INTRODUCTION

Bangladesh is blessed with a large number of different water types like rivers, canals, floodplains, haors, baors, ponds, lakes, estuaries and the Bay of Bengal having 260 fresh water fish, 12 exotic fish, 475 marine fish, 24 freshwater prawn and 36 marine shrimp (FSY 2010). 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 2005). 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 3.74% to the GDP and 4.04% to foreign exchange earnings and provide livelihood to about 12 million people directly and indirectly (DoF 2010). 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 (Ahmed et al 2008).

However, in an attempt to produce more seed to meet the increasing demand, the seed producers did not pay attention to the genetic quality in Bangladesh (Hussain and Mazid 2005). As a result, the growing carp industry has suffered several incidences of much mortality in culture system suspected to be caused by inbreeding negative selection, genetic quality deterioration and some other problems. The carp seed produced from the hatcheries was reported to have poor genetic quality, survival and growth, susceptibility to disease, deformities, etc. Hence, it became essential to carry out research on genetic improvement of these species. Genetically good quality fish seed is a prerequisite for successful aquaculture program and fish culturists show special interest on natural fish seed due to their rapid growth rate, high disease resistance and survival rate. The genetic variation of hatchery population is generally lower than that of wild populations because hatchery
hybrid is possible, the carp aquaculture production can bring a revolution in the country. In this study, attempts were made to produce heterotic hybrid of rohu, *Labeo rohita* by reciprocal strain crossing (intra-specific hybridization) among Padma, Jamuna and Hatchery strains and their growth performances were also tested in ponds.

**MATERIALS AND METHODS**

**Brood collection, transportation and brood stock maintenance**

Sexually mature brood fishes of different strains were collected from three locations. Jamuna strain was collected from Iswardi Fish Seed Multiplication Farm, Padma strain was collected from Department of Fisheries (DoF) Jessore and hatchery strain was collected from Baluhor hatchery, Kotchandpur, Jheinadah. Ten pairs of broods (equal no. of male and female) were collected from each source. The broods were carried by some large plastic drum with aeration and then reared in the brood rearing ponds of “Fish Seed Multiplication Farm (Gallamari, Khulna)” under the Department of Fisheries, Government of the People’s Republic of Bangladesh. Before stocking in the ponds, the broods were kept in a conditioning tank for 24 hours with shower. Then the broods were tagged by fin clipping at the base of the dorsal fin treated with 5ppm KMnO4 to avoid injury or infection. Supplementary feed was given at a rate of 5% of total stocked biomass twice daily.

**Artificial breeding and intra-specific hybridization**

Induced or artificial breeding was performed by using carp pituitary hormone injection at the base of the pectoral fin. The doses were 2mg/ kg and 6mg/ kg body weight for female injected at 6 hours interval while only a single dose was given to male at 2mg/ kg body weight at the time of second dose to the female. Eggs and sperm were collected by manual stripping and were mixed well in clear plastic bowls with physiological saline solution (5% dextrose and 0.9% NaCl) for proper fertilization. Intra-specific hybridization produced 9 different crosses; Cross-1: Padma ♀ X Hatchery♂, Cross-2: Jamuna ♂ X Jamuna ♀, Cross-3: Padma ♂ X Jamuna ♀, Cross-4: Hatchery ♀ X Jamuna ♂, Cross-5: Padma ♂ X Hatchery ♀, Cross-6: Hatchery ♀ X Hatchery ♂, Cross-7: Padma ♂ X Padma ♂, Cross-8: Jamuna ♀ X Hatchery ♂, Cross-9: Padma ♀ X Jamuna ♂. As a result, 6 reciprocal crosses and 3 parental crosses were produced.
**Determination of fertilization and hatching rates**

Immediately after mixing, the fertilized eggs were kept in the round hatching jars of 50 liter water holding capacity. After 30 minutes of mixing the eggs were examined to determine the fertilization rate with the help of a magnifying glass. Eggs were hatched after 18-20 hours of fertilization. To count hatching rate exactly, 200 fertilized eggs were isolated and kept in some small hatching jars of 6 liter water holding capacity (5 replications for each treatment) with continuous flow. Then the actual hatching rate was determined by counting the number of hatchlings in the jars.

**Rearing of the spawns**

The newly hatched spawns were reared in the round hatching jars up to 4th day. After 2 days of hatching, the spawns were fed with egg yolk. Then the spawns were reared in the net hapa for 4 days and released in the prepared earthen nursery rearing ponds of Khulna University.

**Stocking of the larvae in the ponds and feeding**

The 8 day old larvae were stocked in 27 different prepared earthen ponds to check the growth performance of 9 different crosses. The stocking density of the larvae was 2500 decimal⁻¹. Larvae of 9 different crosses were stocked separately in 27 different ponds (3 replications). For the first 4 weeks feed was given at the rate of 8% of total fish biomass and then the rate was 5% of total biomass. The nursery rearing feed EON was collected from the fish feed market and applied twice daily in the ponds. Compost was also applied in the ponds at the dose of 1 kg decimal⁻¹ twice in a month to maintain natural food level.

**Water quality monitoring**

Various aspects of water quality viz. pH, salinity were measured by using pH-meter, refractometer; dissolve oxygen (DO) level, free CO₂ and alkalinity were measured regularly by the standard titrimetric method (Greenberg et al. 1992). Water quality parameters were monitored regularly and attempts were made to maintain suitable condition for the fish larvae. Depth of water was always maintained at 1.5m by regular water exchange.

**Growth performance and heterosis calculation**

Random sampling was done at every 10 days interval to compare growth performance of various crosses. Total body weight in gram (g) was measured for growth performance study. Heterosis was calculated by the equation -

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

**Data analysis**

MS Excel was used to store all the growth data for body weight and also for the presentation of the graphs. One way ANOVA was done for the test of significance at 5% level to obtain the difference in growth performance is significant or not.

**RESULTS AND DISCUSSION**

The experiment was carried out in two phases for 6 months (180 days), from 18/04/09 to 18/10/09. Firstly, brood collection, rearing and induced breeding were done in the “Government Fish Seed Multiplication Farm”, Khulna. Secondly, the spawns were reared in hapa in the hatchery for two days and then in nursery ponds of Khulna University.

**Fertilization rate**

The mean fertilization rates of 6 different reciprocal crosses were 91%, 87%, 90%, 89%, 86%, and 87% respectively but for 3 single strain (parental) crosses 93%, 95% and 94% respectively (Figure 1). In most of the cases, reciprocal crosses showed comparatively lower fertilization rate where as pure Jamuna strain showed the highest fertilization rate (95%). Intra specific crosses in this case showed lower fertilization rate than the pure crosses due to differences in population that probably provided them less fertilization capability. Islam and Shah (2007) obtained mean fertilization rate for the reciprocal intra-specific hybrids of hatchery and Jamuna as 75.75%, for pure Jamuna strain 75.49% and for pure hatchery strain 65.49%. The intra-specific hybridization of rainbow trout provided better fertilization performance than that of the parental members (Moav and Hulata 1975) but for channel catfish the fertilization of intra-specific crosses was lower compared to the parents observed by Wohlfarth et al (1975) and Hulata et al (1985). In all the present study, fertilization rates were considerably higher and satisfying in all the crosses; clearly indicating good quality brood with proper management in the brood stock ponds and in hatchery operation.
Hatching rate

Mean hatching rates of 6 different reciprocal crosses were 71%, 64%, 69%, 66%, 58% and 61% respectively but for pure Padma, Jamuna and hatchery strains 72%, 76% and 70% respectively (Figure 2). The intra-specific hybrids showed lower fertilization and hatching rates the reason of which could most probably be ascribed to general problem of mixing of gametes of the species of two separate populations. Islam and Shah (2007) obtained 71.3% hatching rate of Jamuna strain, 41.8% hatching rate of hatchery strain and 64.5% hatching rate of intra-specific hybrids of rohu. The intra-specific crosses showed higher hatching percentage in common carp (Bakos and Gorda 1999; Wang 2009), Chinook salmon (Barman et al 2003), tilapia (Tave et al 2007), catla and mrigal (Biswas et al 2008). Lower hatching rate was also observed in intra-specific hybrids of guppy (Julie and Yoshihisa 1988), cutthroat trout (Allendorf and Leary 1984), Arctic char (Nilsson 2005), African catfish (Wachirachaikarn 2009). The intra-specific hybridization treatment may provide both the higher and lower fertilization and hatching rate; depending on species and strain, physical condition, environmental condition and management practices during the breeding time. In the present experiment, the overall hatching rates for all of the crosses were very promising.

Growth performance

The mean body weights of 6 different reciprocal intra-specific hybrids and 3 different crosses of pure strains have been presented in the Fig. 3. In the present experiment, F1 hybrids were produced by intra-specific crossing between 3 different strains of *Labeo rohita* with the expectation that there will be increased incidence of heterozygosis through exploitation of $V_D$. Growth performance study for a period of 3 months provided that the final body weight of 6 different reciprocal crosses were obtained at 3.44g, 2.15g, 2.3g, 1.86g, 1.6g and 4.75g respectively. But the final body weight of pure Padma, Jamuna and hatchery strains were 1.56g, 2.45 and 1.55g respectively. The highest body weight 4.75g was obtained for the cross-9 (Padma ♀ X Jamuna ♂) as a result 72% heterosis was obtained.
in this case. The findings of the present experiment clearly indicate that the maternal impact of the Padma strain showed the best result in the offspring. The growth performance of riverine strain was also found better than the hatchery strain indicating probably the accumulation of inbreeding and small effective number of broods in the hatcheries.

There are available references on the use and applicability of strain crossing for increased vigor of the offspring produced (Moav and Hulata 1975; Kirpichnikov 1981; Wohlfarth 1993; Bakos and Gorda 1999; Hulata 1995). Islam and Shah (2007) noticed 55.76% 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, all the strain crossed hybrids also showed better growth performance over their pure parental strains. This is probably due to increase of genetic variation in the offspring and thus hybrid vigor was achieved which was the target of the experiment. Although the hatchery population is in inbreeding condition in Bangladesh, proper management of brood and breeding with distant hatchery populations may provide increased heterozygosity by exploiting dominant genetic variance ($V_D$). Shah (2004) reported that there is a lack in the integration of genetic norm and practice in the hatchery management system of Bangladesh. 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.

There are some deleterious recessive alleles which do not quote for functional proteins. The probability of the 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 nuance (Falconer 1981). In the large natural populations where panmictic breeding takes place, the deleterious recessive alleles remain hidden under heterozygous combinations with dominant alleles and do not get chance for expression in the phenotypes (Falconer 1981). The Additive Genetic Variation ($V_A$) is created by the cumulative action of alleles across the loci. 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. The quality of the hybrid is simply a matter of luck. 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

Figure 3. Mean body weights of 9 different crosses.
hybridization. The intra-specific/strain crossing technique provided 29.84% positive heterosis in common carp (Wang 2009), 17% positive heterosis in tilapia (Tave et al. 2007), 78% positive heterosis in guppy (Julie and Yoshisita 1988) and 28% positive heterosis in African catfish (Wachirachaikarn 2009). The intra-specific hybridization technique may also provide negative result or slower growth performance than the parental strain as it was found in Arctic char (Nilsson 2005) where 15% negative heterosis was obtained. One way to improve the likelihood of producing intra-specific F1 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 F1 hybrids exhibited positive heterosis, but only 30% of hatchery X wild F1 hybrids exhibited positive heterosis (Smitherman and Dunham 1985).

CONCLUSION
The present experiment was successful in producing heterotic hybrid in *Labeo rohita* through strain crossing. Thus, it can 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. The results clearly proved the wide spread claims of the fish-farmers and aquaculturists for the poor growth performance of hatchery produced seed of Indian major carps in Bangladesh. The most plausible causes of lower growth performance of hatchery seeds are poor brood management practice, small size of broods and lack of maintenance of genetic diversity as discussed earlier. If a good attention is paid on these areas in hatchery practices with domestic and international research cooperation, the valuable populations of Indian major carps can be protected mostly from being genetically damaged. Thus, the intra-specific hybridization or strain crossing technique can effectively be introduced to the inland aquaculture of Bangladesh to boost aquaculture production.

REFERENCES


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