines. The number of hyperplastic fibers was found to be significantly higher in line 4 indicating an increase number of muscle hyperplasia rather than hypertrophy. Earlier presence of small diameter fiber indicates improved seed quality. Therefore, the line 4 was superior to other lines in this case. It may be due to the effects of optimum embryonic temperature provided to different lines after fertilization. The optimum embryonic temperature that was provided had suited for the acceleration of the different embryonic stages viz. cleavage, morula, gastrula, blastula of line-4 which ultimately initiated the early growth of muscle fiber of the individuals of the line. This result may also be attributed due to the variations in egg quality and parental growth factors of different lines. Experiments in different years indicate considerable variation in the effects of temperature during development on the number and diameters of embryonic muscle fibers in Clyde herring (Vieira and Johnston, 1992; Johnston, 1993) which may be related to variations in egg quality including the amount of yolk, the amino acid content and the concentration of maternal growth factors. Indirect evidence that embryonic temperature modulates muscle growth characteristics up to 3 weeks after first feeding was obtained in the Atlantic salmon (Nathanailides *et al.,* 1995).

Previous research showed that various species of fish viz. European sea bass, Antarctic teleost, Gilthead sea bream and some marine fishes that experience long periods of hyperplasia reached larger body sizes than those that grow primarily through hypertrophy (Veggetti *et al.,* 1990; Battram and Johnston, 1991; Kundu and Mansuri, 1992; Koumans and Akster, 1993; Rowlerson *et al.,* 1995). The findings of the current investigation were in agreement with this trend, as hyperplasia was found to persist well into the line 4 that attains a relatively large ultimate size compared to the other lines. Considerable evidence therefore suggests that fiber recruitment holds the main role in determining the growth of a fish. In this case, there may be some factors that might trigger the muscle growth related gene by changing their mRNA expression patterns. However, this hypothesis needs further research to characterize *L. ariza* through molecular marker analysis.

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

- Domestication of wild Bhagna, *L. ariza* in pond condition
- Development of Bhagna, *L. ariza* breeding technique using PG extract
- Identification of line bred Bhagna (Kangsha♀×Attrai♂for broodstock development
- Development of Line bred Bhagna can be used as a base population for producing of high quality seeds for better growth performance

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	i) Scanner ii) Laptop	10000 60000	i) Scanner ii) Laptop	10000 60000	100% Achieved
(b) Lab &field equipment	i) Up-down shaker ii) Plastic Tank iii) Ice-box iv) Electric balance iv) pH meter v) DO meter	160000 42000 7000 15000 10000 20000	i) Up-down shaker ii) Plastic Tank iii) Ice-box iv) Electric balance iv) pH meter v) DO meter	160000 42000 7000 15000 10000 20000	100% Achieved
(c) Other capital items					

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
(a) Aquarium repair, renovation and maintenance	18000	Completed			100% Achieved
(b) Pondconstruction and management	22000	Completed			100% Achieved

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

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	20	5	25	1D	A Training on "Seed production of Bhagna*Labeoariza* (Hamilton,1807) through line breeding trial in Bangladesh" 2017/198/BARC held on 23 September2018, organized by the Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh

(b) Workshop	15	9	24	1D	A Workshop on **"**Seed production of Bhagna*Labeoariza* (Hamilton,1807) through line breeding trial in Bangladesh**"** 2017/198/BARC held on 20 September2018, organized by the Department of Fisheries Biology and Genetics, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh.

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	4,16,953	4,16,953	4,16,953	0	100	
B. Field research/lab expenses and supplies	5,37,607	5,37,607	5,37,607	0	100	
C. Operating expenses	1,15,000	1,15,000	1,15,000	0	100	
D. Vehicle hire and fuel, oil & maintenance	50,000	50,000	50,000	0	100	
E. Training/workshop/seminar etc.	75,000	75,000	75,000	0	100	
F. Publications and printing	75,000	75,000	75,000	0	100	
G. Miscellaneous	30,000	30,000	30,000	0	100	
H. Capital expenses	3,24,000	3,24,000	3,24,000	0	100	

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)**
a) Domestication of the wild sources of *L. ariza* for gonadal maturation under captive condition.	• Field visit to collect the wild sources of Bhagna, L. ariza from three geographical areas. • Improve nursery management of collected three population under earthen pond condition; and • Rearing of adult Bhagna for gonadal maturation.	Filed report, periodical reports, PCR, financial statements, bill and vouchers.	Domestication technique of wild Bhagna populations in earthen pond condition are developed

b) Performing Line breeding experiment for improved seed production	• Selection of brood fish through individual selection system. • Study on the Line breeding trials of three population of Bhagna in hatchery condition	Half yearly, annual reports, breeding photographs, photographs of fish fry, PCR, etc.	Line breeding technique of the Bhagna in the laboratory or hatchery conditions are developed
c) Identification of the best line using muscle cellularity analysis through histological observation.	Cellular analysis using histological observation for identification of best line.	Leaflet, PCR	• Improved seed for the hatchery owners and fish farmer. • Strategic plan to promote Bhagna seed production trade in Bangladesh

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		Title of leaflet: বাংলাদেশে লাইনব্রিডিং কৌশলেরমাধ্যমে ভাগনামাছের পোনাউৎপাদন
Journal publication	1		Title of paper: Growth performances and muscle fiber morphometry in line bred Bhagna, *Labeoariza*; Submitted to Aquaculture Journal.
Information development			
Other publications, if any			

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

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

Development of the Bhagna, *L. ariza* breeding technique using PG extract

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

Identification of line bred Bhagna (Kangsha♀×Attrai♂) for broodstock development

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

The technology is being practiced by the trained farmers and they are financially benefited from these activities.

Strategic plan to promote Bhagna seed production trade in Bangladesh

G. Information regarding Desk / Field Monitoring

i) Desk Monitorin:

- CRG Sub- Project: Progress Review Workshop held in BARC, Farmgate Dhaka on 10-11 April 2018 under NATP - 2 under Fisheries Division BARC. In this workshop, various suggestions had been documented and need to incorporate.

These are given below:

- Results should be presented in accordance with objectives
- NationalFishery Policy needs to be review
- Number of sampling needs to be increased.

ii) Field Monitoring:

Time: 07.03.2018

No. of visit: 1

Team members: Dr. Md. Abdul Jalil Bhuyan, Research Management Specialist, PIU-BARC, NATP-2;

and Dr. Mian Sayeed Hassan, Director, PIU-BARC, NATP-2.

Output: Need to review the growth pattern of related species as well as maintain cash book.

H. Lesson Learned/Challenges (if any)

i) Detection of new muscle fiber for characterization of different lines of Bhagna using muscle cellularity analysis.

I. Challenges (if any)

Not Applicable

Signature of the Principal Investigator
Date …….
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J. References:

Aguiar DH, Barros MM, Padovani CR, Pezzato LE, & Dal Pai-Silva M 2005: Growth characteristics of skeletal muscle tissue in *Oreochromis niloticus* larvae fed on a lysine supplemented diet. *Journal of Fish Biology* ***67*****(5)** 1287-1298.

Ahammad AKS, Ahmed MBU, Akhter S, Hossain MK 2018: Landmark-based morphometric and meristic analysis in response to characterize the wild Bhagna, *Labeoariza* populations for its conservation. *Journal of the Bangladesh Agricultural University* ***16*****(1)** 164-170.

Ahammad AKS, Haque MM, Rahman AM, Asaduzzaman M 2015: Morpho-genetic Analysis of Three River Populations of Bhagna, *Labeoariza*(Hamilton 1807) in Bangladesh. *Journal of Aquaculture and Marine Biology***2(3)** 29.

Akhteruzzaman M, Kohinoor AHM, Islam MS, Modak PC 1998: Observations on the induced breeding of indigenous small fish, Bhagna (*Cirrhinusreba* Ham.) in Bangladesh. *Progressive Agriculture***9(1-2)** 281-284.

Alami-Durante H, Fauconneau B, Rouel M, Escaffre AM, Bergot P 1997: Growth and multiplication of white skeletal muscle fibers in carp larvae in relation to somatic growth rate. *Journal of Fish Biology* ***50*****(6)** 1285-1302.

Ali FS, Nazmi HM, Abdelaty BS, El-Far AM, Goda AM 2017: Genetic improvement of farmed Nile tilapia (*Oreochromisniloticus*) through selective breeding in Egypt.

Almeida FLA, Carvalho RF, Pinhal D, Padovani CR, Martins C, Dal Pai-Silva M 2008: Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (*Piaractusmesopotamicus* Holmberg 1887: Serrasalminae, Characidae, Teleostei) during juvenile and adult growth phases. *Micron***39** 1306-1311.

Battram J, Johnston I 1991: Muscle growth in the Antarctic teleost, *Notothenianeglecta*(Nybelin). *Antarctic Science***3** 29–33.

Bentsen HB, Gjerde B, Eknath AE, de Vera MSP, Velasco RR, Danting JC, Dionisio EE, Longalong FM, Reyes RA, Abella TA, Tayamen MM 2017: Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in *Oreochromisniloticus* and the further impact of the project. *Aquaculture* **468** 206-217.

Chattopadhyay NR, Patra S, Giri S, Naskar A, Roy U 2013: Low Cost Innovative Technology for Seed Production of *Cirrhinu sreba* (Hamilton, 1822) at Backyard of Murshidabad District, West Bengal, by using Ovaprim. *International Journal of Advanced Fisheries and Aquatic Science* 49.

Dal Pai-Silva M, Carvalho RF, Pellizzon CH, Dal Pai V 2003: Muscle fiber types in tilapia do Nilo (*Oreochromisniloticus*) from larval to adult: histochemical, ultrastructural and morphometric study. *Tissue and Cell* **35** 179-187.

DoF 2007: Department of Fisheries Annual Report. Department of Fisheries, Ministry of Fisheries and Livestock, Government of the Peoples' Republic of Bangladesh. *Matsha Bhaban, Dhaka, Bangladesh* 1-111.

DoF 2014: Yearbook of Fisheries Statistics of Bangladesh 2013-14. *Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh***30** 1-52.

DoF 2016: Department of Fisheries Annual Report. Department of Fisheries, Ministry of Fisheries and Livestock, Government of the Peoples' Republic of Bangladesh. *MatshaBhaban, Dhaka, Bangladesh* 1-148.

DoF 2017: Yearbook of Fisheries Statistics of Bangladesh 2016-17*. Fisheries Resources Survey System (FRSS), Department of Fisheries. Bangladesh* **34** 129.

FAO 2018: The State of World Fisheries and Aquqcultue – Meeting the sustainable development goals. Rome16-27.

Felts RA, Rajits F, Akhteruzzaman M 1996: Small indigenous fish species culture in Bangladesh. (Technical Brief), IF ADEP sub-project-2. *Development of Inland Fisheries* 41.

FRSS 2015: Yearbook of Fisheries Statistics of Bangladesh 2013-14. Fisheries Resources Survey System (FRSS). *Department of Fisheries*. Bangladesh: Director General. DoF.P. 34-129

Gupta S 1975: Some observations on the biology of *Cirrhinusreba*(Cuvier). *Journal of Fish Biology* **7** 71-76.

Higgins P, Thorpe J 1990: Hyperplasia and hypertrophy in the growth of skeletal muscle in juvenile Atlantic salmon, *Salmosalar*L. *Journal of Fish Biology* **37** 505–519.

Hogan CM 2012: Indus River. In: Saundry P & Cleveland C (Editors), Encyclopedia of Earth. *National Council for Science and the Environment. Washington DC, USA.*

Hossain MAR, Wahab MA, Belton B 2012: The checklist of the riverine fishes of Bangladesh. *The world Fish Center, Bangladesh and South Asia Office, Dhaka.*

Humason GL 1972: Animal tissue techniques. *WH Freeman and Company, Sanfrancisco, California* 614.

Hussain MG, Mazid MA 2001: Genetic improvement and conservation of carp species in Bangladesh. *Bangladesh Fisheries Research Institute, Mymensingh.*

IUCN 2015: Red Book of Threatened of Bangladesh. IUCN-The world conversation union. P. 9-24

Jhingran VG 1991: Fish & Fisheries of India. (3rd edition), *Hindustan Publishing Corporation, Delhi, India. 727.*

Johnston IA 1993: Temperature influences muscle differentiation and the relative timing of organogenesis in herring (*Clupeaharengus*) larvae. *Marine Biology***116** 363– 379.

Johnston IA 1999: Muscle development and growth: potential implication for flesh quality in fish. *Aquaculture***177** 99-115.

Johnston IA, Alderson R, Sandham C, Dingwall A, Mitchell D, Selkirk C, Nickell D, Baker R, Robertson B, Whyte D, Springate J 2000: Muscle fiber density in relation to colour and texture of smoked Atlantic salmon (*Salmosalar* L.). *Aquaculture***189** 335-349.

Johnston IA, Lee HT, Macqueen DJ, Paranthaman K, Kawashima C, Anwar A, Dalmay T 2009: Embryonic temperature affects muscle fibre recruitment in adult zebrafish: genome-wide changes in gene and microRNA expression associated with the transition from hyperplastic to hypertrophic growth phenotypes. *Journal of Experimental Biology* **12(12)** 1781-1793.

Khawaja DK 1966: Biochemical compositions of the muscles of some freshwater fishes during the prematurity phase. *Fisheries Technology***3** 94-102.

Kohinoor AHM, Islam ML, Wahab MA, Thilsted SH (1998) Effect of mola (*Amblypharyngodonmola* Ham.) on the growth and production of carps in polyculture. *Bangladesh Journal of Fisheries Research***2(2)** 119-126.

Koumans JTM, Akster HA 1995: Myogenic cells in development and growth of fish. *Comparative Biochemistry and Physiology Part A: Physiology***110(1)** 3-20.

Kundu R, Mansuri A 1992: Growth of pectoral muscle fibers in relation to somatic growth in some marine fishes. *Netherlands Journal of Zoology* **42** 595–606.

Lashari PK, Narejo NT, Laghari MY, Mastoi AM 2007: Studies on the Gonadosomatic Index and Fecundity of a Carp *Cirrhinusreba* (Hamilton) from Fishponds of District Jacobabad, Sindh. Pakistan. *Pakistan Journal of Zoology* ***39*(2)** 95.

Mahalder, B., &Mustafa, M.G. 2013. Introduction to Fish Species Diversity: Sunamganjhaor region within CBRMP's working area. In: MN Naser& AKM Firoz Khan (Eds.), Community Based Resource Management Project LGED, Agargaon, Dhaka 1207, Bangladesh, p. 75.

Mazid MA 2002: Development of Fisheries in Bangladesh. *Plans and Strategies for Income Generation and proverty Alleviation,* Dhaka, Bangladesh. 176

Moav R, Wohlfarth G 1976: Two-way selection for growth rate in the common carp (*Cyprinuscarpio* L.). *Genetics* **82(1)** 83-101.

Mollah MFA, Roy A, Mamun MSA 2009: Growth performance of larvae produced from vitamin E treated female *Clariasbatrachus* (Linnaeus). *Progressive Agriculture***7(2)** 425-430.

Mookerjee HK, Majumder SR 2006: On the life history, breeding and rearing of *A. testudineus*(Bloch). *Journal of Department Science***2** 101-140.

Nahar Z, Azad Shah AKM, Bhandari RK, Ali MH, Dewan S 2000: Effects of different feeds on growth, survival and production of African catfish (*Clariasgariepinus* Burchell). *Bangladesh Journal of Fisheries Research***4 (2)** 121-125.

Naser NM 2015: *Labeoariza*. In: IUCN Bangladesh. Red List of Bangladesh Volume 5: Freshwater Fishes. *IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh* 101.

Nathanailides C, Stickland NC, Lopez-Albors O 1995: Influence of pre-hatch temperature on the development of muscle cellularity in post-hatch Atlantic salmon (Salmosalar). *Canadian Journal of Fisheries and Aquatic Sciences**52***(4)** 675-680.

Ponzoni RW, Hamzah AST, Kamaruzzaman N 2005: Genetic parameters and response to selection for live weight in the GIFT strain of Nile tilapia (*Oreochromisniloticus).Aquaculture***247(1-4)** 203-210.

QAZI ZH 2001: Induced breeding of the fish *Cirrhinusreba*by pituitary gland extract and survival of Jawn in nursery ponds. *Journal of Asiatic Society of Bangladesh.* **27** 205-213.

Rahman AKA 2005: Freshwater fishes of Bangladesh (2nd edition) *Zoological Society of Bangladesh, Department of Zoology, University of Dhaka, Dhaka*. 394

Rahman MA, Zaher M, Azimuddin KM 2009: Development of Fingerling Production Techniques in Nursery Ponds for the Critically Endangered Reba Carp, *Cirrhinusariza* (Hamilton, 1807). *Turkish Journal of Fisheries and Aquatic Sciences***9(2)** 165-172.

Rahman MM, Habib MA, Shah MS 2007: Induced breeding of *Cirrhinusreba* (Ham.) and *Labeobata* (Ham.). *Khulna University Studies***8(2)** 286-292.

Roberts TR 1997: Systematic revision of the tropical Asian labeon cyprinid fish genus *Cirrhinus*, with descriptions of new species and biological observations on *C. lobatus*. *Natural History Bulletin of theSiam Society* **45** 171-203.

Rowlerson A, Mascarello F, Radaelli G, Veggetti A 1995: Differentiation and growth of muscle in the fish *Sparusaurata*(L): II. Hyperplastic and hypertrophic growth of lateral muscle from hatching to adult. *Journal of Muscle Research and Cell Motility* **16** 223–236.

Rowlerson A, Veggetti A 2001: Cellular Mechanisms of Post-Embryonic Muscle Growth in Aquaculture Species. *Fish Physiology***18** 103-140

Swain DP, Riddell BE, Murray CB 1991: Morphological differences between hatchery and wild populations of Coho salmon (*Oncorhynchuskisutch*): environmental versus genetic origin. *Canadian Journal of Fisheries and Aquatic Sciences***48(9)** 1783–1791.

Talwar PK, Jhingran AG 1991: Inland fishes of India and adjacent countries. *A.A. Balkema, Rotterdam***1** 541

Veggetti A, Mascarello F, Scapolo P, Rowlerson A 1990: Hyperplastic and hypertrophic growth of lateral muscle in *Dicentrarchuslabrax*(L): an ultrastructural and morphometric study. *Anatomy and Embryology* **182** 1–10.

Vieira VLA, Johnston IA 1992: Influence of temperature on muscle-fiber development in larvae of the herring *Clupeaharengus*. *Marine Biology***112** 333– 341.

Walter R 1994: Inland fishes of India and adjacent countries. *Reviews in Fish Biology and Fisheries***4(1)** 135-136.

Weatherley A, Gill H 1984: Growth dynamics of white myotomal muscle fibres in the bluntnose minnow. *Pimephalesnotatus*Rafinesque, and comparison with rainbow trout, *Salmogairdneri*Richardson. *Journal of Fish Biology***25** 13–24.

Wimberger PH 1992: Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus*(Pisces: *Cichlidae*). *Biological Journal of the Linnean Society***45(3)** 197–218.

Wohlfarth GW 1993: Heterosis for growth rate in common carp. *Aquaculture ***113*****(1-2)** 31-46.