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Reproductive Biology of the Amazon Sailfin Catfish *Pterygoplichthys pardalis* from Tempe Lake, South Sulawesi, Indonesia

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ABSTRACT

Pterygoplichthys pardalis is an invasive fish species that grows rapidly in Lake Tempe, Kabupaten Wajo, and has disturbed the existence of native, endemic, and introduced fish. This study aimed to analyze the reproductive biology of this species in terms of population control. The research was conducted from January to April 2023 at Tempe Lake, Wajo Regency, South Sulawesi. Fish samples were caught using gill nets, cleaned, and stored in a coolbox for observation in the laboratory. Reproductive biology observations began with the separation of male and female fish, measuring the length and weight of each with a fish measuring board and analytical scales. Samples were dissected for maturity gonad and fecundity observations. Length-frequency distribution of male fish ranged from10.08- 41.58cm, while that of female fish ranged from 10.08- 35.28cm. The overall sex ratio between male and female fish was 1:1 (balanced), while the sex ratio between mature males and females (maturity stage IV) was 1:2 (polygamy). The growth pattern exhibited a negative allometric trend, with a long spawning season reaching its peak in March. The species is considered a partial spawner.The length at first maturity of gonads (L_m) for male and female fish were 28,99 and 25.98cm, respectively. Moreover, the fecundity ranged from 201– 5,050 eggs with an average of 1,762± 966 eggs.

INTRODUCTION

Pterygoplichthys spp. originates from the South American Amazon River **(Kottelat &Whitten, A.J.,1996;Armbruster & Page, 2006; Bijukumar *et al.*, 2015; Wahyudewantoro, 2018)**. Moreover, ithas invaded waters around the world, both tropical and sub-tropical waters such as Philippines **(Joshi, 1989; Hubilla & Primavera, 2007**), Japan **(Nakabo, 2002)**, Indonesia, Malaysia, Singapore **(Page & Robins, 2006)**, Turkey **(Özdilek, 2007)**, Mexico **(Wakida-Kusunoki *et al.*, 2007; Orfinger & Goodding, 2018)**, Bangladesh **(Hossain *et al.*, 2008)**, Europe **(Keszka *et al.*, 2008;Piazzini *et al.*, 2010)**, Taiwan **(Wu *et al.*, 2011)**, the United States **(Nico *et al.*, 2012)**, Israel **(Golani & Snovsky, 2013)**, Vietnam **(Zworykin & Budaev, 2013)**, Sri Lanka **(Sumanasinghe & Amarasinghe, 2014)**, and India **(Bijukumar *et al.*, 2015)**.

The distribution of *Pterygoplichthys* spp. in Indonesia is extensive, and the population is growing very fast including that found in Lake Tempe, KabupatenWajo. The population has grown rapidly in the last five years and has affected fish productivity **(Hasnidar *et al.*, 2021)**. According to **Hill and Lodge(1999)**, *Pterygoplichthys* spp. is included in the invasive species. Invasive species can be both predators and competitors to native species. The competition between these invasive fish and native species can result in negative impacts on local fisheries **(Keszka *et al.*, 2008)**, reduceing biodiversity and contributing to declining native species populations worldwide **(Keszka *et al.*, 2008**; **Vilà *et al*., 2011; Hossain *et al.*, 2019)**.

Pterygoplichthys spp. has fast growth and high reproductive ability **(Hoover *et al*., 2004; Gibbs *et al.*, 2008)**. Moreover, it has good adaptation to polluted and oxygen-deficient waters **(Sakai *et al.*, 2001;Chavez *et al.*, 2006)**, and has gills as their main respiratory organ. Additionally, it possesses additional respiratory organs, namely the stomach, which has undergone modifications allowing it to absorb oxygen from outside the water **(Armbruster, 1998)**. It is an omnivore, capable of consuming algae, protozoa, microfungi, organic substances (detritus), and other microbes **(Cardoso *et al.*, 2017)**. Moreover, it lacks predators and competitors **(Hill & Lodge, 1999)**, and it is not commonly consumed by the public, and has no other significant uses.

Research on the reproductive biology of *Pterygoplichthys* spp.; namely, length size distribution, sex ratio, length and weight relationship, stage of gonad maturity, size at first maturity, spawning season and fecundity are needed. Information from the results of this study can be a reference for population control of *Pterygoplichthys* spp., especially in Lake Tempe.

MATERIALS AND METHODS

Pterygoplichthys pardalis specimens were collected from Tempe Lake in Kabupaten Wajo, South Sulawesi, Indonesia (Fig. 1).

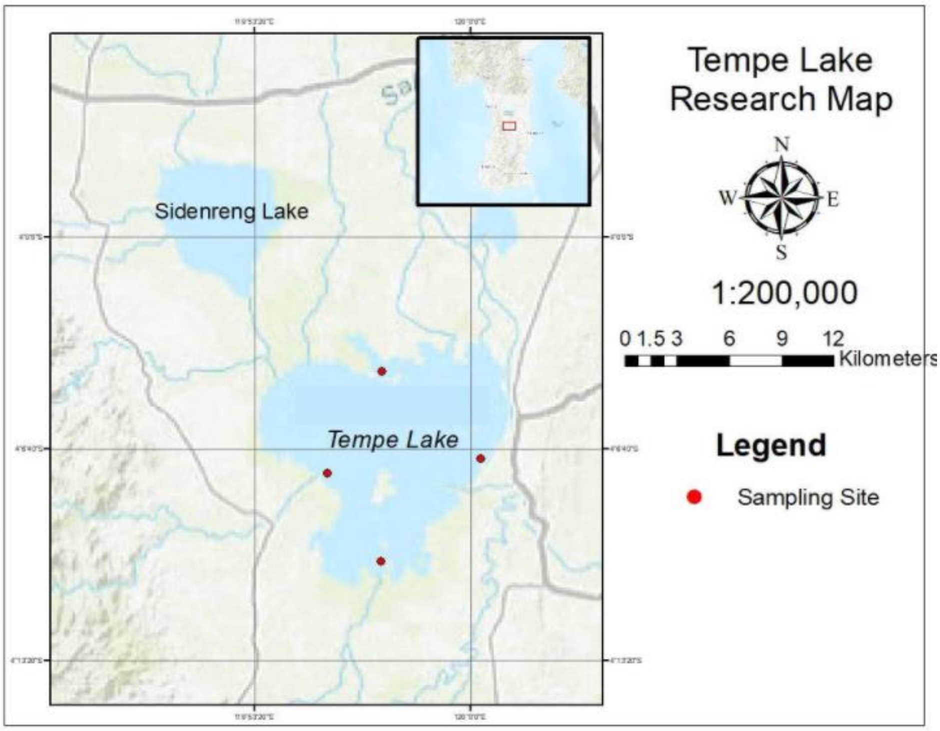

Fig. 1. Map of locations for collecting *Pterygoplichthys pardalis* from Lake Tempe, Wajo Regency, South Sulawesi

Sampling was carried out every week for four months from January–April 2023 using gill nets. Fish samples that were caught were first cleaned, drained, and then put in a cool box and covered with ice cubes. Observation of samples was carried out in the laboratory.

Sample measurement

The measurements taken were: 1) Total length was measured using a measuring board with an accuracy of 0.1cm. 2) Weight of fish was measured using a digital scale with an accuracy of 0.01g. 3) Determination of the stage of gonad maturity was based on the morphology of the gonads (shape, size, color), the position of the gonads in the abdominal cavity **(Wahidin, 2021)**, and the diameter of the eggs was measured using an ocular micrometer on a microscope (magnification 40 x).

Length- frequency distribution

Length-frequency distribution involved determining the number of classes according to Sturges' instructions **(Mahendra & Parmithi, 2015)**.The number of classes was determined using the formula k = 1 + 3.3 log n, where k = the number of classes, and n = the number of data. While class intervals was determined using the equation $\boldsymbol{c = Xn - X1k}$, where c = class interval; Xn = largest data value; X1 = smallest data value, and k = number of classes.

Sex ratio

The sex ratio of male to female individuals was assessed. Sex ratio was estimated as: M/F **(Peña-Mendoza *et al*., 2005)**, where M=Number of males, and F=Number of females.

Length-weight relationship

The length-weight relationship shows the relationship between the weight and total length of the fish using the exponential plot. The exponential plot was done using the expression: $W = aL^b$**(LeCren, 1951)**, where W=Weight of fish (g); L = Length of fish (cm), a = Constant (intercept), b = growth pattern (slope) or exponent showing the length- weight relationship parameters (isometric or allometric). To get this equation, the values of L and W were transformed to logarithms (base 10) as follows: log W = log a + b log L. If the value of b = 3, it indicates an isometric growth (growth in length with weight), if the value of $b \neq 3$, it indicates an allometric growth (b>3 is positive allometric: weight growth is faster than length; b<3 is negative allometric: length growth is faster than weight).

Length at first maturity (L_{m50})

The length at first maturity represents the size at which 50% of the female individuals of the fish species are mature. The L_{m50} was estimated using the log transformed equation of the logistic curve: with : $\frac{p}{1+e^{r(L-l_m)}}$ **(King, 1995)**; dimana p = adjusted population ripe; L = length of fish (cm), and L_m = length at first maturity.

Fecundity

Mature female fish (stage IV) were dissected, and the gonads were removed, and the overall gonad weight (Wg) was measured, then the gonads were taken (anterior, middle and posterior) and the sub-gonad weight (Ws) was measured. Sub gonads were given Gilson's fluid **(Sivashanthini, 2008)**. The gravimetric method, as described by **LeCren (1951)** and **Hasan *et al.* (2020)**, was used to calculate fecundity. This method involves counting the number of eggs contained in the sub-gonad (Fs) and applying the formula F = (Wg / Ws) x Fs, where F represents fecundity in eggs; Wg is the gonad weight in grams; Ws is the sub-gonad weight in grams, and Fs is the number of eggs in the sub-gonad.

RESULTS

1. Length-frequency distribution

The total count of male *P. pardalis* captured was 386 individuals. Based on the length-frequency distribution, the length of the fish caught ranged from 10.08- 41.58cm. The largest proportion, comprising 81 individuals or 20.98%, was observed in the length category of 19.53cm. Conversely, the smallest proportion accounting for 3 individuals or 0.78% was recorded in the length category of 41.58cm (Fig. 2A). Among the captured female *P. pardalis*, totaling 412 individuals, the length-frequency distribution ranged from 10.08 to 35.28cm. The highest number was observed at 25.83cm, comprising 85 individuals or 20.63%, while the smallest proportion, represented by 3 individuals or 0.73%, was found at 35.28cm (Fig. 2B).

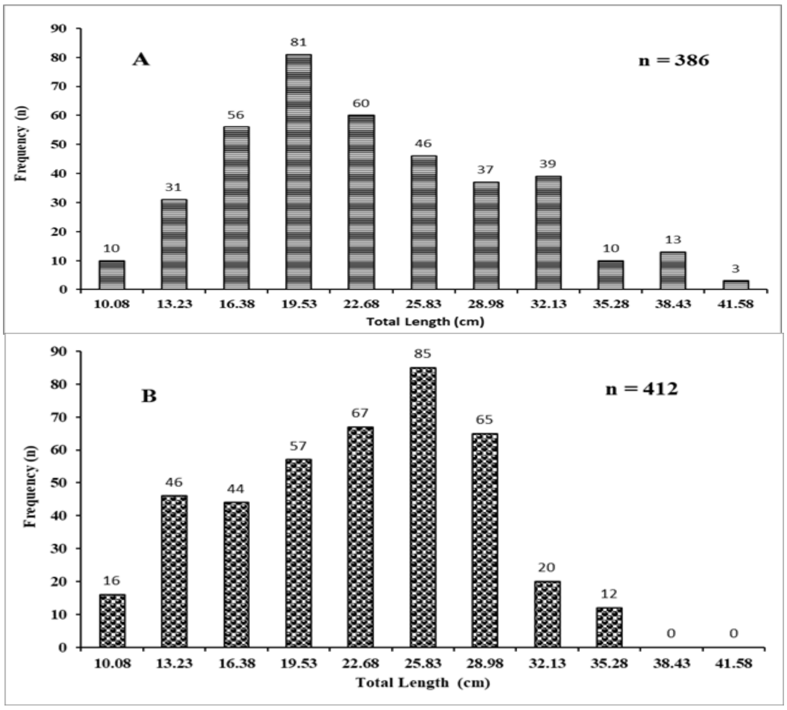

Fig. 2. Length-frequency distribution of **(A)** male and **(B)** female *P. pardalis*

2. Sex ratio

During the study, a total of 798 *Pterygoplichthys pardalis* were observed, comprising 386 males and 412 females.The results of the chi-square test analysis yielded a sex ratio male and female in this study of 1:1 or 48:52% (Fig. 3). Furthermore, the sex ratio from gonad maturity (stage IV) was 38:62% or 1:2 (Fig. 4).

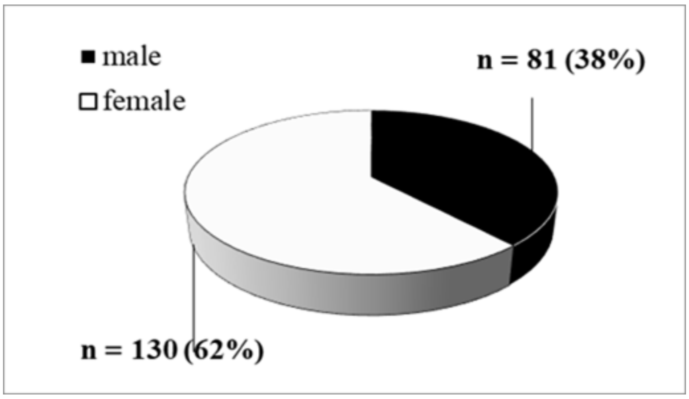

Fig. 3. Number of male and female *P. pardalis*

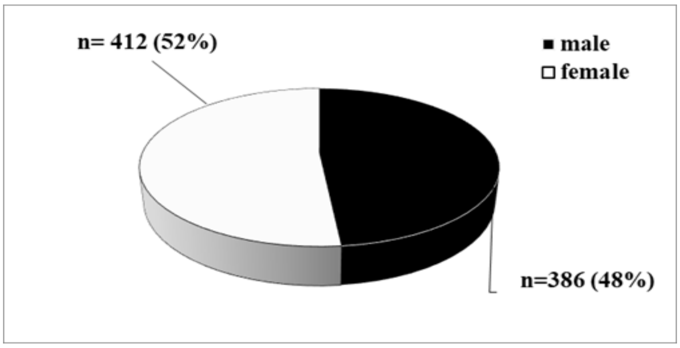

Fig.4. Number of mature male and female *P. pardalis*

3. Relationship of length-weight

The analysis of the relationship between length and weight of *P. pardalis* yielded the following equations: $W = 0.02\ L^{2.7069}$ for males and $W = 0.02\ L^{2.6951}$ for females (Fig. 5).

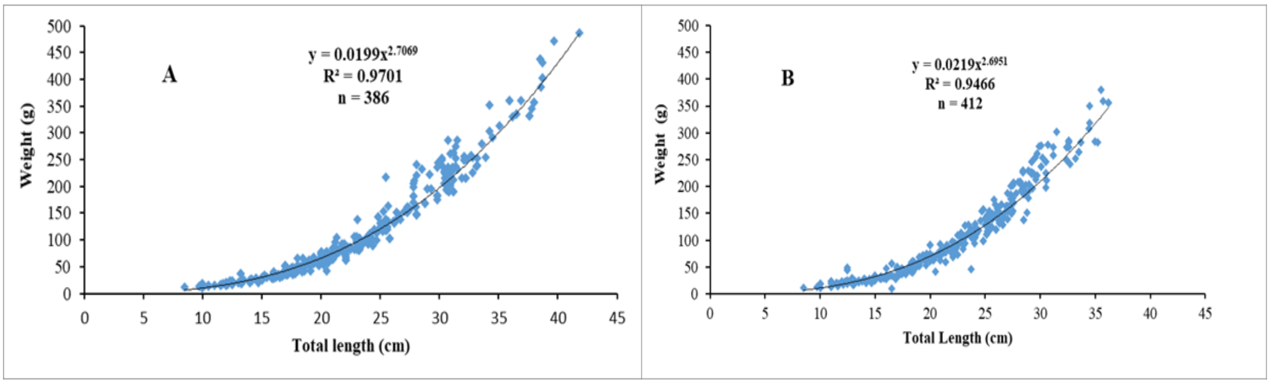

Fig. 5. Relationship of length-weight between**(A)** male and **(B)** female *P. pardalis*

4. Gonad maturity stage

Criteria for the gonad maturity stage male and female *P. pardalis* were based on gonadal morphology (shape, size, color, and position of the gonads in the abdominal cavity) and size (egg diameter) (Table 1).

Table 1. Gonad maturity stage of female and male *P. pardalis*

Stage	Female (Ovary)	Male (Testes)
I (Immature)	Ovaries are small, elongated in shape, translucent white to pink in color, filled with a viscous liquid, the eggs are not visible, it is estimated that <15% fills the abdominal cavity.	Testes are elongated, very small and clear in color
II (Development)	Ovaries are larger in size, light yellow to red in color, the eggs are visible but the size is very small, the diameter of the eggs ranges from 0.20 to 1.60 mm, it is estimated that 20-40% fills the abdominal cavity.	Testes are larger than maturity stage I and are white.
III (Early mature)	Ovaries are large, yellow in color, the eggs are clearly visible but the eggs are still difficult to separate. There are small and large eggs. Small egg diameter ranges from 0.30 – 0.80 mm, large eggs range from 0.80 – 2.50 mm. It is estimated that 30% -60% fills the abdominal cavity.	Testes have a size larger than maturity stage II, the surface of the testes looks rough and white in color.
IV (Mature)	The ovaries are getting bigger, the color is yellow to orange, the eggs are clearly visible and the eggs are easy to separate. There are medium and large eggs. Small egg diameter ranges from 0.50 – 1.70 mm, large eggs range from 1.50 – 2.7 mm. It is estimated that 50-80% fills the abdominal cavity.	Testes are larger than maturity level III, filled with white viscous fluid.
V (spent)	Ovaries are wrinkled and red in color, containing leftover eggs that are not spawned	Testes are wrinkled and look like stage II maturity.

Pterygoplichthys pardalis both male and female were found to reach gonad maturity stage I-V. The highest number of male *P. pardalis* was found at maturity stage II, while females were predominantly at stage IV (Fig. 6).Furthermore, both male and female *P. pardalis* with the highest gonad maturity stage IV were found in March (Fig. 7).

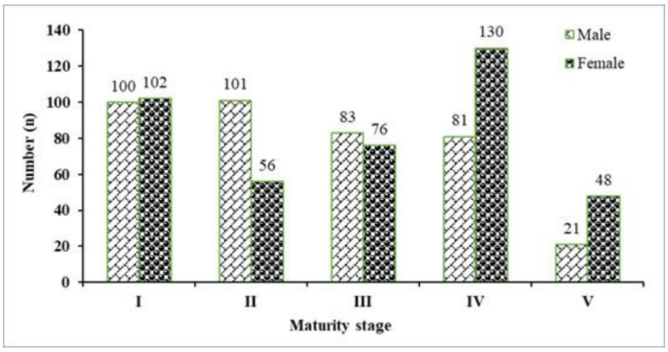

Fig. 6. Number of male and female *P. pardalis* based on gonad maturity stage

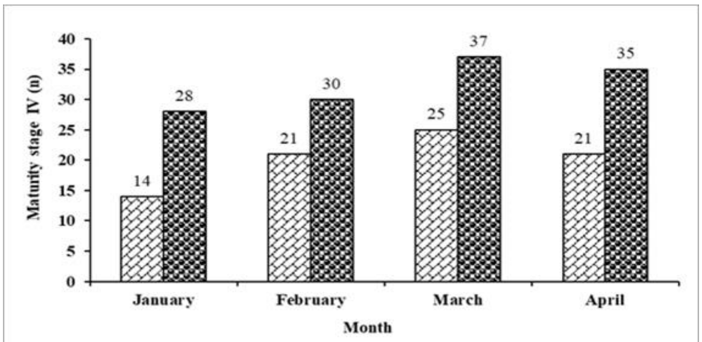

Fig. 7. Distribution of mature (stage IV) male and female in January-April 2022

5. Length at first maturity of gonads (L_{m50})

The number of *P. pardalis* that matured in relation to length showed that males started to mature at 16.23cm and female at 13.23cm. The highest number of mature male and female *P. pardalis* were 32.13 and 29.98cm, respectively (Fig. 8). Furthermore, based on the length at first maturity of gonads (Lm) of *P. pardalis*, males matured at 28.99cm and females at 25.98cm.

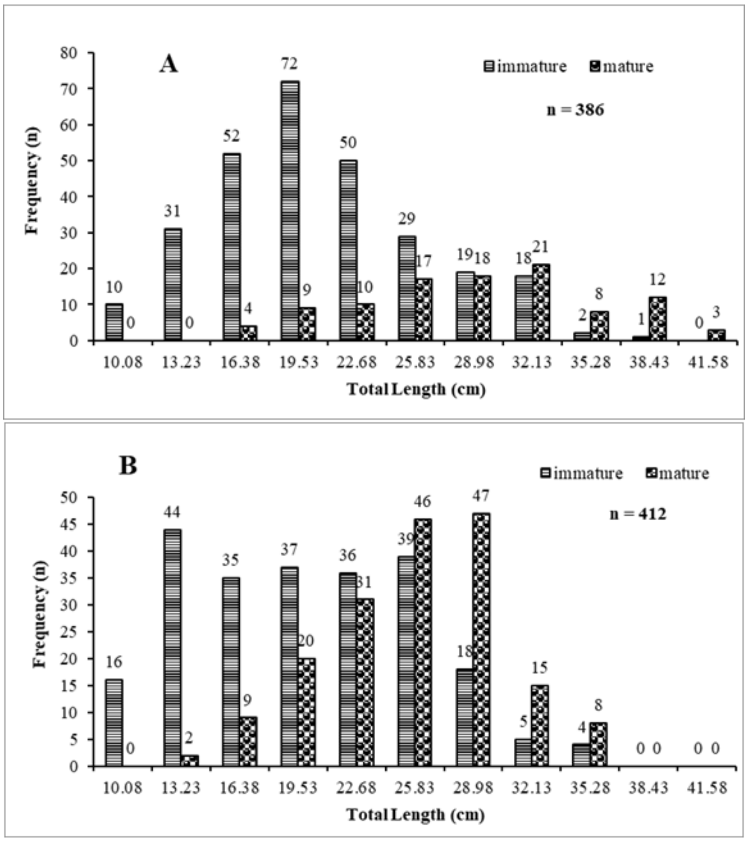

Fig. 8. Number of mature and immature (A) male and (B) female *P. pardalis*

6. Fecundity

During the observation, 130 mature female gonads (stage IV) of *Pterygoplichthys pardalis* were recorded, with lengths ranging from 13.23 to 35.28cm and weights from 28 to 380g. The gonad weight varied between 6.6 and 66.4g. Their fecundity ranged from 201 to 5,050 eggs, with an average of 1,579± 966 eggs.

DISCUSSION

The length-frequency distribution of *Pterygoplichthys pardalis* in Tempe Lake ranged from 10.08 to 41.58cm. This distribution varies across different locations and studies. For instance, in the Ciliwung River at Bogor Botanical Gardens, Indonesia, the reported length range is 27.40– 60.70cm **(Hariandati, 2015)**. In Peninsular Malaysia, it's 10.08– 38.00cm **(Samat *et al.*, 2008)**, while in Siak River in Pekanbaru, Indonesia, it's 5.25– 36.87cm **(Sari *et al.*, 2015)**. In the Amazon River, the reported range is 21.00– 37.00cm **(Sousa *et al.*, 2019a)**.

Moreover, in the Gangga-Brahmaputra River in Bangladesh, it's 33.40– 39.40cm **(Hossain *et al.*, 2018)**. Other studies have reported lengths of up to 40cm **(Geerinckx *et al.*, 2007)**. Moreover, some investigations reported lengths of up to 70 cm **(Kottelat & Whitten, 1996; Nelson *et al.*, 2016; Elfidasari *et al.*, 2022)**. The differences in the length-frequency distribution of *P. pardalis* mentioned above are influenced by their habitats.

The sex ratio of male and female is 1:1 (Fig. 3), meaning that the number of male and female fish is balanced. The ratio of *P. pardalis* in the Air Hitam River, Riau Province, is the same **(Pinem *et al.*, 2016)**. However, it differs from that observed in other locations. In Peninsular Malaysia, the sex ratio is reported to be 1: 2 **(Samat*et al.*, 2008)**, and in the Ciliwung River at Bogor Botanical Gardens, it's also 1:2 **(Hariandati, 2015)**. The sex ratio, which tends to have fewer males than females, is thought to be due to males guarding the nest after spawning. This result is the same as the findings of **Mazzoni and Iglesias-Rios (2002)** and **Liang *et al*.(2005)**, elucidating that male fish are responsible for protecting their eggs and young. In addition, it is also suspected that females are more active than males **(Pinem *et al*., 2016)**. The sex ratio between males and females based on maturity (stage IV) is 1: 2 (Fig. 4). Based on this concept, it is suspected that *P. pardalis* is polygamous, that is, one male is paired with more than one female. The same finding was reported by **Liang *et al*. (2005)** and **Gibbs *et al*. (2008)**, postulating that the number of mature female fish is more than the males in the spawning season. According to **Herjayanto *et al*. (2016)**, each fish species has a different optimal male and female sex ratio for spawning.

The length-weight relationship for both males and females has a negative allometric growth pattern, namely the growth in length is more dominant than weight (Fig. 5). The same is true for *P. pardalis* in Sungai Langat, Malaysia **(Samat *et al*., 2008)** and the Amazon River **(Sousa *et al*., 2019)**. **Froese (2006)** suggested that, in general the growth pattern (value b) depends on the biological conditions of the fish such as gonad development and food availability. In addition to these factors, physiological conditions and environmental conditions, such as temperature, pH, salinity, geographic location, and sampling technique can also play significant roles.

Gonad maturity stage (ovaries and testes) of *P. pardalis* was classified into five stages (Table 1). The maturity classification is based on morphology, gonad position in the abdominal cavity, and egg diameter. The results showed that with higher maturity, there was an increase in both egg diameter and ovary volume. Variations in egg diameter were also found in the ovaries. **Pinem *et al*. (2016)**also classified the gonadal maturity of *P. pardalis* into five histological stages, non-vitellogenic eggs (stage I and II); yolk, non-vitellogenic and vitellogenic eggs (stage III); yolk, early vitelogenic eggs, vitelogenic, atretic eggs (stage IV); atretic eggs and shriveled ovaries (stage V).

Furthermore, according to **Jumawan and Herrera (2014)**, macro and microscopic observations of the gonads of *P. disjunctivus* showed that it was stage I (immature); stage II (the process of maturation, Ø eggs 1-2.5mm); stage III (mature; before spawning; Ø eggs 2.5-3mm); stage IV (spawning); stage V (after spawning, wrinkled ovaries still contain residual eggs); and stage VI (recovery, re-preparation for maturation of leftover eggs). According to **Samat *et al*. (2016)**, in *P. pardalis* from Peninsular Malaysia, the ovaries contain several groups of eggs, with some reaching Ø 3.3cm. Similarly in *P. disjunctivus*, several egg sizes were found in the ovary. Based on the maturity analysis, it is suspected that *Pterygoplichthys* spp. is a partial spawner **(Gibbs *et al*., 2008)**.

The number of male and female *P. pardalis* varied at each maturity stage (Fig. 6). Based on these maturity variations, it is suspected to have a long spawning time. This behavior was also noted in *P. pardalis* from the Air Hitam River, Riau Province, where stage IV fish were observed every month **(Pinem *et al.*, 2016)**. This behavior occurs approximately eight months each year **(Neves & Ruffino, 1998)**. Furthermore, based on the number of mature (stage IV) relationship with the time of observation, the number of mature began to increase in January, the highest was in March and a decrease was detected in April (Fig. 7). Hence, it is suspected that the peak of spawning activity occurred in March. The spawning peaks of *P. pardalis* varied as observed all year round in Florida, USA **(Yamamoto & Tagawa, 2000)**, March-September and sometimes reaching February in the United States **(Liang *et al*., 2005)**, June–July in the Philippine Marikina River **(Gibbs *et al*., 2008;Nico *et al*., 2012; Gibbs *et al*., 2013)**, April or May to September in US Hawaii **(Gestring *et al*., 2010)**, July–September and April-October in Taiwan **(Jumawan & Herrera, 2014; Wickramaratne, 2018)**, and in the Ciliwung River, Bogor Botanical Gardens, July and August in Sri Lanka's Victoria & Kalaweva Reservoir **(Hariandati, 2015)**. According to **Mendoza-Palmero *et al*. (2009)**, the highest spawning of fish in sub-tropical areas usually lasts several months in summer, but in other areas, it can occur throughout the year.

Based on the size distribution of male and female length, the size at maturity of male gonads was 16.38cm and for females it was13.23cm. The highest mature male and female fish were found at 32.13 and 28.98cm, respectively (Fig. 8). Furthermore, the results of the analysis of the length at first maturity of gonads (Lm50) indicated that female fish mature faster (25.98cm) compared to males (28.99cm). The length at first maturity of gonads found by several researchers also varied, including males at 25cm and females at 28cm **(Neves & Ruffino, 1998)**; 25cm (**Mendoza-Palmero *et al*., 2009; Nico *et al*., 2012)**; males at 12.5cm and females at 13cm **(Samat *et al*., 2016)**, and 22.3cm **(Sousa *et al*., 2019)**. The length at first maturity can vary due to environmental factors, fishing gear used, and fishing effort **(Anderson *et al*., 2008)**.

The fecundity of *P. pardalis* in this study is 201–5,050 (1,579±967 eggs). The fecundity of *P. pardalis* in several locations varied, such as from the Michoacán-Guerrero dam in Mexico, with 2,447 eggs (±12.6 eggs/g fish) **(Rueda-Jasso *et al.*, 2013)**; from the Ciliwung River, Kebun Raya Bogor, where the fecundity is 207–1,445 eggs **(Hariandati, 2015)**; from the Air Hitam River, Riau Province, with 5,351–48,980 eggs **(Pinem *et al.*, 2016)**; from Sungai Langat, Malaysia, with 1,297-18,791 eggs, influenced by the brood size **(Samat *et al.*, 2016)**; and from Victoria and Kalaweva Reservoirs in Sri Lanka, recording 956±261 and 1,856±817 eggs, respectively **(Wickramaratne, 2018)**. According to **Hoover *et al*. (2004)** and **Gibbs *et al*. (2008)**, *Pterygoplichthys* spp. are classified as fish with high fecundity; their fecundity ranges from 500-3,000 eggs/brood depending on the size of the brood **(GISD, 2015)**. Variations in fecundity between fish populations are influenced by environmental factors, including water temperature, abundance of food, and different types of species **(Alp *et al*., 2003)**.

CONCLUSION

The length distribution of male fish is larger than that of the female fish. Moreover, the overall sex ratio between male and female fish is balanced, and the spawning pairs of *P. pardalis* are polygamous (one male, two females). The growth pattern is negative allometric; the spawning season is long with the peak in March. *Pterygoplichthys pardalis* is a partial spawner, with the size at first maturity of the male and female gonads being 28.99 and 25.98cm, respectively. Fecundity ranges from 201 to 5,050 eggs, with an average of 1,762±966 eggs.

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