Domestication Strategies of Tinfoil Barb *Barbonymus schwanenfeldii* (Bleeker, 1854): Potential Candidate for Freshwater Aquaculture Development

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**Abstract.** This paper discussed domestication strategies of Tinfoil barb from different populations for evaluation of potential fish candidate for freshwater aquaculture development. Collection and maintenance of wild-caught adult fish was the first step in domestication strategies followed by characterization of fish, broodstocks selection and maturation, control of fish reproduction, and larval rearing. The Tinfoil barb presented an excellent performance to adapt to the new environment. This was indicated by survival rate of the fish acclimated in the captive environment of about 69.28 ± 19.64% for five weeks. Phenotypic characterization revealed that Tinfoil barb collected from Borneo, Java, and Sumatra have different morphological characters. Based on genetic characters, polymorphism percentage and heterozygosity of the Borneo tinfoil barb population provided the highest performance followed by Java and Sumatra. The growth model of all Tinfoil barb populations was allometric negative indicating a faster increase in length than in weight. Reproductive performance presented that the fecundity of Tinfoil barb female from Borneo was the highest (2795-5099 eggs/gram) followed by Sumatra (1822-2976 eggs/gram) and Java (2210 eggs/gram). The artificial reproduction performance of broodstock from Borneo also presented more excellent performance than other population after hormonal treatment. Fertility rate and hatching rate were 94.33+0.29% and 77.44+1.03%, respectively. Java Tinfoil barb had fertility rate 93±0.05% and hatching rate 73.67±3.15%, while it was no fertility rate and hatching rate obtained from Sumatra brood-stock group. Wild-caught adult Tinfoil barb from Borneo population presented the best performance used for potential brood-stock regarding genetic characters, reproductive performances, and response to artificial reproduction. Future studies need to be concerned with the acceleration of gonad maturation and the improvement of larval rearing technology for improvement of domestication strategies of Tinfoil barb as a potential candidate for freshwater aquaculture development.

1. **Introduction**

Prior to taxonomic revision, *Barbonymus* was previously known as *Barbus, Barbodes, Puntius* or *Systomus*. Genus *Barbonymus* has 5 species including *B. altus* (Gunther, 1868), *B. balleroides* (Valenciennes, 1842), *B. collingwoodii* (Gunther, 1868), *B. gonionotus* (Bleeker, 1849), and *B. schawenfeldii* (Bleeker, 1854). Except for *B. altus*, four species are distributed in the Indonesian freshwater ecosystem. Tinfoil barb *Barbonymus schawenfeldii*, locally known as Tengadak or...
Lampan, can be found in Java, Borneo and Sumatra regions. In South East Asia, this species widely distributes in Mekong rivers Vietnam, Chao Phraya rivers Thailand, Peninsula (Pahang, Perak, Kelantan, Terengganu, Selangor), and Sarawak Malaysia [1]. Tinfoil barb is targeted species of inland water for fisheries production. In recent years, the population of fish has decreased significantly due to overexploitation and habitat degradation [2]. Thus, it is necessary to alleviate the pressure on the tinfoil barb wild population through the domestication of this fish for its conservation. Moreover, the domestication of Tinfoil Barb appeared as a key strategy for diversification of aquaculture production. The adult fish can reach 1 kg in weight and 35 cm in length. This species also performed attractively appearance, especially at fingerlings phase. Hence, it is potential for freshwater aquaculture development based on native species targeting for human consumption and ornamental fish market.

Fish domestication is a process of adapting wild fish into culture systems where the breeding, rearing, and feeding are artificially controlled by human [3, 4]. This process leads to development of phenotypic and genetic changes of the fish in response to culture conditions [5]. The success of domestication can be assessed based on the capability of wild fish to adapt in a captive environment, controlled reproduction, and larval production and rearing [6]. Investigation of biometric, growth and gonad maturation in fish also can be used to evaluate potential fish for aquaculture [7]. Therefore, it needs adaptive strategies in domestication program to achieve targeted domestication level as every fish species needs specific domestication techniques [8]. Domestication of fish plays a pivotal role in diversification of farmed species and conservation of the species for sustainable production. This paper discussed domestication strategies of Tinfoil barb from different populations for evaluation of potential fish candidate for freshwater aquaculture development.

2. Materials and Methods

The series of research on tinfoil barb domestication were carried out more than 5 years from 2015 to 2019. The primary data and information were collected from research experiences and publications related to Tinfoil barb domestication program conducted at research facility of Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor, Indonesia. During the research, the collection of wild-caught adult fish, characterization on the potential fish broodstock, selection and maturation of broodstock, induced breeding, and larval rearing were observed and studied to complete the domestication protocol of tinfoil barb in Indonesia.

3. Results and Discussion

Collection of wild-caught adult fish. The purpose of the first step of domestication in wild fish is to enable them to adjust to the new environmental conditions [9]. The brood-stock candidates were collected from river ecosystems in Java, Sumatra, and Borneo islands, Indonesia. Fish were maintained at different earthen ponds which were previously prepared for the growth of natural feed. The tinfoil barb presented an excellent performance to adapt to the new environments. It was indicated by survival rate of the fish acclimated in the captive environment about 69.28 ± 19.64% for five weeks. Mortality mainly occurred in the first week of maintenance probably due to the transportation process [10]. For adaptation to artificial feed, tilapia (Tilapia niloticus) and carp (Cyprinus carpio) were added to the ponds to train Tinfoil barb giving response to commercial feed. Acclimatization period of the fish giving positive responses to artificial feed in the ponds was observed about 10 days. Thus, it can be explained that the first domestication level of tinfoil barb has successfully occurred as the fish can present an excellent acclimatization process in the culture environment. Water quality measured during the acclimated process including temperature, DO, pH and nitrate was 27.1-27.5°C, 7.3-7.6 ppm, 5.8-7.1, and nitrate <0.5 ppm, respectively.

Characterization of tinfoil barb population. Phenotypic characterization. Identification of morphological diversity or phenotype in fish is one of the essential parameters for supporting fish domestication programs determined based on measurement of body shapes and external organs. Morphometric is a measurement conducted by connecting the distance of landmarks created in body
shape and external organs of fish [11]. The method of measurement was conducted by connecting the
distance of landmarks determined on the body shape and external organs. At a population level, this
method is commonly known as truss morphometry. Subsequently, canonical function analysis (CFA)
were applied to evaluate morphological characters. CFA showed that the morphological characters of
Tinfoil barb collected from three different populations (Borneo, Sumatra, and Java) were distributed in
different quadrants (Figure 1). Tinfoil barb from Java was identified in quadrant 2 and 3, and
Sumatran Tinfoil barb was observed in quadrant 1. On the other hand, Borneo Tinfoil barb was
observed in quadrant 4. These results indicate that Tinfoil barb collected from Borneo, Java, and
Sumatra has different morphological characters (Figure 2).

![Figure 1. Canonical function analysis of Tinfoil barb from Borneo, Java and Sumatera [10]](image1.png)

**Figure 1.** Canonical function analysis of Tinfoil barb from Borneo, Java and Sumatra [10]

![a. Borneo](image2a.png) ![b. Java](image2b.png) ![c. Sumatra](image2c.png)

**Figure 2.** Morphological performance of Tinfoil barb from Borneo, Java and Sumatra

**Genotype characterization.** For the success of domestication strategy, genetic diversity information
of fish plays important role in providing high-quality broodstock candidates. Genetic diversity can be
determined based on allele variation and heterozygosity urgently used for the long-term sustainability
of species and population related to adaptation capability on environmental changes. Genotype
characters of fish were analyzed using PCR-RAPD method. Genetic diversity analysis based RAPD
metode was also carried out in native Indonesian fish species such as silver barb *Barbonymus
gonionotus* [12], and snakehead, *Channa striata* [13].

Genotype characters of Tinfoil barb were determined by evaluation of polymorphism, heterozygosity and genetic distances. Polymorphism percentage was determined by the number of amplified DNA fragments. Polymorphism differences identified based on DNA bands depends on the
primary attachment site and can be used to provide information on genetic diversity level in fish population [14]. Comparing polymorphism percentage of wild Tinfoil barb from three different populations shows that Borneo Tinfoil barb population provided the highest performance, followed by Java and Sumatra. Similarly, heterozygosity of Borneo population presented the highest value compared with Java and Sumatra (Table 1). Heterozygosity indicates the potential adaptability of fish to the environment. The higher heterozygosity parameter, the more genes involved in contributing to the fitness level of a population [15].

Table 1. Polymorphism and heterozygosity of Tinfoil barb from Sumatera, Jawa, dan Borneo [10]

| Populations | Polymorphism | Heterozygosity |
|-------------|--------------|----------------|
| Sumatra     | 35.29        | 0.175          |
| Java        | 44.12        | 0.171          |
| Borneo      | 50.00        | 0.206          |

Genetic diversity of cross-breeding Tinfoil barb population (female Java x male Borneo) showed that the highest polymorphism, heterozygosity, and genetic distance was 32.43%, 0.13, and 0.48, respectively. Thus, these values present an excellent potential used for genetic resource for development of Tinfoil barb aquaculture [16]. Investigation of polymorphism and heterozygosity value the wild population (G0), first-generation (G1) and second-generation (G2) was 42.50%; 0.163, 27.50%; 0.105, and 15.00%; 0.071, respectively. Thus, intrapopulation diversity between wild generation (G0) and domesticated population (G2) decreased from 30% to 40% [17]. Genetic distance between wild generation and domesticated population ranged from 0.26 to 0.36 with the closest genetic distances were G1 and G-2 (0.26). This revealed that the domestication process leads to a decrease in the diversity of the fish genotype.

**Growth model of tinfoil barb.** Evaluation of length-weight relationship and factor conditions is an essential factor in aquaculture to predict fish weight from the length to analysis body performance and to compare morphological characters in different populations and regions [18]. The length-weight relationship of Tinfoil barb was 2.811 for Sumatra population, and 2.686 for Borneo population. This value presented that the growth model of Tinfoil barb was allometric negative indicating a faster increase in length than in weight. Negative allometric also indicates that the body shape becomes tinnier as the length increases [19, 20]. Condition factor of Tinfoil barb ranged from 0.99 to 1.002. This range of values indicated that fish was categorized as long and thin performance [21]. Condition factor in fish species can be influenced by age, sex, growth, maturity level, diet, and environmental factors [22, 23]. Bassey and Ajah [24] reported that condition factor increases in line with optimum diets and is mainly used for evaluation of the physical wellbeing of the fish [25]. Length-weight relationship and factor conditions can be used to evaluate the growth performance of fish. The growth performance of cultured fish plays a pivotal role in the determination of economic benefit in commercial aquaculture production [26]. Growth patterns can be useful information in the evaluation of rearing technique of cultured fish for improvement of fish production and business profit. In aquaculture farms, growth is mainly influenced by feed intake and quality of diets, stocking density, culture period, and water quality [27].

**Breeding Technologies.** roodstock selection and maturation. Determination of brood-stock candidates used for a fish reproduction program is an essential factor for the success of Tinfoil barb domestication. Sex identification was the first step in the selection of fish used for potential broodstock. Sex identification was performed to determine different characters between males and females of fish based on external morphological characters. External observation on fish body shape,
size, color, specific appearances, and behavior provided more simple and reliable methods for sex determination in fish [28, 29]. Observation of urogenital appearance also can be directly used to determine the different sex of various fish [30]. External observation of Tinfoil barb broodstock candidate revealed that the male of fish has slimmer body shape, bright body color, reddish fins, flattened abdomen, rough body scales, and the milk-white sperm was easily released as its abdomen was stripped. On the other hand, the female was a rounded abdomen, pale body color, smooth body scales, and reddish-colored urogenital [31]. The mature broodstock has an age of more than 1.5 years. The size of mature females was 150-400 grams, while the male was 200-400 grams.

The quality of fish reproduction was observed by evaluation of gonad maturation especially color, size and diameter of eggs. Broodstocks were weekly monitored by releasing the sample of the eggs in targeted fish using the cannulation method to evaluate gonadal maturation level. The eggs then were observed using a binocular microscope (Olympus) fitted with a micrometer to measure the color and diameter of eggs. Wild fish obtained from Borneo, Sumatra, and Java commonly have already mature gonad levels with gonadosomatic index (GSI) from 3 to 4. The fecundity of Tinfoil barb female from Borneo was 2795-5099 eggs/gram. It was higher than Sumatra and Java Tinfoil barb with fecundity ranging from 1822-2976 eggs/gram and 2210 eggs/gram, respectively. Total fecundity of fish in both groups ranged from 20.168 to 232,040 eggs collected from fish gonad ranged from 9 g to 45.5g [32]. Potential selected male and female broodstocks were separately reared at different ponds to avoid uncontrolled breeding events in the ponds. Broodstocks were fed with commercial feed (protein 30%) about 3% of total weight given three times a day. Stocking density of broodstock reared in the earthen ponds was 2–5 fish/ m².

Induced Breeding. Sexually mature brood-stock were selected for artificial reproduction based on induced breeding technology. Gonadotropin-releasing hormone analog (GnRha) was intramuscularly injected into the dorsal area of the fish with the dose of 0.6 ml/kg bodyweight female and 0.2 ml/kg male. Injections were applied once only both in female and male of brood-stock. The weight ratio between male and female of broodstock was 1:1. Breeding of the fish can be accelerated with ovaprim treated to the broodstock maintained in a tank or pond [33]. Eggs and sperm were released using the stripping method and subsequently mixed in a sterilized media. Fertilization was conducted by mixing sperm and eggs from selected broodstock. The fertilized eggs hatched about 24 hours. Larvae were fed by newly hatched Artemia nauplii for 7 days and then food supply was substituted by tubifex for approximately 2 weeks. The hormonal treatment on wild Tinfoil barb broodstock provided a positively response to gonad maturation. The wild broodstock from Borneo performed fertility rate 94.33±0.29 %, and hatching rate 77.44 ± 1.03 % [10]. The broodstock from Java had fertility rate 93.2±0.05% and hatching rate 73.67 + 3.15%, while it was no fertility rate and hatching rate obtained in the Sumatra brood-stock group.

Kusmini et al. [34] reported that observation on the first generation of Tinfoil barb (G1) found that the first mature females were recorded at the standard length of 15.79±1.23 cm and the males were found at 16.01±1.18 cm. The spawning season for the G1 fish occurred in the rainy season in October and November. It is indicated by the reddish genital papillae and rough body scales in the dorsal area. All male broodstocks were commonly found in mature gonads. Ovulation of G1 fish was observed 10 hours and 44 minutes with the water temperature during spawning 25°C. The eggs hatch in 23 hours and 7 minutes. The fecundity of the first females (217.15 g) was 12,495 eggs, and the second female (197.93 g) was 19,068 eggs. Induced breeding of Tinfoil barb using hCG with the dose of 200 IU/kg showed that fecundity of fish reached 19,021 eggs [35]. The fertility rate of the G1 female fish (331.91 g) and the male (178.2 g) was 99.72% and the hatching rate was 95.29%. The survival rate of larva aged three days after hatching was 100%.

Fertilized eggs were sampled and observed about 24 hours to investigate the embryogenesis of Tinfoil barb using an Olympic Stereomicroscope fitted with a camera and photograph. Embryogenesis of Tinfoil barb has four phases including morula, blastula, gastrula, and organogenesis (Figure 3). Morula phase is the early stage in post-fertilization as the cells are mitotically divided to create a mass of cells. At this phase, the embryos are very sensitive to the shock and the cells are easily released.
from the surface causing the death of the embryo. A small space was formed between the yolk and the cell mass called the segmentation cavity [36].

Figure 3. Development of embryogenesis in Tinfoil barb including the phase of morula, blastula, gastrula, and organogenesis [36].

At this stage, the layer of the main organ was formed in preparation for gastrulation, namely ectoderm (epiblast), endoderm (hypoblast), and mesoderm (mesoblast). Blastula stage was formed about 1 hour and 45 minutes at 30°C, while it needed 1 hour and 47 minutes at 28°C after fertilization. The time needed to complete blastula phase was 2 hours and 20 minutes [37]. The results of observations in the blastula phase of silver barb occurred at 170-230 minutes [38]. The next process is gastrula phase. In this phase, three layers of the cell were formed including ectoderm, mesoderm, and entoderm. Gastrulation is closely related to nerve formation called neurulation. In this stage performed epiboly and emboly as the organ candidates actively take part in the formation of gastrula. The gastrula phase occurred 3 hours and 40 minutes at temperature 30°C, and 4 hours at temperature 28°C [37]. At the silver barb, gastrula phase occurred from 290 to 350 minutes [38]. Organogenesis is a process of embryonic development indicated by the initial forming of organs such as the brain, eyes, digestive organs, glands, and endocrine glands. The time needed for the organogenesis phase in Tinfoil barb was 4 hours and 10 minutes at 30°C, and 4 hours and 30 minutes at 28°C [37]. Alfath et al. [38] reported that in silver barb the organogenesis phase occurred at 410 to 530 minutes which took a longer time as compared to Tinfoil barb.

Larval rearing. Larval rearing can be conducted at tanks, concrete ponds, earthen ponds and net cages. The newly hatched larvae were fed with natural feed including microalgae and zooplankton previously cultured in the circular tank after 2 days of hatching. Feeding frequency was conducted three times a day for 7 days. Commercial pellet powder (protein 34%) was initiated after a week rearing period given 5% of the biomass weight two times a day conducted over 2 months rearing period. The average weight of larvae aged 30 days was 0.03 ± 0.01 g with the length about 10.2 ± 0.21 mm. Observation of larval growth was continued in three different culture media including concrete ponds (2m x 5m x 1m), earthen ponds (2.5m x 4m x 1m), and net cages (2m x 2m x 1m) were used for larval rearing (Table 2). Larvae were reared at stocking density of 13 fish/m² over 120 days rearing periods. Fish were fed with commercial diet (protein 34%) given three times at satiation.

Table 2. Growth performance of larvae B. Schwanenfeldii reared at different culture media [32].
Growth performance of larvae reared at different culture media conducted over 120 days was shown in table 2. There were no significant differences in the growth of fish reared at different culture media (P>0.05). Fish reared in earthen ponds was the highest growth followed by concrete ponds and net cages. The specific growth rate (SGR) of fish reared at net cages, concrete ponds and earthen ponds was 1.74 ± 0.14%, 1.77 ± 0.04% and 1.78 ± 0.03%, respectively [32].

Many studies were carried out to improve the growth and survival rate of Tinfoil barb. The use of commercial feed with protein content of about 32% resulted in a significant impact on growth and FCR than 28 % dan 23 %, while the survival of the fish was not significantly different [39]. Treatments of different salinity combined with calcium showed that salinity of 3 ppt and calcium 20 mg/l was the best concentration for improvement of growth (6,54%) and survival (95,24%), while the growth and survival of control (0 ppt, no calcium) were about 5,61% and 90,23%, respectively [40]. The use of magnesium (20 mg/L) at temperature 28°C also significantly impacted the growth (8,91%) and survival (95,24%) of Tinfoil barb as compared with control [41]. Pulungan [42] reported that the optimum temperature for excellent growth of Tinfoil barb ranged from 25 °C to 30°C, while temperature ranged from 20 °C to 24°C can inhibit the growth of Tinfoil barb [43]. Growth and survival of cross-breeding Tinfoil barb from Borneo (female) and Java (male) showed that the specific growth rate and survival were 4,02% and 57,20%, respectively [34].

These research provided a better understanding in Tinfoil barb domestication program to prepare the excellent second generation of brood-stock prior to releasing the species as potential candidate for aquaculture. For conservation purposes of this species, the domestication success of tinfoil barb outside its natural habitat (ex-situ conservation) needs to be in line with habitat conservation program where the species exist (in-situ conservation) [44].

4. Conclusion
The series of research in the domestication of Tinfoil barb were successful to select potential brood-stocks from three different populations until reproduction of first-generation offspring in a controlled environment. Wild-caught adult Tinfoil barb from Borneo population presented the best performance used for potential brood-stock regarding adaptation process in captivity, genetic characters, reproductive performances and response to artificial reproduction. Future studies need to be concerned for the acceleration of gonad maturation and improvement of larval rearing technology to achieve a more successful domestication program and to provide the Tinfoil barb production completely conducted in captivity for the development of aquaculture based native species.

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