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Original article

Strain crossing in mrigal (*Cirrhinus cirrhosus*): An avenue to persuade heterosis in F_1 generation of wild×hatchery hybrid

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Abstract

While the increase of the yield of commercially trendy farmed fishes are of special priority in aquaculture, a number of handy technologies are indispensable to realize the surplus production aiming to feed the growing population. As a genetic means of doing this, hybridization technology has been being used for years, because it can produce hybrid vigor through inducing heterosis. Similar attempt was made in mrigal, *Cirrhinus cirrhosus*, by crossing the hatchery strain with the riverine strain to investigate the heterotic effect in the progeny the species could generate through such intra-specific hybridization. Mean fertilization and hatching rates in hybrids were found to be lower by 7% and 4% respectively than that in purebreds, which envisioned to be trivial to impact the purpose of crossing. Comparative growth test between purebreds and hybrids showed significant differences ($p< 0.05$) in weight and length, and resulted 30.51% heterosis in hybrid mrigal. This experiment can be considered as a preliminary investigation to motivate the application of strain crossing in mrigal farming and the result found thereof can be used as a clue to optimize this growth boosting technique for the species through further research.

Keywords: Aquaculture genetics, intra-specific hybridization, mrigal, heterosis, hybrid vigor

INTRODUCTION

Aquaculture in Bangladesh, since its initiation, is mostly dominated by the cultivation of Indian Major Carps, *viz*. rohu, catla, mrigal etc. Carp culture technology here has got a considerable development in last couple of years where production of fish has been increased by means of scientific intensification and sophistication. Genetic improvement technology in recent past has paved this way more rapidly and has become popular all around. Such experimentation in Indian Major Carps has brought substantial success in the field of aquaculture and genetics in Bangladesh. While rohu and catla have got prior choice by the most of researchers here in different trials and investigations, mrigal even being an important protein-supplementary species has most often been over-looked. This study got a motivation on that ground. In addition, developed breeding technique and seed availability of mrigal are supposed to enhance the adaptation of any sorts of further improvement and its practice in the field. Introduction of mrigal in genetic research has received a special attention in the current study to make the fish popular for polyculture system and to maintain genetic quality of this species in farming. Getting higher growth rate was an addition to this. Furthermore, due to being a bentho-pelagic feeder in nature, mrigal shows well compatibility in polyculture farming by properly utilizing the target trophic level. On ecological ground, this bottom dweller species helps in re-suspension of nutrients from the sediment by its food searching activity thus facilitating other inhabitants to get nutrients from water. Hence, mrigal with such multifarious significance may revolve into a prospective culturable species and creates a room for its quality improvement through research.

In carp genetic research, hybridization or cross-breeding is a well-developed and widely practiced technology for stock improvement. Results of such cross experiments most often manifested well-performed progeny in survival, growth, fertility, and feed utilization when compared with their parental origin (Tave 1993, Bakos and Gorda 1995). These sorts of manifestations are technically termed as positive heterosis, which is the improvement in biological functioning or genetic quality in a cross-bred generation. To exploit heterosis from hybridization is a means of achieving desirable traits in hybrids. These phenotypic traits are originated from the effects of inter or intra loci gene action and are worthy to be superior over the parental attributes to commend the merit of hybridization (Barelli *et al*. 1998). Thus, heterosis is effected in F_1 generation through individual loci interaction (Yan and Ozgunen 1993).

Strain crossing and realization of positive heterosis has been important basis of carp culture in many countries, *viz*, Israel, Vietnam, Hungary etc. (Hossain 2001). Hybridization had been reported as a means to transfer desirable characteristics from one pool to another or to combine valuable traits from both strains or even to produce sterile individuals (Davidand Pandian 2006). While the agriculture sector in Bangladesh has brought revolution in producing serials, pulses, fruits and vegetables etc. through hybrid seed technology, the fisheries sector cannot lag behind (Shah *et al*. 2011). Therefore, aiming to fold the production of fishes higher is now a challenge, which can be feasible through inducing heterotic effect in the progeny. Strain crossing is a promising technology, which enables an offspring to surpass its parents for one or more traits through procuring heterosis. With these viewpoints, a similar attempt of intra-specific hybridization was made in mrigal, *Cirrhinus cirrhosus*, by cross-breeding the wild and hatchery strains to reconnoiter the heterotic effect in the offspring through comparing their growth performances.

METHODOLOGY

Design of experiment: The experiment was designed to conduct at two distinct phases *viz*, induced breeding with reciprocal hybridization (riverine strain × hatchery strain) and comparative growth test of the hybrids with the parents. Fertilization and hatching rates of control and treatment groups were determined and the hatchlings were reared up to 10 weeks in earthen ponds for subsequent growth test.

Collection, transportation and maintenance of broodstock: Broodstocks of riverine (Padma) strain were collected from Freshwater Research Sub-station, Jessore and those of hatchery strain were sourced from Fish Seed Multiplication Center, Khulna. A total of five pairs of brood from each source were carried to the study site (Khulna) in large plastic containers having arrangements of ice, saline and aeration for expedient transportation. Reaching at experimental station the broods were allowed for a temporary rest in large concrete tanks set under water cascade and after that tagged and released in brood rearing ponds. Supplementary feed was given at a rate of 5% of total biomass.

Induced breeding with hybridization: The matured brood fishes were prepared for hypophysation keeping them under a 12-hour photoperiod in conditioning tank. Shifting in brood tank, the induced breeding was performed by injecting carp PG hormone intramuscularly. Dry PG was crushed, dissolved and centrifuged for collecting its supernatant solution, which was then taken for the injection. The sex ratio of the spawners at '1 (male) : 2 (female)' was maintained. Female broods were treated with a double dose of 2 and 6 mg PG per kg body weight at 6 hours interval while male broods were given a single dose of 2 mg PG per kg body weight at the time of second administration to the female (Shah *et al* 2011). 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. Eggs and milt thus collected were mixed well for proper fertilization. A reasonable amount of 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 stickiness of the eggs. For heterosis production and analysis, two different strains of mrigal were reciprocally hybridized. For this, the artificial inseminations were carried out by Riverine ♂× Riverine ♀ and Hatchery ♂× Hatchery ♀ as control and by Riverine ♂× Hatchery ♀ and Riverine ♀×Hatchery ♂as treatment.

Fertilization and hatching rates: 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. Fertilization rates were then determined as: *Fertilization rate (%) = (Number of fertilized eggs having transparent shells present in a specific volume of water taken for measurement / Number of total eggs present in that volume) * 100*. After passing the hatching period of about 24 hours, hatching rates were calculated in similar way as: *Hatching rate (%) = (Number of hatchlings ascertained by visual observation and present in a specific volume of fertilized eggs set for measurement / Number of total fertilized eggs present in that volume) * 100*.

Culture for growth test: Three days old hatchlings were transferred into eight prepared earthen ponds [for 2 strains × 2 observations (1 control and 1 treatment) × 2 replications of each] and reared for ten weeks. Pond preparation, liming, fertilization, acclimatization, stocking, feeding, water quality maintenance etc. were done according to local standard aquaculture practice. Growth in terms of weight and length were checked at every tenth day through random sampling and recorded for subsequent analyses.

Heterosis calculation and statistical analysis: Heterosis thus produced in the hybrids of the mrigal was measured from a comparative growth test with the parents and its effect was calculated as: *Heterosis (%) = (Mean growth of* F_1 *reciprocal hybrids – Mean growth of parents) * 100*. The results of the various growth trials were statistically compared by One-way ANOVA and using R Programming Language.

RESULTS

Strain crossing was successfully done in mrigal to bring heterotic effects in the hybrid. Hybrid thus produced from intra-specific crossing of *Cirrhinus cirrhosus* specifically between the strain of the river Padma and the strain of the hatchery showed variable fertilization, hatching and growth rates, and finally positive heterotic effect which were analyzed by comparing their growth rates with that of the purebred.

Fertilization rate: The fertilization rates of two reciprocal crossbred groups, 'Padma ♀× Hatchery ♂' and 'Padma ♂× Hatchery ♀' were 83% and 81% respectively and of two purebred groups, 'Padma ♂× Padma ♀' and 'Hatchery ♂× 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 purebred.

Hatching rate: The hatching rates of two reciprocal crossbred groups, 'Padma ♀× Hatchery ♂' and 'Padma ♂× Hatchery ♀' were 69% and 66% respectively and of two purebred groups, 'Padma ♂× Padma ♀' and 'Hatchery ♂× Hatchery ♀' were 73% and 70% respectively. Percent hatching of different breeding products is shown in Figure 1, which reveals lower hatching rates in hybrid than in purebred.

Growth and heterosis: Growth performance of the hybrids recorded in terms of weight and length at different sampling days was compared with that of the purebreds (Table 1 and Figure 2). The analysis showed significant differences ($p < 0.05$) in the growth rates between the hybrid and the purebred. This difference was quantified in terms of heterosis, which yielded 30.51% positive effect in the hybrids, *i.e.* 30.51% higher growth rates were observed in the reciprocal hybrids than the purebreds. This indicated that hybrid vigor in mrigal had been accompanied in the progenies through strain crossing.

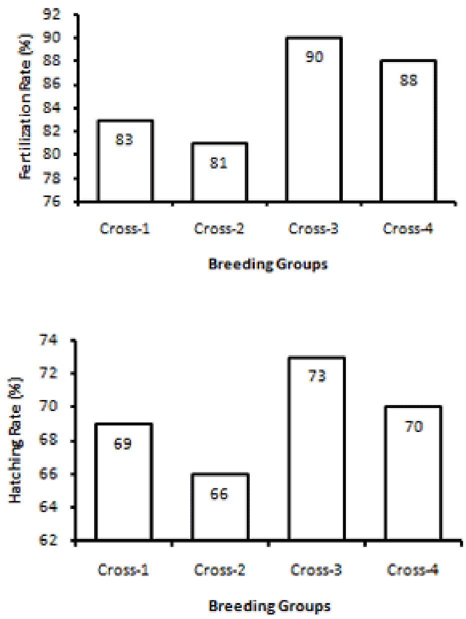
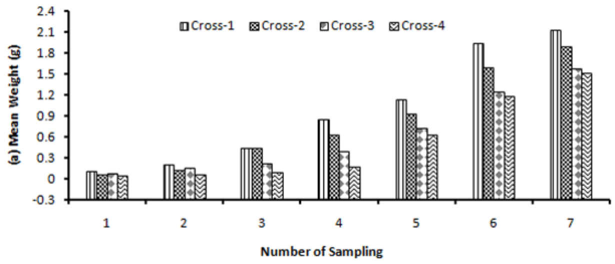


Figure 1: Fertilization rates and hatching rates of different breeding groups (Cross-1: Padma ♀× Hatchery ♂; Cross-2: Padma ♂× Hatchery ♀; Cross-3: Padma ♀× Padma ♂; Cross-4: Hatchery ♀× Hatchery ♂)

Table 1: Average weight and length gained in different breeding groups

Sampling	Mean Weight (g)				Mean Length (cm)			
	Cross-1 Padma ♀× Hatchery ♂	*Cross-2* Padma ♂× Hatchery ♀	*Cross-3* Padma ♂× Padma ♀	*Cross-4* Hatchery ♂× Hatchery ♀	*Cross-1* Padma ♀× Hatchery ♂	*Cross-2* Padma ♂× Hatchery ♀	*Cross-3* Padma ♂× Padma ♀	*Cross-4* Hatchery ♂× Hatchery ♀
1	0.088	0.046	0.062	0.024	-	-	-	-
2	0.184	0.114	0.140	0.053	-	-	-	-
3	0.428	0.423	0.210	0.081	2.640	2.120	1.940	1.250
4	0.832	0.609	0.371	0.152	3.970	3.540	2.860	2.160
5	1.123	0.912	0.712	0.620	4.860	4.040	3.900	3.330
6	1.927	1.576	1.230	1.170	5.400	4.820	4.340	4.120
7	2.130	1.890	1.570	1.510	6.120	5.840	5.460	4.790



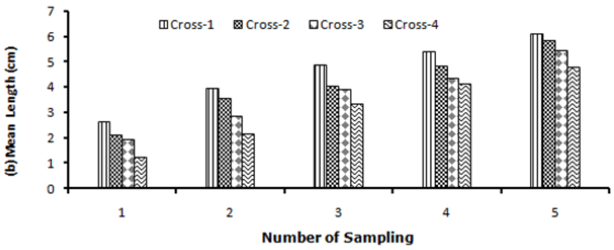


Figure 2: Comparative weight and length of hybrid and purebred (Cross-1: Padma ♀× Hatchery ♂; Cross-2: Padma ♂×Hatchery ♀; Cross-3: Padma ♀× Padma ♂; Cross-4: Hatchery ♀× Hatchery ♂)

DISCUSSION

The experiment was carried out in order to observe the growth performance of intra-specific hybrids of mrigal with the implication to bring this knowledge as a reference of stock improvement of the species and to commercialize the benefit of the outcome of this study if would affect positively. Fertilization and hatching rates were found to be higher in the purebred than in the hybrid. This might happen due to the adaptation of the strains at different ecological niche, which might weaken the natural viability of the gametes while artificially inseminated. Shah *et al.* (2011) mentioned about lower rates of these estimators in strain crossing of other carp species rohu. Nevertheless, the lower rates of fertilization and hatching of the intra-specific hybrids in no way disqualify or dissatisfy the implications of strain crossing in this species. These rates may be affected by various other factors, such as temperature, rate of water flow, water quality etc. and even the condition of the broods, quality of PG, method of insemination etc. Hossain (2001) reported higher fertilization and hatching rates in the hybrid progeny of another species of the same genus of mrigal, namely *Cirrhinus reba*. Alike result was disclosed by Islam and Shah (2007) in the reciprocal riverine-hatchery crossed hybrids of carp species. Breeding season and maturity of brood vary from species to species and may vary from strain to strain also. Intra-specific hybridization of rainbow trout resulted better fertilization performance (Moavet *al.* 1975), while the same in channel catfish denied this (Hulata *et al*. 1985). These rates recorded in the current study for mrigal were not found to be daunting for the production and analysis of heterosis in this species.

Hybrids are generally produced for better growth than their parental strain, which was likely for mrigal also. This was probably due to the transfer of good quality traits of mrigal in the hybrids and thus hybrid vigor was achieved in the species. The crosses between riverine and hatchery strains of mrigal produced 30.51% heterosis. Although the quality of the hybrid is simply a matter of chance, however a number of researches on intra-specific strain crossing faced success in increasing vigor in the progeny thus produced. Sunshine bass was a specific example of the cross of white and stripped bass, which was reported to grow faster than either parent (Smith 1988). Similar other results of strain crossing in different species worldwide were documented in the literature as of being capable to improve overall performance for aquaculture systems (Moav *et al.* 1975, Ibrahim 1977, Kirpichnikov 1981, Senhorini *et al*. 1988, Khan *et al*. 1990, Salami *et al*. 1993, Wohlfarth 1993, Bakos and Gorda 1995, Basavaraju *et al*. 1995, Hulata 1995, Nwadukwe 1995, Knibb *et al*. 1998, Reddy 2000, Islam and Shah 2007, Shah *et al*. 2011). Combination of cross or breeding groups may inspire the likelihood of producing F_1 hybrids. Tave (1993) noticed that the chance of positive heterosis increased from a combination of hatchery × hatchery rather than that of wild × hatchery. Dunham and Smitherman (1983) also found to exhibit the same in channel catfish.

Hussain and Mazid (1997) and Shah (2000) reported about the mostly unknown pedigree of the broodstock and widespread incidence of inbreeding depression in the hatchery-produced seed in Bangladesh. The same was realized in the form of retarded growth, poor reproductive performance, morphological deformities, and increased incidence of disease and mortality of seed in the hatcheries. Hussain and Mazid (1999) suggested on the improvement of broodstock management to address the issues highlighted above; intra-specific hybridization or strain crossing has been proved to be one of the prospective strategies in this area. Population with lost or less amount of V_A can be hybridized with other outbred population to increase heterozygosity as a mean of increasing production performance (Tave 1993).

Stocking of hatchery strains hybridized with local population can produce faster growing hybrids aiming to be harvested by commercial fishermen (Moav *et al*. 1979). In Indonesia, strain development using artificial gynogenesis and sex-reversal resulted in ten common carp inbred lines, which were later used for crossbreeding (Sumantadinata 1995). In Vietnam, eight local varieties of common carp were crossed with Hungary, Ukraine, Indonesia and Czech strains where significant heterosis was observed in F_1 generations of the crossbreds (Thien and Trong 1995). Artificial hybridization among cyprinids of different families and genera was carried out extensively in China (Wu *et al*. 1994).

Crossbreds of different strains of European catfish, *Silurus glanis* were characterized by outstanding adaptability under warm water and mixed diet regimes (Krasznai and Marian 1985). In addition, crosses with the walking catfish, *Clarias macrocephalus*, showed improved resistance to *Aeromonas hydrophila* infections (Prarom 1990). Increased environmental tolerance might 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). 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 (*Clarius 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). All theses references entertain the use and importance of practicing intra-specific strain crossing in aquaculture farms and hatcheries. Therefore, the attempt in the current study and the results found thereof are thought to be promising for stock improvement of mrigal and its commercial farming in the locality.

CONCLUSION

Hybrid of two strains of the same species sourced from different or even distant population often had a higher growth rate than the offspring of either parental strain; this fact was proved by lots of experiments in various fish species worldwide. Similar upshot was evidenced in the current study where the strain-crossed mrigal demonstrated positive heterosis (30.51%) in growth performance. Difference in average fertilization and hatching rates between purebreds and hybrids were too trifling to impede the research target. Hence, the current result concludes the importance of practicing intra-specific strain crossing in mrigal as to be promising for its stock improvement and commercial farming in the locality. Even this prefatory study motivates the application of strain crossing in mrigal culture and offers a hint to optimize this yield raising technique for the species through further research.

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