GROWTH PERFORMANCE OF STRAIN-CROSSED HYBRID OF ROHU (*Labeo rohita*) AMONG TWO RIVERS & A HATCHERY

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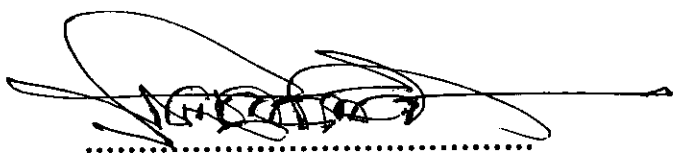

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Declaration

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The results presented in the project thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree.

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DEDICATION

This project-thesis is dedicated to **my beloved parents** who sacrificed for me, inspired and blessed me in every way of my life.

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At first all of my admiration goes to the almighty creator and ruler of the universe, God, for his great mercy to create and to keep me alive and healthy.

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Abstract

Two of the approaches for improvement of growth performance in the aquaculture species are selection and hybridization. Depending upon breeding cycle of a species, selection program can always be time consuming; however, hybridization that exploits dominant genetic variation (V_D), production of F_1 hybrids is quicker and although nicking of dominant allelic action in hybrids is fortuitous, the program is worth trying. With this aim in view, six reciprocal hybrid crosses were made between three strains of rohu (*Labeo rohita*) from the Padma, Halda and from a local Hatchery from Jessore, Bangladesh, together with control crosses of the three single strains. The participating broods were tagged intraperitoneally with digital tags and preserved in the pond facility of Bangladesh Fisheries Research Institute, Jessore campus. The fishes of the different crosses were tested for length and weight for 18 weeks in ponds. The single strain, Padma × Padma showed the highest growth performance followed by one of the reciprocal crosses between Hatchery ♂ × Padma ♀. The lowest growth performance was observed in the single strain Hatchery × Hatchery crosses which were deemed obvious as poor performance of hatchery produced seed is variously claimed by the farmers. Hybrid seed production is an established method in agriculture, in general. Thus the parents of strain crossed foregoing hybrid which were conserved with tag marks in Jessore campus of BFRI can be used for hybrid seed production in future.

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CHAPTER ONE

INTRODUCTION

Chapter 1 :Introduction

Fish and Fisheries play an important role in the social and economic life of Bangladesh in terms of income, nutrition, employment and foreign exchange earnings. The people of Bangladesh depend on fish as the principal source of animal protein. It contributes around 4.39% to the GDP and 2.46% to foreign exchange earnings through export. Fish provide 60% of national animal protein consumption. Fisheries provide livelihood to about 16.5 million people of the country directly and indirectly (DoF, 2012). It is also a major earner of foreign exchange contributing about 25,717 million taka, ranking third in terms of export items. To meet protein deficiency, to let sustain the source of employment and to keep earning of foreign currency, the country has to keep trying for increasing fish production. For this it is important to develop an appropriate method by which the farmers would be interested in raising fish production for socio-economic upliftment.

Bangladesh is situated in such a geographic location that it abounds with a large number of different water types like rivers, canals, floodplains, haors, baors, ponds, lakes, estuaries and the Bay of Bengal. The total inland water area of the country is 45,75,706 hectare (including ponds and ditches, haors, baors, beels, rivers and estuaries, shrimp or prawn farm etc.) and marine waters 1,66,0700 hectare. The waters are rich in biological diversity with 260 fresh water fish, 12 exotic fish species, 475 marine fish, 24 freshwater prawn and 36 marine shrimp species (DoF, 2012). In regard to the wealth of water and fish genetic resources, Bangladesh is the 3rd ranking country in Asia after China and India (Hussain and Mazid, 2002).

Once upon a time fish were used to be easily and sufficiently available to all over the country. But in the recent years the gap between supply and demand is increasing with increasing human population without concomitant increase in fish production. In view of the decline in fish availability, the government of Bangladesh has emphasized for increasing fish production. As a means of promoting fish production, government of Bangladesh has taken up program for increase of production different means.

The demand for supply of quality protein in the diet of the ever increasing global population is increasing. The supply of protein especially from the aquatic sources has been actively sought for. There is the potential for such supply from fisheries sector, however, the prospect from the

capture fisheries is not as promising, as it is thought that the capture fisheries has reached its maximum limit and any increase in pressure in it will bring negative impact under the existing world scenarios of capture fisheries.

For the purpose of meeting the increasing demand of protein, together with increasing harvest from capture fisheries with modern technologies, the role of increased production from aquaculture sector has been given special attention. There has been already excellent achievement in the improvement of aquaculture production through intervention in enhanced management, nutrition, disease prevention, water quality and genetic improvement of the production traits through better quality brood and seed production system. The common avenue for achievement of success in all the above areas of interventions has been the genetics of the species population and in fact many production challenges have so far been met through the application of both active and passive approaches of genetics in aquaculture throughout the world.

Aquaculture in ponds and in other closed waters is contributing increasingly in the total production in the country. Numerous farmers are engaged in pond culture with the Indian major carps, rohu, catla, mrigal, kalibausand some Chinese carps, grass carp, silver carp, bighead carp and other species like Thai punti, tilapiaetc. The three Indian major carp species satisfy all the important requirements of an ideal species for culture. But the growing carp culture industry has suffered several incidences of much mortality of carps in culture system suspected to be caused by microbial disease, inbreeding, negative selection and some other problems.

Until recent past the major sources of carp seed were the river sources. Millions of eggs and spawn of carp were collected from major rivers such as the Halda, the Jamuna, the Padma, and their tributaries during the monsoon season (May- August). About 8.8 billion seed were collected from these rivers annually. However, collection of seed from natural water has declined in recent years. This decline may be due to construction of irrigation and flood control dams, river pollution, over siltation, destruction of breeding grounds, increased use of pesticides for growing high yielding rice etc. With a view to solving this problem government of Bangladesh is laying emphasis on the production of quality seed of fast growing desirable species in hatcheries. Consequently, the carp seed produced from the hatcheries was reported to show signs of poor genetic quality, such as poor survival and growth, susceptibility to diseases, deformities, etc.

Hence, it became essential to carry out research on genetic improvement of the species through various approaches. A glance at the current state of aquaculture genetics throughout the world shows that progresses made in the aquaculture genetics could be grouped mainly in the areas of: inbreeding mitigation and maintenance of genetic quality of the breeding population; intra and interspecific hybridization for production of heterotic hybrids; genetic selection for various aquacultural traits; ploidy manipulation and sex manipulation. Genetically good quality fish seed is a prerequisite for successful aquaculture program. Fish culturists show special interest on natural fish seed due to their rapid growth rate, high disease resistance and survival rate. The hatchery produced seed show lower growth and survivability probably due to inbreeding problem in hatcheries. Although the genetic quality of fish stocks of hatchery is deteriorating day by day in Bangladesh, no good initiative has been taken at all for genetic improvement of the brood stocks in hatcheries. Fish culturists show special interest on natural fish seed due to their rapid growth rate, high disease resistance and survival rate. The hatchery produced seed show lower growth and survival probably due to inbreeding problem in hatcheries. The genetic variation of hatchery population is generally lower than that of wild populations because hatchery populations are necessarily produced from a limited numbers of parents. In addition, successive generations of domestication are accompanied by population bottlenecks and subsequent genetic drift and thus bring further constraints of decrease of genetic variation in hatchery populations. Under the present scenarios of open water fisheries resource constraints and degradations, the contribution from open water catch will continue to decline, and as such, option for increase of production from aquaculture has to be widened. The only genetic option for achieving that under the backdrop of decline of variation under hatchery production system is hybridization in the form of strain /stock crossing. Aquaculture has yet a long way to go in fulfilling the target production from closed waters. Genetic selection work, even if there may be scopes with the wild counterparts of the hatchery engaged populations, it needs longer time, costly resources and expertise to achieve success, thus the only alternative to genetic selection to achieve the goal of quicker increase of production from aquaculture is to embark upon biotechnology of line breeding and hybridization among and between lines of strains/stocks of the cultivable species.

Hybridization is a well-known means of genetic improvement, which has a wide spread application in aquaculture. Commonly, the desired goal is to produce offspring that perform

better than both parental species (hybrid vigor or positive heterosis). Hybridization (or cross breeding) can be divided into two categories: (1) intra-specific (or line crossing); crosses between strains, stocks, land races, geographic populations within a species; (2) inter-specific; crosses between species. Hybridization is practiced to achieve either of two favorable outcomes. These are; (1) heterosis or hybrid vigor, which is defined in a broad sense as an increased performance or value (to the aquabreeder) of progeny above the average of the parental performances or value, and (2) non-heterotic effects which is the performance of the progeny as the result of simple combination of parental genotypes. Biologically species are protected gene pools and hybridization that is defined as interbreeding of two good species is opposing to that rule. However, as it is obvious, hybridization is the result of occasional break down of isolation of species and thus it is regarded as a rare and incidental phenomenon in nature. Hybridization is more common in fish than in any other groups of vertebrates.

The biotechnological approaches of genetic screening, selection and crossbreeding or hybridization within species (crossing between different strains) often produces good heterosis. Of these approaches, genetic selection could be applied but it takes longer time in producing expected gains over several generations especially in species having longer generation time of 2-3 years in the local carps. Again selection can bring outcome only in presence of suitable amount of heritability and heritability is rapidly reduced under inbreeding in hatchery production system. Cross breeding in fish has been proved successful in growth increase in many studies. Also the approach is quicker in response than in any other approaches. The inter-strain cross breeding biotechnology in rohu, can bring a revolution in pond aquaculture in Bangladesh creating a long lasting impact in fish production increase in the country.

Interbreeding of two good species is denoted as hybridization. Hybridization is the mating of genetically differentiated individuals or groups and may involve crossing individuals within a species (also known as intra-specific hybridization or line crossing or strain crossing) or crossing individuals between separate species. This breeding technique is used by aquaculturists in the hope of producing aquatic organisms with desired traits. Commonly, the desired goal is to produce offspring that perform better than both parental species (hybrid vigor or positive heterosis). Hybridization may also be used to transfer other desirable characteristics from one group or species to another, to combine valuable traits from two species, and to produce sterile

individuals. Although crossbreeding is a tried-and-true method of increasing yields, the results of crossbreeding programmes are impossible to predict (unless the mating has been made previously). Genetic variation had three sub-components, V_A, V_D and V_I (Additive, Dominant and Epistatic genetic variation respectively).

When a little or no V_A exists and it is either difficult or impossible to improve a phenotype by selection, the breeding technique that can be used to improve the productivity is hybridization. Hybridization improves productivity by exploiting V_D. It is possible to use hybridization to exploit V_D even when a large number of V_A exists. V_D is created anew and in different combination each generation and its effect are basically those based on luck. Therefore, production of superior F_1 hybrid is a hit or miss proposition. Sometimes someone can be lucky or sometimes he/she may not. He/she can mate two superior fish and produce nothing but culls or he/she can mate two culls and produce a grand champion. Fish that are superior because of certain interaction are superior because of fortuitous combination of alleles. Unfortunately these fish can not transmit their superiority to their progeny. Consequently, the goal in a cross breeding program is to discover which combination of parents produces the combination of alleles that produces the desirable interaction in the progeny, and thus improves productivity (Tave, 1993). Of the different components of variations for improvement of fish production, the V_A, additive genetic variation or breeding value is exploited by genetic selection, however, if inbreeding is frequent, the additive genetic variability is reduced to such an extent that selection cannot provide easy option for improvement and in that case the only option that remains open for the breeder is to go for cross breeding / strain crossing program to resort to exploiting V_D, dominant genetic variation created by dominant action of alleles in individual loci (Tave, 1993). The Indian major carp species concerned, all have been in aquaculture and seed production system in the hatchery in the country for several years since. These species have their natural counterpart populations in the rivers; however, seldom they are exchanged with the natural stocks. It is thus apprehended that all the populations employed at different pocket areas of the country have attained certain degrees of genetic distinctness which might offer chances for obtaining heterotic effect in intra strain crossing.

Fish hybridize relatively easily, and the variety of species crosses and even wider taxonomic combination is vast. Hybridization can be incorporated into a selection program as a final step to

produce animals and plants for grow out. In this case, fish are selected in two lines to produce good hybrids; after the selection program selected lines are hybridized. Hybridization may also be used to transfer other desirable characteristics from one group or species to another, to combine valuable traits from two species into a single group, and to produce sterile individuals.

Another use of hybridization is found for the production of new breeds or strains. Hybridization can also be used to produce uniform products. Processing plants and consumers often desire uniform products, and hybridization is the most efficient method of producing uniform progeny. Production of monosex population can be accomplished by hybridization. Hybridization is also effectively used for the production of hybrid to be stocked in natural bodies of water that are unable to maintain self reproducing populations. In crop agriculture, hybrid seed technology has already brought a revolution in Bangladesh in increase of production in cereals, pulses, roots/tubers, fruits and vegetables etc. Fish, in this case, cannot lag behind. Presently rohuis cultured in Bangladesh with two to three other Indian major carps species under polyculture system with hatchery produced seed; the seeds are inbred under hatchery production system and thus the rate of production is extremely low; the average production per hectare is only about 5 tons. With hybrid seeds, with heterotic effects, it is easily possible to increase the production to several folds higher. If the production of heterotic hybrid is possible, the carp aquaculture production can bring a revolution in the country. The pond and many other closed water aquaculture is done by the Indian major carps and rohu being the premier species in the sector, numerous marginal fish farmers in the country will be benefited from this technology. So, the research on strain crossing for F_1heteroticrohu will help increasing production by using heterotic hybrid seed lessening the demand of seed production for culture with the single species like presently being done.

In this study, attempts were made to produce heterotic hybrid of rohu that would contribute in the increase of production in the aquaculture sector of the country because this sector now requires quick growing; low valued but popular fish species in the market. With this aim in view, six reciprocal hybrid crosses were made between three strains of rohu (*Labeo rohita*) from the Padma, Halda and from a local Hatchery ofJessore, Bangladesh, together with control crosses of the three single strains. The participating broods were tagged intraperitoneally with electronic tags and preserved in the pond facility of Bangladesh Fisheries Research Institute, Jessore

campus. The fishes of the different crosses were growth tested for length and weight for 18 weeks in ponds. The single strain, Padma × Padma showed the highest growth performance followed by one of the reciprocal crosses between Padma♀ × Hatchery♂. The lowest growth performance was observed in Hatchery × Hatchery crosses which was deemed obvious as poor performance of hatchery produced seed is variously claimed by the farmers. The study was also aimed to determine the heterosis of the strains from the better growing crosses by molecular techniques using microsatellite and RAPD marker; however, those findings are not part of this paper. Thus heterotic hybrid production technique can be introduced in Bangladesh to improve the genetic quality of fish which will enhance aquaculture production and culture efficiency as well.

Objectives of the study

- To produce F_1 hybrid by reciprocal hybridization among different strains of rohu.
- To compare the growth performance of newly produced crosses.

CHAPTER TWO

LITERATURE REVIEW

Chapter 2: Literature Review

Many researchers carried out hybridization in fishes in different parts of the world; in recent time there has been growing awareness among researcher in Bangladesh to study about Hybridization and growth performance of the hybrids, especially in Indian major carps species. Webber and Riordan (1976), stated that successful implementation of culture based capture fisheries depends on the availability of sufficient quantities of good quality fish seed. Regular supply of fish seed is the pre-requisite for the development of aquaculture.Ahmed *et al.* (2008) showed that inland aquaculture in Bangladesh is mainly consisted of the Indian major carps (Catla, Rohu and Mrigal) which are commercially important fish species widely cultured in the entire Indian sub-continent.

Simonsen *et al.*(2005); Ahmed *et al.* (2008) showed that the three Indian major carp species Catla (*Calta catla*), Mrigal (*Cirrhinus cirrhosus)* and Rohu (*Labeo rohita*) are major contributors and potential candidate species to the aquaculture and inland fisheries production of Bangladesh and India having a high demand in the domestic market. Hussain and Mazid (2001) stated that the three species of Indian major carps are reared in polyculture in ponds, based on assumption that the different species have the different performance for water depth, thereby utilizing different parts of water column.

Hussain and Mazid (2005) showed in an attempt to produce more seed to meet the increasing demand, the seed producers did not pay attention to the genetic quality.Hussain and Mazid (2001) showed that in recent years production from open water catch registered decline due to wide spread habitat degradation, while experiencing a rise in aquaculture production; the present aquaculture production accounts for nearly 40% of total fish production in Bangladesh.

Shah (2004) stated that the genetic variation of hatchery population is generally lower than that of wild populations because hatchery populations are necessarily produced from a limited numbers of parents, negative selection and smaller brood size at first maturity. Shah (2000) indicates research needs in fish breeding and genetics in Bangladesh and points out the problems on genetic management of the brood stock and hatchery in Bangladesh. Shikano *et al.*(2008) stated that both the qualitative and quantitative genetic characteristics of hatchery produced individuals can differ from those of wild individuals due to population bottlenecks and

subsequent genetic drift, as well as unintentional selection through successive generations of domestication. Such genetic alterations in qualitative and quantitative traits may affect the fitness and growth of hatchery-produced-individuals.

Yan and Ozgunen (1993) stated thatheterosis achieved through interspecific and/or intraspecific strain crossing enables offspring to surpass its parents for one or more traits. Heterosis is affected in F_1 generation through individual loci interaction. It was accompanied by heterosis in growth rate and viability, especially when fish from different highly inbred groups are crossed. Chaudhuri (1999) and Kowtal (1987) reviewed all the hybridization works carried out in India with indigenous and exotic carps and listed the promising hybrids.

Das *et al.*(1980) stated that the performance of the best Indian Major Carp's hybrids are compared with their parent species which was again intermediate in performance, being better than Rohu but not better than catla. Dunham and Smitherman (1983) and Dunham (1996) reported 55% increase in growth rate of channel catfish intraspecific hybrid; in rainbow trout the crossbreds had 22 percent increase in growth rate. Dunham *et al* (2001) state that strain crossing and realization of positive heterosis is now an important basis of common carp aquaculture in Israel, Vietnam and Hungary. Tave (1993) suggested hybridization of closely related species producing better results.

Basavaraju *et al.* (1995) said that in India they did not get better performing hybrids in reciprocal crosses between rohu and mrigal; however, they observed *Labeorohita*♂× *Cirrhinamrigala*♀ hybrid growing faster than the reciprocal ones. Ibrahim (1977) suggested that rohu female ×mrigal male hybrid had better growth than the parents.

Bartley *et al.* (2004) reported that hybridization is widely used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase disease resistance, and to improve environmental tolerance. Hybrids may also be used to exploit degraded aquatic environments.David and Pandian (2006) stated that in general, hybridization between species often results in offspring that are sterile or have diminished reproductive capacity. According to Tave (1993) it is a quick and dirty method of improvements before selection is employed. Any efforts for improvements of production traits in fish require exploitation of genetic variation.

Islam and Shah (2007) produced 55 percent heterosis in crossbred progenies between hatchery originated strain and Padma River originated stock of rohu. Hussain and Mazid (2002) report crosses of three strains of Thai sharpunti, *Barbodes gonionotus* from Bangladesh, Thailand and Indonesia; growth rate of the females was obtained at 23 percent higher than the parental lines. Moav *et al.* (1978, 1979) and Wohlfarth (1983) outlined program in which hatchery strain can be stocked to hybridize with local population in order to produce faster growing hybrids that can be harvested by commercial fisherman.

CHAPTER THREE

METERIALS AND METHODS

Chapter 3: Materials and Methods

3.1. Experimental Design

The experiment was carried out in two phases; production of reciprocal hybrids by interspecific crossing of different strains and second stage of the experiment was rearing the spawn in earthen nursery ponds. Growth performance data of the crosses were collected by sampling fortnightly. The first phase of the experiment was conducted at the hatchery facilities of Basepara Fish Seed Multiplication and Extension Farm in Jessore on 29/06/2013, 08/07/2013, and 30/07/2013. The 3 days hatchlings were carried in oxygenated poly-bags to the experimental ponds of Khulna University Campus. The second phase of the experiment, rearing of fry was carried out in the earthen ponds at Khulna University campus. Culture of the crosses was continued for 18 weeks with regular basis of management and sampling.

3.2. Brood collection and maintenance

Three different strains of the broods were collected from two rivers and a hatchery *viz.*, the Padma, the Halda and the hatchery, Basepara Fish Seed Multiplication Farm, Jessore. The river strains were collected from brood bank of Jessore Freshwater Sub-station of BFRI. Five pair broods were collected from each source with equal number of sex. All the broods were carried to the hatchery in oxygenated poly-bags. The broods were kept in separate tanks in the hatchery.

3.3. Brood Selection

Fish having matured gonads were treated as brood fish. Selection of brood fish was the main task on which successful spawning mostly depends on. Improper selection of brood may results poor quality eggs or fish may not spawn at all. The mature spawners were selected based on the following criteria: females have prominent, bulged and soft abdomen; genital opening swollen, protruding, pink to reddish color; vent swollen and reddish; smoothness of pectoral. However, the males have oozing of milt by soft pressure on abdomen; roughness of pectoral fin.

3.4. Induced breeding

The selected broods collected from the stocking pond were placed in rectangular spawning (cistern) tank. Continuous water flow was provided in the tanks by showering of water. The fish were kept in the tank for about 24 hours. Showering of water made the fishes ready for spawning. The sex ratio of the spawners was 1:1 of male and female respectively. Ovulin was used for artificial propagation as an extrulizing agent. Hormone was given to the brood fish as a rate of 1cc/6 kg fish.Spawners were obtained ready for spawning within a few hours after injection. Time required for induced breeding depends on the maturity condition of the spawners, hormone quality and environmental condition. It was taken 6 hours in the experiment.

3.5. Stripping, hybridization and fertilization

The male and females were stripped very carefully by applying gentle downward pressure with the thumb and index fingers from just below the pectoral fins up to the genital opening of the fish. At first female was stripped and eggs were collected in a bowl. In the mean time respective male were stripped and collected milt was mixed well with previously collected eggs. From three strains, six reciprocal hybrid cross and three controls were performed. The inseminated eggs were then transferred into nine incubation tank provided with continuous water flow. Fertilized eggs hatched in this condition.

The crosses were as follows:

Cross-1	Hatchery♂ X Padma ♀	Tank 1
Cross-2	Halda ♂ X Halda ♀	Tank 2
Cross-3	Padma ♂ X Halda ♀	Tank 3
Cross-4	Halda ♂ X Hatchery ♀	Tank 4
Cross-5	Padma ♂ X Hatchery ♀	Tank 5
Cross-6	Hatchery ♂ X Hatchery ♀	Tank 6
Cross-7	Padma ♂ X Padma ♀	Tank 7
Cross-8	Hatchery ♂ X Halda ♀	Tank 8
Cross-9	Halda ♂ X Padma ♀	Tank 9

3.6. Experimental ponds

The experiment was conducted in experimental ponds of Khulna University campus. The size of the rearing ponds was two decimal. The ponds were rectangular in shape and all the ponds were given identity with respect to stocked crosses.

3.7. Pond preparation

It is essential to prepare the ponds before stocking of fish. Better condition of pond is essential for the better growth as well as for the survival of fry. To ensure the adequate primary production in the ponds before stocking, pond preparation was done three weeks before stocking of fry. Steps of pond preparation were as follows-

3.7.1. Drying

Experimental ponds were dried to improve the condition of the ponds and to remove the aquatic weed, bottom mud, and unexpected fauna.For drying purpose the water of the pond was first pumped out and then dried in sun at least for two weeks.

3.7.2. Liming

After 2-3 days of drying liming was done in the ponds. Lime controls the pH that balances acidity and alkalinity of soil and water. The dose depends upon soil conditions of the ponds. Calcium carbonate ($CaCO_3$) was used as a liming agent. In the experimental ponds liming was done at a dose of 1.5 kg / decimal.

3.7.3. Watering

After proper drying and liming, the ponds were filled with water up to the depth of 3 feet. The level of optimum depth of water in the ponds was maintained by a shallow water pump, situated at the farm site.

3.7.4. Predator control

In order to control predator and other undesirable fishes, amphibians, reptiles, insects, frogs' etc, rotenone was used at the rate of 25gm/decimal. Bushes on the banks of the ponds were removed

manually to destroy the habitat of other predators like otters, snakes, etc. The total area of the ponds was fenced so that undesirable organisms cannot enter into the pond from outside.

3.8. Transportation of fry

The control and reciprocal hybrids of three different strains produced in the hatchery were transferred to the rearing ponds at Khulna University campus in oxygenated poly-bags.

3.9. Stocking

Fry was stocked in the morning. Before releasing in the pond, acclimatization of fry in the new environment was done by keeping the poly-bags in pond water for a while and then gradually mixing pond's water with fry containing water, which was very important to reduce mortality. Each of the ponds was 2 decimal and the stocking density was 6500-7000 pcs/2dec.

3.10. Post stocking management

Post stocking management is important for the better survival and growth of fry. After stocking fry in the rearing ponds, adequate primary production was maintained to ensure proper natural feed supply to the fry. It was also tried to maintain good water quality throughout the rearing period. The post stocking management included the following steps:

3.10.1. Feeding

After ensuring enough primary production, fry was stocked to the ponds. Fry was reared eighteen weeks in the ponds. The feed was given four times in a day. At the first week 100gm/2dcml feed was given. Then after every 5 days the amount increased by 5 gm.Three types of feed were given, they are Finisher feed, mega feed and floating pellets.

3.10.2. Monitoring

To maintain the water quality of the ponds at an optimum level all sorts of measures were taken. The green vegetation in the pond was removed manually, the floating leafs and other materials were removed by netting, the toxic gases in the ponds were removed through harrowing in the ponds. Liming was done for pond hygiene maintenance by reducing acidity of water. Water level of the ponds was maintained optimum by pumping water whenever necessary

3.10.3. Diseases and parasites

Disease often causes havoc in the experimental ponds. So, its control is essential. However there was no disease and parasites found in the fishes during experimental period.

3.11. Sampling

The objective of the study was to compare the growth of normal spawns and the reciprocal hybrids of different strain. Therefore sampling was done to measure the weight and length of rearing fishes. Sampling was done fortnightly by drawing small mesh net in the pond and then sample was taken randomly from the whole collection. Same technique was followed in each pond. In the first three samplings only the average weight of the fishes was taken because during this period the fishes were small and not allowed to take the length properly. In the next samplings fry was measured in both weight and length individually. An electric balance with up to 0.1 mg denomination and a centimeter scale up to 1mm graduation were used for weight and length measurement respectively. The growth data were recorded in the sampling sheet.

3.12. Data analysis

Qualitative and quantitative analysis of all kinds of data were carried out. MS Excel was used to store all the growth data for length and weight. MS Excel was also used for presentation of the tables and graphs from of different types of data.

CHAPTER FOUR

RESULT

Chapter 4: Results

The experiment was carried out in two phases for five months from 29/06/13 to 23/11/13. Firstly, induced breeding was done in the "Basepara Fish Seed Multiplication and Extension Farm in Jessore". Hybrid was produced from intra-specific crossing among different strains of *Labeo rohita* from the Padma, the Halda and hatchery strain. Secondly, the spawn were reared in hapa in the hatchery for two days and transferred to the experimental ponds in Khulna University campus for culture of the crosses. Growth was measured by checking weight and length data fortnightly. The study revealed that pure cross Padma ♂ × Padma♀ performed the better growth rather than that of other crosses. Moreover, one of the hybrid strains Hatchery♂ × Padma♀ showed good performance, ever better than Halda×Halda prure cross. In addition, Hatchery♀ × Hatchery♂ showed the worst performance among all the crosses.

4.1. Growth Measurement

The second phase of the experiment for growth check of the crosses was performed in the experimental ponds of Khulna University for culture of the crosses. Growth of the crosses was checked fortnightly throughout the culture period. The study revealed that the better growth was in the pure cross of Padma ♂ × Padma ♀ and the highest observed mean value was found to be 34.39 g. The lowest value at the last sampling was found to be 3.21 g for the other pure cross Hatchery ♀ × Hatchery♂. The hybrid cross Hatchery♂ × Padma♀ showed the best growth performance, 26.706 g among all the hybrid crosses. Last sampling of other crosses presented the weight of 24.81 g, 5.411 g, 6.415 g, 5.97 g, 12.503 g, 6.139 g for the crosses of Halda♂ × Halda♀, Padma♂ × Hatchery♀, Hatchery♂ × Halda♀, Halda♂ × Hatchery♀, Halda♂ × Padma♀ and Padma♂ × Halda♀ respectively (Table 4.1).

Table 4.1 Average weight (g) of hybrids and single strain crosses of rohu

No of sampling	Padma ♂ × Padma♀	Halda♂ × Halda♀	Hatchery♀ × Hatchery♂	Hatchery♂ × Padma♀	Padma♂ × Hatchery♀	Hatchery♂ × Halda♀	Halda♂ × Hatchery♀	Halda♂ × Padma♀	Padma♂ × Halda♀
1	0.017	0.013	0.78	0.067	0.103	0.143	0.073	0.163	0.105
2	0.282	0.062	0.186	0.265	0.197	0.313	0.149	0.429	0.192
3	0.6	0.232	0.37	5.824	0.667	1.451	0.243	3.521	1.394

4	7.189	8.744	0.553	8.589	2.249	1.726	0.383	6.115	3.011
5	13.962	13.195	0.66	17.766	3.167	3.188	3.269	9.497	3.419
6	24.817	17.18	1.57	20.203	3.521	4.052	4.05	10.519	5.307
7	34.39	24.81	3.21	26.706	5.411	6.415	5.97	12.503	6.139

4.2. Length measurement

In the second phase another dimension of growth measurement was recording of lengths during all the sampling except sampling 1-3 for the smaller size of fry. The study revealed that the better length was found to be 15.16 cm during the last sampling in the pure cross of Padma ♂ × Padma ♀.The lowest value at the last sampling was found to be 6.13 cm for the other pure cross Hatchery ♀ × Hatchery♂. The hybrid cross Hatchery♂ × Padma♀ showed the better growth performance, 13.14 cm among all the hybrid crosses. Last sampling of other crosses presented the length of 13.97 cm, 7.62 cm, 8.45 cm, 8.48 cm, 9.82 cm, 7.62 cm for the crosses of Halda♂ × Halda♀, Padma♂ × Hatchery♀, Hatchery♂ × Halda♀, Halda♂ × Hatchery♀, Halda♂ × Padma♀ and Padma♂ × Halda♀ respectively (Table 4.2).

Table 4.2 Average length (cm) in hybrids and single strains crosses of rohu

No of sampling	Padma♂ × Padma♀	Halda♂ × Halda♀	Hatchery♀ × Hatchery♂	Hatchery♂ × Padma♀	Padma♂ × Hatchery♀	Hatchery♂ × Halda♀	Halda♂ × Hatchery♀	Halda♂ × Padma♀	Padma♂ × Halda♀
1	7.64	8.15	3.97	7.48	3.24	3.62	3.47	5.55	3.24
2	10.1	9.2	4.18	8.55	5.61	4.03	6.36	6.98	5.61
3	12.65	12.79	4.95	10.75	6.42	6.14	6.97	8.37	6.42
4	14.04	12.85	6.13	11.81	7.13	6.71	8.48	9.09	7.13
5	15.16	13.97		13.14	7.62	8.45		9.82	7.62

4.3. Water quality parameters

Different water quality parameters such as temperature, pH, free CO_2, alkalinity, acidity, hardness and salinity were collected fortnightly during the study period. Temperature of the experimental ponds ranged between 31.3-36.9 °C; whereas pH was ranged from 7-8.5. Moreover, other important water quality parameters of the experimental ponds were within the acceptable range of the culture (Table 4.3 and 4.4).

Table 4.3 Range and mean value of environmental parameters throughout the culture period of different crosses of rohu

Parameters	Range	Mean Value
Water Temperature(°C)	31.3-36.9	33.78369
Alkalinity(mg/l)	150-500	275.947917
Hardness(mg/l)	200-980	585.9611
Free CO2(mg/l)	13.20-66	35.01874
pH	7-8.5	7.083646
Acidity(mg/l)	15-80	40.41005

Table 4.4 Average value of the environmental parameters in the ponds of the corresponding to cross

Cross No.	pH	Temperature (°C)	FreeCO_2 (mg/l)	Alkalinity (mg/l)	Acidity (mg/l)	Hardness (mg/l)	Salinity
1	7.27407	35.2222	18.9037	222.2222	21.48148	942.222	<0.5
2	7.307407	35.44074	18.41481	164.8148	20.92593	776.4815	<0.5
3	7.274074	35.2222	18.9037	222.2222	21.48142	591.1111	<0.5
4	7.67142	33.3714	51.3711	254.0427	53.8095	479.286	<0.5
5	7.22381	32.0877	48.6048	314.2857	59.5238	363.3332	<0.5
6	7.41429	33.3714	53.3143	238.0998	65.2381	362.3312	<0.5
7	7.32450	35.04077	35.10031	235.1582	65.5214	336.7123	<0.5
8	7.29403	34.3176	28.49035	216.1270	25.1083	845.1590	<0.5
9	7.30241	35.19802	20.5403	312.1041	26.1035	744.2381	<0.5

CHAPTER FIVE

DISCUSSION

Chapter 5: Discussion

In Bangladesh Indian major carps breeding technologies have appeared to extent well at the farmers' level. There has been a revolution in this sector. Numerous hatcheries have been established in the private sector. The hatchery operators do not have strict knowledge about genetic norm and practice of brood stock management and fish breeding. Most of the hatcheries maintain small number of broods and there are also the tendencies for breeding small sized broods. Under such a situation, inbreeding, negative selection and introgression are very much likely.

In Bangladesh Indian major carps constitute the principal species for pond culture. Prior to 1990, most of demand for carp-seed was met from naturally collected seeds and they used to grow well. But for the last two decades or so, when a large number of hatcheries were established in different parts of the country, situation began to alter. During this period a wide spread claim has been recorded from the fish farmers that hatchery originated seed do not grew well, having less survival and less production performance etc.

Hussain and Mazid (1997) reported the widespread incidence of inbreeding depression in the hatchery-produced seed in Bangladesh; the same is realized in the form of retarded growth, poor reproductive performance, morphological deformities, and increased incidence of disease and mortality of seed in the hatcheries. The study of Hussain and Mazid (1999) does not appear to have formally based on looking the amount of genetic variation in the hatchery stock, rather popularly writing about the existing management status of the hatchery and brood stock. Shah (2000) reported that there is a lack in the integration of genetic norm and practice in the hatchery management system of Bangladesh. The brood fish are not managed with proper records as to their origin. More over the hatcheries in the country are run in more or less isolated conditions; there is replenishment in the stocks, but their identity as to their origin are rarely correctly known and recorded.

Inbreeding occurs when mating takes place between related individuals. There are some deleterious recessive alleles which do not quote for functional proteins. The probability of pairing of the deleterious recessive alleles increases with the increase of the relationship between the parents. The pairing of the deleterious recessive alleles and their subsequent expression in the

phenotypes is the prime reason why inbreeding is given a bad connotation (Falconer 1981). The Additive Genetic Variation (V_A) is created by the cumulative action of alleles across the loci and Tave (1993) suggests that in an inbred population with very little amount of V_A or no V_A present at all, making it difficult to bring changes in production performance through selection, the only option that is left to increase of production is hybridization. Through hybridization, Dominant Genetic Variation (V_D) is exploited.

In the present study F_1 hybrid was produced by intraspecific crossing among different strain of *Labeo rohita* with the expectation that there will be increased incidence of heterozygosis through exploitation of V_D. In this experiment, the mean body weights of 9 different crosses reached at 34.39g, 24.81g, 3.21g, 26.706g, 5.411g, 6.415g, 5.97g, 12.503g and 6.139g respectively (Table 1). Cross-1 (Padma ♂X Padma ♀) showed the best growth performance among of all the crosses. The best growth performance of Cross-1 could be most probably ascribed to the pure heterozygosity in the offspring produced from crossing of same riverine strain. The growth performance of riverine strain was also found better than the hatchery strain indicating the slowest growth rate of hatchery strains probably due to accumulation of inbreeding and small effective number of broods. In the present study the second highest growth was found in reciprocal cross Hatchery ♂X Padma♀. In the experiment, the mean body lengths (total length) of 9 different crosses reached at 15.16cm, 13.97cm, 6.13cm, 13.14cm, 8.45cm, 9.82cm, 7.62cm, 7.82cm and 8.48cm rspectively(Table 2). At the backdrop of the prevailing complaints by the pond culturists of Bangladesh about the poor performance of the hatchery originated fish seed of the Indian major carps, an experiment was conducted by Shah (2004) on the growth comparison of the three species *Labeo rohita, Cirrhinus cerrhosus*and *Catla catla* from two major rivers Jamuna and Padma with Hatchery. Three experiments were carried out for 18 months which revealed that riverine sources' fry has a better growth than the hatchery sources. The present experiment supports the idea and findings of the results of Islam and Shah (2004). Poor performance of the hatchery-produced seed in comparison with the riverine sources, in the present experiment might imply that the broods in the hatchery are under inbreeding pressure.

There are available references on the use and applicability of strain crossing for increased vigor of the offspring produced (Moav and Wohlfarth, 1974;Kirpichnikov, 1981;Wohlfarth, 1993; Bakos and Gorda, 1995;Hulata, 1995). Islam and Shah (2007) noticed better growth performance

in the reciprocal intra-specific hybrids of hatchery and Jamuna strains of rohu. They also noticed that "Jamuna female x hatchery male" had comparatively better growth increment. Biswas *et al.* (2008) obtained better growth performance of riverine strains of Indian major carps over the hatchery strains. Dunham and Smitherman (1983) reported 55% increase in growth rate of channel catfish intra-specific hybrid and Dunham (1996) reported 22% increase in growth rate of rainbow trout crossbreed.

In this experiment some hybrids also generally showed better growth but not more than their parental strain. This is probably due to the surpass of good quality straits of parents in their hybrids. Eighteen weeks comparative growth study of F_1 hybrid and normal spawns revealed the higher growth rate of normal spawns than hybrid.

From the experiment it was found that Padma ♂X Padma ♀ showed better growth than all other that was 34.39 gm. On the other hand, Hatchery♂ XPadma ♀, showed better result which was 26.706 gm but their reciprocal hybrid Padma ♂ X Hatchery ♀ produced 5.41g only. Though the hatchery population is in inbreeding condition, yet the successful management of brood and if they are breed with distant population, there is a possibility to increase the heterozygosity by exploiting dominant genetic variance (V_D). This can be possible even crossing two distant hatchery populations.

The quality of the hybrid is simply a matter of luck. Chappel (1979) found that certain intraspecific hybrid catfish were as good as their more famous cousin, the blue ♂ X channel ♀hybrid. Tave (1993) suggests that if the V_A that contributes to the selection response, is lost or present in less amount, there cannot be any means of increasing the production performance, but definitely such population can be hybridize with other out bred population to increase heterozygosity. Hatchery strains will be stocked to hybridize with local population in order to produce faster growing hybrids that can be harvested by commercial fishermen. (Moav *et al.*, 1978, 1979).

Dunham *et al.*, (1986) studied the growth rates of the *Cirrhinusmrigala*♂× *L. rohita* ♀ and the reciprocal hybrids and compared them with that of the parental species, which were also reared along with the hybrids. This study has shown that the parental species, *L. rohita* and *C. mrigala* grew faster than both the hybrids. Again, among the two hybrids the hybrid between *L. rohita*

♂× *Cirrhinusmrigala* ♀ grew faster than the reciprocal *Cirrhinusmrigala*♂× *L. rohita* ♀ hybrid. Whereas the observations of Ibrahim (1977) have shown that growth rate of the *Cirrhinusmrigala*♂× *L. rohita* ♀ was better than the parent species. Likewise the rohu*(Labeorohita)* x catla (*Carla catla*) hybrid grows almost as fast as pure catla, but has the small head of the rohu considered desirable in Indian aquaculture (Reddy, 2000). *Catlacatla* x *Labeo fimbriatus* (fringe-lipped peninsula carp) hybrids have the small heads of *L. fimbriatus,* plus the deep body and growth rate of catla (Basavaraju *et al.*, 1995).

In the culture period some water quality parameters were measured which was in the acceptable range. Fish is poikilotherms. So, growth, reproduction and other biological activities of fish are controlled by temperature. For 1°C rise of water temperature metabolic rate of fish increase 10%. In very low temperature fish growth stops and in extremely high temperature it will die. Temperature of water was found to vary from 31.3° C to 36.9° C. Maximum temperature 36.9°C was recorded in July where minimum temperature was 31.3° C in November. Ali *et al.* (1982) stated that 20.5°C-33.5°C is favorable for fish culture. As result of generally high temperature the metabolic rate of the fishes increased, but there feeding rate was same. So it may be effect the growth rate.

pH is considered as an important factor in fish culture. It indicates the acidity-alkalinity condition of a water body. The range of water pH in different ponds during the study period was varied from 7 to 8.5. Sarker (2000) noted that the range of pH value 7-8.5 is suitable for fish culture but fish died when pH reached more than 11. Andrew *et al.* (1972) stated that waters having a pH range from 6.7 to 8.6 are generally support a good fish population.

Transparency is one of the complex environmental factors that have profound effect on all the aquatic organisms. Penetration of light in an aquatic habitat mainly depends on the clearness of the water as it is essential for photosynthesis of aquatic plants. In the present experiment, the range of water transparency was found to be 25-34cm throughout the rearing period and this range is suitable for growth of aquatic plants in ponds Ali *et al.* (1982).

CHAPTER SIX

CONCLUSION

Chapter 6: Conclusion

Hybridization is a process by which heterogygosity in the progenies can be produced and thus hybrid vigor or heterosis is achieved. In the present experiment F_1 generation of Padma female X Padma male of rohu showed better growth than either of the single strain. But the Hatchery male and Padma female showed acomperatively better growth than other reciprocals. This result can be considered as promising especially at the backdrop of the decline of the performance of the hatchery breed seed of the species. From the present study it could be concluded that though the production of heterotic hybrid is a chance proposition, however, if the populations employed happens to be inbred, the chances for obtaining the superior hybrid increases. Incase of inbreeding problem the heterogygosity is lost because of close mating. So it is necessary to carry out hybridization in the strains to overcome inbreeding problem and to achieve heterosis for better growth and production in the aquacultural systems in the country.

CHAPTER SEVEN

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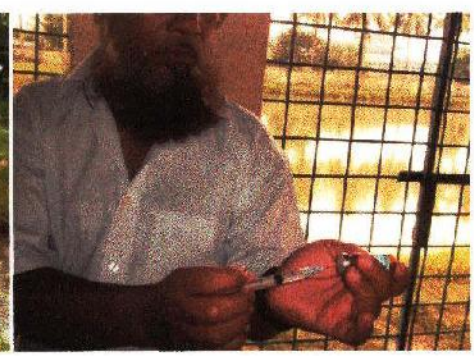
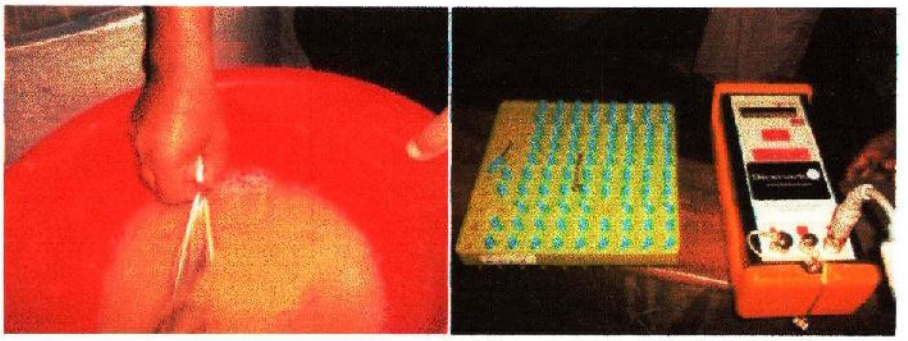
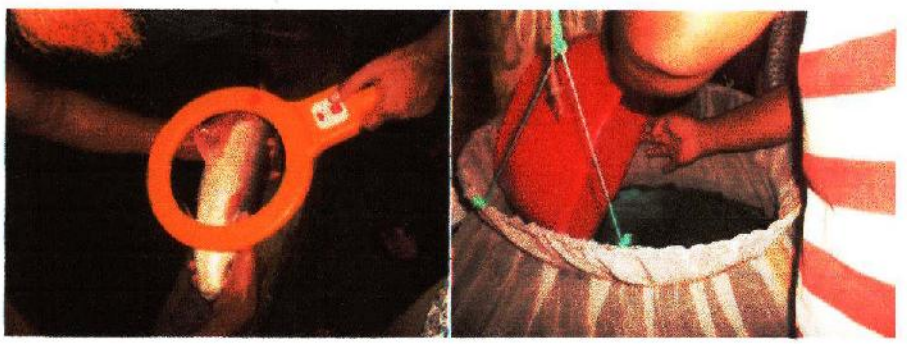
PHOTOGRAPHIC PLATES







Sampling sheet

TITLE:

Growth Performance of Strain-Crossed Hybrid of Rohu (*Labeo rohita*) Among Two Rivers & A Hatchery

.Date of sampling:

Name of the strain: Rohu (*Labeo rohita*) &HYBRID.

Type of stocking:

- ❖ Normal Spawn :
- ❖ Hybrid :

Pond No:1-type of cross

Sl no	*Tw (Gm)*	*Tl (cm)*	*Sl no*	*Tw (Gm)*	*Tl (cm)*
1			16		
2			17		
3			18		
4			19		
5			20		
6			21		
7			22		
8			23		
9			24		
10			25		
11			26		
12			27		
13			28		
14			29		
15			30		

N.B.: SL- Serial no, Tw- Total weight, Tl- Total length.

PLACE: KHULNA UNIVERSITY CAMPUS