HETEROSIS ANALYSIS IN HYBRIDS BETWEEN DIFFERENT STRAINS OF MRIGAL (*Cirrhinus cirrhosus*)

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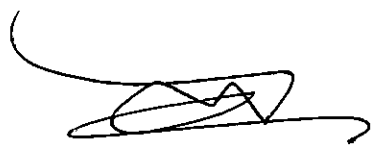

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Declaration

HETEROSIS ANALYSIS IN HYBRIDS BETWEEN DIFFERENT STRAINS OF MRIGAL (*Cirrhinus cirrhosus*)

The results presented in the 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 thesis is dedicated to **my beloved parents** who sacrificed for me, inspired and blessed me in every way of my life.

Acknowledgement

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

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ABSTRACT

Hybridization is conducted for the production of hybrid vigor and often regarded as quick method of increasing production. A 72 day study was carried out to determine heterosis in Mrigal (*Cirrhinus cirrhosus*) by comparing growth of the hybrid (strain of the river Padma X strain of hatchery) with that of the pure-bred. The fertilization and hatching rates were assayed and found to be lower in hybrid than in parents. Hatchlings were reared in earthen nursery ponds for 70 days. Growth in weight and length were measured for comparison and the analysis showed significant differences ($P < 0.05$) in the growth rates between the hybrid and the pure-bred, which produced 30.51% heterosis. The results obtained can be considered as preliminary in strain crossing and needs further work in this line in future.

Key words: Strain crossing, mrigal, heterosis.

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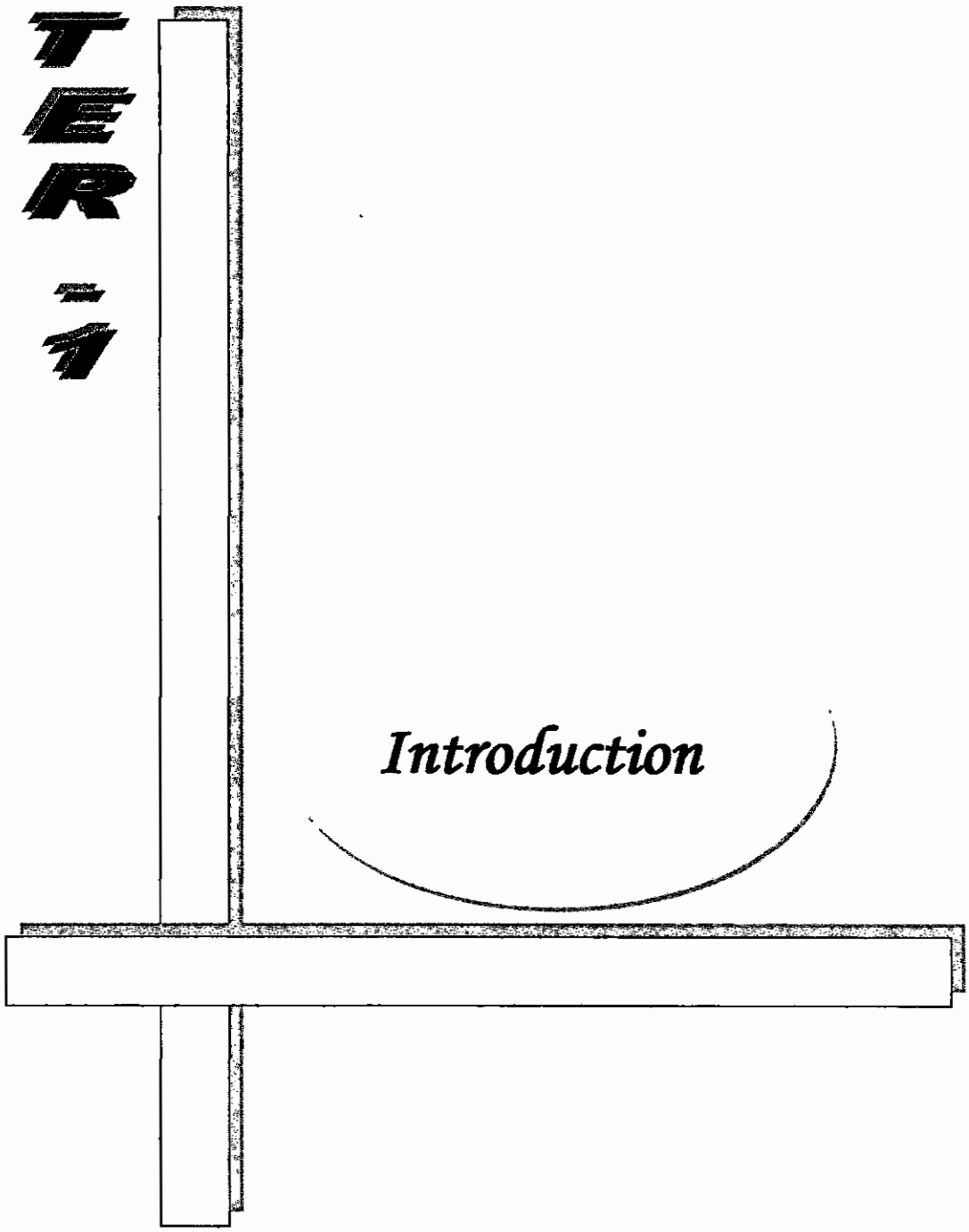
CHAPTER 1



Introduction

INTRODUCTION

Bangladesh, a country of deltaic plains dominated by the main major river systems like the Ganges, the Brahmaputra and the Meghna, is endowed with unique water resources comprising both inland and marine waters. Along with potential water resources, the country is also rich in the diversity of various fish species. 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). And the fisheries resources of Bangladesh represents about one percent of the total world fish catch (FAO, 1997).

The country is blessed with a vast expanse of inland waters in the form of rivers, canals, depressions, reservoir, ox-bow lakes, ponds, tanks, seasonally flooded areas, and others. According to an estimation, the inland fisheries resources cover an area of 4.47 million hectares in which perennial and seasonal water body constitute an area of about 2.83 million ha and 1.53 million ha respectively and of this area 90% comprises capture fisheries and 10% closed water culture fisheries (DoF, 2007). The bulk of fish production (more than 75 % of the total fish production) comes from inland fisheries and it was 1.85 million mt. in 2005-2006 (DoF, 2007).

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. It contributes around 3.74% to the GDP and 4.04% to foreign exchange earnings through export. Fish is the principal source of food, supplying some 58% of animal protein in the diet of our population. Fisheries provide livelihood to about 12 million people of the country directly or indirectly (DoF, 2009). To meet up the protein deficiency as well as to increase the foreign currency we must have to increase the total fish production.

Once upon a time fish were used to 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, regular supply of fish seed is the pre-requisite for the development of aquaculture (Webber and Riondan, 1976). Aquaculture accounts for nearly 40% of total fish production in Bangladesh (Hussain and Mazid, 2001). The Indian major carps *Labeo rohita, Cirrhinus cirrhosus* and *Catla catla* and some species of Chinese carps are the principal species for pond aquaculture in Bangladesh. We also need to have variety of species in this aspect of the development of aquaculture in our country to meet the increasing demand. 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; inbreeding, negative selection and introgressive hybridization are the common problems in the carp hatcheries of the country. So, genetically good quality fish seed is a prerequisite for successful fish culture.

Hybridization is a well-known means of genetic improvement, which has application in aquaculture. Heterosis or Hybrid vigor enables an offspring to surpass its parents for one or more traits. Heterosis can be obtained with respect to growth rate and viability, especially when fish from different highly inbred groups are crossed. In the recent time, hybridization programme was conducted in some area, because it was another approach for producing genetically superior hybrid by using hybrid vigor. Kowtal (1987) reviewed all the hybridization works carried out in India with indigenous and exotic carps and listed the promising hybrids. The performance of the best Indian Major Carp's hybrids are compared with their parent species (Das *et al.*, 1980), which was intermediate in performance, being better than rohu but not better than catla. Common carp crossbreds generally express low levels of heterosis (Moav *et al.*, 1979; Moav and Wohlfarth, 1976;

Wohlfarth, 1993; Hulata, 1995). However, those that exhibited positive heterosis are now the basis for carp aquaculture in Israel, Vietnam, China and Hungary. The crossing of common carp lines in Szarvas, Hungary (Bakos and Gorda, 1995) is a good example of the relative success of crossbreeding. During the last 35 years, more than 140 crosses have been tested. Three were chosen, based on 20 percent improvements in growth rate (and other qualitative features), compared to parent and control carp lines, for culture purposes. Now approximately 80 percent of common carp production comes from these "Szarvas" crossbreds. Production of gynogenetic female lines and gynogenetic sex-reversed inbred male lines from common carp with the best combining ability was an important part of the Hungarian crossbreeding programme. A higher heterosis was expected from crossing inbred lines, but the growth rate of F_1 crossbreds was only 10 percent higher than controls (Bakos and Gorda, 1995).

In Indonesia, strain development using artificial gynogenesis and sex-reversal resulted in 10 common carp inbred lines, which were later used for crossbreeding (Sumantadinata, 1995). In Vietnam, eight local varieties of common carp, along with "Hungary", "Ukraine", "Indonesia" and "Czech" strains are maintained, with significant heterosis observed in F_1 generations of crossbreds. Double crosses among Vietnamese, Hungarian and Indonesian strains have subsequently been used for carp selection and crossbreeding programmes throughout Vietnam (Thien and Trong, 1995).

Intraspecific crossbreeding (crossing of different strains) may increase growth rate but heterosis (differences between offspring and parents) may not be obtained in every case. Increases of 55 percent and 22 percent in growth rate of channel catfish and rainbow trout crossbreeds, respectively, were achieved using this technique (Dunham and Smitherman, 1983). Chum salmon crossbreeds, however, have shown no increase in

growth rates compared with parent strains (Dunham, 1996a). Under various rice-field conditions, growth rates of different strains of Nile tilapia and their crosses showed that the crosses were superior to pure Senegal strains. Breeding programmes are also under development in several countries for the Java or silver barb, Barbodes (formerly *Pontius gonionotus*), and another economically important fish species in Southeast Asia (Hussain and Mazid, 1999). The Bangladesh programme used three strains: "Bangladesh", "Thailand" and "Indonesian". The growth rate of females from six crosses was 23 percent higher than the average growth rate of the parent strains. Even higher growth rates (35 percent improvement) were found in the three crosses using the Thailand strain as either the sire or dam. In the Vietnamese breeding programme, six different strains were used to produce a population ideally suited for culture.

Cross-breeds of different strains of European catfish, *Silures glanb* are characterized by outstanding adaptability under warm water holding conditions and mixed diet feeding regimes (Krasznai and Marian, 1985). In addition, crosses with the walking catfish, *Clarias macrocephalus,* have shown improved resistance to *Aeromonas hydrophila* infections (Prarom, 1990). Studies on "domestic a" x "domestic b" channel catfish also showed greater heterotic growth rates than "domestic" x "wild" crosses (Dunham and Smitherman, 1983). The same was found in rainbow trout crosses (Kincaid, 1981).

Channel catfish females x blue catfish, males ("channel-blue") is the only cross (between 28 catfish hybrids examined) that exhibits superiority for growth rate, growth uniformity, disease resistance, tolerance of low oxygen levels, dressing percentage and harvestability (Dunham *et al*, 1986). However, mating problems between the two species have prevented commercial utilization.

The "sunshine" bass is a cross between white bass, *Morone chrysops*, and striped bass, *M. saxatihs,* and grows faster, with better overall culture characteristics than either parental species (Smith, 1988). In addition, crosses of the silver carp *(Hypophthalmichthys molitrix)* and bighead carp *(Aristichthys nobilis),* black crappie *(Pomoxis nigromaculatus)* and *P. annularis* and African catfish hybrids *(Clarias gariepinus, Heterobranchus longlis and H. blsorsah (*Salami *et al.*, 1993; Nwadukwe, 1995) all show faster growth than parent species. Numerous crosses of common carp with rohu, mrigal *(Cirrhinus cirrhoses)* and catla *(Catla catla)* (Khan *et al.*, 1990); tambaqui *(Colossoma macropomum)* and *Piaractus brachypoma* and *P. mesopotamicus* (Senhorini *et al.*, 1988), and gilthead seabream *(Spares aurata)* with red seabream *(Pagrus major)* (Knibb *et al.*, 1998a), have also resulted in improved overall performance for aquaculture systems. Hybridization in commercial Thai oysters *(Crassostrea belcheri, C. lugubris and Saccostrea cucullata)* was carried out to explore the possibility of producing hybrid oysters with superior traits.

Interspecific hybridization has been used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase disease resistance, improve tolerance of environmental extremes, and improve a variety of other traits that make aquatic animal production more profitable. Although interspecific hybridization rarely results in an F_1 suitable for aquaculture application, there are a few significant exceptions.

Increased environmental tolerance may also be imparted to hybrids where one parent species has a wide or specific physiological tolerance or due to increased heterozygosity (Nelson and Hedgecock, 1980; Noy *et al.*, 1987). Hybrid red tilapia, *O. mossambicus*, (high salinity tolerance) and Nile tilapia, *O. niloticus*, (low salinity tolerance) show enhanced salinity tolerance. *O. niloticus x O. aureus* hybrids also show enhanced salinity

tolerance (Wohlfarth, 1993).

Chromosome-set manipulation (e.g. Polyploidization) can be combined with hybridization to increase the viability of fishes and provide increased developmental stability during' early life history stages. Polyploid hybrid salmon appear to be better suited for culture than either polyploid or hybrid salmon are on their own. Although many diploid salmonid hybrids are not used for culture, triploidization of the hybrids may confer increased viability on the hybrids (viability refers to the ability of the hybrid to survive and grow, but does not infer that the hybrid is fertile or capable of reproducing).

As the domestication of fish species increases, the possibilities to increase production through appropriate hybridization will also increase. New hybrids will need to be tested as to their performance, viability (their ability to grow), and fertility (their ability to reproduce). The Kuwait Institute of Scientific Research has produced a hybrid bream, *Acanthopagus latus* (Sheim) x *Sparidentex hasta* (sobiaty) that is currently being investigated. It has good growth and body quality and appears to be fertile.

Hybridization is a method of improving productivity of culturable species, however, Tave (1993) opined 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. Genetic variation has three sub-components V_A, V_D and V_I (Additive, Dominant and Epistatic variations). V_D is exploited by the hybridization programme because it is created by the interaction between alleles at each locus; it is a function of diploid state and cannot be inherited beyond F_1generation.The production of superior F_1 hybrid is a hit or miss proposition. Sometimes someone can be successful or sometimes may not. We mate two superior fish and produce nothing but culls, or we can mate two culls and produce a grand champion. Basically what we do is to discover a cross that

nicks. (Produce superior hybrid progeny). When we have found the right combination then it can be used for increasing fish production.

Fish hybridize relatively easily, and the variety of species crosses and even wider taxonomic combination is vast. List of such hybrids occurring naturally and/or produced artificially have been published by different researchers. Some indication of the use of hybrids is found to generate genetic variance, to assess the genetics of sex determination, to improve productivity etc. 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. 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. And this is done for research purpose.

There are many examples which show that hybridization helps to improve productivity. Chappell (1979), Islam and Shah (2007) find that some hybrid improve yield by 10-55%. Chappell (1979) also finds that hybridization improve seinability and food conversion. Hickling (1968) shows the most famous and most important example of production of monosex population by hybridization. He produces all males of tilapia. Das *et al. (1980)* observed an intermediate performance of the best Indian rohu-catla hybrid. However, the hybrid was better than rohu, but not better than catla. Dunham and Smitherman (1983) reported 55% increase in growth rate of channel catfish intraspecific hybrid. Strain

crossing and realization of positive heterosis is now an important basis of common carp aquaculture in Israel, Vietnam and Hungary (Dunham *et al.,* 2001).

In Bangladesh there are 31 commercial important species of which 5 are exotic (Rahman, 1989). Among them the Indian Major Carps are much popular and farmers usually select them as rearing species. *Cirrhinus cirrhosus* is locally known as mrigal, it belongs to the family Cyprinidae and remains in the group carps. The breeding process of mrigal is similar to other Indian major carps (Chattergi and Siddiqui, 1979). It is one of the most popular food fish among bata species and once was widely distributed throughout the rivers, haors, baors, beels, canals and pond of Bangladesh. Mrigal is a common Indian major carp and it is found in all over Bangladesh. Its growth rate is high and has a good market demand.

The Indian major carp species are known to be able to hybridize, and hybrids are fertile and can be backcrossed to the parental species. Furthermore, hybridization has been shown to have a significant impact on production related traits (notably growth). Contemporary hypotheses have highlighted that hybridization as an important mechanism for adaptive radiation and diversification of animals and hybridization is believed to be more common in fishes than in other vertebrates. Hybridizations between vertebrate species are most common between freshwater fishes with external fertilization and have been documented in a variety of ecological settings (Das *et al.*, 1980).

Reciprocal crosses are the two possible mating between two groups: female A X male B and female B X male A. This is necessary because reciprocal hybrids were seldom the same. For example, channel female X blue male hybrid catfish is superior to its reciprocal: It is more uniform (Brooks, 1977), grows faster, is more seinable, exhibit more feeding vigor, has a better dressing, has a better food conversion (Chappell, 1979),

is more catch-able by hook and line (Tave *et al.*, 1981; Dunham *et al.*, 1986), and is easier to produce (Tave and Smitherman, 1982).

In this investigation, attempts were made to produce a good quality F_1 hybrid. This was done by reciprocally mating Mrigal (*Cirrhinus cirrhosus*) from Gollamari fish seed multiplication farm, Khulna and from the source of Padma. The fish were bred artificially by PG injection. The rates of fertilization and hatching were calculated. Then the fry were stocked in hapa and they were given food, and then were transferred to earthen nursery pond for further rearing. In the experiment, the fertilization and hatching rates were obtained lower in hybrids than in case of single strain. The growth rates of the hybrids were higher than single strain.

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 mrigal being the premier species in the sector, numerous marginal fish farmers in the country will be benefited from this technology.

OBJECTIVES OF THE STUDY

- To produce heterosis in the offspring of mrigal through crossing a riverine strain with a hatchery strain.
- To determine the heterosis by testing growth.

CHAPTER - 2

Material and Methods

MATERIALS & METHODS

2.1. Design of Experiment

The experiment was designed to be conducted in two distinct phases. Firstly, induced breeding with reciprocal hybridization was made and fertilization and hatching rates were determined. Secondly, newly hatched individuals were reared for 10 weeks in earthen nursery ponds located in Khulna University Campus and growth of hybrids was compared with that of the parents.

2.2. Collection, Transportation and Maintenance of Brood stock

Brood stocks were collected from two different locations, one, *the riverine strain*, from Jessore Fish Farm (DoF) and the other, *the hatchery strain*, was sourced from Fish Seed Multiplication Center (FSMC), Khulna. A total of five pairs of brood were transported from each sources using large plastic container with ice, saline and aeration. Coming at experimental station the broods were allowed for rest temporarily in large concrete tanks set under water cascade and after that those were released in brood rearing ponds at FSMC with fin clipping for future identification. Supplementary feed was given at a rate of 5% of total biomass.

2.3. Induced Breeding with Hybridization

The mature brood fishes were collected from brood ponds and kept under a 12 hour photoperiod in conditioning tank. Then the broods were shifted for induced breeding into a concrete brood tank provided with continuous shower. Induced breeding was performed by using carp pituitary hormone injection intramuscularly. Dry PG preserved in vial was bought from the market. Then the required amount of PG was at first crushed and then diluted by distilled water to dissolved it and centrifuged it for precipitation. The

supernatant solution of hormone was then taken for the injection. The sex ratio of the spawners was 1:2 of male and female respectively. The doses were 2mg/kg and 6mg/kg body weight for female administered at 6 hrs interval while male was given a single dose at 2mg/kg body weight at the time of second dose to the female. The stripping was done 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. At the mean time respective male were stripped and collected milt was mixed well with previously collected eggs. Eggs and milt were collected separately by hand stripping and were mixed well in clear plastic bowl with a clean and sterile feather for proper fertilization. Physiological saline solution (5% dextrose and 0.9% NaCl) was used during fertilization to increase the viability of eggs and sperm and also to reduce the sticky condition of eggs.

For two different strains of mrigal, artificial insemination was made with two reciprocal hybridizations and two controls.

The crosses were as follows:

Cross-1	:	Padma ♀ X Hatchery ♂	Tank 1
Cross-2	:	Hatchery ♀ X Padma ♂	Tank 2
Cross-3	:	Padma ♂ X Padma ♀	Tank 3
Cross-4	:	Hatchery ♀ X Hatchery ♂	Tank 4

2.4. Fertilization, Incubation and Hatching

The inseminated eggs were then transferred into four incubation tanks of 50 liter water holding capacity connected to water circulating system to facilitate gentle movement of the developing eggs at all times for proper hatching. After a certain period (1-2 hrs) the

2.10. Feeding Management

After stocking, a nursery feed was used named EON which was marketed by Eon Animal Health Products Ltd. The composition of the feed was as follows.

Crude protein	42% (minimum)
Crude fat	8% (minimum)
Energy	3200 KCal
Moisture	10% (maximum)

Other elements like essential amino acid, phosphorus, calcium, mineral, vitamin, ascorbic acid etc. were present in equal ratio.

The feed was given three times in a day. The amounts of feed were as follows:

Table 1. Daily amount of feed for fry

Age of fry (days)	Daily amount of feed for 1000 fry (g)
1-7	10
8-15	20
16-30	50
31-49	100

After seven weeks mega feed was used which contained above 35% protein and other essential elements. The feed was used at a rate of 150 gm / 1000 fry. The feeding frequency was three times/day. Cow-dung was also applied once a week at a rate of 2 kg per decimal to increase the primary production in the ponds.

2.11. Water quality monitoring

Different aspects of water quality viz. temperature, pH, turbidity, and dissolved oxygen (DO) level were measured and monitored at regular interval and kept in a balanced

condition to maintain a healthy environment in the ponds. Depth of water was always maintained at 1.5m by regular water exchange.

2.12. Sampling and Growth Check

To assess the growth performance and feed adjustment, sampling was done at every tenth day by drawing small mesh net in the pond and then sample was taken randomly from the whole collection. In the first two sampling only the average weight of the fishes was taken. From subsequent sampling growth performance were measured in terms of both weight and length. An electronic balance capable to quantify at least 0.1 mg was used to measure the weight and a centimeter scale marked at regular 1mm grade was used to measure the length. The growth data were recorded in the sampling sheet.

2.13. Heterosis calculation

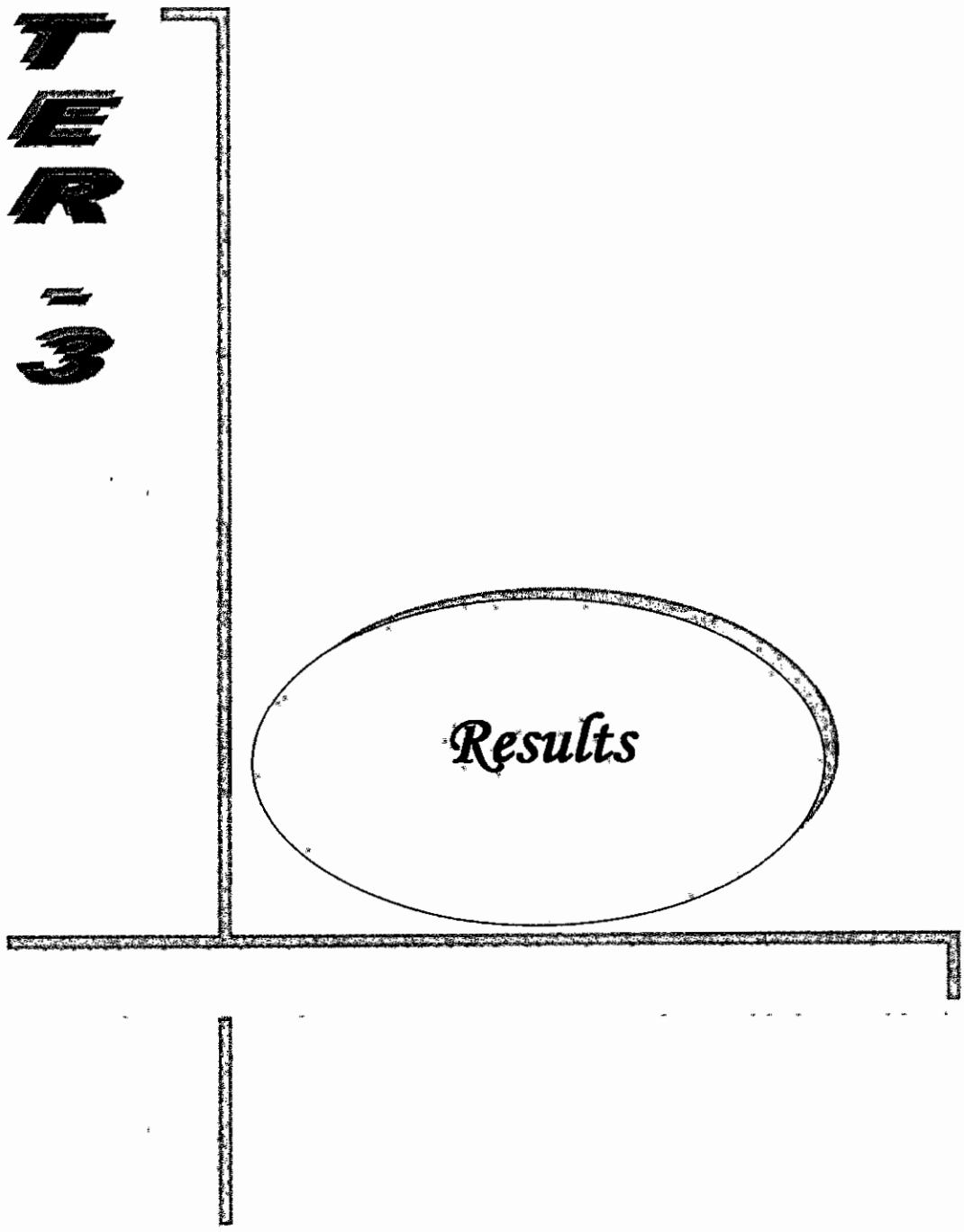
Heterotic effect was calculated by using the following equation:

$$H = \frac{\text{Mean reciprocal F1 hybrids} - \text{Mean parents}}{\text{Mean parents}} \times 100$$

2.14. Statistical analysis

The results of the various growth trials were statistically compared using One Way ANOVA. These statistical analyses were performed with the aid of the computer software SPSS (Statistical Products for Service Solution). Test of significance was done at 5% level of significance.

CHAPTER - 3



RESULTS

Strain crossing was successfully done in Mrigal to bring heterotic effects in the hybrid analyzed by comparing their growth rates with that of the pure-bred. The study took a total period of 18 weeks covering brood collection, maturation, hybridization, and rearing the breeding products for growth performance. Main parts of the research work took 72 days consisting of breeding program and rearing of fish. Hybrid was produced from intra-specific crossing of *Cirrhinus cirrhosus* between the strain of the river Padma and the strain of the Hatchery. Afterward, the spawns were transferred in nursery ponds and reared for 10 weeks. Fertilization and hatching rates of the breeding groups were documented and growth performance of the fishes was recorded in terms of weight and length. Heterosis effected in hybrids was calculated using the referenced formula.

3.1. Fertilization Rate

The fertilization rates of two reciprocal crossbred groups, 'Padma ♀ X Hatchery ♂' and 'Padma ♂ X Hatchery ♀' were 83% and 81% respectively and of two pure-bred groups, 'Padma ♂ X Padma ♀' and 'Hatchery ♂ X Hatchery ♀' were 90% and 88% respectively. Percent fertilization of different breeding products is shown in figure 1 which reveals lower fertilization rates in hybrid than in pure-bred.

3.2. Hatching Rate

The hatching rates of two reciprocal crossbred groups, 'Padma ♀ X Hatchery ♂' and 'Padma ♂ X Hatchery ♀' were 69% and 66% respectively and of two pure-bred groups, 'Padma ♀ X Padma ♂' and 'Hatchery ♂ X Hatchery ♀' were 73% and 70% respectively. Percent hatching of different breeding products is shown in figure 2 which reveals lower hatching rates in hybrid than in pure-bred.

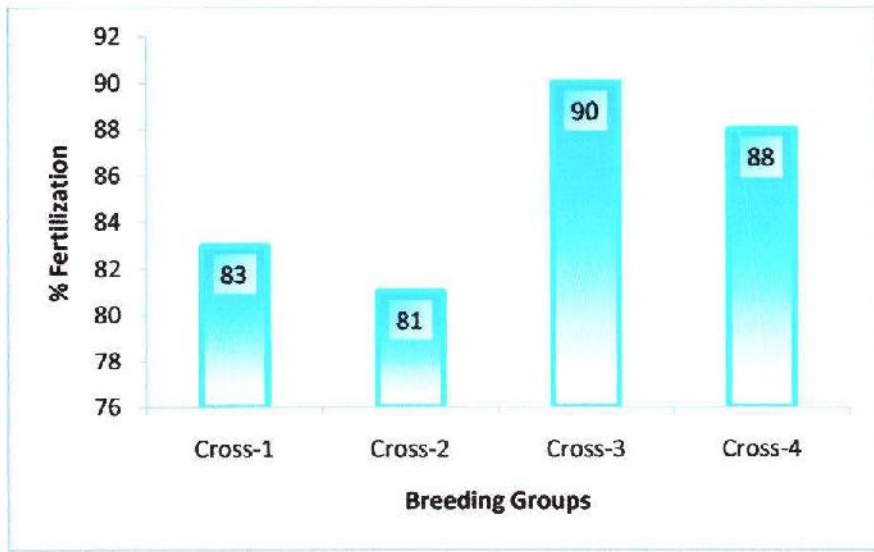


Fig 1. Percent fertilization of different breeding products

Cross-1 : Padma ♀ X Hatchery ♂

Cross-2 : Hatchery ♀ X Padma ♂

Cross-3 : Padma ♀ X Padma ♂

Cross-4 : Hatchery ♀ X Hatchery ♂

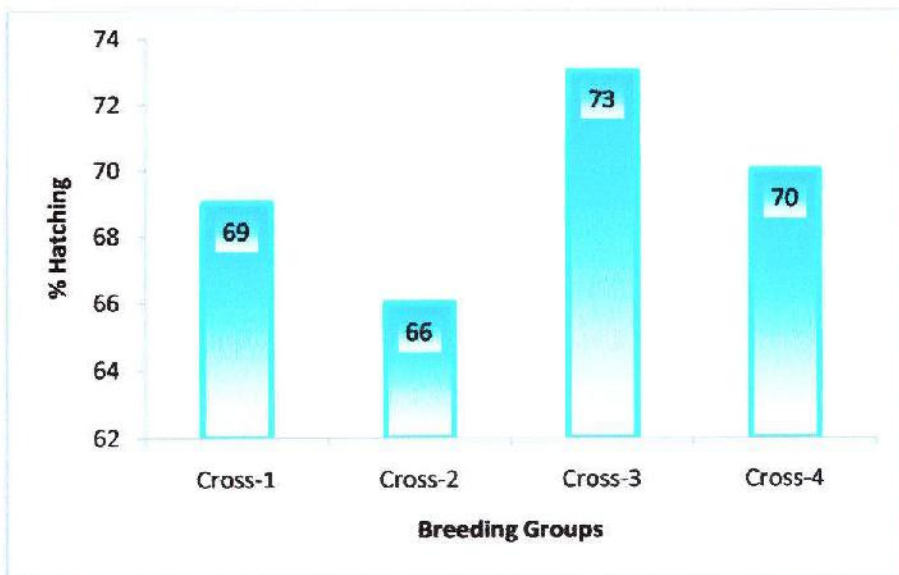


Fig 2. Percent hatching of different breeding products

3.3. Testing of Growth and Analysis of Hetrosis

After 10 weeks of rearing growth performance of the hybrid (strain of the river Padma X strain of hatchery) was compared with that of the pure-bred in terms of both weight and length, which is presented in figure 3 and figure 4 respectively. The analysis showed significant differences ($P < 0.05$) in the growth rates between the hybrid and the pure-bred, which produced 30.51% heterosis. Seven sampling were performed for weight test and five for the length as having undersize to measure the larvae during former sampling. Higher growth rates were found in reciprocal hybrids than pure-bred. This indicated that hybrid vigor had been accompanied in the progenies. Growth data are presented in table 2 and table 3.

Table 2. Average weight in different breeding groups

No. of Sampling	Mean Weight (g)			
	Cross 1 (Padma ♀ X Hatchery ♂)	***Cross 2*** (Padma ♂ X Hatchery ♀)	***Cross 3*** (Padma ♂ X Padma ♀)	***Cross 4*** (Hatchery ♂ X Hatchery ♀)
1	0.088	0.046	0.062	0.0245
2	0.184	0.114	0.14	0.053
3	0.428	0.423	0.21	0.081
4	0.832	0.609	0.371	0.152
5	1.123	0.912	0.712	0.62
6	1.927	1.576	1.23	1.17
7	2.13	1.89	1.57	1.51

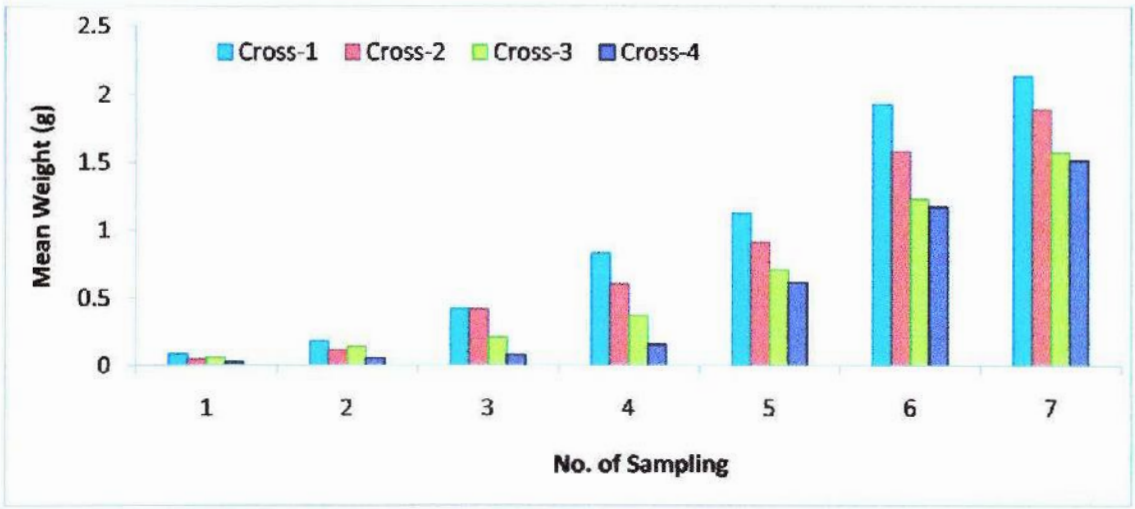


Fig 3. Comparative weight (g) of hybrid and pure-bred

Table 3. Average length in different breeding groups

No. of Sampling	Mean Length (cm)			
	Cross 1 (Padma ♀ X Hatchery ♂)	***Cross 2*** (Padma ♂ X Hatchery ♀)	***Cross 3*** (Padma ♂ X Padma ♀)	***Cross 4*** (Hatchery ♂ X Hatchery ♀)
1	2.64	2.12	1.94	1.25
2	3.97	3.54	2.86	2.16
3	4.86	4.04	3.9	3.33
4	5.4	4.82	4.34	4.12
5	6.12	5.84	5.46	4.79

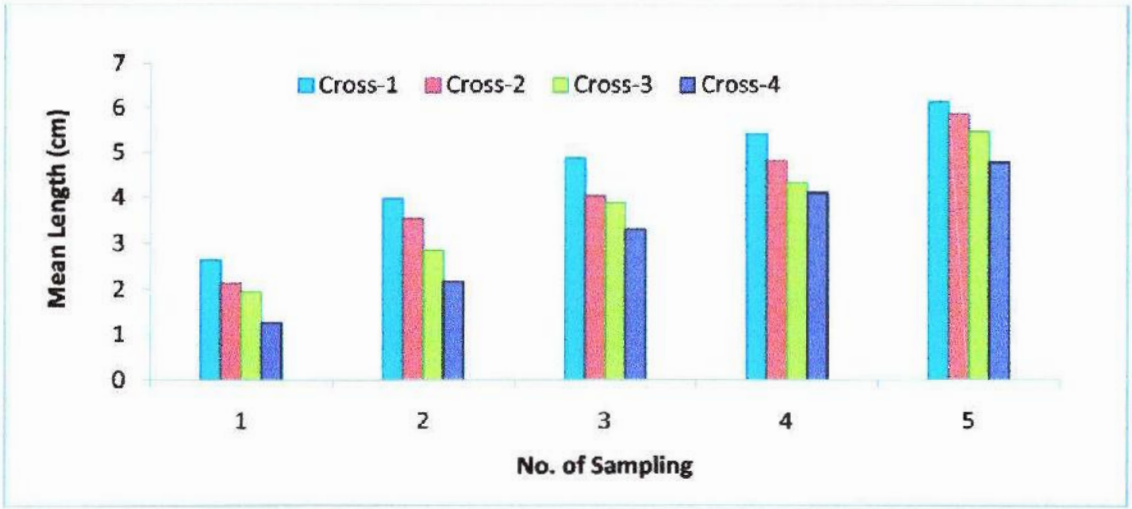


Fig 4. Comparative length of hybrid and pure-bred

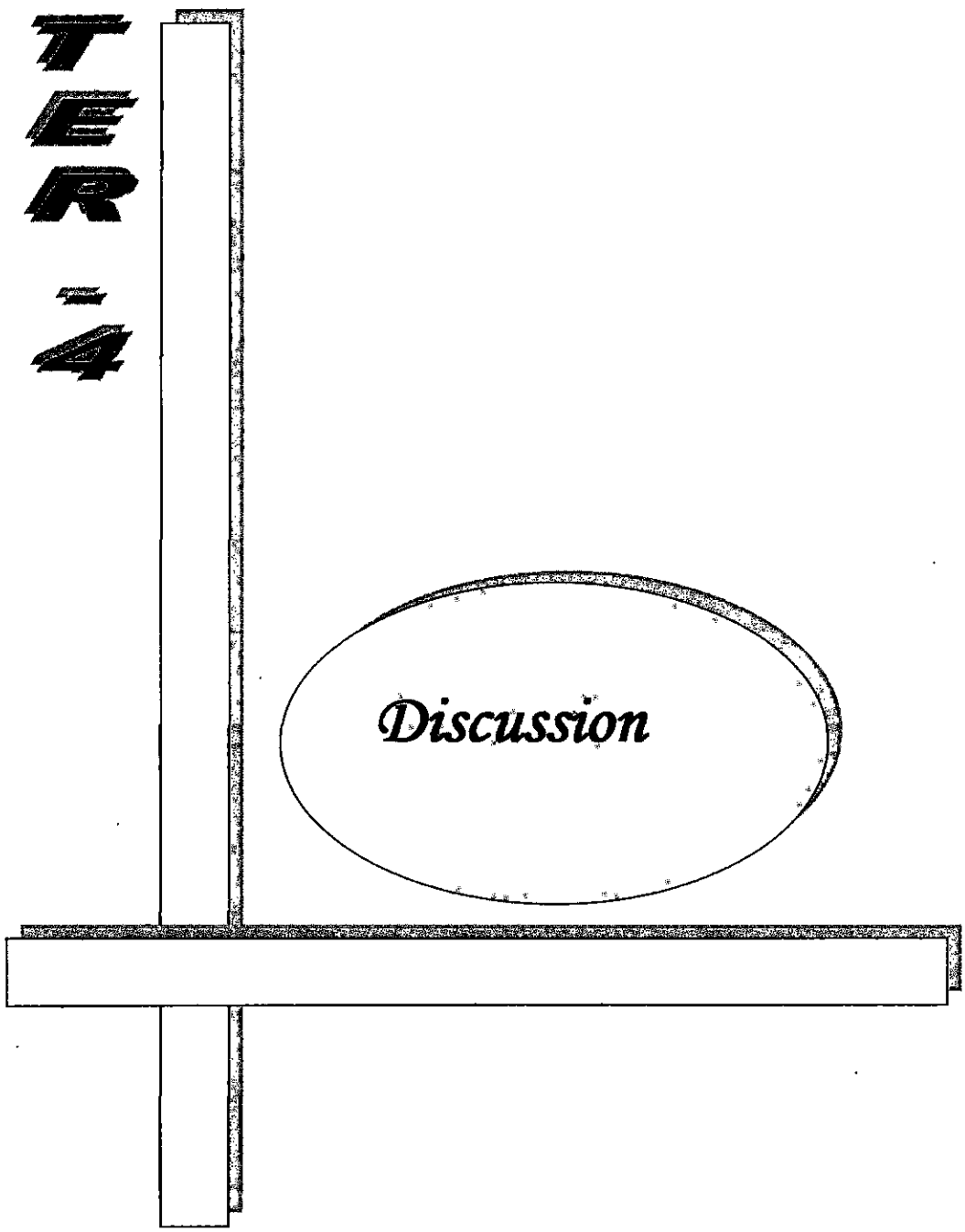
3.4. Water quality parameters

The table 4 depicts the overall results of the water quality parameters obtained from the regular water quality monitoring in the ponds throughout the rearing period.

Table 4. Water quality parameters of the rearing ponds

Parameters	Value	
	Range	**Mean ± SD**
pH	6.5-7.5	7.2 ± 0.3
Temperature (°C)	27-32	30 ± 1.6
Turbidity (cm)	25-36	29.4 ± 5.3
DO (mgL^{-1})	5.8-6.9	6.2 ± 0.4

CHAPTER - 4



DISCUSSION

The experiment was carried out in order to observe the growth performance of intra-specific hybrids of mrigal and growth performance of the hybrids were compared with single strain. For this purpose F_1 hybrid was produced by cross breeding between Padma strain and Hatchery strain of Mrigal in a government hatchery at Gallamari, Khulna. The spawn were then transferred in Khulna University campus for nursery rearing. In seventy days culture period, growth was measured at every ten days interval. At Gallamari fish farm fertilization and hatching rates were counted.

Fertilization and hatching rates were found better in single strain than in the hybrid. The hatching rate may be affected by various factors, such as temperature, rate of water flow, water quality etc. The optimum temperature range for rohu, catla, mrigal, kalibaus is 27^0 C to 29^0 C (Haque, 1997). Activity of hormone secretion in fish body is affected at a water temperature below 16^o C and above 31^oC. Fertilization and hatching rates also differ with the condition of brood. It is impossible to programme the maturation in of gametes and supply of quality seeds and thus ensure an all year round production of table fish of consistent size and quality unless and until a detailed control on reproduction and brood stock management of the species concerned is established. The result obtained between the trails could also be due to inappropriate hormone doses and bad quality of pituitary glands. The pituitary glands are preserved in absolute alcohol, air tight vials should be used and after being kept in this medium for 24 hours, old alcohol should be replaced by new absolute alcohol to ensure preservation.

Hussain (2001) reported the fertilization and hatching percentages of *C. reba* to be at 92 and 86, respectively with the doses of injection at 2 and 6 mg PG/ kg body weight as first and second injection, respectively, for females at an interval of 6 hour and a single

injection of 2 mg PG/ kg body weight for males after 3 hours of the first injection for females. Breeding season and maturity of brood vary from species to species. Response to hormone injection for breeding is always optimum at the peak of the breeding season.

In the present experiment hybrid generally showed better growth than their parental strain, mrigal. This is probably due to the inheritance of good quality straits of mrigal in the hybrids and thus hybrid vigor was achieved. Seventy days comparative growth study of F_1 hybrid and normal spawns revealed the higher growth rate of hybrid than normal spawns. The crosses of these two strains produced 30.51% heterosis which was the highest result in this experiment. Thus hybrid vigor was achieved in F_1 hybrid which was the target of the experiment.

The quality of the hybrid is simply a matter of luck. Chappel (1979) found that certain intra-specific hybrid catfish were as good as their more famous cousin, the channel catfish ♀ X blue ♂ hybrid. One advantage of intra-specific hybridization is the fact that one should have little trouble producing progeny, something which is often a problem with inter-specific hybridization. One way to improve the likelihood of producing intra-specific F_1 hybrids that exhibit hybrid vigor (positive heterosis) on fish farms is to hybridize hatchery strains rather than wild stocks (Tave, 1993). For example, hybridization studies with channel catfish showed that 80% of hatchery X hatchery F_1 hybrids exhibited positive heterosis, but only 30% of hatchery X wild F_1 hybrids exhibited positive heterosis (Dunham and Smitherman, 1983).

The "sunshine" bass is a cross between white bass, *Morone chrysops*, and striped bass, *M. saxatihs,* and grows faster, with better overall culture characteristics than either parent species (Smith, 1988). In addition, crosses of the silver carp *(Hypophthalmichthys molitrix)* and bighead carp *(Aristichthys nobilis),* black crappie *(Pomoxis nigromaculatus)*

and *P. annularis* and African catfish hybrids *(Clarias gariepinus, Heterobranchus longifilis* and *H. blsorsalis* (Salami *et al.*, 1993; Nwadukwe, 1995) all show faster growth than parent species. Numerous crosses of common carp with rohu, mrigal *(Cirrhinus cirrhosus)* and catla *(Catla catla)* (Khan *et al.*, 1990); tambaqui *(Colossoma macropomum)* and *Piaractus brachypoma* and *P. mesopotamicus* (Senhorini *et al.*, 1988), and gilthead seabream *(Spares aurata)* with red seabream *(Pagrus major)* (Knibb *et al.*, 1998a), have also resulted in improved overall performance for aquaculture systems.

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.

Eknath and Doyle, (1985 a, b) demonstrated that hatcheries in Karnataka, India, are inadvertently breeding slow-growing and late maturing individuals corroborative evidence for this observation come several small hatcheries where younger individuals are infect exhibiting relatively slow growth rates, late maturity and also smaller size at first spawning. It was also reported that small hatcheries appear to rapidly inbreed their

stock, the tendency to breed slow growing and late maturing individuals was also observed in some hatcheries.

Tave (1993) suggest 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 hybridized 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.*, 1979).

Reciprocal crosses are the two possible mating between two groups: female A X male B and female B X male A. In our experiments mating was conducted between Padma ♀ X Hatchery♂, Padma ♂ X Hatchery ♀, Padma ♂ X Padma ♀ and Hatchery ♂ X Hatchery ♀. This is necessary because reciprocal hybrid were seldom the same. From the experiment it was found that Padma ♀ X Hatchery♂ showed better growth than all other that is 2.13 gm. But their reciprocal hybrid (Padma ♂ X Hatchery ♀) showed 1.89 gm. The crosses of these two strains produced 30.51% heterosis which was the highest result in this experiment.

Basavaraju *et al.*, (1995) studied the growth rates of the *L. rohita* (female) × *Cirrhinus mrigala* (male) 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 *Cirrhinus mrigala* grew faster than both the hybrids. Again, among the two hybrids the hybrid between *Cirrhinus mrigala* female × *L. rohita* male grew faster than the reciprocal *L. rohita* female × *C. mrigal* male hybrid. Whereas the observations of Ibrahim (1977) have shown that growth rate of the rohu (female) × mrigal (male) was better than the parent species.

In Indonesia, strain development using artificial gynogenesis and sex-reversal resulted in 10 common carp inbred lines, which were later used for crossbreeding (Sumantadinata, 1995). In Vietnam, eight local varieties of common carp, along with "Hungary", "Ukraine", "Indonesia" and "Czech" strains are maintained, with significant heterosis observed in F_1 generations of crossbreeds. Double crosses among Vietnamese, Hungarian and Indonesian strains have subsequently been used for carp selection and crossbreeding programmes throughout Vietnam (Thien and Trong, 1995).

Artificial hybridization among cyprinids of different families and genera was carried out extensively in China (Wu, *et al.*, 1994). Hybrids embryos have been obtained in almost any combination attempted, but hybridization across families was not successful. Several of the hybrids between sub-families survived beyond hatching, although the rate of abnormalities was high and all fish were sterile due to triploidy (Wu *et al.*, 1994).

Cross-breeds of different strains of European catfish, *Silures glanb* are characterized by outstanding adaptability under warm water holding conditions and mixed diet feeding regimes (Krasznai and Marian, 1985). In addition, crosses with the walking catfish, *Clarias macrocephalus*, have shown improved resistance to *Aeromonas hydrophila* infections (Prarom, 1990). Increased environmental tolerance may also be imparted to hybrids where one parent species has a wide or specific physiological tolerance or due to increased heterozygosity (Nelson and Hedgecock, 1980; Noy *et al.*, 1987). In case of an intra-specific hybridization of rohu a positive heterosis of 55.76% was obtained in hybrid (Islam and Shah, 2007). Hybrids between marine species, and between marine and freshwater spawning species, have produced surprising results. Hybrids between *Sparus aurata* and *Pagrus major* (both belonging to the *Sparidae*) developed vestigial gonads at two to three years and were sterile (Knibb *et al.*, 1998a).

Sometimes an inter-specific hybrid does not exhibit heterosis for specific traits, but may still be important for aquaculture if it expresses other useful traits from the parent species. The main catfish cultured in Thailand is the hybrid between African *(Clarias gariepinus)* and Thai (*C. macrocephalus)* catfish. This combines the fast growth of the African catfish and the desirable flesh characteristics of the Thai catfish (Nwadukwe, 1995).

Likewise the rohu *(Labeo rohita*) x catla (*Catla catla*) hybrid grows almost as fast as pure catla, but has the small head of the rohu considered desirable in Indian aquaculture (Reddy, 2000). *Catla catla* 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 case of hybridization, proper planning and maintenance are two most important matters. Without proper planning and maintenance hybridization is not possible to make hybridization successful, attention should be paid to phylogenic tree, karyotypes and biology and reproductive behavior of two species. If the species are distantly related or dissimilarities between their karyotypes are found or their reproductive and biological behavior are different that means one species spawns at March and another at June or one spawn in running water and another at stagnant water then hybridization is not possible. By taking into account all this important thing good hybridization is easy to perform.

ANOVA tests were performed to test the significance of the difference between single species and F_1 hybrid. ANOVA test at 5% level of significance showed that there was significant difference between the parental strain and F_1 hybrid.

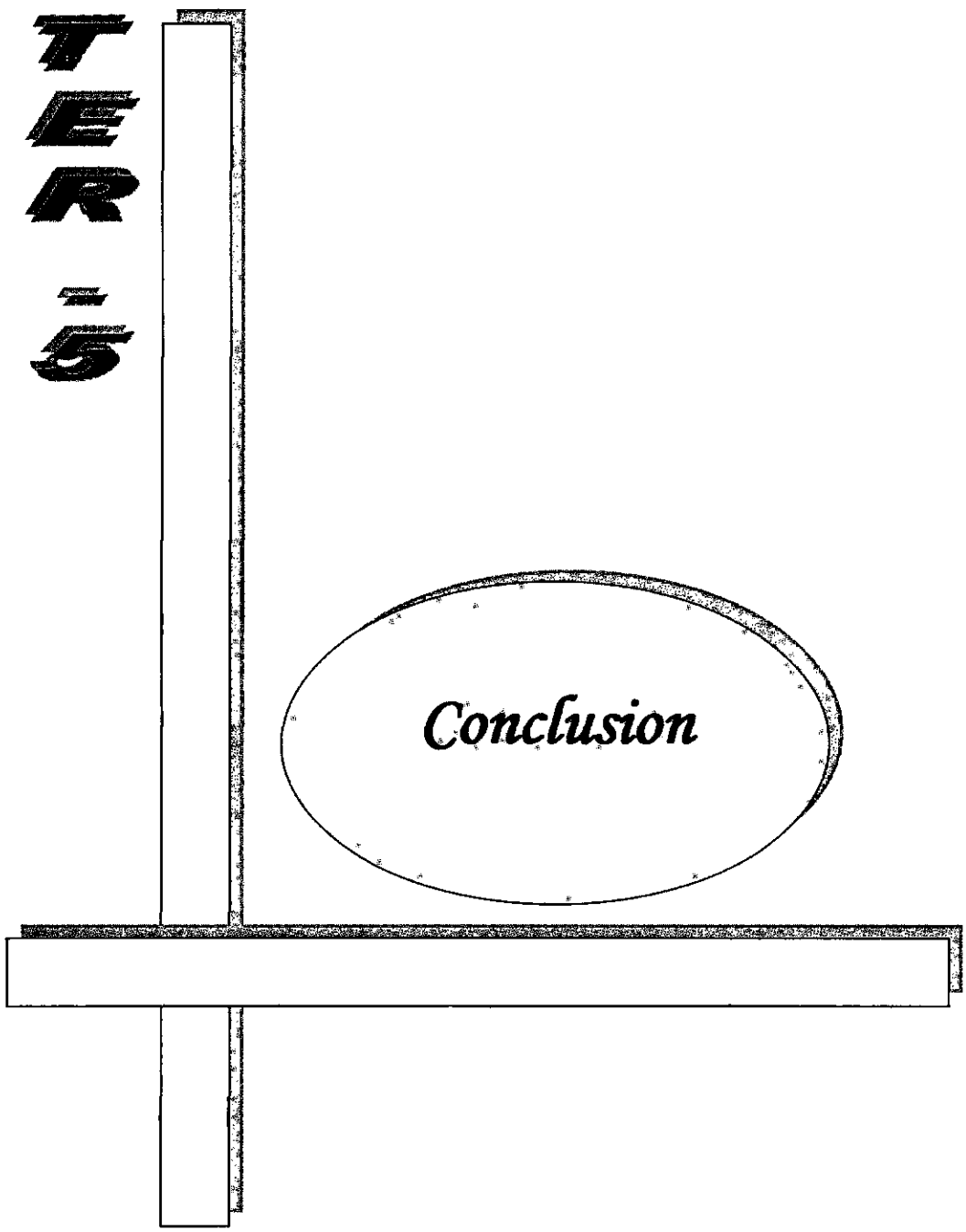
In the culture period some water quality parameters were measured which was in the acceptable range. Fish is poikilotherms. So, growth, reproduction and other biological activities of fish are controlled by temperature. For 1°C rise of water temperature

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 25° C to 35° C. Maximum temperature 35°C was recorded in July where minimum temperature was 25°C in October. Ali *et al.* (1982) stated that 20.5°C-33.5°C is favorable for fish culture.

The monthly average Dissolve Oxygen at the experimental ponds varied from 5.5 and 7.3 mg/L. Pahwa and Mehrotra (1966) stated, the dissolve oxygen values are maximum during winter and minimum of the same July and August. The high concentration of dissolve oxygen content during winter was possibly due to low temperature, low rainfall and low pH. Due to the less temperature and low rainfall, the decomposition of organic matter was reduced with low production of carbon dioxide and less consumption of dissolve oxygen.

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 7.2 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.

CHAPTER - 5



CONCLUSION

Hybrid of two different strains often has a higher growth rate than the single strain; this fact has been proved by several experiments. And through hybridization heterozygosity in the progenies can be produced and thus hybrid vigor or positive heterosis is achieved. In the present experiment, F_1 hybrids of 'Padma ♀ X Hatchery ♂' and 'Padma ♂ X Hatchery ♀'showed better growth performance than the single strain, which produced 30.51% positive heterosis. Thus the study experiences the necessity of inter or intra-specific hybridization in commercial aquaculture of the country to overcome inbreeding problem and to achieve heterosis for increased production. However, further trials are needed for improving the percentage of heterosis in the hybrid.

CHAPTER - 6

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CHAPTER - 7

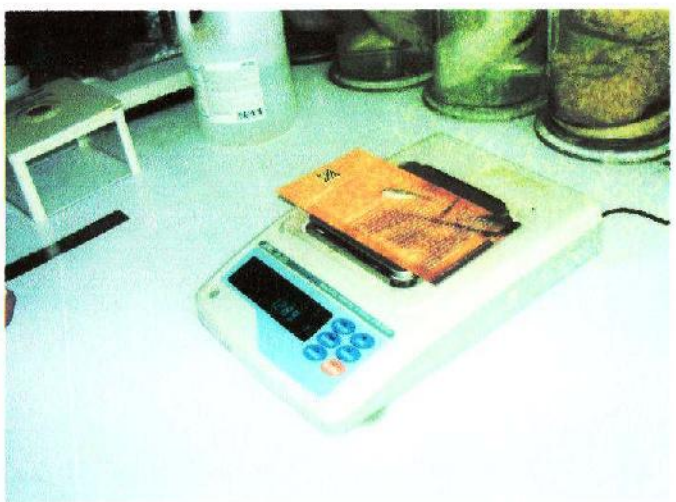
Appendices

Brood Fish

Fin clipping

Hapa settings

Study pond



Weight measurement

Length measurement

Figures: Glances of the activities carried out in the project work

Table 5: Results of One-way Anova test for the mean body weight of 4 different crosses.

ANOVA

Mean body weight of fishes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.832	3	1.944	2.926	.041
Within Groups	39.861	196	.664		
Total	45.693	199			

TITLE:

HETEROSIS ANALYSIS IN HYBRIDS BETWEEN DIFFERENT STRAINS OF MRIGAL (*Cirrhinus cirrhosus*)

Date of sampling:

Name of the strain: Mrigal (*Cirrhinus cirrhosus*) & HYBRID.

Type of stocking:

- ❖ Pure-bred :
- ❖ Hybrid :

POND-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					
12					
13					
14					
15					

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

PLACE: KHULNA UNIVERSITY CAMPUS