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## Production of cross koi through crossing between local and Thai koi (*Anabas testudineus*, Bloch)

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### Abstract

The present study was undertaken for production of cross koi through crossing between local and Thai koi (*A. testudineus*). Four crossing groups viz. parent Thai (P-T), parent local (P-L), Thai female and local male (F<sub>1</sub>-A), and local female and Thai male (F<sub>1</sub>-B) were designed to produce the cross koi. The ovulation rate was found to be the highest (100%) in (P-T) crossing group followed by (P-L), (F<sub>1</sub>-B) and (F<sub>1</sub>-A) crossing groups (80, 80 and 60% respectively). On the other hand, the fertilization rate was significantly ( $P<0.05$ ) higher in (P-L) than (P-T), (F<sub>1</sub>-A) and (F<sub>1</sub>-B) cross groups. The highest hatching rate was also observed in (P-T) cross group which was significantly ( $P<0.05$ ) different from that of the other groups. However, the highest survival rate was found in (F<sub>1</sub>-A) followed by (P-T), (P-L) and (F<sub>1</sub>-B) crossing groups. The highest growth (length and weight) was found in (P-T) cross group followed by (F<sub>1</sub>-A), (F<sub>1</sub>-B) and (P-L) cross groups after 28 days of rearing. Results indicate that cross koi might have important implication for our aquaculture production.

**Keywords:** Cross koi, local and Thai koi, *Anabas testudineus*

### Introduction

*Anabas testudineus* (Bloch) locally known as koi, is a small indigenous fish in Bangladesh (Rahman, 1989). It inhabits haors, paddy fields, ponds and ditches (Siddiqua *et al.*, 2000) and it can withstand adverse environmental conditions (Alam *et al.*, 2006). It contains high amount of physiologically available iron and copper, essentially needed for hemoglobin synthesis (Shah, 1971). But the production of this fish is alarmingly reducing due to various reasons. To overcome the situation, Thai koi has been introduced recently. Typically, Thai koi are gray to green in color with edge of their scales and fins brightly colored (Sterva, 1973). Another distinguishing character is the number of vertebrae of Thai koi is 26 whereas it is 25 in case of local koi (Noor, 2005). Thai koi is a quick growing fish but it is not as tasty as the local one.

Crossbred has commercial importance in aquaculture due to its faster growth, better feed conversion, tolerance of low oxygen, increased resistance to many diseases, tolerance to crowded growth conditions in ponds, uniformity in size and shape, higher dress out percentages, increased harvest ability by seining and increased vulnerability to angling (Brain Bosworth *et al.*, 1966). The reciprocal crosses (hybrid) generally perform better than parental species for several important production traits including survival, growth, disease resistance, and carcass yield (Dunham and Argue, 1998).

Crossbreeding between *C. gariepinus* and *C. batrachus* was done in Bangladesh in 1990s (Mollah and Karim 1990). Rahman *et al.*, 1995 similar hybridization was done between *C. macrocephalus* Gunther and *C. batrachus* (Boonbrahmp *et al.*, 1977), between *C. batrachus* or *C. macrocephalus* and *Pangasias sutchi* Cuvier and Valenciennes (Tarnchalanukit 1986), between *C. batrachus* and *Heteropneustes fossilis* (Bloch) (Mukhopadhyay and Dehadrai

1987) and *C. gariepinus* and *Heterobranchus longifilis* Valenciennes (Hecht and Lublinkhof 1985). Several hybrids have been produced in the Mediterranean between red seabream and common dentex (*Dentex dentex*) and the hybrid produced were found to be fast growing in cage culture (Colombo *et al.*, 1998). Therefore, the present work to cross local koi with Thai koi through hormone-induced spawning was an attempt to produce cross koi that is supposed to have enormous potential to aquaculture production.

## Materials and Methods

The experiment was conducted in the Backyard hatchery of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh from 1 May to 3 June 2007. Live adult local and Thai koi weighing 2 kilograms were procured from Brahmaputra Fish Seed Complex, Shambhuganj during December, 2006 and were reared for 15 days in two 280 m<sup>2</sup> earthen rearing ponds. Before stocking, the ponds were fertilized with cowdung @ 750 kg/ha/week. The fish were fed with supplementary feed comprising of 60% rice bran, 25% mustard oil cake and 15% fish meal. 20 females of local and Thai koi having average weight of 53.0±9.500 g and 61.70±21.045 g, respectively, and 20 males of local and Thai koi having average weight of 30.35±5.207 g and 33.35±6.207 g, respectively were stocked in four fiber glass tank each having 30 L of tap water. Four crossing groups viz. (P-T), (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B) having three replications each were designed. The possible crosses, crossing groups and number of brood used in each cross are presented in Table 1.

**Table 1. Possible crosses, crossing pattern and number of fish used in each crosses**

Crosses	Crossing pattern	No. of brood		Cross symbol
		Female	Male	
Conspecific cross	T♀×T♂	5	5	Purebred (P-T)
Conspecific cross	L♀×L♂	5	5	Purebred (P-L)
Heterospecific cross	T♀×L♂	5	5	F <sub>1</sub> hybrid (F <sub>1</sub> -A)
Heterospecific cross	L♀×T♂	5	5	F <sub>1</sub> hybrid (F <sub>1</sub> -B)

The ripeness of female breeders was identified by soft bulging abdomen and that of males by the oozing of milt, when the belly near the genital opening was gently pressed. PG extract was injected in the muscle near the base of the pectoral fin. The females were given injection of 6 mg PG/kg body weight while the males with single injection of 2 mg PG/ kg body weight. After injection, both female and male breeders were carefully kept in the rectangular spawning tank having the same volume of tap water provided with artificial aeration at the sex ratio of 1:1. Ovulation time, water temperature and number of eggs ovulated by different crossing groups were recorded.

Immediately after fertilization the eggs were collected from tank by small beaker and kept into the bowl and used for the determination of fertilization rate. The number of live eggs in each bowl were estimated within 2-3 hrs after fertilization. Hatching rate was calculated by collecting and counting the hatched larvae. The yolk of hard boiled chicken egg was then supplied to the larvae up to 4 days. The physico-chemical parameters such as temperature, dissolve oxygen (DO) and pH were estimated weekly during study period with the help of Aqua Mate Water Testing Kit (Model WAKQ-1A).

The larval rearing was done in the Wet Laboratory of the Fisheries Faculty, Bangladesh Agricultural University, Mymensingh for a period of 28 days in 12 glass aquaria (45x25x24 cm<sup>3</sup>) each containing 17 L of deep tube-well water. 150 4-day old spawn (average length 2.0±0.15 mm and weight 3.5±0.05 mg) were stocked in each aquarium and were reared for 28 days. ARC-Z feed was administered at the rate of 3% of the body weight. The left over feed was siphoned from the aquarium twice daily at 0800 and 1600 hrs before feeding. The larvae were sampled at weekly intervals to determine the increase in their size (length and weight). Ten (10) fishes were randomly collected from each aquarium and then weight (mg) was taken in an analytical balance (College B204S, Switzerland) and length (mm) was measured by placing the larvae on a Petri dish on a 1 mm graph paper. The experiment was terminated on the 28<sup>th</sup> day and the larvae were harvested from the aquaria and survival of fries was estimated.

Fertilization rate, hatching rate, length gain (mm), weight gain (mg), SGR (%/day) and survival rate found in (P-T), (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B) were subjected to statistical analysis, ANOVA and Duncan's New Multiple Range Test (DMRT) to identify significance differences among means were performed with the computer software SPSS and MS Excel software program.

## Result and Discussion

The ovulation rate, fertilization rate, hatching rate and survival rate as obtained from the four crossing groups are shown in Table 2.

**Table 2. Average performance traits (ovulation, fertilization, hatching and survival rate) in *A. testudineus* brood for producing cross koi under different crossing groups**

Crossing group	Weight (g)		Dose of PG/kg body weight (mg)		Crossing performance			
					Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
	Female	Male	Female	Male				
(P-T)	82.0±5.70	36.0±3.74	6	2	100	92.94±1.29 <sup>b</sup>	90.58±1.71 <sup>a</sup>	76.53±6.61 <sup>a</sup>
(P-L)	41.4±1.01	24.2±3.18	6	2	80	96.87±1.19 <sup>a</sup>	75.57±1.91 <sup>c</sup>	71.54±2.23 <sup>b</sup>
(F <sub>1</sub> -A)	83.0±3.46	37.4±1.01	6	2	60	86.17±1.69 <sup>c</sup>	80.43±1.07 <sup>b</sup>	79.29±0.76 <sup>a</sup>
(F <sub>1</sub> -B)	40.4±1.019	35.8±3.96	6	2	80	94.05±2.69 <sup>b</sup>	71.88±2.27 <sup>d</sup>	70.20±1.94 <sup>b</sup>

\*Average±SD; Figures in each column having different superscripts (a, b and c) differ significantly ( $P<0.05$ )

The ovulation rate as recorded from (P-T), (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B) were 100, 80, 60 and 60%, respectively. The highest ovulation rate (100%) was recorded in (P-T) crossing group while the lowest (60%) in (F<sub>1</sub>-A) crossing group. The probable reasons might be due to the broods that were collected from different sources. Wonarovich and Hovarth (1980) reported that the eggs in the ovary after completion of the vitellogenesis remain in dormant stage, the induced breeding is then more effective and the eggs became naturally ready for further development to be triggered by gonadotropin for ovulation. Nash and Shehadeh (1980) suggested that success of spawning by hypophysation depends ultimately on the selection of suitable recipient fishes at their proper stage of ovarian development.

The fertilization rate of 92.94, 96.87, 86.16 and 94.05% was obtained from (P-T), (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B), respectively. A significantly ( $P<0.05$ ) higher fertilization rate was observed in (P-L). The hatching rate of 90.58, 75.57, 80.46 and 71.88% was found in (P-T), (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B), respectively. The hatching rate as obtained from (F<sub>1</sub>-A) was significantly ( $P<0.05$ ) higher than that of the other crossing groups (Table 2). The highest (90.58%) hatched out rate of larvae was observed in (P-T) followed by (P-L), (F<sub>1</sub>-A) and (F<sub>1</sub>-B). The hatched out rate as obtained from four crossing groups in the present study was relatively higher due measures taken to the keep the water quality favorable like continuous aeration resulted in higher dissolved oxygen content and also removal of waste material from the bowl.

The length of fry at harvest was found to be the highest in (P-T) crossing group ( $20.0\pm0.02$  mm) followed by (F<sub>1</sub>-A), (F<sub>1</sub>-B) and (P-L) crossing group ( $19.0\pm0.21$ ,  $16.0\pm0.41$  and  $15.26\pm0.49$  mm, respectively). The length of (P-T) cross fry was significantly ( $P<0.05$ ) different from that of the other three crossing groups viz. (F<sub>1</sub>-A), (F<sub>1</sub>-B) and (P-L). In case of weight at harvest, fry of (F<sub>1</sub>-A) was found to be the highest ( $120.0\pm3.15$  mg) followed by those the (P-L), (P-T) and (F<sub>1</sub>-B) crossing groups ( $112.0\pm2.05$ ,  $110.0\pm1.25$  and  $98.0\pm1.85$  mg, respectively). There was also a significant variation ( $P<0.05$ ) among the four crossing groups. The highest SGR ( $17.49\pm0.80$ ) was also observed in (F<sub>1</sub>-A) followed by (P-L), (P-T) and (F<sub>1</sub>-B) crossing groups ( $13.55\pm0.20$ ,  $13.28\pm0.07$  and  $10.12\pm0.4$ , respectively). The SGR value of (F<sub>1</sub>-A) and (F<sub>1</sub>-B) was significantly ( $P<0.05$ ) different from (P-T) and (P-L) but there no significant variations between them (Table 3). The highest survival rate of fry was observed in (P-T) (13.36%) followed by (P-L), (F<sub>1</sub>-B) and (F<sub>1</sub>-A) (13.23%, 13.10% and 12.98% respectively) but group to group variation was not significant (Table 3). The survival rate as obtained in the present study was quite low due to non-availability of a balanced diet for larval rearing of cross bred koi.

**Table 3. Range and average $\pm$ SD in parenthesis of growth parameters and survival rate of cross koi (*Anabus testudineus*) fry after 28 days of experiment**

Cross group	Initial size		Final size		SGR (%/day)	Survival rate (%)
	length (mm)	Weight(mg)	Length(mm)	Weight (mg)		
(P-T)	(1.0-2.1) ( $2.0\pm0.15$ )	(2.1-3.0) ( $3.5\pm0.05$ )	( $20.0\pm0.02$ ) <sup>a</sup>	( $110.0\pm1.25$ ) <sup>b</sup>	( $13.28\pm0.07$ ) <sup>b</sup>	( $13.36\pm1.25$ ) <sup>a</sup>
(P-L)	(1.1-1.9) ( $2.0\pm0.15$ )	(1.5-3.0) ( $3.5\pm0.05$ )	( $15.26\pm0.49$ ) <sup>b</sup>	( $112.0\pm2.05$ ) <sup>b</sup>	( $14.55\pm0.20$ ) <sup>b</sup>	( $13.23\pm0.69$ ) <sup>a</sup>
(F <sub>1</sub> -A)	((1.2-1.9)) ( $2.0\pm0.15$ )	(1.8-3.0) ( $3.5\pm0.05$ )	( $19.0\pm0.21$ ) <sup>a</sup>	( $120.0\pm3.15$ ) <sup>a</sup>	( $17.49\pm0.80$ ) <sup>a</sup>	( $12.98\pm0.58$ ) <sup>a</sup>
(F <sub>1</sub> -B)	(1.1-2.2) ( $2.0\pm0.15$ )	(1.4-2.6) ( $3.5\pm0.05$ )	( $16.0\pm0.41$ ) <sup>b</sup>	( $98.0\pm 1.85$ ) <sup>c</sup>	( $10.12\pm0.40$ ) <sup>c</sup>	( $13.10\pm0.22$ ) <sup>a</sup>

(Average $\pm$ SE); values of the parameter in each column with different superscripts (a, b and c) differ significantly ( $P<0.05$ ).

Temperature, DO and pH of water of experimental aquaria were found to range from 26.8 to 27.5°C, from 8.5 to 8.9 and from 5.7 to 5.8ppm, respectively (Table 4). The cross bred individuals took longer time to hatch than the purebred ones although water temperature of all the aquaria was similar. This might be due to embryogenetic stress resulting from cross breeding.

Table 4. Range and average  $\pm$  SD in parenthesis of water quality parameters during experimental period

Cross groups	Temperature ( $^{\circ}$ C)	pH	Dissolve oxygen (DO) (mg/L)
Conspecific cross	(26.0-27.7) (27.5 $\pm$ 0.02) <sup>a</sup>	(7.1-9.0) (8.9 $\pm$ 0.03) <sup>a</sup>	(5.1-6.5) (5.70 $\pm$ 0.01) <sup>c</sup>
Conspecific cross	(25.8-27) (26.8 $\pm$ 0.04) <sup>a</sup>	(7.4-8.7) (8.5 $\pm$ 0.04) <sup>b</sup>	(5.2-6.7) (5.75 $\pm$ 0.02) <sup>b</sup>
Heterospecific cross	(25.2-27.8) (27.0 $\pm$ 0.05) <sup>b</sup>	(7.9-9.1) (8.8 $\pm$ 0.02) <sup>a</sup>	(5.3-6.7) (5.80 $\pm$ 0.03) <sup>a</sup>
Heterospecific cross	(26.3-27.1) (27.1 $\pm$ 0.06) <sup>b</sup>	(8.1-8.9) (8.7 $\pm$ 0.01) <sup>b</sup>	(5.4-6.8) (5.74 $\pm$ 0.04) <sup>b</sup>

Figures in each column having different superscripts (a, b and c) differ significantly ( $P < 0.05$ )

An attempt was made to produce hybrids of Thai and local koi, and indicated some potentials. Further trials with much more variables are needed to arrive at a conclusion.

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