Outbreeding Performance of Tinfoil Barb *Barbonymus schwanenfeldii* from Java and Kalimantan for Aquaculture Development

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Abstract. Aquaculture development of tinfoil barb farming is needed to increase production. This study aimed to evaluate the reproductive performance, growth and heterosis value on outbreeding of two tinfoil barb populations (Java and Kalimantan) reciprocally. Spawning was conducted by induced breeding using hormone stimulation (at dose of HCG 500 IU/Kg and dose of LHRH analog 0.6 mL/Kg). Spawning was carried in the aquarium with 1 x 0.5 m length and 50 cm height of fresh water as full sib ratio 1:1 (one female: one male). The measurement of hybrid in each population was repeated three times. The data were analyzed using analysis of variance (ANOVA), followed by Duncan test. Result showed that fingerlings resulted from ♀ Kalimantan and ♂ Java exhibited the highest reproductive and growth performances ($P<0.05$) with fertilization rate (94.17%), absolute length gain (2.85 cm), absolute weight gain (0.33 g), specific growth rate of length and weight (2.35% and 4.02%), and survival rate (57.20%).

1 Introduction

Tinfoil barb is known as a native species in Indonesia with good economic value (IDR 25,000-40,000/kg) [1]. This species is popular for consumption due to its taste. Tinfoil barb culture in Indonesia has been carried out since 2010. However, it has not been implemented intensively. So far in aquaculture activities, the adaptability of tinfoil barb in aquaculture environments is relatively low with the survival value of 69.28 ± 19.64% and growth of 1-2 cm at the age of 50-60 days [2]. The low adaptability of this species on aquaculture environment is probably due to availability of brood fish which would increase the inbreeding. Inbreeding causes in lowering the genetic diversity which would decrease adaptability and productivity [3].

Aquaculture development of tinfoil barb was carried out through a genetic approach with outbreeding to increase the genetic diversity of cultured populations. Outbreeding is generally carried out between different individual strains or population stocks to enrich the allele variety of populations and increase heterozygosity, so that it has implicated in the

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improvement of adaptability and production performance [4]. Outbreeding is also a way to improve survival and growth. Some outbreeding activities have been carried out on several native fish species, such as mahseer [5], giant gourami [6] and climbing perch [7]. In general, the seedlings produced from the outbreeding show better performance and adaptability. This study aimed to evaluate reproductive performance, growth, and heterosis values of the outbreeding results of two tinfoil barb populations (Java and Kalimantan) for aquaculture development.

2 Materials and Methods

2.1 Experimental fish

Fish used in this study were two populations originating from Java (Center for Conservation of General Aquatic Fisheries, Department of Marine and Fisheries, West Java Province) with environmental characteristics of temperature: 24-30ºC, pH: 5-7 and dissolved oxygen: 4-5.6 ppm and from Kalimantan (Central for Fish Culture, Anjungan, West Kalimantan) with environmental characteristics of temperature: 29-33ºC, pH: 5-7.4 and dissolved oxygen: 4.5-6.6 ppm. Fish (length: 15.2 ± 1.1 cm; weight: 124.2 ± 28.5 g) has gonad maturity level of IV and it was selected by the cannulation method.

2.2 Outbreeding

The outbreeding process on tinfoil barb was carried out at the Germplasm Research Station, the Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor. Spawning was carried out by induced breeding using hormone stimulation (HCG at a dose of 500 IU/Kg and LHRH analog at a dose of 0.6 mL/Kg). Spawning was carried out in an aquarium (dimension: 1.0 × 0.5 m, water level: 0.5 cm) with a female: male ratio of 1:1. The outbreeding of each population was repeated three times. The breeding scheme is presented in Table 1.

Table 1. Schematic outbreeding of tinfoil barb from Java (J) and Kalimantan (K)

Population		Male (♂)	
		J	K
Female (♀)	J	J x J	J x K
	K	K x J	K x K

2.3 Reproductive performance

The parameters observed in reproductive performance were fecundity (eggs/fish), fertilization rate (%), hatching rate (%), latency time of ovulation (hour) and survival rate of larvae (%). A total of 250 fertilized eggs from four populations outbreeding the tinfoil barb from Java and Kalimantan were stocked into a plastic bowl (15 cm × 10 cm × 5 cm) with a water level of 2 cm. Plastic cups (n=12) were used, representing four treatments with three replications. Reproductive parameters were calculated using a formula according to [8] and [9].

2.4 Growth performance

In this experiment, growth parameters observed were length gain (cm), weight gain (g), and survival rate (%). Tinfoil barb used in the experiment was at the larval stage (after the yolk sac was finished) which consists of four outbreeding populations (Java and Kalimantan). Larval rearing was carried out using aquariums (dimension: 40 cm × 30 cm × 20 cm; water level: 10 cm), each aquarium was aerated with the same intensity. Each aquarium was stocked with 250 fish. Fish were kept for 90 days and fed according to age (size) with different types. At the age of 3-30 days, the larvae were fed by chlorella (ad-satiation). At the age of 31-60 days, the seedlings were fed by Artemia (ad-satiation). Meanwhile, at the age of 61-90 days, the seedlings were fed by commercial feed (10% of fish biomass). Feed was given three times a day (morning, afternoon, and evening). In addition, water quality management was treated by syphoning and change the 50% of water every five days. As the supporting data, water quality measurement were carried out during the experiment, including pH, DO and temperature. Fish mortality was observed every day. The standard length and weight of fish were measured every month during the study.

2.5 Heterosis value

Heterosis values were calculated based on the equation from [10]. Heterosis value is a value that describes the increase in parameters measured from hybrid results (outbreeding) compared to pure lines.

2.6 Data analysis

The effect of treatment on tested parameters was analyzed by using ANOVA at a 95% confidence interval. The Duncan test with SPSS version 18 was conducted when the ANOVA resulted the significant different among the treatments.

3 Results and discussion

3.1 Reproductive performances on four populations of tinfoil barb resulted from outbreeding

Reproductive characterization on the broodstock of tinfoil barb from Java and Kalimantan which includes length, weight, fecundity, egg diameter, somatic index and latency time of ovulation are presented in Table 2. Meanwhile, the reproductive characterization of outbreeding which include the fertilization rate, hatching rate and survival rate are presented in Table 3.

Based on our observations of length and weight characters of tinfoil barb broodstocks, the size of female broodstocks from Java population was 16.13 cm and 144.80 g, respectively, while the size of male broodstocks from Java population was 14.42 cm and 86.87 g. In Kalimantan population, the female broodstocks was 16.48 cm and 171.13 g, while the male broodstocks was 16.12 cm and 140.86 g. In general, female broodstocks were bigger than male broodstocks. The weight on the female broodstocks affected their gonad weight. Female broodstocks from Kalimantan were 20% larger than the female broodstocks from Java. Java populations had an ovo somatic index of 12.26% with fecundity of 73,421 eggs. Meanwhile, broodstocks from Kalimantan have an ovo somatic index of 11.83% with fecundity of 61.152 eggs. The eggs from both populations had relatively equal diameters from 0.4 to 1.2 mJm. According to [5], the egg numbers produced by female broodstocks was strongly influenced

by its conditions, such as age and size. The greater size of broodstocks will further increase the fecundity.

Table 2. Characterization of reproductive performances of the broodstock of tinfoil barb from Java and Kalimantan

Reproductive characters	Tinfoil barb			
	♀ J	♀ K	♂ J	♂ K
Standard length (cm)	16.13 ± 1.64	16.48 ± 0.80	14.42 ± 1.04	16.13 ± 0.99
Weight (g)	144.80 ± 51.44	171.13 ± 23.33	86.87 ± 22.08	140.86 ± 20.30
Fecundity (eggs/kg)	73.421 ± 6.069	61.152 ± 1.760	-	-
Egg diameter (mm)	0.6 – 1.2	0.4 – 1.0	-	-
Somatic index (%)	12.26 ± 5.49	11.83 ± 2.42	-	-
Latency time of ovulation (hour)	12 ± 2	12 ± 1	-	-

Table 3. Reproduction characterization of outbreeding results tinfoil barb fish from J and K reciprocally.

Reproductive characters	Tinfoil barb			
	J x J	K x K	J x K	K x J
Fertilization rate (%)	93.00 ± 0.50[a]	94.33 ± 0.29[b]	92.00 ± 0.87[a]	94.17 ± 0.58[b]
Hatching rate (%)	73.67 ± 3.15	75.44 ± 1.03	76.41 ± 6.05	76.08 ± 5.39
Survival rate at day-3 (%)	26.60 ± 5.98	24.81 ± 12.40	29.61 ± 4.93	30.15 ± 7.40

Remarks: J=Java, K=Kalimantan, initial population=female, final population=male
*) Numbers followed by the same in this column indicates no significant difference

On reproductive characters (Table 3) showed significant different results ($P<0.05$) only at fertilization rate. Tinfoil barb hybrid from ♀ Kalimantan x ♂ Java exhibited the highest fertilization rate of 94.17%. The fertilization rate was evaluated from the egg color. Unfertilized eggs were milky white within two hours after the fertilization process. In addition, the yolk sack duration and egg incubation time from each spawning population were not significantly different (3 ± 0 days and 20 ± 1 hours). In this study, the eggs were incubated under the same environment conditions (temperature: 25-27ºC; pH: 6-7), so it could be assumed that the hatchability of most eggs was inherited. According to [11], the increase in reproductive performance in outbreeding fish occurred because there was an increase in heterozygosity and outbreeding. In addition, according to [12], reproductive performance could be influenced by size, condition and viability of the female broodstocks.

3.2 Growth performance on four populations of tinfoil barb from outbreeding

The length gain, weight gain and survival rate of tinfoil barb from each reciprocal outbreeding population during the study are presented in Table 4. Based on the observations on the growth characters (Table 4), the highest value of length gain, weight gain, specific growth rate and survival rate were found in the outbreeding from population ♀ Kalimantan x ♂ Java with the value of 2.85 cm, 0.33 g, 2.35%, 4.02% and 57.20%, respectively. Their growth was significantly different ($P<0.05$) compared to other populations, while the survival rate character showed in significantly different results between the populations from outbreeding ($P>0.05$). Superiority on growth of the hybrid from the outbreeding ♀ Kalimantan x ♂ Java was due to their high genetic diversity. Outbreeding increases heterozygosity [3]. Populations with high genetic diversity have a higher life chances, because many alternative or combinations of genes are available to cope with the environmental changes [13-14]. The outbreeding population is adaptable to rearing

environment, so that it improves their growth performance and disease resistance [15]. Genetic variety is a key parameter on population fitness which guaranteed its sustainability and the ability to passively respond the natural or artificial selection [16]. Genetic diversity reflected the number of allele variations associated with flexibility in the adaptation process. Potential genetic resources for aquaculture were required to have responsive genetic diversity on environmental changes, so that it could contribute to maintain their generations. In general, the growth pattern of tinfoil barb depends on their rearing environment. [17] reported that the growth patterns of tinfoil barb which maintained in floating nets are isometric. In concrete ponds, they are allometric positive. Meanwhile, they are allometric negative in earthen ponds.

Table 4. Growth characteristics (length and weight), specific growth rate and survival rate of outbreeding tinfoil barb from J and K reciprocally.

Growth parameters	Tinfoil barb			
	J x J	K x K	J x K	K x J
Initial length (cm)				0.39 ± 0.01
Initial weight (g)				0.09 ± 0.02
Final length (cm)	0.39 ± 0.01	0.39 ± 0.01	0.39 ± 0.01	3.24 ± 0.03
Final weight (g)	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.34 ± 0.01
Absolute length gain	2.56 ± 0.11	2.96 ± 0.03	2.72 ± 0.03	2.85± 0.03[d]
(cm)	0.17 ± 0.02	0.26 ± 0.01	0.23 ± 0.01	0.33 ± 0.02[d]
Absolute weight	2.17 ± 0.11[a]	2.57 ± 0.03[b]	2.33 ± 0.03[c]	2.35 ± 0.01[d]
gain (g)	0.16 ± 0.02[a]	0.25 ± 0.01[b]	0.22 ± 0.01[c]	4.02 ± 0.06[d]
Specific growth rate	2.09 ± 0.05[a]	2.25 ± 0.01[b]	2.16 ± 0.01[c]	57.20 ± 4.60[b]
of length (%)	3.27 ± 0.13[a]	3.74 ± 0.41[b]	3.57 ± 0.52[c]	
Specific growth rate	48.7 ± 3.10[a]	53.33 ± 9.20[b]	52.27 ± 2.60[b]	
of weight (%)				
Survival rate (%)				

Remark: J=Java, K=Kalimantan, initial population=female, final population=male
*) Numbers followed by the same in this column indicates no significant difference

3.3 Heterosis value

Heterosis values on reproductive characters and growth of the outbreeding of tinfoil barb from Java and Kalimantan obtained during the study are presented in Tables 5 and 6.

Table 5. Heterosis value for reproductive character from reciprocal outbreeding of tinfoil barb from J and K.

Reproduction characters	Heterosis value (%)	
	J x K	K x J
Fertilization rate	-1.78	0.54
Hatching rate	2.49	2.05
Survival rate (3-day)	15.19	17.29

Remarks: initial population=female, final population=male

Based on the results, heterosis value on fertilization rate, hatching rate and survival rate resulted from spawning of tinfoil barb hybrid ♀ Kalimantan x ♂ Java showed positive values. The heterosis value showed an increase in heterozygosity of 0.54 (fertilization rate), 2.05 (hatching rate) and 17.29 (survival rate). The values obtained in this study were relatively low. However, the value could provide information that reproductive characters could still be improved. Low value of heterosis did not mean that the generation resulted from outbreeding were poor. However, it explained a condition of comparison between the average descendants with their parents.

Table 6. Heterosis value for growth resulted from reciprocal outbreeding of tinfoil barb from J and K

Growth characters	Heterosis Value (%)	
	J x K	K x J
Length	-1.69	20.25
Weight	7.32	60.98
Survival rate	2.46	12.12

Remarks: initial population=female, final population=male

The biggest positive heterosis value on length gain, weight gain and survival were shown by the fingerlings resulted from ♀ Kalimantan x ♂ Java with the value of 20.25%, 60.98% and 12.12%, respectively. The heterosis value was very significant compared to the heterosis value obtained in fingerlings resulted from ♀ Java x ♂ Kalimantan with the value obtained was -1.69%, 7.32% and 2.46%, respectively. Heterosis value was a description of the genes compatibility inherited from both parents to their generations. Heterosis value arises because of the combination of new genes, so that it was expected to produce better performance than their parents [18].

3.4 Water quality

The results of water quality measurements in the aquarium during experiment are shown in Table 7.

Table 7. The water quality in the aquarium during the experiment

Variable (Water quality)	Range	References	
		Susanti (2014)	Kottelat (1993)
Temperature (°C)	25 - 28	25 - 30	28.1 – 28.4
pH	5 - 7	5 - 7	6.5 - 7
Dissolved oxygen (ppm)	41 - 6	3 - 8	7 - 7.3

In addition to genetic factors, fish performance was also influenced by environmental factors. Growth is strongly influenced by temperature, pH and dissolved oxygen [19]. In general, water quality data (temperature, pH and DO)during experiment in the aquarium were still within the tolerance limit for fish growth and survival.

4 Conclusion

Outbreeding population ♀ K and ♂ J showed better performance on fertilization rate (94.17%), absolute length gain (2.85 cm), absolute weight gain (0.33 g), specific growth rate on length and weights (2.35% and 4.02%) and survival rate (57.20%). Heterosis value on fingerlings resulted from outbreeding ♀ K and ♂ J was positive. Male broodstocks from J and female broodstocks from K have the potential as genetic sources of donors for the development of tinfoil barb culture.

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