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Evaluation of Reproductive Traits of Four Strains of Silver Barb (*Barbodes gonionotus* Bleeker)

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Abstract

Two wild strains of *Barbodes gonionotus* obtained from Indonesia and Thailand and an existing stock of Bangladesh were used to develop F_1 crossbred generation through selective breeding using a 3 x 3 diallele crossing method. Reproductive traits i.e. gonado-somatic index (GSI), fecundity, egg fertility, hatchability, percentage of normal, abnormal and dead embryo, egg size, larval size and sperm concentration were evaluated in these strains. The crossbred produced a significantly ($P<0.05$) higher number of larger eggs than that of the other three strains. The crossbred was also found to be significantly higher in GSI and fecundity than the other strains and had larger larvae. There were no significant differences in fertilization rate and hatching rate among the strains. Sperm concentration was not also significantly different among the strains. The combination of several desired characters in the crossbred suggests that this stock may be economically advantageous to culture in Bangladesh.

Introduction

Selective breeding is a breeding technique in which the breeder chooses the next generation's broodstock, based on some predetermined criteria. The process by which crosses are accomplished between the parental stocks representing different strains of the same species is referred to as crossbreeding, and can also be called intraspecific hybridization. Crossbreeding can produce strains of superior performance by introducing greater genetic variability, and by producing novel combination of genes.

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Silver barb is a popular species among fish farmers in Bangladesh and elsewhere in the Southeast Asia. The Bangladesh Fisheries Research Institute (BFRI) introduced two wild germplasms of *B. gonionotus* from Indonesia and Thailand in 1994 and an existing strain, which was also introduced from Thailand in 1977. Six groups of crossbred and three groups of control were developed from three wild stocks through 3 x 3 diallele crossing and selective breeding. Six groups of crossbreds were communally reared and selective breeding was performed up to F_3 generation (Hussain et al. 2002). There were some genetic differences between the two wild germplasms and the existing stock because these were imported from different ecological systems at different times.

There is a need to evaluate the breeding value of these three stocks and the produced crossbreed generation. The purpose of the study is to evaluate the genetic differences of the three strains and F_1 crossbred group, with respect to reproductive parameters such as egg number, egg size, fertility, hatchability and the number of normal and deformed embryos.

Materials and Methods

Two wild germplasms of *B. gonionotus* obtained through ICLARM from Thailand and Indonesia in 1994 and one existing stock were reared for genetic improvement in the Freshwater Station, BFRI. These three stocks had been bred to produce a base population and the next F_1 generation crossbreed was obtained through selective breeding and line crossing techniques as described by Hussain et al. (2002).

A total of nine genetic groups consisting of six different crosses and three control progenies were derived from diallele crosses using the three founder stocks. The six genetic crossbred groups were reared communally. In 1997 three full sib groups of spawn produced from three pairs of broods within each crossbred group, only 375 (125 x 3) spawns were taken and as such a total of 18 (3 x 6) full sib families from 18 pairs of broods, 2250 (125 x 18) spawns were nursed communally in two ponds (Hussain et al. 2002). This F_1 crossbred generation and the pure parental strains were used in the present study.

The stocking density, feeding, and water quality of each experimental pond were constant during the study period. The reproductive characters of these four strains, which were obtained in the same environmental condition, were evaluated in BFRI.

The male and female brood fish were collected from a 400 m^2 earthen pond at a density of 1 fish·m^{-2} and transferred to the hatchery. Commercially available acetone dried pituitary glands were used for hormone induction. The males and females were kept separately in two hatchery holding cisterns in hapas. Injection was applied intramuscularly at the base of the dorsal fin (Hussain et al. 1987). The total length of each male and female was measured before injection. Then both male and female breeders were carefully transferred to the spawning hapa (6 ft x 4 ft) facilitated with continuous aeration enhancing the activity of natural spawning at the injected breeders. Ovulation took place within 4 to 6 hrs after injection at which time they were ready for stripping. After eggs and milt were stripped and mixed in a bowl using a feather, 0.85% NaCl (physiological solution) was added at 5 to 10% of the total volume of egg and kept for 1 min after which pure water was added and stirred for 1 to 2 min to ensure that all eggs were completely fertilized.

Before fertilization, total egg weight of each fish, and the total volume of eggs were measured. After fertilization, the egg of a fish was kept in a beaker (500 ml) and the volume was recorded. Then the number of eggs was determined by counting a subsample of 1 ml. Fecundity was estimated using the following formula:

$$\text{Fecundity} = \frac{\text{No. of eggs obtained in subsample x total volume of egg}}{\text{Volume of subsample}}$$

One to two drops of milt were collected from each male fish to assess sperm concentration. At first the semen was diluted twice in a solution of 0.85% NaCl. For counting in a haemacytometer, 0.1 ml samples of this diluted solution were transferred into the cell of haemacytometer and the numbers of spermatozoa were counted in 16 x 5 = 80 units of a haemacytometer. Sperm concentration (sperm cell ml^{-1}) was estimated as follows:

$$\text{Sperm concentration (sperm cell ml}^{-1}) = \frac{\text{No. of sperms counted x dilution x 4000 x 1000}}{\text{No. of sperm squares counted}}$$

The GSI of females was calculated using the ratio of the mean ovary weight to body weight of the fish and expressed using the formula:

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Total body weight of fish}} \times 100$$

B. gonionotus egg diameters were obtained by randomly sampling 100 eggs per ovary and by measuring their diameter using an ocular micrometer. Fertilization and hatching rates were recorded by collecting several subsamples of developing eggs and hatchlings.

Statistical differences within groups for each character were determined through the LSD test for means with unequal numbers of replication. Statistical analysis was performed using the SPSS program (Version 7).

Results

The average body length, pre and post ovulation body weight of the Bangla strain were significantly ($P<0.05$) lower than those of the other strains (Table 1). However, no differences were observed among crossbred, Thai and Indo strains for body length and pre and post ovulation body weight. Highest values for these parameters were recorded in the Indo strain followed by the Thai strain and the crossbred and lowest in the Bangla strain. The mean egg weight, GSI and fecundity of crossbred were significantly (P<0.05) higher than those of the other strains. Highest GSI value was recorded in crossbred, about 30.64% per body weight followed by Bangla (19.44%) and Indo (14.78%) and lowest in the Thai strain (9.01%). The highest value for average fecundity for crossbred was 269364 per mature fish (20 to 24 cm total length) followed by Bangla, 117778 (15 to 19.7 cm total length) and Indo, 138553 (20.5 to 29.4 cm total length) and lowest in Thai, 35597 (20 to 24 cm total length).

The mean egg diameter was significantly (P<0.05) lower (95.77) in Thai than that of the other strains. The highest value was observed in Bangla and crossbred, 113.35 mm and 111.40 mm, respectively.

The percentage of normal, abnormal or deformed and dead embryos, as recorded during 0 to 3 h of post fertilization period, showed significant variations (P<0.01) among the four experimental strains. The percentage of dead embryos was significantly (P<0.05) higher in the crossbred than that of the other three strains. The percentages of normal embryos were significantly higher in Bangla and Thai strains compared to the crossbred and Indo strains. The percentage of abnormal embryo recorded in Indo strain was significantly higher than that of the other strains and crossbred recorded the lowest value for this parameter. The fertilization rate and hatching rate of the four experimental strains were not significantly different. The highest value for fertilization rate was recorded in crossbred (92.89%) and the lowest value (73.76%) was observed in Bangla. The highest value (61.11%) for hatching rate was recorded in crossbred and the lowest value (52.31%) was observed in Thai. The diameter of egg and nucleus of fertilized eggs immediately after fertilization was measured from each strain, which are presented in table 2. The egg diameter of Bangla strain had a higher value (2.83±0.11mm) than that of the other strains. The nuclear diameter through the major and minor axes was slightly higher in crossbred. There was a significant difference in larval size (3 day old)

Table 1. Reproductive performance of the four different strains of female *B. gonionotus*

Parameter	Strain				CV (%)
	Bangla (n=5)	Thai (n=4)	Indo (n=3)	Cross (n=5)	
Body length (cm)	19.72[a]	24.00[b]	29.36[c]	24.02[b]	17.20
Body weight (g)	100.40[a]	206.37[b]	261.66[b]	200.32[b]	38.79
Post ovulation weight (g)	85.16[a]	180.30[b]	215.96[b]	156.00[b]	40.54
Egg weight (g)	19.55[a]	18.60[a]	37.53[b]	59.32[c]	53.99
Egg diameter (mm)	113.35[b]	95.77[a]	106.87[b]	111.40[b]	9.92
Gonado somatic Index	19.44[a]	9.20[a]	14.78[a]	30.64[b]	49.20
Fecundity	117778[b]	35597[a]	138553[b]	269364[c]	72.91
Fertilization (%)	73.76	91.77	91.46	92.89	33.32
Hatching (%)	58.73	52.31	52.76	61.11	35.45
Normal embryo (%)	71.80[c]	64.00[c]	36.42[a]	50.94[b]	25.85
Abnormal embryo (%)	14.40[a]	18.50[a]	46.85[b]	12.00[a]	71.42
Dead embryo (%)	13.80[a]	17.50[a]	16.73[a]	37.06[b]	53.89
Larval size (3-days old)	4.25[b]	3.40[a]	3.48[a]	4.15[b]	16.20

Values in the parenthesis indicate sample size in each strain.
[a,b,c]Mean values having different superscripts differ significantly (P<0.05)

among the strains. Crossbred and bangla strains had significantly higher values than the other two strains.

Table 3 represents the body length, pre and post breeding body weight, and sperm concentration of the four different strains of male *B. gonionotus*. The differences between pre and post breeding body weight were found to be higher in Indo strain than that of the other strains. Significant differences in sperm concentration among the strains were also observed.

Relationship between egg sizes and hatching rates of the four strains showed that there was a positive correlation between egg size and percent hatch in all the strains except for the Thai strain, which showed a negative relationship. The 'r' values of the Bangla, Thai, Indo and crossbred strains were 0.336, -0.589, 0.999 and 0.333, respectively (Fig. 1).

Discussion

Reproductive traits of both female and male breeders are important criteria upon which to judge the performance of crossbred and purebred strains of *B. gonionotus*. The characters that differed most among the strains, were fecundity, GSI, sperm concentration, egg size, hatchability and percentage of deformed individuals.

Crossbreds produced more eggs than purebreds. At 24 cm body length, females from Thai strain produced 35597 ova. Length of crossbred was about 24 cm and fecundity, relative to length was several times higher than that in Thai (269364). Higher fecundity was also observed in mass selected crossbreds

Table 2. Diameter of egg and nucleus of ova measured after 3hr of fertilization in the four different strains of *B. gonionotus*.

Name of the strain	Egg diameter (mm)	Nucleus diameter (mm)	
		Major axis	Minor axis
Crossbred	2.78±0.22	0.978±0.02	0.663±0.02
Bangla	2.83±0.11	0.637±0.02	0.611±0.05
Thai	2.39±0.26	0.686±0.02	0.621±0.08
Indo	2.51±0.55	0.667±0.01	0.654±0.01

[SED]Standard error of differences

Table 3. Reproductive characteristics of different strains of the male *B. gonionotus*.

Parameter	Strain				CV (%)
	Bangla (n= 5)	Thai (n= 4)	Indo (n= 3)	Crossbreed (n= 5)	
Body length(cm)	21.64[a]	20.50[a]	24.83[b]	20.86[a]	7.74
Body weight(g)	114.40[a]	108.75[a]	187.00[b]	105.02[a]	27.27
Post breeding weight(g)	111.40[a]	107.45[a]	182.16[b]	102.74[a]	27.04
Sperm conc./ml	$3.9X10^{10}$	$5.2X10^{10}$	$8.9X10^{10}$	$5.7X10^{10}$	$67.8X10^{10}$

Values in the parenthesis indicate sample size in each strain
[a,b,c]Mean values having different superscripts differ significantly (P<0.05)

of channel catfish (Dunham et al. 1983). Weak fish in North Carolina did not reach sizes beyond 45 cm but had fecundity relative to length, several times higher than the Northern weak fish (Merriner 1976). In this study, the variable fecundity for *B. gonionotus* species in different geographic areas may be the specific physiological response to different environmental conditions.

There were also differences between crossbred and purebred in egg size. Egg size may influence the hatchability and growth rate of fry. Several investigators suggest that egg size depends on the size of the female parent (Gall 1974; Islam et al. 1973). Selection of larger egg size may be an important index because a larger egg produces a larger fry (Gall 1974; Fowler 1972; Dunham et al. 1983). Larger fry size has been associated with higher rates of survival in several species (Kincaid et al.1977).

The mean value for GSI was significantly different among the strains. Crossbred was found to be higher in GSI than that of other strains. GSI value is an important reproductive trait in evaluating a strain.

Meryl et al. (1981) found that the highest level of deformed channel catfish was in an out cross between the two domestic strains. They also reported that environmental factors seem to have overshadowed the genetic ones. In the present study, crossbreds produced significantly higher percentages of dead embryos and lower percentages of abnormal embryo. This indicates two possible causes: one may be inbreeding depression, another could be environmental factors that overshadowed any genetic characters.

The observed strain to strain variation in both fertilization rate and hatchability of eggs were not statistically significant and the values obtained were similar to those obtained by Tangtrongpiros et al. (1990) and Meryl et al.

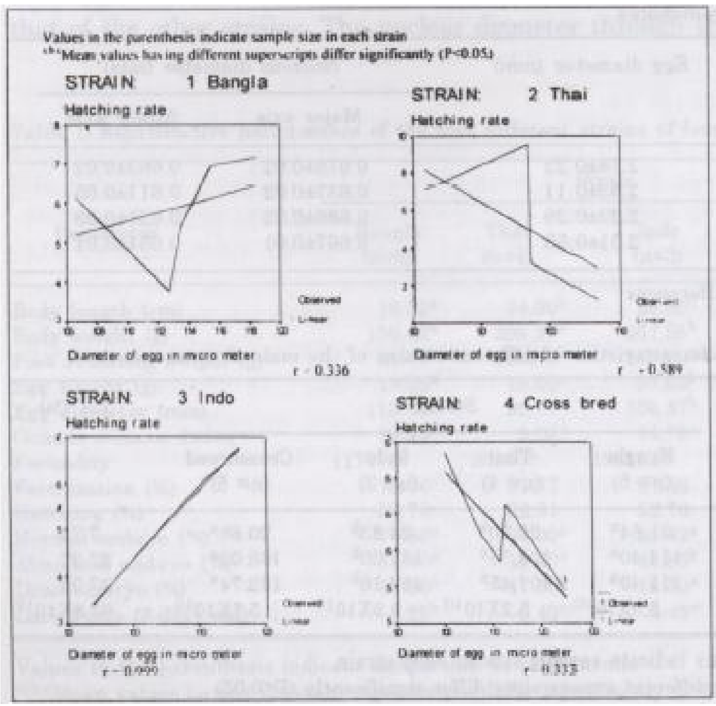

Fig.1. Relationship between egg diameter in micro meter and hatching rate of four different stains of *Barbodes gonionotus*

(1981). Crossbreds did not exhibit any superiority for fertilization rate and hatching rate. Dunham et al. (1983) reported that there were low maternal effects on hatchability in crossbred and purebred brood stocks of channel catfish where hatchability was not significantly different.

No superior effect in hatching rate due to line crossing was found in the crossbred. Bakos (1979) reported that successful fertilization and subsequent hatch were lower in inbred line of common carp than in outbred lines. Aulstud et al. (1972) reported lower hatchability in inbred lines of rainbow trout than in control lines. However, hatching rate recorded in this study could be improved by providing better environmental conditions (proper supply of water circulation, hygienic incubation unit, etc.) Some types of genotype environmental interaction occurred between inbred and control lines of channel catfish and the observed differences in hatchability may not occur under ideal hatchery conditions (Meryl et al. 1981).

There were significant differences in the length of 3 day old larvae between the strains, crossbred and Bangla strain showed significantly higher length than the other strains. In general, larger fry results to larger adult fish. The observed differences between strains were probably due to variations in egg size.

The present study revealed that better genetic gains in terms of growth and reproductive performance (i.e. higher fecundity, greater egg size, greater GSI, larger fry and lower percentage of abnormal fry) with no loss in fertilization capacity can be obtained from crossbred population compared to purebred stock through several generations of selection. This suggests that hatchery operators should have genetically improved breeds of *B. gonionotus*. Further study in this regard is included in the program of FS, BFRI.

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