Reproductive and larval performance of artificially spawned javaen barb *Systomus orphoides* from two populations

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Abstract. Indonesian freshwaters are rich in fish biodiversity, one of them is javaen barb. In terms of domestication, it is important to ensure the fish breeding and larval rearing in ex-situ environment. Therefore, studies on artificial spawning are needed to strengthen the information about reproduction of javaen barb in ex-situ environment for domestication and aquaculture purposes. This study aimed to evaluate the reproductive and larval performance in artificially spawned javaen barb within two populations. Broodfish were obtained from West Java (Tasikmalaya and Cianjur population), kept on the fish nets (2×2×1 m) inside the large ponds for two months of adaptation. Broodfish were attached by RFID chips and periodically observed their size and gonad maturity level. Ripped broodfish were artificially spawned. Several reproductive parameters were observed during the experiment, such as egg diameter, fecundity, fertilization rate (FR), hatching rate (HR), and hatching time. Moreover, larval performance was also observed. The results showed that javaen barb successfully spawned with 11 hours of latency period. Javaen barb from Tasikmalaya population had a significantly higher fecundity than that of the Cianjur population. Meanwhile, other parameters observed in relation with reproductive and larval performance were not significantly different.

Keywords: artificial spawned; fecundity; larval performance; *Systomus orphoides*; reproductive

1. Introduction

Indonesia has rich in native freshwater fish biodiversity, one of them is javaen barb (*Systomus orphoides*). This species is known for consumption purposes. Javaen barb belongs to the family of Cyprinidae and is widely distributed in tropical areas with a water temperature range of 22-25°C especially in Asia such as Chao Phraya, Mekong, and Mae Khlong Valley, the Malay Peninsula, and Indonesia. It is less commercialized and the market price is still unknown. *S. orphoides* also has a lifespan between 1.4-4.4 years [1]. According to the IUCN [2], javaen barb is included in the red zone of threatened species.
Previous research has carried out efforts to domesticate javaen barb. One of very important stages in domestication is ensuring that fish can reproduce. Sugiharto et al. 2009 [3] studied the reproductive profile of Systomus spp. on Serayu River. Meanwhile, Sobari 2005 [4] studied the reproductive patterns of Osteochilus hasselti and P. orphoides in Pelus River, Banyumas. However, those studies were only observed their reproduction in natural habitat. Setyaningrum and Nuryanto 2006 [5] successfully implemented artificial breeding technology of javaen barb through hormonal induction. However, nursery yields are still not optimal because gonad maturity still depends on the spawning season which is heavily influenced by the season. Where when it is not at the peak of the breeding season, the seeds are obtained only a little. Periodic hormonal induction could stimulate the physiological processes of gonad development to final maturation and spawning synchronization during the outspawning season [6]. However, it must be emphasized that environmental signals also played an important role in reproduction process by stimulating pituitary (central nervous system) to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH) on adult fish, especially at the spawning stage. The stimuli of environmental factors will be captured by the fish sensory organs such as skin, eyes, and nose. Then, it will be transmitted to the hypothalamus through nerve fibers [7]. Environmental signals, especially temperature and photoperiod, could stimulate the central nervous system in the brain (pituitary) to release gonadotropin releasing hormone (GnRH) which drives the pituitary to produce gonadotropin hormone (GtH), namely FSH. Thus, the gonads produce 17β-estradiol which controls vitellin production in the liver for vitellogenesis and LH for final ripening. In this case, the changing of seasons played a role as a triggering factor for spawning [8]. although mature fish are found every month, the peak spawning occurs in August and September which is the peak of the summer near the start of the rainy season [9].

According to that information, studies related to artificial spawning of javaen barb are needed to strengthen the information about reproduction, especially on cultivated habitat environment which is useful for domestication and aquaculture purposes. This study aims to evaluate the reproductive and larval performance in artificially spawned javaen barb from two populations.

2. Methods
This research was conducted at Research Station for Freshwater Fisheries Germplasm, Cijeruk, Bogor, West Java, Indonesia. Two populations of javaen barb were originated from West Java (Tasikmalaya and Cianjur), which was wild capture. Fish were reared and adapted in the fishnets (2×2×1 m) inside a large pond for two months. Then, each fish was tagged with RFID chip and periodically measured the length and weight. Furthermore, gonad maturity observation was conducted by using the cannulation method in female fish and the stripping method in male fish.

Ripen female broodfish are injected using the hormone LHRHa + antidopamine (Ovaprim) with the dosage of 0.6 mL/kg of fish weight, while ripen male broodfish were not injected. After the injection, the fish were incubated while in the fiber bath and examined at 8, 9, and so on every hour until the fish spawned to determine their latency period. Fish were spawned by stripping. The striped eggs were sampled as much as 1 g (three samples of each fish), counted, and converted into the formula for calculating fecundity (eggs g⁻¹) based on the gravimetric method. Additionally, egg samples (100 eggs) were also collected to measure the diameter of the eggs at ovulation stage. Then, the eggs were fertilized with collected sperm and hatched in the aquarium. In order to observe the reproduction parameters, eggs were stocked in aquariums (30×40×20 cm) as many as 250 eggs/aquarium with three replications for each population. Moreover, the parameters observed were the fertilization rate, hatching rate, and hatching time.

After hatching, the larvae were kept in aquarium (30×40×20 cm) inside a recirculation system with 15 cm of water level and moderate aeration for each treatment. Each aquarium contained of 250 javaen barb larvae with three replications for each treatment. Observation of length measurement was performed to 25 larvae in each replication. During larval rearing, the egg yolk absorption rate was observed. Fish were not fed until the egg yolk full absorbed. Water quality is maintained by siphoning...
and changing 50% of water every day. Observation was performed to 25 larvae in each replication. The data were analyzed using Software PAST [10, 11].

3. Results and discussion

Of all the fish used, all of them succeeded in spawning both males and females. The spawning duration from induction ranged from 16-18 hours (table 1).

Table 1. Results of artificial spawning on javaen barb *S. orphoides* through hormonal induction.

| Parameters                          | Population       |
|-------------------------------------|------------------|
|                                     | Tasikmalaya      | Cianjur         |
| **Female broodfish (injection)**    |                  |                 |
| Standard length (cm)                | 16.1±2.1         | 16.0±0.9        |
| Weight (g)                         | 158.1±73.5       | 120.9±15.6      |
| Number of broodfish (fish)         | 8                | 10              |
| Number of ripen broodfish (fish)   | 8                | 10              |
| Number of successfully spawned fish (fish) | 8     | 10              |
| **Male broodfish**                 |                  |                 |
| Standard length (cm)               | 16.0±1.4         | 14.5±1.1        |
| Weight (g)                         | 131.5±9.1        | 83.6±17.2       |
| Number of broodfish (fish)         | 5                | 10              |
| Number of ripen broodfish (fish)   | 5                | 10              |
| Number of successfully spawned fish (fish) | 5     | 10              |
| **Spawning**                       |                  |                 |
| Latency period (hours:minutes)     | 11:23            | 11:04           |

The response to gonad maturation on javaen barb was the same within two populations. It can be seen from the whole broodfish that are successfully ripen. Of all the ripen female broodfish, all of them managed to spawn successfully. Meanwhile, total spawned male broodfish from Cianjur population are 10 fish because their size was smaller than Tasikmalaya population (5 male broodfish were used). This number adjusts the ratio of broodstock in javaen barb fish spawning is 1:1 bodyweight.

The latency period of javaen barb was 11 hours after the second injection within 24-25°C of water temperature. Latency period is defined as the period between injection and ovulation. The latency period in this study is not significantly different from previous studies. Javaen barb from Banjaran River were successfully spawned in the pond by injecting synthetic hormones with the latency period of 10 hours [5].

Temperature and hormones have influence on the latency period. The effect of temperature and hormones on the latency period depends on fish species. The latency period for gouramy to spawned is 8–10.5 hours by using ovaprim [12]. The artificial spawning of African carp (*Labeo parvus*) resulted in a latency period of 11.0–12.2 hours [13]. Meanwhile, in tinfoil barb, the latency period is between 10.23 hours and 12.54 hours [14]. These studies were conducted at the incubation temperature of 24-28°C. At incubation temperature of 24.5–26°C, the latency period of hard-lipped barb was 10.8 hours on average [15]. Maifitri 2004 [16] reported that injection of ovaprim at doses of 0.5 and 0.7 mL/kg body weight against Long-barbell sheatfish (*Cryptopterus limpok*) at a temperature range of 24-27°C obtained a latency time of 8.5 and 9.5 hours. Meanwhile, Kahkesh et al. 2010 [17] stated that giving the ovaprim hormone 0.5 mL/Kg in *Barbus sharpeyi* (Cyprinidae) fish has a latency period of up to 25 hours. This shows that optimization of reproductive activity with the help of the hormone ovaprim requires the correct dosage. If the dose is insufficient, the gonadotropin content in the body is not sufficient to
stimulate ovulation, especially if there is no external stimulation that can increase gonadotropin levels in the fish's body [18].

The results of reproductive performance in two populations of javaen barb showed that the egg diameter during ovulation was not significantly different. In addition, fecundity, hatching time, fertilization rate, and hatching rate were not significantly different within two populations (p>0.05) (table 2).

Table 2. Reproductive performance of javaen barb from two populations.

| Parameter                              | Population       |
|----------------------------------------|------------------|
|                                        | Tasikmalaya      | Cianjur          |
| Female parent (injection)              |                  |                  |
| Standard length (cm)                   | 16.1±2.1a        | 16.0±0.9a        |
| Weight (g)                             | 158.1±73.5a      | 120.9±15.8a      |
| Egg                                    |                  |                  |
| Diameter (mm)                          | 1.05±0.47a       | 1.30 ± 0.04a     |
| Fecundity (egg g⁻¹)                    | 572.9±179.3a     | 245.5±35.3b      |
| Hatching time (hours:minutes)          | 22:23            | 22:41            |
| Fertilization rate (%)                 | 100.0±0.0a       | 100.0±0.0a       |
| Hatching rate (%)                      | 84.3±7.4a        | 73.2±3.8a        |
| Larvae                                 |                  |                  |
| Size of hatched larvae (mm)            | 3.22±0.16a       | 3.22±0.16a       |
| Time when yolk sac is empty (hours:minutes) | 73:46            | 71:53            |
| Time when larvae start swimming to the surface (hours:minutes) | 22:37            | 22:37            |

The data presented are ± SD from three replications. Different superscripts in different columns showed significant differences (p<0.05).

The egg diameter of javaen barb at ovulation has not been deeply studied. Several studies related to eggs were focused on oocyte diameter to analyze their distribution. Thus, it can predict the spawning patterns [19, 20]. Eggs are considered uniform if the sizes are close to each other 70% or more. As in the research of Cestraceus plicatilis which measures the average egg diameter, at least 7 out of 10 fish samples (20 each) are close to the average, so the eggs are considered uniform [21]. In this study, Cianjur population had a more uniform of egg diameter average than Tasikmalaya population. Although there were differences in the level of uniformity, the egg diameter of two populations was not significantly different. This is certainly in line with other reproductive parameters. Hence, it can be considered as good results.

A significant difference was found in fecundity. Fecundity from Tasikmalaya population was significantly higher than Cianjur population (table 2). This value is thought to be the result of a very high fecundity (123,000 eggs/fish) from one of female broodfish in Tasikmalaya population, whereas the others were only ranged from 73,000 to 75,000 eggs/fish. Meanwhile, the fecundity of female broodfish from Cianjur population were ranged from 34,000 to 36,000 eggs/fish. The variation in fecundity is probably due to different condition of the fish from wild capture in terms of age, size, and gonad maturity level. However, this value is still greater compared to previous studies, namely 4,097-32,794 eggs [20] and 3,222-32,673 eggs [22]. The fecundity of javaen barb is smaller than several species in family Cyprinidae, such as “lalawak” Barbonymus balleroides from Cimanuk River, West Java with the fecundity ranging from 12,224-207,261 eggs [23], and with “ilelan” Osteochilus wandersii from Lake Singkarak which ranges from 28,140-129,042 eggs [24].

The results of this study indicated that there are many similarities between Tasikmalaya and Cianjur population with the significant difference only occurred in fecundity. The difference is presumably due
to the unpredictable condition of the broodstock captured as well as its age. Cahyanti 2018 [25] stated that Indonesian mahseer with different species, age, origin, and size also produced different egg diameters and fecundity. Muchlisin et al. 2011 [26] stated that fecundity variation between species and individuals depends on parent conditions, such as size (length, weight, and age), genetics, food availability, and environmental factors.

At 24-25°C, the eggs of javaen barb hatched almost simultaneously (Tasikmalaya: 22 hours 23 minutes; Cianjur: 22 hours 41 minutes). In general, javaen barb’s eggs hatched after 36 hours [5]. Indeed, the hatching time in this study was faster, this was due to the more controllable water quality of incubation media which was carried out in a recirculating system. Prabowo et al. 2016 [27] stated that water quality that is stable and in accordance with standards affects the success of fish spawning, hatching, and rearing. The fertilization rate values obtained in this study both reached 100% with the hatching rate (HR) above 70%. The value of hatching rate is smaller than that of Setyaningrum and Nuryanto 2006 [5] which obtained at 82%. In addition, the use of hormones also has an important role [28]. Hormonal induction (sGnRHa + dopamine) not only triggered the female to ovulate but also improved the fertilization rate, hatching rate, and larval survival rate [29].

Kunlapapuk and Kulabtong 2011 [30], Rachmatika et al. 2005 [31], and Boyd 1982 [32] stated that water quality parameters are one of the important factors for eggs and larval rearing. Good water quality will result in a good hatching rate and survival rate. Water quality management is essential in larval production. The temperature in natural habitat for javaen barb is between 22-25°C [1]. This value is suitable for farming conditions, especially for broodstock management and breeding activities. Although the recorded temperature in egg incubation and larval rearing was higher (+27°C), the results of fertilization rate and hatching rate was still considerably good. The temperature range is still within a tolerance limit to support fish life in the tropical area, which is around 25-32°C [33].

4. Conclusion
Javaen barb from Tasikmalaya population produced significantly higher fecundity than Cianjur population. Meanwhile, other parameters related to reproductive and larval performance were not significantly different. Further study on seedling production should be carried out to improve broodstock management and larval rearing of this species.

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