Comparison of growth performance of gynogenetic female, gynogenetic neo-male and normal mixed-sex silver barb (*Barbonymus gonionotus*) in earthen ponds

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Abstract. The experiment was conducted to compare the growth performance of gynogenetic females and gynogenetic neo-males with mixed-sex silver barb (*Barbonymus gonionotus*) in six earthen ponds for a period of 120 days. Three treatments i.e. T1 (Gynogenetic female), T2 (Gynogenetic neo-male) and T3 (mixed-sex, control) each having two replicates were stocked with 60 fish/decimal. During stocking the mean length and weight of fishes were 5.83 ± 0.12 cm and 2.72 ± 0.10 g, 5.85 ± 0.12 cm and 2.77 ± 0.12 g and 5.79 ± 0.16 cm and 2.66 ± 0.16 g, in T1, T2 and T3, respectively. The fishes were reared with a commercial fish feed (Mega Feed) of different types such as pre-starter (36% crude protein) for the first 42 days, starter (32% protein) for the second 42 days and grower (32% protein) for the last 36 days. Water quality parameters were measured and found in optimal levels for fish culture. A significantly highest ($p < 0.05$) weight gain was observed in T1 and lowest in T3, and T2 was much closer to T1 and not significantly ($p > 0.05$) different. Similar trend was also observed in other growth parameters such as final length, final weight, % weight gain, average daily gain and specific growth rate among the treatments. However, no significant difference ($p > 0.05$) was observed for survival among the treatments. The significantly highest ($p > 0.05$) gross and net production was observed in T1 (1219.62 ± 23.29 and 1178.83 ± 22.33 kg/ha/120 days) and lowest in T3 (1029.38 ± 22.44 and 989.54 ± 21.30 kg/ha/120 days) and no significantly different ($p > 0.05$) was observed for the production between T1 and T2. Considering the growth performance of silver barb, it can be concluded that the gynogenetic females and neo-males possess higher production potentiality and thus generation of all-female population using neo-males would be an effective approach for accelerating fish production.

Keywords: Gynogenetic female, Gynogenetic neo-male, *Barbonymus gonionotus*

Introduction

Silver barb *(Barbonymous gonionotus)* is one of the most important aquaculture species commercially cultivated in freshwater ponds in Thailand. It was introduced in Bangladesh from Thailand in 1977. It is a herbivorous fish feeding mainly on aquatic plants, grasses and algae (Phaohorm 1970, Srisuwantach 1981). It has high market price and become increasingly popular for its bright silvery appearance and good taste. The main reasons for its introduction to Bangladesh are that silver barb has faster growth rate than the local barb and is suitable for culture in the natural environment of Bangladesh. It has high culture potentiality in seasonal ponds, ditches and road-side canals where the major carps do not perform well. It is the most suitable species for culture in rain-fed ponds where water remains for a maximum 4-5 months. Though silver barb usually grows well but the females of silver barb grows faster (at least 20-

30% more) than the males. This faster growth characteristic of females should be exploited by any biotechnological means and gynogenesis could be the best option in this regard. On the other hand, sex-reversed gynogenetic males (XX) (also known as neo-males, are genotypically female but phenotypically male) produced using masculinised hormone (17 α-methyl testosterone) would produce all-female population upon crossing with genetic females (XX) (Pongthana *et al.* 1999). Thus production of gynogenetic females and neo-males of silver barb would definitely have positive impact on total fish production. However, information on the growth performance of gynogenetic fish in culture system is very limited. Therefore, the present study was designed to investigate the growth performance of gynogenetic females and neo-males of silver barb (*B. gonionotus*) in earthen ponds.

Materials and Methods

Study area and experimental design

At first the meiotic gynogenetic fry were produced by fertilizing eggs with UV-irradiated sperm followed by cold shock and heat shock treatment at $2^{o}C$ at 10 min and $40^{o}C$ for 1 min after 1.5 min of fertilization respectively. After that, half of the gygogenetic fry were fed with hormone (17 α-methyl testosterone) treated feed starting from first feeding stage up to 28 days to produce sex-reversed male or neo-male (XX). To compare the growth performance among gynogenetic female, gynogenetic neo-male and normal mixed-sex silver barb, the experiment was conducted for a period of 120 days from 01 July to 28 October, 2016 in six earthen ponds situated at the Field Laboratory complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The surface area of each pond was 81 m^2 (2 decimal) with an average depth of 1.2 m. To investigate the growth rate, survival rate and production potential of *B. gonionotus,* three treatments were used with two replicates each. Treatment1 (T1) was denoted for gynogenetic female, Treatment 2 (T2) for gynogenetic neo-male and Treatment3 (T3) for normal mixed-sex groups. Stocking density of fish was maintained same for all treatments i.e. 60 fish/decimal.

Pond preparation, stocking and management

All the experimental ponds were rectangular in shape and possessed similar size, depth, basin configuration and bottom type. They have inlet and outlet system. The ponds were dried, and the aquatic vegetation was cleaned manually. After drying, Quick lime ($CaCO_3$) was applied at the rate of 1kg/decimal. After 7 days of liming, the ponds were filled with underground water and then fertilized with urea and TSP at the rate of 200 g and 100 g per decimal respectively. After that, the ponds were stocked with silver barb @120 fish/pond. The average length and weight of fish stocked in T1, T2 and T3, were 5.83 ± 0.12 cm, 5.85 ± 0.12 cm and 5.79 ± 0.16 cm and 2.72 ± 0.10 g, 2.77 ± 0.12 g and 2.66 ± 0.16 g respectively. Fish were fed with commercially available pelleted feed (Mega feed) twice a day, at 9:00 am and 5:00 pm. At the beginning of the experiment,

feed was supplied at 15% of the total body weight of fish and the feeding ration was gradually reduced to 13%, 11%, 9%, 7%, 5%, 3% and 2% with the progress of culture period and increment of body weight. The feeding ration is shown in Table I.

Table I. Feeding ration of different types of feed during experimental period

Culture period	Types of feed	Feeding rate (% of body weight)
1-14 days	Pre-starter	15%
15-28 days	Pre-starter	13%
29-42 days	Pre-starter	11%
43-56 days	Starter	9%
57-70 days	Starter	7%
71-84 days	Starter	5%
85-98 days	Grower	3%
99- 120 days	Grower	2%

Water quality parameters

The water quality parameters such as water depth, transparency, temperature, dissolved oxygen (DO) and pH were monitored weekly between 09.00 am and 10.00 am throughout the experimental period. Air and water temperature (^{0}C) of the ponds were measured with the help of a Celsius thermometer. Dissolved oxygen (mg/L) was measured directly by a digital DO meter (Model-HI 9146, Romania), pH by a digital pH-meter (Milwaukee pH meter, Model-PH55/PH56, USA), and water transparency (cm) was measured by using a Secchi disc and measuring tape.

Sampling of fish and measurement of growth parameters

Fish samples were fortnightly collected with a seine net in the morning between 8:00 am to 9:00 am to check the health condition, growth, survival of fish and to adjust the feed ration. The weight of fish was taken by using a portable balance (Model HI 400EX). The length of fish was measured in centimetres (cm) while weight of the fish was measured in nearest gram (g). After measurement, fishes were released immediately into their respective ponds.

Weight gain (g) = Mean final weight (g)-Mean initial weight (g)

$$\% \text{ Weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$$

$$\text{Specific Growth Rate, SGR (\% /day)} = \frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{Number of experimental days}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{No. of fish harvested}}{\text{No. of fish stocked}} \times 100$$

Production= No. of fish harvested × Average final weight of fish

Statistical analysis

All the data were analyzed by using the SPSS program. The data on the growth, survival, and production was analyzed using one-way analysis of variance (ANOVA). The Duncan's Multiple Range test was used to determine differences among the treatment means and the differences were considered at 0.05%.

Results and Discussion

Water quality parameters

Gynogenetic and normal mixed-sex fish were reared in ponds under different treatments and the water quality parameters in different treatments were measured. The mean values of water quality parameters during the 120 days rearing period of silver barb are given in Table II. The mean values of water temperature were recorded as 29.47 ± 0.68, 29.01 ± 0.87 and 29.38 ± 0.69^0C in T1, T2 and T3 respectively during the experimental period and the range of temperature (27^0C to 31.5^0C) was found suitable for fish rearing (Kohinoor *et al*. 1994 and Wahab *et al*. 1996). The mean dissolved oxygen (DO) was 5.29 ± 0.44 (T1), 5.24 ± 0.49 (T2) and 5.09 ± 0.52 mg/L (T3) with a range between 4.10 and 5.97 mg/L and it was found suitable for fish culture as Bhatnagar and Singh (2010) and Bhatnagar *et al*. (2004) DO level >5 ppm is essential to support good fish production. Similar trends of dissolved oxygen concentrations were also reported by Saha *et al*. (1988), Ahmed (1993), and Rahman and Rahman (2003a, b) in various carp and barb nursery ponds. Oxygen depletion in water leads to poor feeding of fish, starvation, reduced growth and more fish mortality, either directly or indirectly (Bhatnagar and Garg 2000). The pH values were recorded as 6.38 ± 0.63, 6.53 ± 0.46 and 6.87 ± 0.62 in T1, T2 and T3 respectively with a range of 5.1 to 7.8. This pH range indicates the productive condition of the ponds as according to Santhosh and Singh (2007) the suitable pH range for fish culture is between 6.7 and 9.5 and ideal pH level is between 7.5 and 8.5 and above and below this is stressful to the fishes. Ideally, an aquaculture pond should have a pH between 6.5 and 9 (Wurts and Durborow 1992, Bhatnagar *et al*. 2004). The findings of the present study also agreed with those obtained by Kohinoor *et al*. (2001), Chakraborty *et al*. (2003) and Rahman and Rahman (2003b). The water transparency value which normally indicates the primary productivity (planktonic concentration) of ponds was measured as 23.67 ± 2.01, 23.43 ± 2.25 and 25.46 ± 2.24 cm in T1, T2 and T3, respectively with a range of 19 to 30 cm. The average water transparency in T3 was slightly higher than those in T1 and T2. The observed transparency values indicated productive condition of the experimental ponds as these values are well supported by Wahab *et al*. (1994) who measured the water transparency between 15.0 and 74.0 cm in polyculture ponds located at BAU campus. Rahman (1992) suggested that the transparency of productive water bodies should be 40 cm or less.

Table II. Water quality parameters (mean$\pm$SD) in the three treatments during the experimental period

Parameter	Treatments		
	T1 (Gynogenetic female)	T2 (Gynogenetic neo-male)	T3 (Normal mixed-sex)
Water temperature (°C)	29.47$\pm$0.68	29.01$\pm$0.87	29.38$\pm$0.69
Dissolved oxygen (mg/L)	5.29$\pm$0.44	5.24$\pm$0.49	5.09$\pm$0.52
pH	6.38$\pm$0.63	6.53$\pm$0.46	6.87$\pm$0.62
Transparency (cm)	23.67$\pm$2.01	23.43$\pm$2.25	25.46$\pm$2.24

Growth performance and yield of Barbonymous gonionotus

The growth performances in terms of length and weight gain of silver barb, *B. gonionotus* under the three treatments are given in Table III. The fishes in different treatments grew well and significantly ($p < 0.05$) highest growth (88.53$\pm$3.38 g) was observed in T1 followed byT2 (87.70$\pm$1.24) and T3 (71.86$\pm$0.54 g). However, there was no significant difference between T1 and T2. The percent weight gain between 2707.25$\pm$57.25 to 3254.91$\pm$47.79 was observed in different treatments. The significantly ($p < 0.05$) lowest percent weight gain was observed in T3 while the highest percent weight gain was observed in T1 and it was not significantly different from T2. The average daily gain (g) recorded indifferent treatments was between 0.63$\pm$0.01 and 0.74$\pm$0.03. The significantly ($p < 0.05$) lowest average daily gain was 0.63$\pm$0.01g observed in T3 while the highest average daily gain was 0.74$\pm$0.03 g observed in T1 and that was0.73 g in T2.The specific growth rate in different treatments ranged from 2.78$\pm$0.020 to 2.93$\pm$0.010 and the highest specific growth rate was observed in T1 while that was lowest in T3 (Table III).

Table III. Growth parameters ($\pm$SE) of silver barb (*Barbonymous gonionotus)* under three treatments during the experimental period

Parameters	Treatments		
	T1 (Gynogenetic female)	T2 (Gynogenetic neo-male)	T3 (Normal mixed-sex)
Initial length (cm)	5.83$\pm$0.12[a]	5.85$\pm$0.12[a]	5.79$\pm$0.16[a]
Final length (cm)	18.26$\pm$0.28[a]	17.80$\pm$0.21[ab]	17.24$\pm$0.29[b]
Initial weight (g)	2.72$\pm$0.10[a]	2.77$\pm$0.12[a]	2.66$\pm$0.16[a]
Final weight (g)	91.25$\pm$1.81[a]	90.46$\pm$1.19[a]	74.52$\pm$2.28[b]
Weight gain (g)	88.53$\pm$3.38[a]	87.70$\pm$1.24[a]	71.86$\pm$0.54[b]
% Weight gain	3254.91$\pm$47.79[a]	3168.91$\pm$3.79[a]	2707.25$\pm$57.25[b]
Av. daily gain (g)	0.74$\pm$0.03[a]	0.73$\pm$0.01[a]	0.63$\pm$0.01[b]
SGR (%/day)	2.93$\pm$0.010[a]	2.91$\pm$0.005[a]	2.78$\pm$0.020[b]
Survival (%)	89.17$\pm$1.67[a]	88.75$\pm$1.25[a]	92.08$\pm$1.25[a]

Values in the same row having different superscripts are significantly different ($p < 0.05$).

Growth performance such as final length (cm) and weight (g), weight gain (g), % weight gain (g), average daily gain (g) and specific growth rate or SGR (% per day) of *B. gonionotus* were significantly higher ($p < 0.05$) in T1 and T2 compared to T3. Although the stocking density was same and same food was supplied to all the treatments at an equal ratio. All these growth parameters in T3 were significantly lower ($p < 0.05$) than T1 and T2 but T1was not significantly different from T2. T1 and T2 contained gynogenetic females and gynogenetic neo-males and both originated from the same maternal parent. So, their growth performance was more or less same though gynogenetic females showed slight superiority over the neo-males as the females of silver barb have higher growth than the males (Pongthana *et al.* 1999). Gynogenetic neo-males have been produced by using masculinizing hormone and this hormone could suppress the growth of sex reversed males (Pongthana *et al.* 1999). T3 showed lower growth compared to both T1 and T2 as because it contained normal mixed sex population. The survival rate of silver barb was obtained highest (92.08 ± 1.25) in T3 and that was lowest in T2 (88.75 ± 1.25) but there was no significant difference ($p > 0.05$) among the treatments.

The gross production of silver barbof the three treatments were 1219.62 ± 23.29, 1204.57 ± 34.07 and 1029.38 ± 22.44 kg/ha and the net production were 1178.83 ± 22.33, 1163.06 ± 33.43 and 989.54 ± 21.30 kg/ha in T1, T2 and T3, respectively (Fig.1). Both the gross and net production was highest ($p < 0.05$) in T1 and lowest in T3 but no significant difference was observed between T1 and T2 for the parameters.

The gross and net productions of silver barb during the 120 days experimental period were significantly ($p < 0.05$) highest in T1 and lowest in T3 (Fig. 1). The gross and net production in T2 was much closer to T1 and no significant difference ($p > 0.05$) was observed between them.
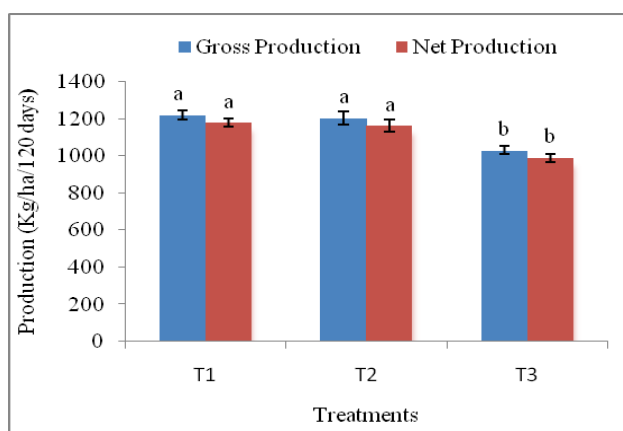

Fig. 1. Showing the gross and net production (Kg/ha) of silver barb after 120 days of culture. Bar denoting the same letter has no significant different ($p < 0.05$)

Pongthana *et al.* (1999) obtained production of monosex females and mixed sex silver barb of 1762 and 1369.25 kg/ha after 5 months of rearing respectively which is comparable to the present study. Kohinoor *et al.* (1993) obtained 2384.26 kg/ha production of *B. gonionotus* as in fertilized pond along with supplemental feeding and 2,129.72 kg/ha with only supplementary feed as rice bran after 6 months of culture period. Hussain *et al.* (1989) obtained *B. gonionotus* production of 1,952 kg/ha after 5 months of rearing with only supplemental feed as rice bran and 689 kg/ha with only fertilizers for the same culture period. Moniruzzaman and Mollah (2010) obtained gross and net production of *B. gonionotus* as 888.39 to 1292.85 kg/ha after 120 days of rearing with fertilizer and rice bran at a stocking density of 57 fish/decimal. In the present study, the gross and net production of gynogenetic females or neo-males of *B. gonionotus* were much higher than the findings of Moniruzzaman and Mollah (2010) with the same culture period (120 days) and similar stocking density (60 fish/decimal). Even in the present study, the gynogenetic fish showed significantly ($p < 0.05$) higher production than that of normal mixed-sex (control) silver barb in the same management. Therefore, it can be concluded that the gynogenetic silver barb have higher growth rate and generation of monosex all-female population through crossing with neo-males (XX) will increase the fish production.

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