BIODIVERSITAS
Volume 23, Number 3, March 2022
Pages: 1511-1519

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230340

Reproductive aspects of javaen barb fish, *Systomus orphoides* in the initial domestication program

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Manuscript received: 10 February 2020. Revision accepted: 24 February 2022.

Abstract. *Susatyo P, Lestari W, Sugiharto, Chasanah T. 2022. Reproductive aspects of javaen barb fish,* Systomus orphoides *in the initial domestication program. Biodiversitas 23: 1511-1519.* The population of javaen barb, *Systomus orphoides* (Valenciennes, 1842) in the Serayu River Central Java has declined, and domestication is one of the most critical conservation initiatives. This research aimed to evaluate the reproductive aspect of javaen barb, *Systomus orphoides* in an initial domestication program. Furthermore, fish samples were collected from the Serayu River and reared in traditional ponds. The bred broodstocks were injected with artificial hormones before spawning, and several reproductive aspects were measured and analyzed descriptively according to the literature. Fish specimens had gonadosomatic indexes ranging from 11±0.3% to 16.2±0.9%. The values represented all phases of oogenesis and spermatogenesis in females and males. The reproductive hormone titer consisted of 854.85-1058.06 pg/mL of 17ß estradiol, 0.29-0.72 ng/mL of progesterone, 10.87-15.68 mIU/mL of follicle-stimulating hormone, and 4.18-9.92 ng/mL of testosterone. These findings demonstrated that the Serayu River's wild javaen barb population reached all reproductive phases and that broodstocks can be domesticated in the traditional pond.

Keywords: Conservation, domestication, gametogenesis, hormone, *Systomus orphoides*

INTRODUCTION

Fish reproduction is essential for conserving species and sustainable fisheries (Soborido-Rey and Trippel 2013). There are several stages in the reproduction process of fish species, e.g., gametogenesis (Ahmad et al. 2018). In males, gametogenesis is known as spermatogenesis, while in females, it is known as oogenesis (Domagala et al. 2015; Pasha et al. 2016). This reproductive process consists of several phases (Krivokapic 2015). Spermatogenesis starts with spermatogonia and ends with sperm in mature stages, while oogenesis begins with the chromatin nuclear stage and ends with the ovum as the final product (Domagala et al. 2015).

Physical and chemical aspects are used to estimate oogenesis and spermatogenesis stages. Physical aspects include gonadosomatic index (GSI) (Hossain et al. 2011) and gonad histology, while chemical characteristics refer to reproductive hormone profiles. GSI is calculated by dividing the total weight of the gonads by the total weight of the body (Hossain et al. 2011; Islam et al. 2012). The histology of the gonads can be assessed through histochemical assay (Gholib et al. 2016).

Systomus orphoides (Valenciennes, 1842) often known as the javaen barb, is a cyprinid species that contribute to the variety of Indonesian fish (Hasan et al. 2015). This Cyprinid species is widely distributed in Asian tropical rivers, such as Chao Phraya, Mekong, Mae Khlong Valley, the Malay Peninsula, and Indonesia (Baumgartner et al. 2012; Nuryanto et al. 2012; Panprommin et al. 2019; Roesma et al. 2019; Dhin et al. 2020; Ut et al. 2020). Sundaland, Sumatera, Java, and Borneo in Indonesia are home to this species (Djumanto et al. 2013; Herawati et al. 2020). Several investigations found *S. orphoides* in the Serayu River in Central Java, Indonesia (Sustyo et al. 2016).

The javaen barb, *S. orphoides* is listed as having Not Evaluated conservation status (IUCN 2021). Nevertheless, conservation is still an important effort to protect this species because habitat alteration due to human activities might cause population decline. Conservation could be done outside the natural habitat through domestication. According to Fabrice and Pascall (2014), domestication that includes growing in traditional ponds is required to preserve certain fish species. This effort requires preliminary data, such as biological and reproductive characteristics. Reproductive characters of fish could be predicted using fish behavior but an inaccurate prediction. It is because some species showed reproductive plasticity and it is common that some species might be unable to complete the entire reproductive cycle (La et al. 2014).

Periodic hormonal induction stimulates the physiological processes of gonad development to final maturation and spawning synchronization during the spawning season (Selvaraj et al. 2012). However, environmental signals also played an essential role in the reproduction process by stimulating the pituitary gland to secrete stimulating follicle hormone (FSH) and luteinizing hormone (LH) on adult fish (Christensen et al. 2012; Chi-Hoon et al. 2017). Previous research carried out efforts to domesticate javaen barbs (Hadisusanto and Suryaningsih 2011; Dewi et al. 2014). Those studies were reported

reproductive characteristics of *S. orphoides* from nature and did not examine histological development of the gonad. Moreover, Setyaningrum and Nuryanto (2006) successfully implemented artificial breeding technology of javaen barbs through hormonal induction but only focused on hatching rate, growth rate, and survival of the fry. In this study, we reported more aspects of the reproductive characteristic of domesticated *S. orphoides*, including gonadosomatic index (GSI), relative proportions of gametogenesis stages, gonad histology, and reproductive hormone profile.

This research aimed to examined reproductive aspects of *S. orphoides* based on gonadosmatic index, relative proportion of gametogenesis, gonad histology, and hormonal profile during the domestication period in traditional ponds. The data is preliminary needs prior to domestication and conservation of javaen barb *S. orphoides*, especially in the Serayu River Central Java, Indonesia.

MATERIALS AND METHODS

Research location and sampling sites

Fish samples were collected from Serayu River, in Purbalingga and Banyumas Districts, Central Java Province, Indonesia. The three different locations were selected as sampling sites representing upstream (Congot Hamlet, Kedungbenda Village, Purbalingga District; -7.490617412930653, 109.33771820917866), middle stream (Kedung Uter Village, Banyumas District; (-7.516871762547014, 109.30307538385844), and the downstream region (Serayu Moveable Weir, Kebasen Village, Banyumas District; -7.52531237704913, 109.20088205441911) (Figure 1).

Fishing activities were conducted in Serayu River, which flows through Congot Hamlet, Kedungbenda Village, Purbalingga (upstream); and Kedung Uter Village, Banyumas (midstream). Serayu River flows into the southwest region of Cilacap after passing through Serayu Moveable Wieir, Kebasen Village. Furthermore, three fishing locations were selected to obtain a sufficient number of samples of javaen barb fish for the experiments.

Procedures

Fish rearing

All fish specimens collected in the Serayu River were reared in traditional ponds for acclimatization. This step was conducted for one month in two 10×10 m^2 ponds with inlets and outlets for adequate water supply. Additionally, 32 javaen barb broodstock pairs were successfully acclimatized and ready for reproductive aspects assessment. The broodstocks had body weights ranging from 250 g to 400 g per individual (Figure 2).

For the initial domestication program, all broodstocks were continuously reared in separated ponds with a 2.5 × 2.5 m^2/plot (Figure 3). This research performed a domestication program for a maximum of eight months. During these periods, the broodstocks were divided into four different domestication durations of 1, 2, 4, and 8 months, Pb1 (March to April), Pb2 (April to June), Pb3 (February to June), and Pb4 (February to October), respectively, with four replications. Two broodstock pairs were reared in each domestication pond, which means 32 broodstock pairs were utilized during this research. Different domestication times were performed to observe reproductive cycles from early gametogenesis to spawning, while hormonal profiles were observed at the end of the domestication program. A pair of broodstock in each plot was sacrificed to evaluate several reproductive parameters at the end of each domestication period. Others were kept alive until the eight-month domestication treatment was completed. Before spawning, all the remaining broodstocks were injected with gonadotropin-releasing hormone (GnRH) analog into mature male and female parents using ovaprim 0.5 cc/kg body weight (DiMaggio et al. 2013). Then the fish were fed with pellets *Alocasia* and Cassava leaves during domestication.

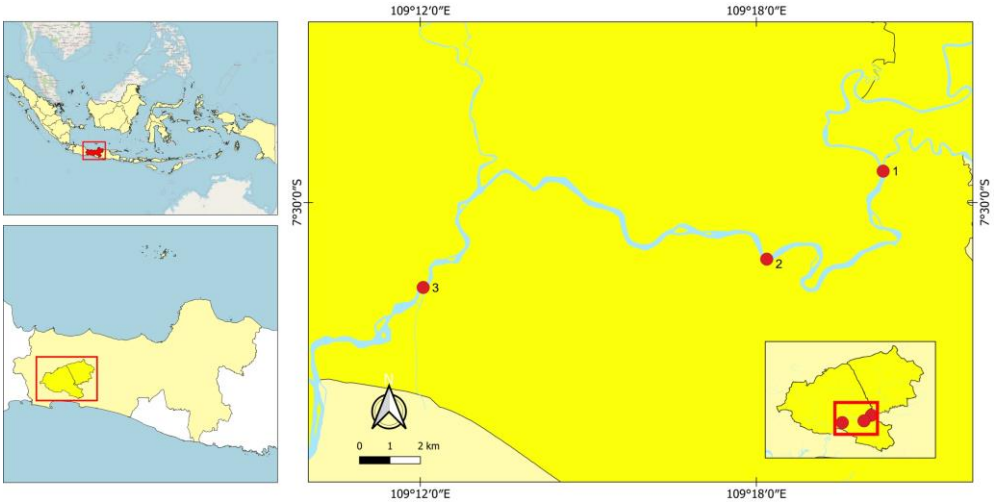


Figure 1. The three regions from which Javaen barb fish were collected are Serayu River, in Purbalingga and Banyumas District, Central Java Province, Indonesia. Note: 1. Congot Hamlet, Kedungbenda Village, upstream region; 2. Kedung Uter Village, midstream region; 3. Serayu Moveable Weir, Kebasen Village, downstream region

Figure 2. General morphology of javaen barb, *Systomus orphoides*

Figure 3. Experimental domestication ponds

Parameter

Several research parameters were measured to evaluate the reproductive aspects of javaen barb, *S. orphoides* in the initial domestication program. The parameters are gonadosomatic index (GSI), relative proportions of each gametogenesis stage, hormone profile, histology of the ovaries and testes.

Gonadosomatic index (GSI)

Fish body weight was measured before dissection, which started from the anus following ventral areas of the abdomen to the anterior part behind the operculum. Dissection continued from the anus to the dorsal region, following the body cavity. Then the scissors were turned out to the anterosuperior region to the dorsal position behind the operculum. The flesh was cut to expose the internal organs before harvesting the gonads. Meanwhile, GSI was calculated using the formula from Flores et al. (2015).

$$GSI = G/W$$

Where, GSI: Gonadosomatic index; G: gonad weight; W: Total body weight (includes gonad).

Relative proportions of spermatogenesis stages

The relative proportion of each spermatogenesis stage was computed as a ratio between each stage and gametogenesis. For example, the relative proportion of spermatogonium (SPG) was calculated.

$$SPG = [SPG / (SPG + PS + SS + SPT + SP)] \times 100\%$$

Where, SPG: Spermatogonium; PS: Primary spermatocyte; SS: Secondary spermatocyte; SPT: Spermatid; SP: Spermatozoa.

Relative proportions of oogenesis stages

The relative proportion of oogenesis stages was computed as the ratio between each phase and total oogenesis. For example, the relative proportion of chromatin nuclear stage (CNS) was calculated.

$$CNS = [CN S / (CNS + PS + CAS + VS + MS)] \times 100\%$$

Where, CNS: Chromatin nuclear stage; PS: Perinuclear stage; CAS: Cortical alveolar stage; VS: Vitellogenin stage; MS: Mature stage.

Ovarian and testicular histology preparations

Ovary and testes were removed surgically from the abdominal cavity and fixed in 4% formalin solution for 24 h at room temperature. Furthermore, the organs were dehydrated in graded 70% to absolute alcohol solutions, dealcoholized in xylol solution, infiltrated in xylol, and blocked in paraplast (Sigma p3558). Histological samples were paraffin-embedded and stained with Mayer's hematoxylin-eosin (James et al. 2020). Paraffined testes and ovaries were sliced transversely to observe spermatogenesis and oogenesis stages.

Specific tissue slices were attached to a 1% gelatin-coated object glass and stained with Mayer-haematoxylin-eosin, and the oocyte diameter was measured to identify the current phase (Edward et al. 2012). The oocytes were classified into five stages: the chromatin nucleolar stage (cns), perinucleolar stage (ps), cortical alveolar stage (cas), vitellogenic stage (vs), and mature stage (ms). In addition, spermatogenesis in teleost was divided into spermatogonia, primary, secondary, and spermatozoa stages (Papah et al. 2013).

Reproductive hormone profile

Four reproductive hormones of the domesticated javaen barb specimens were evaluated, namely estradiol, progesterone, and follicle-stimulating hormone (FSH) for females and testosterone for males. Reproductive hormones were measured from blood obtained from the posterior part of the linea lateralis. A free anticoagulant syringe was used to obtain a 0.5 mL - 2 mL blood volume. The blood was placed in an Eppendorf tube and kept at room temperature for 30 minutes until freezing. Then, it was cooled in the refrigerator for 8 hours to optimize blood clotting. The blood sample was then centrifuged for 15 minutes at 3000 rpm. Next, blood serum was transferred into a new Eppendorf tube (1.5 mL) and stored in a refrigerator (8°C - 10°C) to measure hormone levels.

Calibration was conducted according to the procedure specified by the Kit Instructions. Then, hormone levels were measured using the immunoassay (EIA) method (Gholib et al. 2016), with the kit's catalog EIA-estradiol kit (for estradiol), EIA-progesterone kit (for progesterone), and EIA-testosterone kit (for testosterone). The assay was

performed using a Microplate Reader-LB-6200 Labotron machine (Labotron, Haryana, India).

Data analysis

Quantitative data such as GSI, the relative proportion of gametogenesis stages, and hormone profile were analyzed statistically using variance analysis (ANOVA) in SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). A significant difference was defined based on a 5% confidence level. In addition, histological data were analyzed descriptively by comparing them to previously published references.

RESULTS AND DISCUSSION

Gonadosomatic index (GSI)

The acclimatization stage was successfully conducted, proving that all broodstocks survived. The success was also shown by gonad development, as demonstrated in the increasing GSI. GSI ranged from 11±0.3% after one month of domestication to 16.2±0.9% after eight months. The AMOVA showed significantly different GSI values among domestication periods. However, the Duncan test proved that a significant difference was only observed between P_1b and others (P_2b, P_4b, and P_8b) as indicated by overlapping standard deviation values among these domestication periods (Figure 4).

GSI is the ratio between gonad weight and total body weight (Flores et al. 2015; dos Santos et al. 2020). Individual and gonad development phases will enhance GSI (Islam et al. 2012; Dopeikar et al. 2015; Aberkane et al. 2018) to reach the average values ranging from 10% to 25% of body weight (Effendie 2002). According to Ridho's statement (2021), this situation will continue until spawning is conducted. In line with the maturity level, the gonads will increase in weight and size and reach their maximum size when the fish are ready to spawn. As a result, a significant difference was observed in GSI between domestication periods. The gonad proliferated and converted spermatogonia to spermatozoa in the final stages. A similar phenomenon was also observed in the female individual where oogonia were converted to ovum (Hossain et al. 2011). This research showed that grew fast from one-month domestication (Pb1) to two months (P_2b). After that, the gonad grew slowly, as indicated by gonad development from Pb2 to Pb8 (Figure 4). During these domestication periods (Pb2 to Pb8), the gonad underwent maturation rather than growth. Therefore, the GSIs were not significantly different among the three domestications periods of P_2b, P_4b, and P_8b. Data from spermatogenesis and oogenesis during gonadal development supported the phenomenon. The number of spermatozoa increased significantly from one month to two months of domestication. Similarly, such phenomena were not observed among the other domestication periods, i.e., P_2b, P_4b, and P_8b (Figure 5). Similar phenomena have been observed during oogenesis (Figure 6). GSI had risen due to a high number of spermatozoa and ovum. Spermatozoa and ovum cells were more abundances in the late stages of gametogenesis than other cells in the early stages. The gonad histology showed that spermatozoa and ovum are more prominent in late spermatogenesis and oogenesis, respectively (Figures 8 and Table 1). Similar phenomena were also reported in other fish species (Zeyl et al. 2014; Domagala et al. 2015; Pasha et al. 2016; Ahmad et al. 2018).

Relative proportion of spermatogenesis stages

Five stages of spermatogenesis were observed during the domestication of *S. orphoides*. The stages were spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoa. The relative proportion of each stage was different among domestication periods. However, the dominant stage in all domestication periods was the same, i.e., the spermatozoa stage or mature stage (Figure 5). The condition occurred because the fish was in the early reproduction phase in a one-month domestication period. The Gonad is still in the maturity process at this stage, as indicated by a lower number of spermatozoa. Then, the Gonad continued to develop until Gonad reached maximum maturity at the end of the four-month (P_4b) domestication period (June). The highest spermatozoa demonstrated this during the P_4b domestication period (Figure 5). The condition of the gonads was very good because June is the middle of the rainy season, and most tropical Cyprinids spawn at this time (Muchlisin 2014; Mujtahidah et al. 2019). The Gonad shrinks after eight months of domestication. It was indicated by a lower spermatozoa proportion than the P_4b domestication period (Figure 5). The spawning process, in which spermatozoa are released from the Gonad, causes this condition. It had been previously reported by Christensen et al. (2012) and Chi-Hoon et al. (2017) that the GSI was significantly lower during the spawning season because the sperms were released.

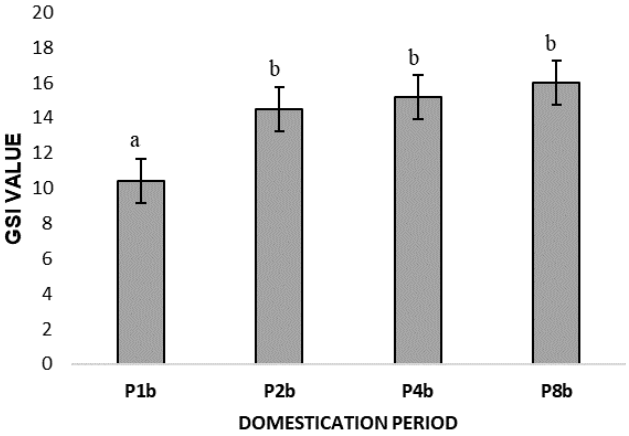


Figure 4. Gonadosomatic index of javaen barb on domestication period. Note: similar letter indicates no significant differences among domestication periods

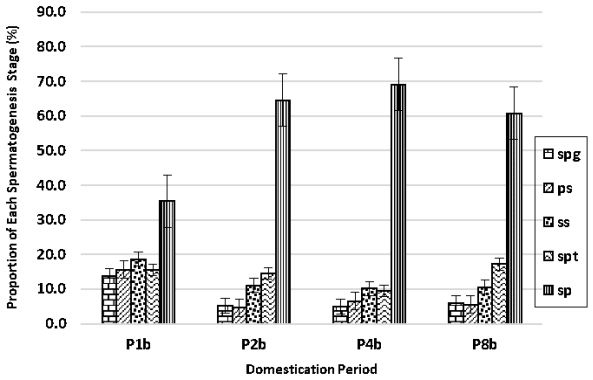


Figure 5. Relative proportions of spermatogenesis stages in javaen barb fish during each domestication period. Note: spg: spermatogonium; ps: primary spermatocyte; ss: secondary spermatocyte; spt: spermatid; sp: spermatozoa

Relative proportion of oogenesis stages

Ovarium development of javaen barb (*S. orphoides*) during reproduction cycles consisted of five stages. These stages include chromatin nuclear stage (CNS), perinuclear stage (PS), cortical alveolar stage (CAS), vitellogenic stage (VS), mature stage (MS). There was a significant alteration in the proportion of each oogenesis stage during domestication periods. The significant difference between treatments was more pronounced on CNS and MS stages. However, these two stages demonstrated contradictory trends. The CNS stage was maximum in the early domestication period and lowered in the next.

The observed phenomena were similar to previous research on other cyprinid species (Choi et al. 2014; Hamzaoglu et al. 2015; Krivokapic 2015; Kim et al. 2017). In addition, those reported that all oogenesis stages were observed over the year. However, the dominant cells changed following each step. In contrast, the mature stage starts with a minimum proportion in the early domestication period and becomes maximum at the end (Figure 6).

The difference between the present research and Hamzaoglu et al. (2015) was also observed. The present research found that oocytes were dominant in September (Figure 6), while Hamzaoglu et al. (2015) reported that oocytes of *Alburnus istanbulensis* (Cyprinidae) were dominant in mid-June. The differences were reasonable because the present research used *S. orphoides*, while Hamzaoglu et al. (2015) used *A. istanbulensis*. Two species have distinct characteristics, including reproductive traits. To cope with the environment, species living in different climates use reproductive strategies, specifically photoperiod (Giannecchini et al. 2012).

Histology of testes

Javaen barb fish have one pair of elongated testes (single or double-pronged) attached to the hindgut through the lining of the dorsal mesentery. The testes are transparent or translucent and enveloped by fat (Figure 7). This was similar to testes morphology in other cyprinid fish species, such as *Puntius sarana sarana* (Ahmad et al. 2018) and *Squalius platyceps* (Krivokapic 2015). Therefore, the observed testes in *S. orphoides* showed a typical morphology in Cyprinidae. Moreover, similar testes morphology was reported in other fish species, such as in *Pomadasys stridens* (Amtyaz et al. 2013) and *Prochilodus brevis* (Gurgel et al. 2012).

This research documented the histological features of the javaen barb during the ripening (maturing) period. Three cell types were observed during the ripening period, i.e., secondary spermatogonia, spermatogonia, and spermatozoa (Figure 8). They were also reported in the ripening phase on spermatogenesis of *Puntius sarana sarana* (Ahmad et al. 2018); Hamzaoglu et al. (2015) used *A. istanbulensis* and *Squalius platyceps* (Krivokapic 2015).

A histological examination of bony fish testes revealed that these organs are made up of germinal and interstitial compartments separated by a basement membrane, thus, the cells in the two compartments do not mix (Uribe et al. 2014). The germinal compartment comprises the germinal epithelium, containing germ and somatic epithelial cells (the germinal ridge). Sertoli cells are supported by a layer (the basement membrane) that separates the epithelium from the subjacent interstitium (Martins et al. 2010). The germinal cells are between or surrounded by Sertoli cells but are not in contact with the basement membrane (Fishelson et al. 2013; Uribe et al. 2014). Germ cells include spermatogenic cells in all stages of differentiation, and the mitotic proliferation of spermatogonia initiated spermatogenesis. This was followed by two rounds of meiosis and ended with spermiogenesis, which produced haploid spermatids transforming into spermatozoa.

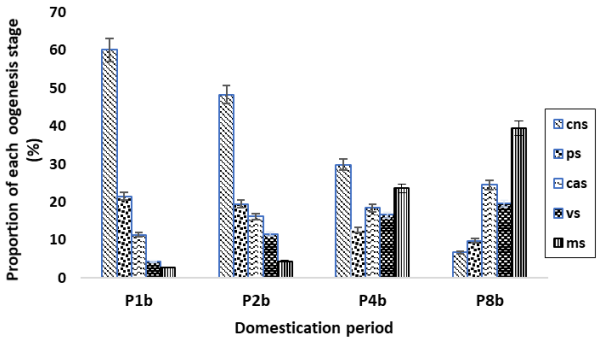


Figure 6. Proportions of oocytes by developmental stage and domestication period. Note: cns: chromatin nuclear stage; ps: perinuclear stage; cas: cortical alveolar stage; vs: vitellogenic stage; ms: mature stage

Figure 7. Testis morphology of javaen barb, *Systomus orphoides*

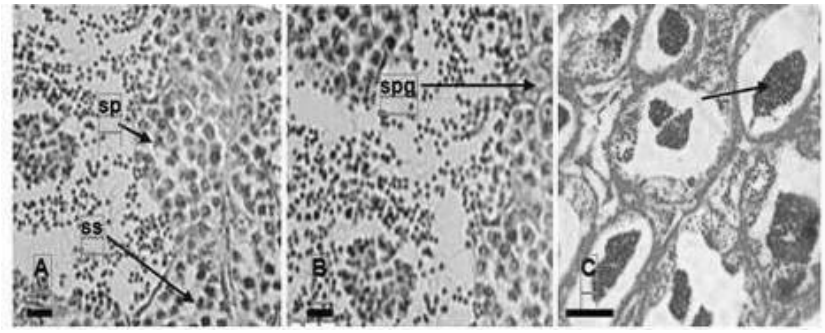


Figure 8. Photomicrograph of Javaen barb testes during ripening. Hematoxylin-eosin staining. Note: A. ss: secondary spermatocyte (scale bar 100 μm); B. spg: spermatogonia (scale bar: 100 μm), C. sp: spermatozoa in the lobular lumen (scale bar: 30 μm)

Spermatogenesis in bony fishes has both similarities and apparent differences in other vertebrates. It proceeds within a cystic structure, in which all germ cells develop as clones surrounded by Sertoli cells (Uribe et al. 2014). The testes are composed of an anastomosed lobule containing the main sperm duct/ductus spermatic with two visible zones of cross-sectional appearance. Seminiferous lobules dominate the outer region of the testes, and the lobules contain epithelial cells and spermatogenic germinal. Furthermore, the inner part of the lumen is composed of lobules filled with spermatozoa (Parenti et al. 2010) (Figure 8C).

The testes cells were in line with the maturity sequence of a screening test for active endocrine substances in fish (Vazquez et al. 2012), where (1) spermatogonia are characterized by vesicular nuclei with a nuclear membrane and nucleoli; (2) primary spermatocytes are dominant over secondary spermatocytes; (3) spermatids are the smallest cells, containing a solid nucleus and an acidophilic, ring-shaped area in the cytoplasm; and (4) spermatozoa appear as mature flagellated cells with a round nucleus and dark color. Groups of spermatogenic cells are scattered randomly in the testes (Vergilio et al. 2012). In addition, several groups of cells of the similar spermatogenic stage were found within the lobular lumen of the fish with Sertoli cells on the walls of lobules and other germinal cells (Uribe et al. 2014) (Figure 8C).

Histology of ovarium

The ovaries of *S. orphoides* are fused in the anterior region, and it has a white to yellowish-orange color depending on the development stage (Figure 9). The observed ovarium morphology was similar to the characteristics of ovarium of Cyprinidae as reported in *Puntius sarana sarana* (Ahmad et al. 2018), *Schizothorax plagiostomus* (Pasha et al. 2016), and *Squalius platyceps* (Krivokapic 2015). Furthermore, similar ovarium morphology was observed in *Pomadasys stridens* (Amtyaz et al. 2013) and *Prochilodus brevis* (Gurgel et al. 2012). Therefore, the observed ovarium morphology of *S. orphoides* described the general morphology of ovarium in fish.

This research examined the histological features of female gonads of *S. orphoides* for all ovarium development stages following the five steps of oogenesis. The stages were chromatin nuclear, perinuclear, cortical alveolar formation, vitellogenin, and mature (Table 1). The characteristics of each stage are described in Table 1.

Histological observations showed that the oogenesis stages of *S. orphoides* were similar to other cyprinid species (Montchowui et al. 2012; Dopeikar et al. 2015; Hamzaoglu et al. 2015; Jyrwa and Bhuyan 2017). Previous research stated that oogenesis consists of chromatin nuclear stage, perinuclear, cortical alveolar formation, vitellogenesis, and mature stages**.** These researches also noted that the first stage was the nucleolar/perinucleolar stage. In contrast, cortical alveoli and yolk characterized the second stage, with the late globular and mature stages. Similar oogenesis stages were observed in other fish groups, such as in *Monopterus albus* (Susatyo et al. 2018) and *Pomadasys stridens* (Amtyaz et al. 2013).

Reproductive hormone profile

The reproductive hormone profile of javaen barb, *S. orphoides* was checked at the end of 1, 2, 4, and 8 months. The titer of each reproductive hormone is presented in Table 2.

The highest concentration of Est was observed at the end of P_4b, and the phenomenon was logical since Est stimulates vitellogenin synthesis in the liver. It is then transported to the gonads through the bloodstream and internalized by oocytes during vitellogenesis (Marinela et al. 2011). Vitellogenesis increases the oocyte volume (Christensen et al. 2012; Edward et al. 2012). Although no vitellogenin content was measured in this research, Est activities were expressed by an increase in vitellogenic stages, as shown in Figure 6.

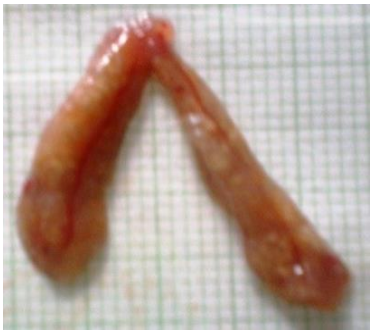


Figure 9. Ovarium morphology of javaen barb, *Systomus orphoides*

Table 1. Histological structure of oocytes from female Javaen barb gonads in each developmental stage revealed using a paraffin method and Meyer's hematoxylin-eosin staining

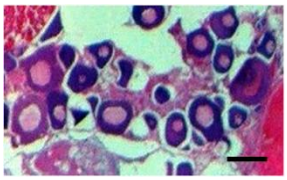
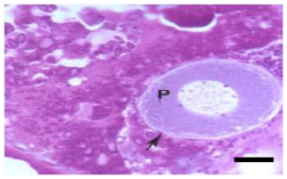
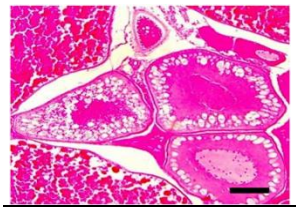
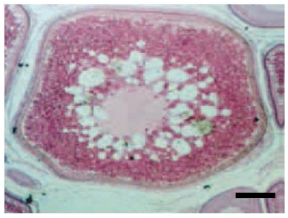
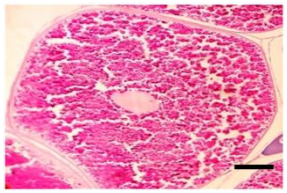
Stage of oocyte development	Oocyte structure
	**Chromatin nuclear stage** Oocytes appear as small spherical cells with a central nucleus containing one to four nucleoli and having a chromatin webbing. The cytoplasm is a thin, strongly basophilic layer, as revealed by alkaline staining. Follicular cells are difficult to observe. Bar: 30 µm
	**Perinuclear stage** The number of nucleoli increases and they are arranged along the inner side of the nuclear membrane. The nucleus is large and surrounded by an increased amount of cytoplasm that is weakly basophilic. Follicular cells are arranged as a single layer of flat cells enclosing the oocyte. Bar: 30 µm
	**Cortical alveoli stage** The cytoplasm is filled with bright vesicles (cortical alveoli), which begin to accumulate in the periphery of the oocyte. The nucleus remains perinuclear. The nuclear membrane begins to appear "tangled". Acidophilus is present and the zona radiata is visible at the edge of the nucleus. The follicular layer is visible at the edge of the oocyte. Bar: 30 µm
	**Vitellogenic stage** The oocyte size increases. Small yolk granules appear as an acidophilic, ring-shaped area in the cytoplasm. The nuclear membrane is still tangled. The radiata zone appears as a bright, non-cellular band that is highly acidophilic. Well-developed follicular (columnar or cuboidal) layers are covered by a thecal layer of flat cells. Bar: 30 µm
	**Mature stage** Enlarged cortical alveoli and yolk granules are seen. The oocyte size increases. Migration of the nucleus to the periphery or edge of the oocyte occurs. The zona radiata layer appears bright. The cells and follicles are surrounded/enclosed by a thin thecal layer. Bar: 30 µm

Table 2. The average titer of each reproductive hormone at the end of the 1-, 2-, 4-, and 8-month domestication periods

Sex	Hormone	Mean ± standard deviation hormone levels			
		1 month (P_1b)	2 months (P_2b)	4 months (P_4b)	8 months (P_8b)
Female	Est (pg/mL)	1293.59 ± 45.44	1021.63 ± 27.56	1541.08 ± 72.52	1493.27 ± 183.09
	Prog (ng/mL)	0.31 ± 0.02	0.51 ± 0.03	0.47 ± 0.19	0.84 ± 0.03
	FSH (mIU/mL)	10.75 ± 0.10	13.64 ± 0.62	13.82 ± 1.08	14.09 ± 1.45
Male	Test (ng/mL)	5.07 ± 0.61	7.50 ± 0.70	9.10 ± 0.17	8.95 ± 0.87

Table 2 showed that testosterone and FSH levels gradually increased during the domestication periods from P_1b, P_2b, P_{4b}, to P_8b. The phenomena were common because javaen barbs (*S. orphoides*) are a cyprinid species. In addition, previous research reported that fish of the Cyprinidae family could spawn in the year. Therefore, it is reasonable that Testosterone and FSH concentration will increase with the development of the gonads until the mature phase (Zhiwei et al. 2015).

Based on gonadosomatic index, relative proportions of gametogenesis stages, and gonad histology, male and female individuals of *S. orphoides* domesticated in traditional ponds showed normal gonadal development and passed all gametogenesis stages. The reproductive hormonal was also showed a typical profile during the domestication periods. These results were vital preliminary data for *S. orphoides* cultivation which is essential for its ex-situ conservation efforts.

ACKNOWLEDGEMENTS

The authors are grateful to the Indonesian Directorate of Research and Society Services for a research grant provided under the Basic Research Incentive Program; the Faculty of Biology, Jenderal Soedirman University, Purwokerto, Indonesia for technical support; and Nabila Gina Irwanti for her assistance with the statistical analysis.

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