The reproductive biology of Waanders's hard-lipped barb, *Osteochilus waandersii* in the Landak River, Indonesia

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Abstract. This study refers to the general reproductive biology of *Osteochillus waandersii*, and provides information about the sex ratio, gonad development stages, gonado-somatic index (GSI), length at maturity, fecundity, and growth pattern. The fish samples were collected monthly, between March and August 2018, from the Landak River, Kalimantan Province, Indonesia. A total of 234 specimens were collected using gillnets and dipnets, 136 males and 98 females, resulting in a 1.39:1 sex ratio, which is significantly different from the expected ratio of 1:1 ($P<0.05$). Males reached sexual maturity at a lower size than females, with a length at first maturity estimated at 137.9 mm for males and 140.3 mm for females. Analysis of the macroscopic and histological observations showed that testicles could be classified into four stages: immature, maturing, mature and spent. The GSI ranged between 1% and 9.54% and 1% and 11.67% for males and females, respectively. The ovaries were classified into five stages: immature or resting, maturing, mature, ripe, and spawned-recovering. The absolute fecundity varied from a minimum of 2130 to a maximum of 13640 eggs and a mean of 7165 eggs, corresponding to fish with total length between 145 to 177 mm and a weight between 30 to 67 g.
Key Words: fecundity, gonadosomatic index, length maturity, sex ratio.

Introduction. *Osteochilus* is a genus that belongs to the Cyprinidae family. This genus consists of 32 species. All of these species can be found in Sumatra, Java, Borneo and even in China. Sixteen species of this genus can be found in Western Indonesia and Sulawesi. Amongst the species found in Indonesia, one is *Osteochilus waandersii*, also known as the "bantak fish". *O. waandersii* is characterized by the presence of one or three hard tubules on the snout and a colored line starting from the operculum to the base of the caudal fin. The fish presents 27-35 filter rackers on the first gill arch, the caudal peduncle is surrounded by 16 rows of scales, and there are 10-13½ branched rays in the dorsal fin. The mouth type is subinferior (Kottelat et al 1993). These fish are found in aquatic habitats with strong currents and clear water. Bantak is used as human food, but it is also an ornamental fish. Even though its exploitation rate is high, the reproductive biological information is still not widely known.

Reproductive biology studies, among others, the reproductive system and sexual organs. Reproductive success is what allows a species to survive, being very important for some populations (Lowerre-Barbieri et al 2011). Some studies have described the reproductive biology of some species of the Cyprinidae family, like *Rasbora rubrodorsalis* (Morioka et al 2014), *Garra regressus* (Geremew et al 2015), *Hampala bimaculata* (Soetignya et al 2016), etc. The results of these studies have described some of the specific characteristics of each species, such as the sex ratio, gonadosomatic index, the size of the first maturity and fecundity.

Biological aspects, especially the reproductive biology, are one of the important issues for fisheries biology. This issue is very critical for predicting the population stability and its fluctuations and it is used in fisheries management in establishing prohibitions on fishing areas or fishing seasons (Njiru et al 2006). Thus, the aim of this study is to describe and determine some aspects of the reproductive biology of *O. waandersii*, from Landak River, West Kalimantan. Some of the reproductive biology aspects analyzed in this study are the sex ratio, gonad development, gonadosomatic index, the size of the fish at first maturity and fecundity.

Material and Method

Sample collection. Fish samples were collected from the upstream part of the Landak River, Landak Regency, West Kalimantan Province (00°45'4.14"S-110°06'34.3"E and 01°18'23"S-110°06'35"E). The frequency of sampling was six times, once a month, from March to August 2018. Samples were collected by using gillnets and cast nets operated at the sampling locations, which were determined by the purposive sampling method. Fish samples were measured, and the total length and standard length were recorded. The total body weight was measured with an electric scale (0.1 g precision). Each specimen was dissected laterally, and the sex was determined macroscopically according to methods presented by Nunez & Duponchelle (2009) and Soetignya et al (2017). The weighed gonads were measured and preserved using Gilson's solution for further observation.

Sex ratio. The sex ratio is expressed by comparing the number of male to female fish. Sex ratios were analyzed with the Chi-square test, for verifying if the proportion of males and females differed from the expected ratio of 1:1 (Lanes et al 2012; Soetignya et al 2016).

The gonadal maturation stages. The gonadal maturation stages were described and classified using macroscopic and microscopic characteristics developed by Nunez & Duponchelle (2009) and Soetignya et al (2017). The gonadal maturation stage was determined by macroscopic criteria, like color and shape of the gonads, transparency, degree of occupation of the coelomic cavity and the degree of visualization of the gonads (de Souza et al 2011). For the microscopic analysis, tissue samples were collected from the anterior, middle and posterior parts of the gonads. The histological procedures in this study followed the method of Brewer et al (2007). The fish gonads were fixed in 10% buffer neutral formalin solution for 48 h before being processed for histology. Each piece of tissue was embedded in paraffin, sectioned (5-7 μm) and subsequently stained with haematoxylin and eosin for the gonad maturation histological determinations.

The gonadosomatic index (GSI). The GSI was determined by reporting the gonad weight to the body weight.

$GSI = 100 \times W_G \times W^{-1}$

Where: WG - gonad weight; W - total body weight of fish (Albieri et al 2010; Ramos et al 2016; Soetignya et al 2017).

The length of the fish at first maturity is considered the length at which 50% of the fish have reached maturity. In order to estimate the size of the fish at first maturity, the total length was plotted on 50% of the mature individual frequencies during the spawning season (Shalloof & Salama 2008). The mature ovaries were immersed in Gilson's solution to estimate the fecundity of the fish. For consistency, oocytes were taken from the anterior, middle and posterior parts of the right lobe of the each ovary (Ma et al 2012).

Fecundity. The absolute fecundity was determined by multiplying the number of ripe eggs in the sample by the ratio of the ovary weight to the sample weight (Shalloof & Salama 2008; Solomon et al 2011).

Fa = (ovary weight x number of eggs in the sub-sample)/sub-sample weight

The relative fecundity (Fr) was expressed by dividing the absolute fecundity (Fa) with the fish body weight. The result was the number of eggs per gram of body weight (Langroudi & Sabet 2018.

Fr = Fa/Fish body weight

Results and Discussion

Sex ratio of O. waandersii. The total number of males was greater than the number of females with a sex ratio (male:female) of 1.39:1. However, the difference in the sex ratio was significantly different from the expected sex ratio of 1:1 (P<0.05) (Table 1).

Table 1

The sex ratio of *Osteochilus waandersii* in the study period

Months	*Number of fish observed*		*Sex ratio*	*P value*
	Male	*Female*		
March	18	16	1.3:1	0.73
April	22	17	1.29:1	0.42
May	26	13	2:1	0.04*
June	25	12	2.08:1	0.03*
July	20	24	1:1.2	0.55
August	21	14	1.5:1	0.24
Total	136	98	1.39:1	0.013*

Note: * - significant difference (95% confidence level).

Gonad maturity stage of O. waandersii. Based on macroscopic observations, the maturity of the testes for bantak can be divided into four stages: immature or resting period, maturing (almost mature), mature, and spent (Table 2; Figure 1).

Table 2

Testicular maturity stages, corresponding morphological and histological features of *Osteochilus waandersii*

Stages	*Classification*	*Macroscopic characters*	*Histological features*
1	Immature or resting	These are narrow, occupy around 5% of the abdominal cavity, are string-like in shape, long, translucent, whitish.	They present small seminal lobules with the presence of spermatogonies.
2	Maturing	They occupy 10-20% of the abdominal cavity, whitish in color, and extend until the end of the swim bladder (Figure 1A).	The seminal lobules contain mainly spermatozoids, as well as spermatides (Figure 1D).
3	Mature	They are more turgid, occupy around 40% of the abdominal cavity, white in color. It was possible to observe semen when the testicle was pressed (Figure 1B).	The seminal lobules are full of spermatozoids (Figure 1E).
4	Partially spent or Spent	The testis occupy on average 15-25% of the abdominal cavity, are hemorrhagic and flaccid and present white to reddish coloring (Figure 1C).	They are characterized by the presence of empty and non-uniform seminal lobules, but still have some seminal lobules with a few spermatozoids (Figure 1F).

Note: source - modified after Nunez & Duponchelle (2009).

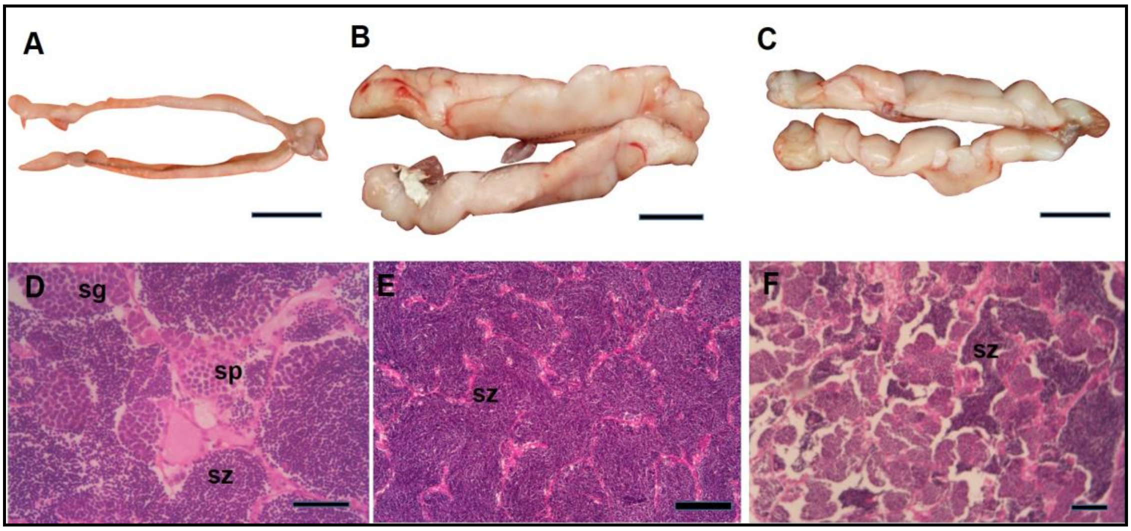

Figure 1. Morphological (A-C) and histological (D-F) appearance of the testicles of *Osteochilus waandersii* with 100X objective. A - maturing stage, scale bar=10 mm; B - mature stage, scale bar=10 mm; C - spent stage, scale bar=10 mm; D - maturing stage (sg - spermatogonia, sp - spermatid, sz - spermatozoa); E - mature stage, full spermatozoids (sz) in the seminifer tubulus, scale bar = 50 μm; F - partially spent stage, lobulus with spermatozoids and empty seminal lobulus, scale bar = 50 μm.

Ovarian maturity stages. Macroscopic observations of the ovaries showed that the fish have a homogeneous pair of gonads with the main blood vessels clearly visible in the internal section. The observations also indicate that ovarian development can be classified into 5 stages: immature or resting, maturing, mature, ripe, and spawned-recovering (modification of Nunez & Duponchelle 2009; Soetignya et al 2017) (Table 3). The ovaries were bi-lobed, elongated, and joined at the posterior part to form a short gonoduct leading to the urogenital pore. The maturity stage of ovaries based on the macroscopic and histological analysis are classified in five stages: immature, maturing, mature, ripe and spawned and recovering (Figure 2; Table 3; Figure 3).

Figure 2. Morphological profiles of ovaries of *Osteochilus waandersii*. A - maturing stage; B - mature stage; C - ripe stage; D - spawned. Scale bar of A and B - 10 mm; scale bar of C and D - 5 mm.

Table 3

Ovarian maturity stages and corresponding morphological and histological features of *Osteochilus waandersii*

Stages	*Classification*	*Macroscopic characters of ovarium maturity*	*Histological features*
1	Immature or resting	The ovaries were small, no oocytes were visually noticeable, occupy around 5% of the abdominal cavity, thin, partly transparent, reddish, sometimes whitish.	All the oocytes in the ovary were in the primary growth stage (chromatin and perinucleolar phase) and well organized (Figure 3A).
2	Maturing	These occupy between 15% and 20% of the abdominal cavity, are opaque, with color varying from orange to brown. Many small and average-sized oocytes were observed (Figure 2A).	They have oocytes at different phases of development. They have a weak basophilic cytoplasm with small lipid droplets. Yolk vesicles appeared gradually maturing (Figure 3B).
3	Mature	The ovaries are larger and more voluminous, occupying around 50% of the abdominal cavity and extending into the anterior part. The ovaries appeared opaque, with coloring ranging from orange to greyish. Oocytes can be seen clearly through the ovary wall (Figure 2B).	Three types of oocytes can be seen: the primary oocytes, the tertiary stage oocytes and oocytes entering FOM (Final oocytes maturation) (Figure 3C).
4	Ripe	Fully distended with granular surface occupying 70-80% of the abdominal cavity; greyish, opaque, sack-shaped (Figure 2C).	This stage starts with the nucleus migration phase followed by the oocyte hydration (Figure 3D).
5	Spawned and recovering	Ovary was not fully empty, with residual oocytes present. Flaccid and greyish to brown in color. The ovary is occupying less than 50% of the abdominal cavity (Figure 2D).	The ovaries are still relatively large and flaccid with remaining empty spaces, atresia, post-ovulatory follicles (POF), and new batches of developing vitellogenic oocytes.

Note: sources: Nunez & Duponchelle (2009) - modified; Soetignya et al (2007).

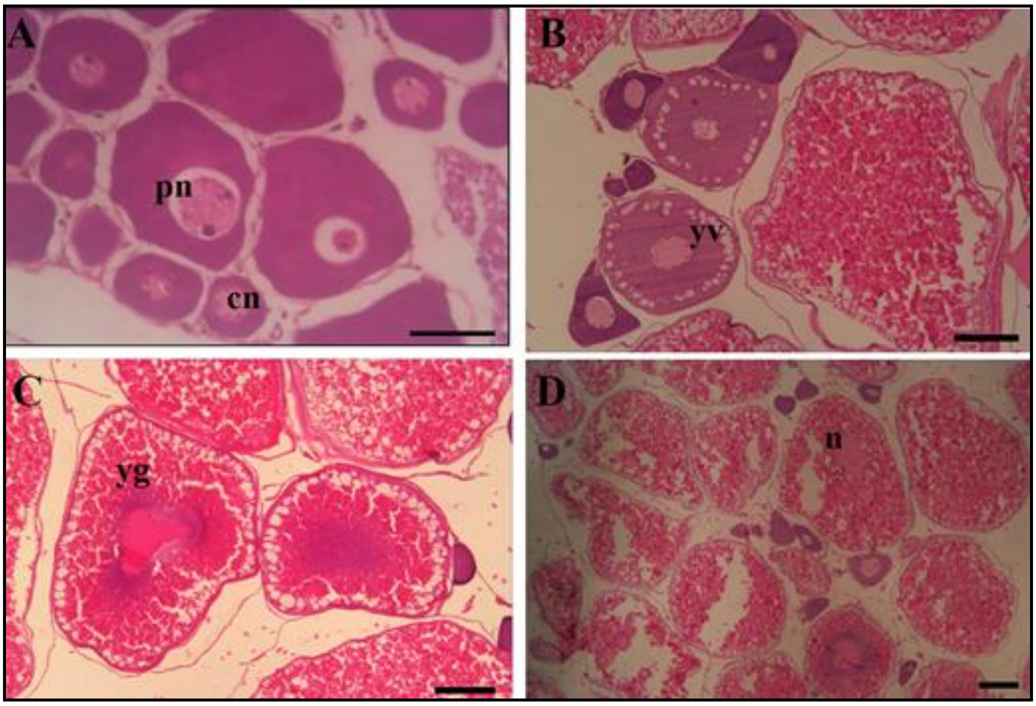

Figure 3. Histological ovary sections of *Osteochilus waandersii* during the gonadal development. A - immature stage, containing germ cells, nucleolar (cn), and perinucleolar chromatin (pn), scale bar = 50 μm, 400X objective. B - maturing stage containing yolk vesicles, scale bar = 100 μm, 100X objective. C - mature stage, ovary containing yolk granules and lipid vitellogenesis oocytes, scale bar = 100 μm, 100X objective. D - ripe stage ovary containing lipid and protein vitellogenesis; nucleus (n) migrates to the periphery of the ooplasm, scale bar = 100 μm, objective 400X.

Gonadosomatic index (GSI). GSI was often used as a substitute for gonad maturity stages to estimate the reproductive season. In this study, the GSI of males ranged from 1 to 9.54, while the female GSI ranged from 1 to 11.67. Overall, the GSI of males was smaller than the GSI of females. The highest GSI values were found in fish at the spawning stage or near spawning stage (stage III and IV for females and stage III for males). The mean size of the GSI presented monthly variation during the study period. Substantially, the results also revealed that July is a month with a minimum GSI for both males and females. The peak of spawning was suspected to occur from March to May, where the GSI has a higher value than in the following months (Figure 4).

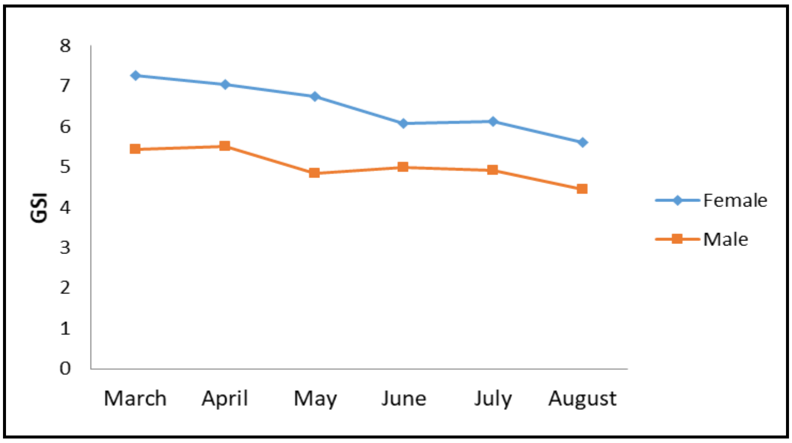

Figure 4. Monthly variation of the gonadosomatic index (GSI) in *Osteochilus waandersii* from Landak River, Indonesia.

Length at first maturity. The sexual maturity logistic curve for *O. waandersii* is presented in Figure 5. Estimated L_{m50} values for sexually mature individuals considering both males and females were 137.9 and 140.3 mm, respectively. The proportion of mature males was 56.62% (77 of 136 individuals), and the proportion of mature females was 40.82% (40 of 98 individuals during the study period).

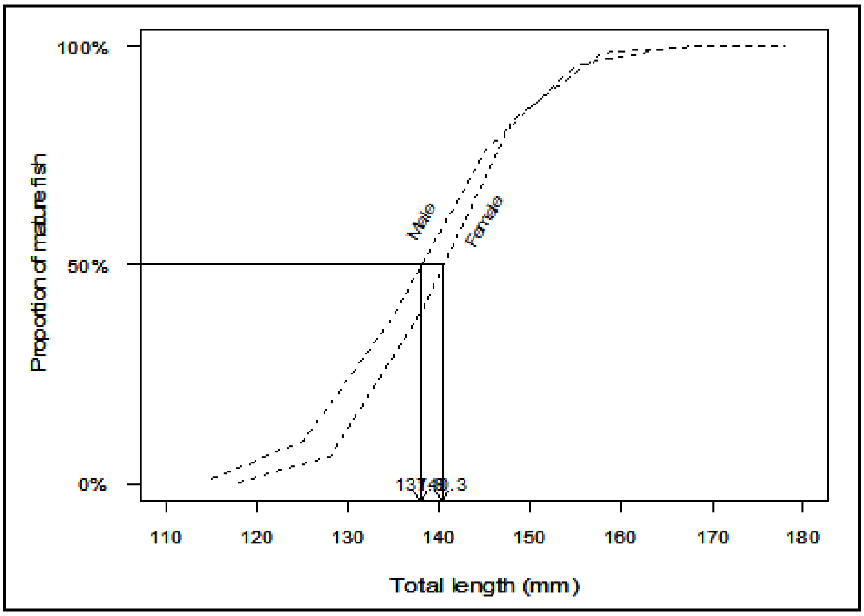

Figure 5. Relationship between the proportion of maturity and the mean total length of *Osteochilus wandersii* females during the sampling periods.

Fecundity. The absolute fecundity varied from a minimum of 2130 to a maximum of 13640 eggs, with a mean of 7165 eggs, corresponding to fish of a total length ranging from 145 to 177 mm and a total weight from 30 to 67 g. Relative fecundity, expressed as eggs g^{-1} of body weight of the females, spanned from a minimum of 67.67 eggs g^{-1} to a maximum of 206.27 eggs g^{-1}. The relationships between absolute fecundity and the parameters under study showed that absolute fecundity is significantly correlated to the total length and total body weight (Figure 6).

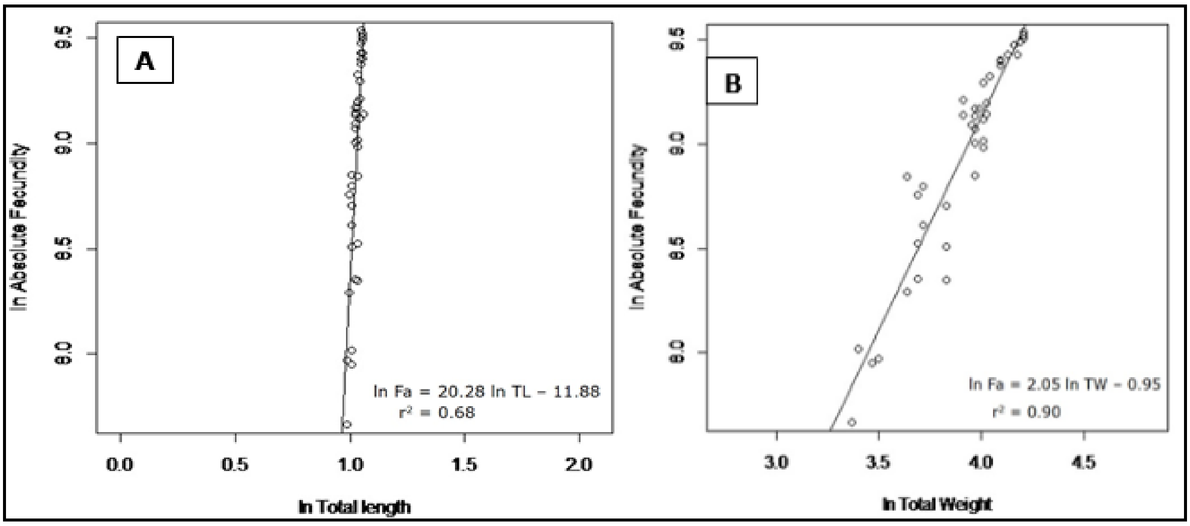

Figure 6. Linear regression function of *Osteochilus waandersii* for absolute fecundity - total length (A) (ln Fa = 20.28 ln TL – 11.88; r^2 = 0.68; P<0.0001); absolute fecundity - total weight (B) (ln Fa = 2.05 ln TW – 0.95; r^2 = 0.90; P<0.0001). TL - total length; TW - total weight.

In this study, the proportion of the total number of males was higher than that of females in the monthly catch data, except for July. However, the difference in the sex ratio between males and females was significantly higher from the expected sex ratio of 1:1, this being consistent with what occurs for the majority of species where the sex ratio is close to 1. This sex ratio showed that the number of males and females is in a balanced state and it is possible that it relates to the needs of a partner, where one male is matched with one female. This condition is also ideal for maintaining the preservation in nature. The sex ratio was considered to vary from species to species, and when the 1:1 sex ratio tends not to occur, some factors will modify the balance. The sex ratio was influenced by some factors including differences in age, growth and sampling methodology (Liao & Chang 2011; Ma et al 2012). The sex ratio may also be influenced by differences in catching factors related to the season and schooling movements in nursery and spawning areas, as well as to the natural mortality that occurs between the sexes (Zhu et al 2008) and food availability factors.

The size at first maturation is a key parameter to identify changes in the life history traits for fishery management. Size at first maturation and mortality directly influence the reproductive potential of a fish population. Environmental factors greatly influence the sexual maturity of fish (Ma et al 2012). According to our findings, males and females attained the mean length of 137.9 and 140.3 mm TL at first sexual maturity, respectively. This indicates that *O. waandersii* is characterized by relatively fast maturation. According to Liu et al (2011), for cyprinid fish from low-elevation rivers, sizes at first maturation were estimated to be much smaller and the fish younger.

The classification of maturation stages is essential for fishery assessment. It is difficult to determine the immature stages of males and females, as well as to distinguish the immature and resting stages macroscopically, including for *O. waandersii*. By histological observation, it is possible to identify each stage accurately. This misclassification has an impact on the estimation of the mature proportion of the stock, because fish in the resting stage have already contributed to the spawning biomass of that year and are macroscopically considered immature.

The gonadosomatic index (GSI) has been widely used as an indicator of fish spawning periods, but its use in regeneration biology studies was more appropriate when associated with other reproductive indicators such as macroscopic observation and histological techniques. During this study, the mean value of the GSI showed monthly variation. Starting with July especially, the GSI had minimum values for both males and females. The fish were seen entering a period of silence after July. However, during those months, there were still fish with mature gonads and this indicates that the fish can spawn throughout the year. According to Esmaeili et al (2017), spawning was influenced by several factors including physicochemical, bioecological and weather factors, which play a direct and indirect role in influencing the maturity of fish.

Estimated fecundity of 40 females collected between March and August varied from 2130 to 13640 eggs. Our findings indicate that *O. waandersii* has lower absolute fecundity than recorded for *O. vittatus* (up to 21350 eggs) from Koto Panjang Reservoir (Syandri et al 2015). According to Langroudi & Sabet (2018), differences in egg numbers between species and/or populations can be related either to the effects of different environmental conditions or to differences of studied species. The fecundity may vary between different species, whereas it is generally dependent on various factors, such as condition and age of the fish, space and the food availability (Soetignya et al 2016; do Carmo Silva et al 2016). In addition, the potential fecundity is strongly affected by female size, trade-off between egg size and egg numbers, reproductive strategy and spawning pattern of the species (Lambert 2008). The fecundity of *O. waandersii* increases with fish size (total length and total weight). Several studies also reported that there is a linear relationship between fecundity and the total length and total weight (Abedi et al 2011; Khaironizam & Zakaria-Ismail 2013; Soetignya et al 2016). Our observations showed that the regression coefficient for the relationships between fecundity and total weight was higher than for the total length. Similar correlations have been reported for other Cyprinids, like *Garra rufa* (Abedi et al 2011) or *Neolissochilus* *soroides* (Khaironizam & Zakaria-Ismail 2013). This suggests that the eggs number in the ovaries may describe the ovary weight increases of the fish.

Conclusions. The study is one of the first to provide information on the reproductive biology of *Osteochilus waandersii.* The population of *O. waandersii* in Landak River, Indonesia, indicated a significantly higher proportion of males compared to females. The females displayed high absolute fecundity. There was a significant relationship between fecundity and fish size (total length, total body weight). Estimated L_{m50} values for sexually mature males and females were 137.9 and 140.3 mm, respectively. The observed results may be used to assist the development of management strategies and conservation of the species.

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