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INDUCED BREEDING AND LARVAL REARING OF LOCAL AND THAI KOI (*Anabas testudineus*, BLOCH, 1792)

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ABSTRACT

Suraiya S, Haq M, Khan MMR (2012) Induced breeding and larval rearing of local and Thai Koi (*Anabas testudineus*, Bloch, 1792). *J. Innov. Dev. Strategy.* 6(1), 63-68.

Four combinations of crossbreeding were conducted to study the effects of hybridization between Local and Thai *Anabas testudineus* on the performance of F_1 progeny. The experiment was carried out with four treatments T_1 - L♂ × L♀, T_2 - T♂× L♀, T_3 - L♂ × T♀ and T_4 - T♂ × T♀ with each having three replications. There was a significant differences among four treatments in the viewpoint of ovulation and hatching rate and the results revealed that T_4 was significantly ($P<0.05$) higher than that of T_1, T_2 and T_3. The results also revealed that there was a significant ($P<0.05$) difference in fertilization rate and survival rate of breeding and significantly ($p<0.05$) higher values were observed in treatment T_1 compared to the other three treatments T_2, T_3 and T_4. Cross between Local and Thai *A. testudineus* resulted variation in survival rate of hatchlings. Higher net weight gain was observed in T_4 (0.910±0.550 g) followed by T_3 (0.875±0.508 g.), T_2 (0.866±0.420 g.) and T_1 (0.738±0.650g.) which indicates that there was significant difference ($P<0.05$) in mean weight gain among four treatments. The total length increments of *A. testudineus* hatchlings in four treatments during the study period of 28 days was observed in T_4 (22.56±3.50 mm) followed by T_3 (21.70±3.00 mm), T_2 (21.50±3.30 mm) and T_1 (19.60±2.57 mm) which indicate that there was significant difference ($P<0.05$) in total length gain among four treatments. Water quality did not differ between the treatments having optimum level for culture. The growth rate regarding weight gain and total body length of T_3 is higher than that of T_1 and T_2. It is crystal clear from the experiment that T_4 showed highest growth rate that is desired almost the entire farm owner but due to the consumers' demand of Local koi, T_3 may be rational to produce seeds for commercial farms.

Key words: *breeding, local, thai, hybrid, offspring, rearing*

INTRODUCTION

The climbing perch, *Anabas testudineus* also popularly known as *'Koi'* is a well known traditionally popular fish, inhabiting fresh waters and brackish waters of India, Pakistan, Bangladesh, Nepal, China, Myanmar, Thailand, Cambodia, Philippines, Indonesia, Singapore, and Sri Lanka (Misra 1959; Hora and Pillay, 1962). It is also becoming a highly demandable fish day by day due to its high nutrition, taste, faster growth and having ability to withstand harsh environmental condition (Alam *et al.* 2006). Although *A. testudineus* is an important food fish for local inland communities, the species has recently come under considerable pressure and is nearly extinct in some areas due to environmental changes (including urbanization and increasing land use for cropping) and over-fishing (Sverdrup-Jensen 2002 and Mijkherjee *et al.* 2003). Degradation of natural water bodies, disease and over fishing in the natural stock of koi cause the population reduction significantly. One decade ago the contribution of koi was 2.83% in the total pond catch of Bangladesh (DoF 1992) but later it turned down to 0.85% (DoF 1999). Harvests of wild sources of koi cannot keep up with the demand presented by the growing consumers. Fundamental aquaculture trials for *A. testudineus* are ongoing in several countries (e.g., India and Vietnam) on the basis of recent studies on artificial breeding, seed production and nursing technologies (Trieu and Long, 2001; Tuan *et al.* 2002; Mijkherjee *et al.* 2003 and Sarkar *et al.* 2005).

In 2002, Thai koi was introduced in Bangladesh for culture purpose. The high growth rate and bigger size of Thai koi pursue the fish farmer enthusiastic for its artificial breeding and culture in Bangladesh (Hasan *et al.* 2010). Therefore, culture of Thai koi is started to meet the consumers demand. The expansion of Thai koi culture results the demand of seed which gears the hatchery production. But, the consumers are not completely satisfied with the Thai koi as it lacks natural odor and taste. On the other hand, the growth rate of our native koi is slow taking more time to attain marketable size. In this circumstance, the present study was undertaken to get superior koi seeds after crossing Local and Thai brood fishes for seed production.

MATERIALS AND METHODS

Brood fishes collection and rearing

Mature 30 male and 30 female of *Anabas testudineus* (Local) having average body weight of 25 ± SDg. and 45 ± SD g. respectively and mature 30 males and 30 females of *Anabas testudineus* (Thai) having average body weight of 35 ± SD g and 80 ± SD g respectively were collected from fisherman of Shambhugonj on 02 March 2007. The brood stocks were reared in the rectangular ponds of size 18 x 14 m^2 and an average depth of 1.3 m situated in the Fisheries Complex of Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Commercial diets of SABINCO specially prepared for Koi was initiated in early March and continued up to the completion of breeding activity up to May 2007. The feed was supplied at 5% body weight of the brood fish two times a day at 08.00 am and 5.00 pm, respectively.

Breeding trails

Four breeding trails using two species of Local and Thai *Anabas testudineus* were carried out to produce four populations such as Local (T_1 - L♂ × L♀), Hybrid (T_2 - T♂× L♀), Hybrid (T_3 - L♂ × T♀) and Thai (T_4 - T♂ × T♀). Each population having three replications comprised of 5 pairs of male and female for each experiment.

Inducing agent and injecting the broods

Just prior to hypophysation, the selected breeders (male and female) were put in two separate cemented circular tanks of Fisheries Complex hatchery. Locally available acetone dehydrated carp pituitary glands (PG) were used as an inducing agent. At first, the pituitary glands were gently removed from the vial with a pair of forceps and dried by using the filter paper for 2-3 minutes and then weighted by an analytical electronic balance (College B204-S, Switzerland). The PG dose applied to *Anabas testudineus* (Local) and *Anabas testudineus* (Thai) for male 4 mg/kg body weight and for female 8 mg/kg body weight. The PG injection was administered using 10 ml size syringes in to the muscular basal part of the pectoral fin.

Collection of fertilized eggs and transferring to hatchery tanks

After injection the fish were transferred to spawning tanks. The broods were ovulated after 7-8 hours of injection. Then the fishes were subjected to strip and the fertilized eggs were transferred into hatching tank with continuous water flow for proper aeration.

Determination of ovulation, fertilization and hatching rate

For determination of fertilization and hatching rate, approximately 100 eggs were placed in a bowl of 1.25 L capacity with three replications each having water flow from porous PVC pipe and outlet facility. At first, the number of fertilized and unfertilized eggs of each bowls was counted with naked eyes. After approximately 19-21 hours of fertilization, when the hatching almost completed, the number of hatchlings in each bowl was counted.

Study of breeding parameters

$$\text{Ovulation rate (\%)} = \frac{\text{No. of fish ovulated}}{\text{Total no. of fish injected}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. eggs (fertilized and unfertilized)}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

Experimental design for larval rearing

The experiment was conducted in the Wet Laboratory of Fisheries Faculty, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. After 42 - 46 hours of hatching, the larvae were fed boiled yolk chicken egg at the rate of 60% per body weight. Five day old spawn having an average length of 0.52±0.06 cm and weight of 0.015±0.008 g of four populations (Local, reciprocal hybrids and Thai) were stocked in glass aquaria (45 × 25 × 24 cm^3) containing 17.0 L of deep tube well water in each. The experiment was designed with 4 treatments (T_1, T_2, T_3 and T_4) for four populations and each treatment had three replications (R_1, R_2 and R_3). Five hundred larvae of each (Local, reciprocal hybrids and Thai) were stocked in their each replication and were reared for 28 days.

Feeding of larvae

Spawn were fed twice daily with SABINCO nursery feeds for the first 14 days and starter-1 feed for days 15 to 28. The rate of feeding was 50% of the estimated body weight of spawn for the first 2 weeks and 40% for the second 2 weeks.

Rearing procedure

The water of each aquarium was exchanged by half of the volume with fresh deep tube-well water once in a day. The fecal output and leftover feed were removed from the aquarium by siphoning. Aeration to the aquarium was provided for 22 hours. Everyday from aerators but during feeding time the aerators was stopped for 1 hour. During cleaning the aquariums, dead fry if any, was removed and the number was recorded.

Sampling procedure

Ten (10) fries were randomly collected from each glass aquarium during each sampling (7 days interval). The weight (g.) was taken in an analytical balance up to 3 decimal points and the length (mm) was measured by

placing the larvae on a Petri dish having underneath a 1 mm. graph paper. Sampling was usually done before application of feed to avoid the biomass of weight due to presence of excessive food.

Growth parameters

Length gain (mm) = Average final length – Average initial length.
Weight gain (mg.) = Average final weight – Average initial weight.

Survival rate and health condition

The survival rate of fish was calculated at the end of the experiment or at 28 days, the number of total live larvae in each aquarium was counted desperately for calculating the survival rate.

Physico-chemical condition of water

The physico-chemical parameters (e.g. temperature, dissolved oxygen p^H and alkalinity) were recorded on sampling dates in the morning from 08.00 to 10.00 a.m. Temperature was determined by a Celsius mercury thermometer. Dissolved oxygen of water was determined by a digital DO meter (YSI, Model 58, USA) and p^H were recorded by a portable p^H meter (Jenway-Model-3020).

Statistical analysis

The length gain (mm.), weight gain (g.) and survival rate of larvae during experimentation with different populations were all tested using one way analysis of variance (ANOVA). Significant result ($p<0.05$) were further tested using Duncan's New Multiple Range Test (DMRT) to identify significant differences among the means.

RESULTS AND DISCUSSION

Brood stock management

At the end of proper brood stock management the fish at the pond were found to be fully mature and ready to spawn. All of the fish did not mature at a time and first growing fishes were found to mature in early breeding season followed by others.

Ovulation, fertilization and hatching rate of different hatchlings

Corresponding data representing the effects of different treatments on ovulation, fertilization, hatching and survival rate are shown in Table 1.

Table 1. Performances of different treatments on induced breeding of *A. testudineus*

Treatments	Ovulation rate (%) M ± SD	Fertilization rate (%) M ± SD	Hatching rate (%) M ± SD	Survival rate (%) M ± SD
T_1	73.33^{c}±00	85.65^{a}±5.78	68.75^{b}±7.44	63.54^{a}±4.25
T_2	80.00^{b}±00	75.50^{c}±3.46	62.47^{d}±3.84	51.47^{b}±4.21
T_3	80.00^{b}±00	70.54^{d}±6.57	65.37^{cb}±4.82	62.35^{a}±1.15
T_4	100.00^{a}±00	80.45^{b}±7.49	71.45^{a}±4.56	62.74^{a}±2.38

^{abc} Means in with different superscripts in each column differs significantly ($P<0.05$)

Ovulation rate

The variations in average ovulation rate with different treatments are shown in Table 1. The highest average ovulation rate (100%) was recorded in T_4 and the lowest value (73.33%) was found in T_1. These variations in ovulation may be due to different types (Local and Thai) of brood fishes and their adaptability to induce breeding. The results indicated that there was a significant ($P<0.05$) difference among four treatments in the viewpoint of ovulation rate and revealed that T_4 were significantly ($P<0.05$) higher than that of T_1, T_2 and T_3. Chaudhuri (1960) have observed that induced breeding experiments yield better results when the donor and recipient fishes are of the same species. The variations in the results obtained between the trials may also be due to environmental parameters and quality of brood fishes.

Fertilization rate

During the experimentation with four treatments the average fertilization rate were recorded as 85.65± SD, 75.50± SD, 70.54± SD and 80.45± SD% under the treatments T_1, T_2, T_3, and T_4, respectively (Table 1). The highest fertilization rate (85.45%) was recorded in T_1 whereas the lowest fertilization rate (70.54%) was found in T_3. Additionally, some variations may arise due to physiological differences of the pair of fishes. The results indicated that there was a significant ($P<0.05$) difference among four treatment T_1 was significantly ($P<0.05$) higher than that of treatments T_2, T_3 and T_4.

Hatching rate

The highest average hatching rate was found 71.45 in T_4 and lowest average hatching rate was found in T_2 (Table 1). The results indicated that there was a significant ($P<0.05$) difference among hatching rate of four different breeding of *Anabas testudineus*. It was found that hatching rate in T_4 was significantly ($P<0.05$) higher

than that of T_1, T_2 and T_3. It can determine the status of how many fry can be produced from a number of fish and how many are lost and why. It helps to improved the hatchery product and thereby production.

Survival rate

The survivality of four different breeding of *Anabas testudineus* were 63.54± SD, 51.47± SD, 62.35± SD and 62.74± SD% in T_1, T_2, T_3 and T_4, respectively after 28th days of experimental period (Table 1). The results revealed that there was a significant ($P<0.05$) difference in survival rate of breeding and a significantly ($p<0.05$) higher survival rate was observed in treatment T_4 compared to the other three treatments T_1, T_2 and T_3. Cross between Local and Thai *Anabas testudineus* resulted variation in survival rate of hatchlings. The survival rate of hatchlings is very much potential for successful aquaculture.

Growth performance of hatchlings

To evaluate the growth performance of hatchlings in different treatments in terms of mean weight gain and mean length gain were calculated and are presented in Table 2 and 3.

Table 2. Average weight of hatchlings in experimental period

Treatments	Weights (g.)				
	1st day	7th days	14th days	21st days	28th days
T_1	0.001	0.010^{a}±0.005	0.069^{d}±0.045	0.325^{c}±0.253	0.738^{c}±0.650
T_2	0.001	0.014^{a}±0.010	0.072^{c}±0.032	0.401^{b}±0.300	0.866^{b}±0.420
T_3	0.001	0.013^{a}±0.012	0.075^{b}±0.055	0.415^{b}±2.742	0.875^{b}±0.508
T_4	0.001	0.016^{a}±0.012	0.080^{a}±0.040	0.430^{a}±4.056	0.910^{a}±0.550

^{abc} Means in with different superscripts in each column differs significantly ($P<0.05$)

Table 3. Average total length of hatchlings in experimental period

Treatments	Lengths (mm)				
	1st day	7th days	14th days	21st days	28th days
T_1	1.00^{c}±0.12	7.50^{c}±0.25	12.20^{b}±1.10	15.50^{c}±0.90	19.60^{d}±2.57
T_2	0.90^{ab}±0.10	8.15^{b}±0.30	13.00^{b}±1.00	16.75^{b}±1.25	21.50^{c}±3.30
T_3	1.00^{ab}±0.08	8.20^{b}±0.40	13.80^{a}±1.20	17.50^{ab}±1.40	21.70^{b}±3.00
T_4	1.12^{a}±0.15	8.85^{a}±0.25	14.50^{a}±1.50	18.25^{a}±1.20	22.56^{a}±3.50

^{abc} Means in with different superscripts in each column differs significantly ($P<0.05$)

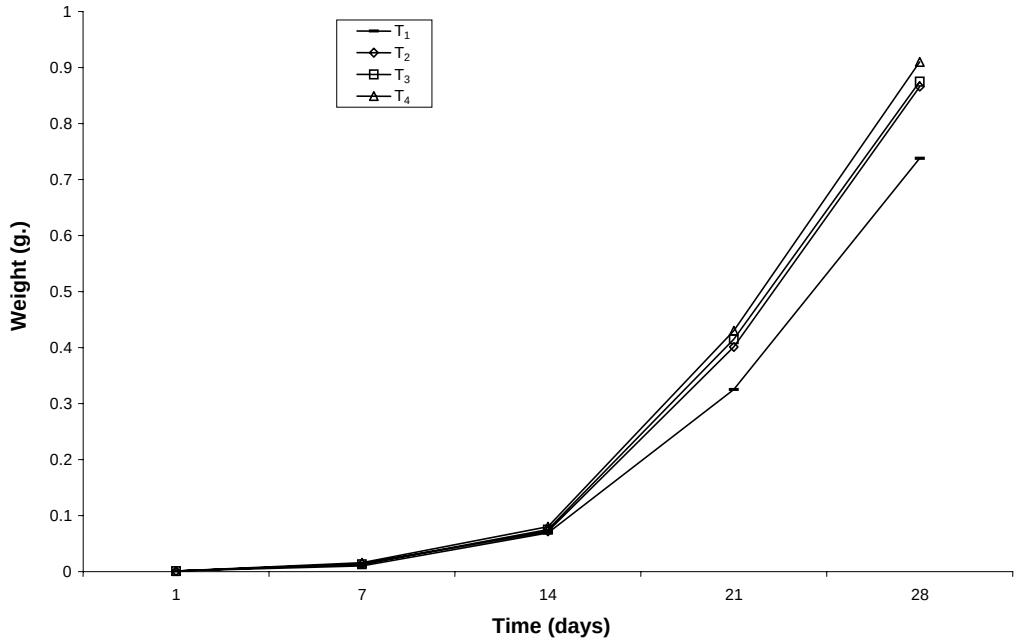

Fig. 1. Comparison in Weight gain of *A. testudineus* in rearing period of 28 days

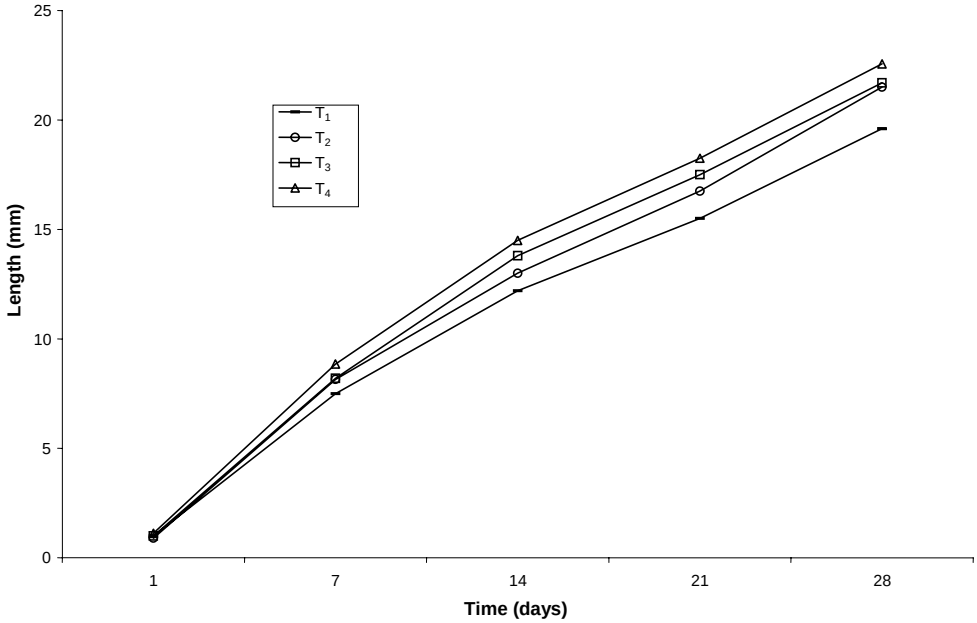

Fig. 2. Comparison in Length of *A. testudineus* in rearing period of 28 days

The weight increments of *A. testudineus hatchlings* in four treatments during the study period of 28 days are presented in Fig. 1. Highest weight was observed in T_4 (0.910±0.550g) followed by T_3 (0.875±0.805 mm), T_2 (0.866±0.420) and T_1 (0.738c±0.650) of 28 days rearing period which indicates that there was significant difference ($P<0.05$) in mean weight gain among four treatments due to different types of breeding performed between Local and Thai *A. testudineus.* Trieu *et el.* (2001) found the net weight gain of Climbing perch larvae 0.879± 0.788 in 30 days rearing period with the stocking density 500 larvae/m^3 which support the weight gain of the present study. The total length increments of *A. testudineus* hatchlings in four treatments during the study period of 28 days are presented in Fig. 2. Higher length was observed in T_4 (22.56±3.50 mm) followed by T_3 (0.875±0.508 g), T_2 (21.50±3.30 mm) and T_1 (19.60±2.57 mm) which indicates that there was significant difference ($P<0.05$) on the basis ofmean weight gain among four treatments due to different types of breeding performed between Local and Thai *A. testudineus.* Sarkar *et al.* (2005) reported larval length larger than 7 mm on day-5 and 22.4 mm a month after hatching. The BL (mean±SD) observed in this study on days-7 and 28 were 8.175± 0.675 mm and 21.08±1.48 mm, respectively (Table 3), which is similar with the finding of Sarkar *et al.* (2005).

The present study was performed to view the incidents of breeding and larval growth patterns among four populations of *A. testudineus* such as Local (T_1 - L♂ × L♀), Hybrid (T_2 - T♂× L♀), Hybrid (T_3 - L♂ × T♀) and Thai (T_4 - T♂ × T♀). T_4 showed the highest ovulation rate (100.00±00%) and hatching rate (71.45±4.56%) where as T_1 showed highest fertilization (85.65±5.78%) and survival rate (63.54±4.25%). It can be opined here that the native breeders (Local × Local) or (Thai × Thai) showed better performances in breeding. In case of growth performances such as weight gain and total body length, T_4 showed best performances (0.910±0.550 g. and 22.56±3.50 mm, respectively). The lowest values in weight gain and total body length were observed in T_1. The growth rate regarding weight gain and total body length of T_3 is higher than T_2. It is crystal clear from the experiment that T_4 showed highest growth rate that is desired almost the entire farm owner but due to the consumers' demand of local koi, T_3 may be rational to produce seeds for commercial farms. Here, 50% of the genotype participates from Local koi that may satisfy consumers' satisfaction and increase market price. Further study is suggested to follow up the growth rate of the four types of seeds up to table size fish. The effects on market price and post cooking panel test for the organoleptic quality and taste are also recommended of the four types of seeds.

Physico-chemical condition of water

The water quality parameters such as temperature, p^H and dissolved oxygen of all the treatments were monitored weekly during the experimental period. The values of water quality parameters (temperature, pH and dissolved oxygne) recorded in the experimental period were 30.0±0.5-30.30±50^0C (temperature), 7.00±0.2-7.10±0.2 (pH) and 5.65±0.75-6.35±0.55 mg/L (DO), respectively.

Ahmed (1997) reported that the minimum water quality to maintain fish health should be 5 ppm, 6.7-8.6, <3 ppm, <0.02 ppm and >20 ppm for DO, p^H, free CO_2, ammonia and alkalinity respectively. From the above discussion it can be said in short, that the ovulation, fertilization and hatching rate and also the survival rate of larvae differs mainly due to the variation of brood as well as brood stock management, quality of brood, quality of eggs, seasonal variation, water flow during incubation, quality of hatchery water and handling procedure of the broods. But upon all consideration Hybrid (T_3 - L♂ × T♀) may be recommended for induced breeding and seed production of *A. testudineus* in hatchery.

CONCLUSION

Breeding of Local and Thai *A. testudineus* and their larval rearing was successfully achieved. The impacts of different breeding trails, the survival rate and growth were studied which showed best result in the breeding between Thai male and Thai female *A. testudineus.* But breeding between Local male and Thai female *A. testudineus* for mass seed production is suggested. Further study can be performed on the growth trail of four different seeds to get table size fish and the organoleptic taste after cooking.

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