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BREEDING PERFORMANCE OF RIVERINE ROHU (*Labeo rohita*) AND GROWTH PERFORMANCE OF F1 PROGENIES REARED IN HAPAS

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Abstract: The present study was carried out to evaluate the breeding performance of riverine rohu, *Labeo rohita,* and growth performances of F1 progenies reared in hapas. Two reciprocal crosses of brood fish from Halda and Padma rivers: Halda♀ X Padma ♂ (cross 1), Padma ♀ X Halda ♂ (cross 2), and one parental cross (cross 3, control): Hatchery ♀ X Hatchery ♂ were obtained in the present study. After 3 days of hatching, the spawns were transferred and reared in hapas for 107 days to observe growth performances in terms of weight and length. Cross 1 and cross 2 showed lower fertilization rates of 86% and 83%, and hatching rates of 66% and 61%, respectively compared with 90% fertilization rate and 71% hatching rate of cross 3. Growth performance study revealed no significant differences ($p > 0.05$) in weight and length between the reciprocal and parental crosses. The Halda♀ X Padma ♂ and Padma ♀ X Halda ♂ crosses showed higher weight of 4.64 g and 4.56 g, and length of 8.63 cm and 8.11cm, respectively compared with 4.47 g and 7.55 cm of parental cross. Fry survival rates in hapas 1, 2, 3, 4, 5, 6, 7, 8 and 9 were 31.25±3.18, 37.50±3.21, 33.33±1.57, 31.25±3.18, 29.41±0.99, 25.00±0.78, 26.67±0.87, 25.00±0.67 and 23.53±0.02, respectively. Hapa 2 showed the highest survival and yield whereas hapa 9 showed the lowest survival and yield.

Keywords: Riverine rohu, *Labeo rohita*, breeding performance, F1 progenies, Bangladesh.

Introduction

Aquaculture is one of the fastest emerging food producing sector that plays a significant role in economy (Devi *et al.,* 2019). Bangladesh is the world's largest flooded wetland, and it is one of the most suitable regions for fisheries. It is the third ranked country in Asia after China and India in terms of aquatic biodiversity (Hussain & Mazid, 2005; Shamsuzamman *et al.,* 2017). Bangladesh is now the third largest fish aquaculture country in the world after China and India (FAO, 2018). Inland aquaculture in Bangladesh is mostly dominated by the cultivation of Indian major carps e.g., catla, rohu and mrigal that are commercially important and broadly cultured in the Indian sub-continent (Ahmed *et al.,* 2008). Rohu is the most delicious and economically affordable fish for local consumers, and fetches high market value (Devi *et al.,* 2019). The agro-based economy of Bangladesh including fisheries and aquaculture sectors provides food and nutrition, elevates poverty, creates employment and earns foreign currency (Islam *et al.,* 2018).

Rohu (*Labeo rohita*), also known as rui, is a fish species of the cyprinidae family found in the rivers in South Asia. In India, Pakistan, Bangladesh, Nepal and Myanmar, this large omnivore species is extensively used in aquaculture. Rohu has specific food preferences at different life stages. During the early stages of its life, it eats mainly zooplankton, but as it grows, it eats more phytoplankton. The juveniles and adults are herbivorous column feeders, eating mainly phytoplankton and submerged vegetation. Rohu has modified, thin hair-like gill rakers, suggesting feeding habit by sieving the water. In carp genetic research, hybridization or cross-breeding is a well-developed and broadly practiced technology for fish stock improvement. The results of such crossing experiments most often expressed in well-performed progeny in terms of growth, survival, fertility and feed utilization compared with their parental origin (Tave, 1993; Bakos & Gorda, 1995). Hybridization has been used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase resistance to disease and improve tolerance to environmental extremes and a variety of other traits that make aquatic animal production more profitable (Dunham *et al.,* 2001). Hybridization may also be used to carry the desirable characteristics from one group or species to another group or species, to combine valuable traits from two species and to produce sterile individuals (David & Pandian, 2006).

Aquaculture sector is flourishing day by day in Bangladesh, leading to increasing seed demand. The seed producers in Bangladesh paid no attention to the genetic quality of carp seed (Hussain & Mazid, 2005), although there were related studies on estimation of genetic parameters for growth and survival in rohu (Gjerde *et al.,* 2019), dietary iron requirement of rohu fingerling based on growth and body composition (Musharraf *et al.,* 2019), effects of fertilization and supplementary feeding on growth performances of rohu, catla and common carp (Abbas *et al.,* 2014), genetic improvement and conservation of carp species in Bangladesh (Hussain & Majid, 2001), and genetic improvement of common carp strains using intra-specific hybridization (Bakos & Gorda, 1995). The genetic variation of hatchery population is generally lower than that of the wild populations because of the limited numbers of parents, negative selection and smaller brood size at first maturity (Shah, 2004). The rate of inbreeding in India is particularly high for rohu, catla and mrigal (Eknath & Doyle, 1990). Shah (2004) reported lack of integration of genetic norm and practice in the hatchery management system and the hatchery-produced seeds of Indian major carps (IMC) have significantly lower growth rate than the seeds originated from the natural sources of river in Bangladesh. In Bangladesh, almost all the hatchery facilities do not have adequate number of ponds to raise their brood fishes (Biswas *et al.,* 2008). As the hatchery-produced seeds resulted in lower growth and survivability probably due to inbreeding problem in hatcheries, fish culturists show special interest in natural fish seed for their faster growth rate, higher disease resistance and survival rate. Therefore, the present study was conducted in an attempt to improve the quality of rohu seeds. The crossbreed between Padma and Halda Rivers was selected due to their brood quality, as Halda River is a well-known natural breeding ground of common carps among South Asian countries. Nevertheless, this is the first report on breeding and growth performances of F1 progenies from Padma and Halda Rivers in Bangladesh. The main objectives of the present study were to evaluate the breeding performances of two riverine rohu, and the growth performances of their F1 progenies reared in hapas.

Materials and Methods

The experiment was carried out in two phases to evaluate the breeding performances of two riverine rohu, and the growth performances of F1 progenies. Induced breeding was conducted on the brood fish collection in Niribili Fish Hatchery and Farm Complex in Jessore. The spawns were reared in hapas in the hatchery for 107 days. The growth performances of the hybrids were recorded in terms of weight by digital analytical balance, and length by centimeter scale at 15-day interval.

Brood Fish Collection and Transportation

The origins of the brood fish were the two major rivers of Bangladesh, Padma and Halda. Two pairs of brood fish (equal number of male and female) were collected from each source. The fish were transported in large plastic containers with aeration. Average length and weight of male brood was 32 cm and 1.57 kg, respectively. The female brood fishes average length and weight was 31.5 cm and 1.54 kg, respectively. Before artificial breeding, the brood fish were kept in a conditioning tank for 24 hrs with aeration. The fish were tagged by fin clipping at the base of the dorsal fin, and treated with 5 ppm of $KMnO_4$ to present infection.

Artificial breeding and intra-specific hybridization

Brood fish were acclimatized in cement tank with flushing water for about 6 hrs. After acclimatization, induced breeding was performed on female fish using carp pituitary gland extract (PGE) injected at the base of the pectoral fin at 2 mg/kg body weight, followed by a second dose 6 hrs later at 6 mg/kg. At the time of second injection for females, a single dose of 2 mg/kg was applied to male fish. When the females ovulated 6 hrs after the second injection, eggs were collected by stripping method. The eggs from a female were collected in a steel bowl and fertilized with sperm collected from a male. When mixing the egg and sperm mass with a clean chicken feather, a small amount of 0.85% NaCl solution was added to activate the sperm. Finally the eggs were washed with clean water and placed in the hatching jar. To perform pair mating, milt from one male was used to fertilize eggs from one female to produce a full sib family. Equal volume of fertilized eggs of about 100 g from each mating pair of selected broods was incubated in separate circular cement jar with fine mesh glass nylon cloth (1.4 m in height). The jars were placed one after another in cement tanks, and the fertilized eggs were kept inside the circular jar with water flushing and showering. The progenies produced from a pair mating kept in a jar were considered full sibs of a single family. Fertilization was observed after 60–72 hrs incubation. A total of 9 reciprocal crossbreed families from Padma ♀ X Halda ♂ cross and Halda ♀ X Padma ♂ cross were produced from the two starting base populations of Padma and Halda rivers. All the families were considered as F1 generation. Three intra-specific hybridizations i.e., reciprocal crosses 1 (Halda ♀ X Padma ♂) and 2 (Padma ♀ X Halda ♂), parental cross (cross 3, Hatchery ♀ X Hatchery ♂) were carried out.

Fertilization and Hatching Rates

Immediately after mixing the sperm with eggs, they were kept in the 50L round fertilization jars connected to water circulating system for proper fertilization. After 30 min, they were examined to determine the fertilization rate using a magnifying glass. The fertilized eggs were differentiated and separated from the unfertilized eggs by the presence of transparent shell with gray spot within the egg shell compared with opaque appearance of the unfertilized eggs. Fertilization rate was determined as follows: Fertilization rate (%) = (Number of fertilized eggs / Number of total eggs)×100. Generally, the eggs were hatched after 18-24 hrs of fertilization. Approximately 200 fertilized eggs were isolated and kept in 6L hatching jars with continuous flow of water for counting hatching rate. The hatching rate was determined by counting the number of hatchlings in the jars as follows: Hatching rate (%) = (Number of hatchlings / Number of total fertilized eggs)×100.

Larvae stocking in hapa and feeding

The 3-day-old larvae were stocked in different hapas to check the growth performance of 3 different crosses. Approximately 1,700–1,800 fry were stocked in the hapas of 13 m^3 for the first 27 days. The density was later reduced to 1,000 for last the 80 days. Larvae of 3 different crosses were stocked separately in 9 different hapas (3 replicates). Supplementary feed prepared with egg mixing in water was administered in the first two days. The feed was administrated three times a day. In the 2nd week, artificial rotifer powder mixed with water was applied in the morning, and commercial feed mixing with water was applied in the afternoon. In the 3rd week, commercial feed were administered to all the 9 hapas. These feeds were applied two times a day. In the 4th week, 100 g commercial feed was applied in hapa 1 (35g in morning, 30 g at noon and 35 g in afternoon). In hapa 2, 80 g commercial feed was applied at the ratio of 20 g, 40 g and 20 g for morning, noon and afternoon, respectively. Likewise, 75 g commercial feed (25 g, 25 g and 25 g) was applied to hapas 3 and 4; 100 g (35 g, 30 g and 35 g) were applied to hapa 5; 80 g (27 g, 26 g and 27 g) were applied to hapas 6, 7, 8 and 9.. This feeding regime was applied for 7 days, and later changed according to different hapas. From 5th week onwards, the feed was reduced to 5% of total biomass for the next 80 days.

Water Quality Monitoring

Water temperature (ºC), dissolved oxygen (mg/L) and pH were measured twice a month. Dissolved oxygen and pH were measured using multi-parameter water quality meter (Model-HQ40D, USA) and temperature was measured by conventional mercury thermometer.

Data Analysis

Statistical analyses of growth performances i.e., weight and length, were performed using MS Excel. One-way ANOVA was performed at 5% significance level to determine the difference in growth performance.

Results

Fertilization and Hatching Rates

The fertilization rates of reciprocal cross 1 (Halda♀ X Padma ♂) and cross 2 (Padma ♀ X Halda ♂) were 86% and 83% respectively, compared with 90% of parental cross (cross 3, Hatchery ♀ X Hatchery ♂) in the present study. The hatching rates of reciprocal crosses 1 and 2 were 66% and 61% respectively, compared with 71% of parental cross. The fertilization and hatching rates of 3 different crosses are shown in Figure 1.

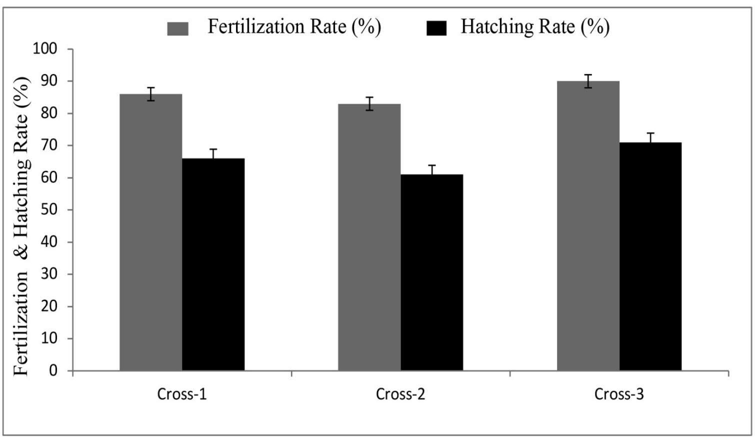

Figure 1: Fertilization and hatching rates of 3 different crosses (Cross-1: Halda ♀ X Padma ♂, Cross-2: Padma ♀ X Halda ♂, Cross-3: Hatchery ♀ X Hatchery ♂)

Table 1: Statistical comparison of mean weight among 3 families

Family	Cross	No. of samples	Mean±SD	P value
F1	Halda (♀) × Padma (♂)	8	2.55±1.54	0.01*
	Padma (♀) × Halda (♂)	8	2.44±1.47	
F2	Halda (♀) × Padma (♂)	8	2.53±1.57	0.01*
	Padma (♀) × Halda (♂)	8	2.37±1.45	
F3	Halda (♀) × Padma (♂)	8	2.39±1.53	0.02*
	Padma (♀) × Halda (♂)	8	2.36±1.43	

* Significance at 0.05 level

Growth Performance and Survival Rate

The mean body weights of 2 different intra-specific reciprocal crosses and the parental cross are presented in Figure 2. The analysis showed no significant differences (*p*> *0.05*) in growth rates between the reciprocal and the parental crosses. In the present study, F1 hybrids were produced by intra-specific crossing between 2 different strains viz. Halda♀ X Padma ♂, Padma ♀ X Halda ♂, and 1 parental cross viz. Hatchery ♀ X Hatchery ♂ of *L. rohita*. Growth performance study for a period of 107 days obtained final body weight of 4.64 g and 4.55 g for reciprocal crosses 1 and 2 respectively, compared with 4.47 g of parental cross. The mean lengths were 8.63 cm, 8.11 cm and 7.55 cm for cross 1, cross 2 and cross 3, respectively (Figure 3).

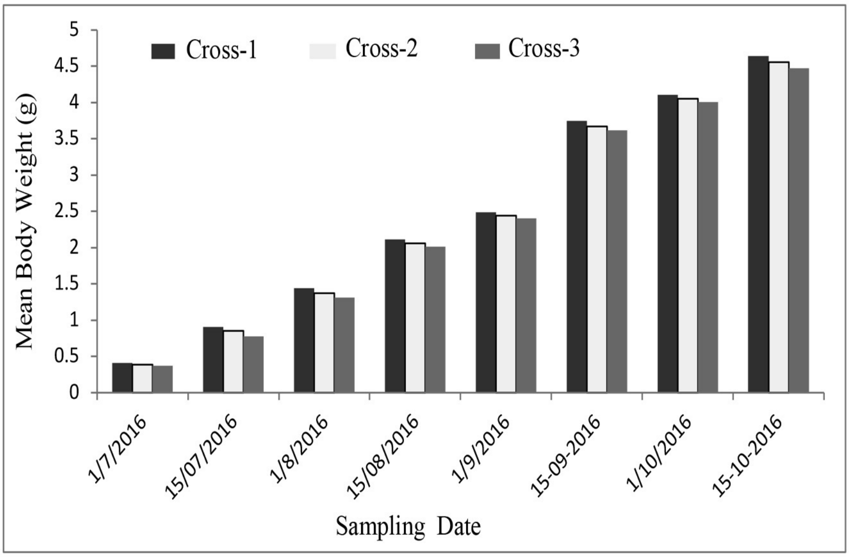

Figure 2: Mean body weight of 3 different crosses (Cross-1: Halda ♀ X Padma ♂, Cross-2: Padma ♀ X Halda ♂, Cross-3: Hatchery ♀ X Hatchery ♂)

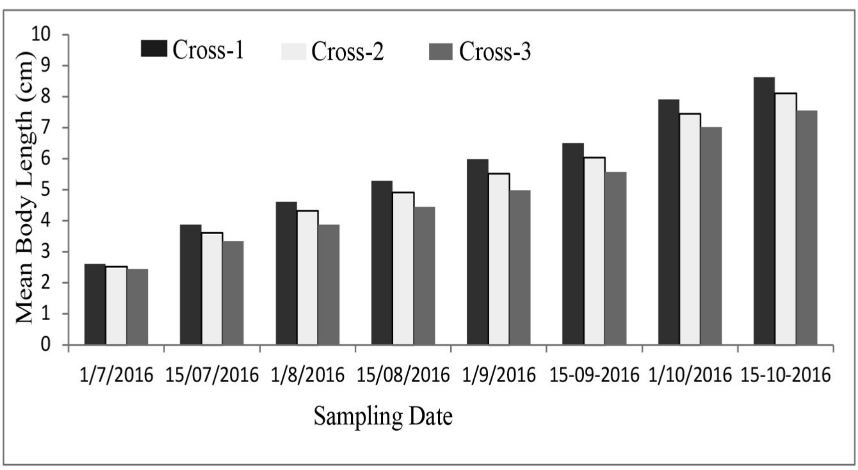

Figure 3: Mean body length of 3 different crosses (Cross-1: Halda ♀ X Padma ♂, Cross-2: Padma ♀ X Halda ♂, Cross-3: Hatchery ♀ X Hatchery ♂)

Table 2: Comparative specific growth and survival rates of fry in hapas

Hapa	SGR (%)	Survival rate (%)	
		Mean	M±SD
1	1.09	31.25	31.25±3.18
2	1.04	37.50	37.50±3.21
3	1.02	33.33	33.33±1.57
4	1.02	31.25	31.25±3.18
5	1.09	29.41	29.41±0.99
6	1.02	25.00	25.00±0.78
7	1.01	26.67	26.67±0.87
8	1.02	25.00	25.00±0.67
9	0.97	23.53	23.53±0.02

Table 3: Ranges of water quality parameters of the rearing hapas

Parameters	Value Ranges	Mean ± SD
Temperature (°C)	26.45-31.32	28.7 ± 1.92
Dissolved oxygen (mg/L)	4.81-5.27	5.02 ± 0.25
pH	7.15-7.92	7.30 ± 0.53

Table 2 indicates the growth and survival rates of fry reared in hapas. The growth and survival rate patterns were almost the same in all hapas except hapa 2 that showed slightly higher survival rate, and hapa 9 that showed comparatively lower survival rate.

Water Quality Parameters

The water quality parameters i.e., temperature (°C), dissolved oxygen (mg/L) and pH of the rearing hapas are shown in Table 3.

Discussion

Recently, it has been reported that hatchery-produced carps had shown reduction in growth and reproduction performances, and higher disease prevalence and mortalities. This is probably due to genetic deterioration in the hatchery stocks as a result of poor fish broodstock management and inbreeding depression (Hussain & Mazid, 2001; Mahapatra *et al.,* 2017). Such poor broodstock management and close mating of breeders result in highly homozygous inbred hatchery populations leaving adverse effects on aquaculture production. However, genome manipulation and selective breeding have a very high potential for improving the genetic makeup of fish in aquaculture production because of high fecundity and high heritabilities of commercially important traits (Mahapatra *et al.,* 2017). In India, 'Jayanti' rohu has shown a genetic gain of 18% per generation for growth trait after eight generations of selection. In this study we evaluated the breeding performances of 2 riverine rohu and the growth performances of their F1 progenies reared in hapas.

The present study demonstrated that the fertilization and hatching rates of 2 reciprocal crossbreeds were lower than the parental cross (Hatchery ♀ X Hatchery ♂). Our results are in good agreement with those reported recently for common carp by Shah *et al.* (2011) and Sayeed (2015). Intra-specific hybridization may lead to higher or lower fertilization and hatching rates depending on the species and strain, physical condition, environmental condition and management practices during the breeding time. The rates of fertilization and hatching may be affected by various other factors such as temperature, rate of water flow, water quality, condition of the broods, quality of PGE and method of insemination. The reciprocal crosses showed significantly lower fertilization and hatching rates compared with parental cross due to differences in population, changes of habitat, stress of new environment and transportation. A study of Mishra *et al.* (2017) showed that significantly higher fertilization (74%) was achieved when the oocytes were stored using artificial carp ovarian fluid, and 65% ovarian fluid under different temperatures. Coating of tanks with CuO and ZnO nanoparticles or direct addition to tank has been exhibited to enhance the percentage of hatching for rohu. The highest survival of rohu fry was also observed 15 days post hatching in CuO coated tanks (Swain *et al.,* 2016). However, in the present study, the higher fertilization and hatching rates in all the crosses were the results of the brood fish management in hatchery operation.

The reciprocal crosses showed relatively higher growth performances than the parental cross (F, Hatchery ♀ X Hatchery ♂) with no significant differences ($p > 0.05$) probably attributed to several factors such as rearing in hapa, little or no natural food available in hapas, solely dependent upon artificial diet as no compost was applied to maintain natural food level. Shah *et al.* (2011) and Sayeed (2015) observed higher growth performances in reciprocal crosses than the parental crosses reared in ponds. The results of the present study were similar to the findings of Islam and Shah (2007). Biswas *et al.* (2008) obtained better growth performance of riverine strains of Indian major carps over the hatchery strains. The results attained from this investigation revealed that the seed of intra-specifically crossed or riverine crossed rohu performed better than the hatchery strain in terms of growth performance. The present study showed better growth and survival rate of fry in all hapas except hapa 9, probably due to the supplementary and artificial feeds. Abbas *et al.,* (2014) found that supplementary and artificial feeds along with organic manure resulted in better growth and yield of common carp. The brood fish should be collected from divergent sources, and the pedigree record must be well kept. A better management practice must be performed as well in hatcheries to produce good quality seed for increasing production.

Conclusion

Present study investigated the growth performances of 2 reciprocal crossbreeds (Halda♀ X Padma ♂ and Padma ♀ X Halda ♂) of rohu compared with its parental cross (Hatchery ♀ X Hatchery ♂). The results revealed that the seed of intra-specific riverine crosses performed better than the pure hatchery strain in terms of growth performance. In order to produce more fish seed to meet increasing demand with the growing feed demand, special attention must be given to maintain a large stock of broods. If a good attention is paid to better management practice in hatchery with domestic and international research collaborations, the valuable populations of Indian major carps can be largely protected from being genetically damaged. Thus, the intra-specific hybridization technique can effectively be introduced to the inland aquaculture to boost aquaculture production.

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