Reproductive biology of rivulated peacock flounder (*Pseudorhombus argus* Weber, 1930) in Wallace Line, Spermonde Islands

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Abstract. In Wallace Line at Spermonde waters, *Pseudorhombus argus* is an ecologically and economically important flatfish species. For sustainable management and conservation, understanding its reproductive biology is essential. However, there is insufficient information on the reproductive components of *P. argus* in Spermonde waters. This study aimed to study the reproductive biology of *P. argus* in Spermonde waters, including sex ratio, gonad maturity stage, gonadosomatic index (GSI), and size at first sexual maturity. Samples were taken monthly from December 2023 to November 2024. Sex ratio, gonad maturity stage, gonad index, and size at first sexual maturity were all reproductive parameters considered. Based on sampling period, gonad maturity stage, and size class, *P. argus* was dominated by females. In addition, females had larger body sizes than males. Morphological differences in the gonad maturity stage indicated a balance between males and females. The highest GSI was recorded in April, indicating the beginning of the dry season. For females, sexual maturity occurs at 20.70 cm, and for males at 20.25 cm. However, the size of the first sexual maturity and the spawning season of *P. argus* differ from that of some other flatfish species. Such data are essential in developing a sustainable *P. argus* resource management plan.
Key Words: flatfish, first maturity, gonadal index, sex-ratio, stage of maturity.

Introduction. Ecologically, the Spermonde waters, famous for their extraordinary marine biodiversity, are part of the Wallace Line. The Wallace Line is a biogeographic transition zone separating Asian fauna from Australia. The economy of coastal communities in the Spermonde waters area, especially small-scale fishermen, is greatly supported by the diversity of fish species (Roberts et al 2002; Carpenter et al 2008). The existence of flatfish in the Wallace Line, Spermonde waters shows an interesting and unique evolutionary adaptation (Tresnati 2001) that needs further research, especially bioecological aspects, to support sustainable management.

Several species of flatfish were found in the Spermonde waters by Ramla et al (2021). These include *Grammatobothus polyophthalmus* and *Engyprosopon grandisquama* from the Bothidae family; *Pseudorhombus arsius* and *Pseudorhombus dupliciocellatus* from the Paralichthyidae family; *Cynoglossus lingua* from the Cynoglossidae family, *Psettodes erumei* from the Psettodidae family, and *Liachirus melanospilus* and *Pardachirus pavoninus* from the Soleidae family.

Pseudorhombus argus, also known as the rivulated peacock flounder, is a flatfish species that has attracted attention due to its economic potential and role in the demersal ecosystem. However, there is still few data on how *P. argus* lives in the Spermonde waters. Understanding aspects of fish reproduction, such as spawning season and gonad maturity size, is very important for the conservation and sustainable management of fish resources (Jennings et al 2014; Tesfahun 2019). These reproductive data are used to determine the allowable catch size, establish no-take zones during the spawning season, and form aquaculture programs (Froese & Pauly 2000).

P. argus is a member of the Paralichthyidae family, with a flat, oval, and bilaterally symmetrical morphology. The upper side (ocular) is brown with black spots and rings, and the lower side (blind) is white (Liew et al 1988) (Figure 1). The peacock fish has not yet contributed to high economic value, but it can be used as an export commodity or domestic consumption material (Adela et al 2016).

This study focuses on the reproductive biology of *P. argus* in the Wallace Line, Spermonde waters. The results are expected to provide complete scientific data on the reproduction of *P. argus* and become a basis for building a sustainable management model to maintain ecological functions and optimize the economic benefits of flatfish resources in Spermonde waters. *P. argus* is a member of the Paralichthyidae family, with a flat, oval, and bilaterally symmetrical morphology. The upper side (ocular) is brown with black spots and rings, and the lower side (blind) is white (Liew et al 1988) (Figure 1). The peacock fish has not yet contributed to high economic value, but it can be used as an export commodity or domestic consumption material (Adela et al 2016).

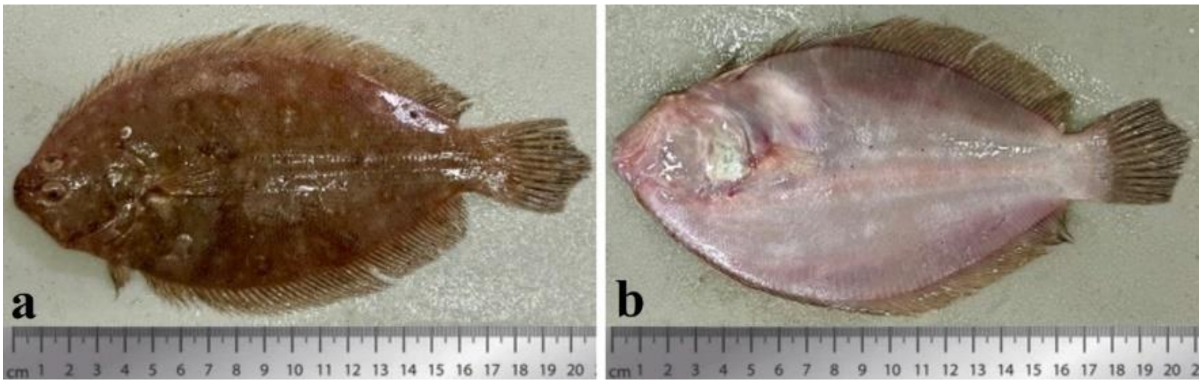

Figure 1. Rivulated peacock flounder *Pseudorhombus argus*: a - left side, b - right side.

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Material and Method. The *P. argus* samples were obtained from fishermen at the Paotere Fish Landing Site, Makassar City. Sampling was carried out for 12 months to get one annual cycle from December 2023 to November 2024. Fishermen caught *P. argus* around coastal waters of the Labakkang Sub-district, Karanrang Island, Sarappolompo Island, Podangpodang Island, Sanane Island, Pajenekang Island, Barranglompo Island, Kodingareng Island, and Barrangcaddi Island (Figure 2).

The parameters measured were the total length of the fish with an accuracy of 1 mm and the width and length of the gonad with an accuracy of 0.01 mm each. The total weight of the fish, gonad, and stomach contents were weighed with an accuracy of 0.01 grams. The morphologic structure of the gonad and the maturity stage (MS) were observed by referring to previous studies in Spermonde waters (Tresnati et al 2019; Yanti et al 2019; Tresnati et al 2020a), who divided the MS into five stages of development: stage I (immature), stage II (early maturation), stage III (maturation), stage IV (mature), and stage V (spawning).

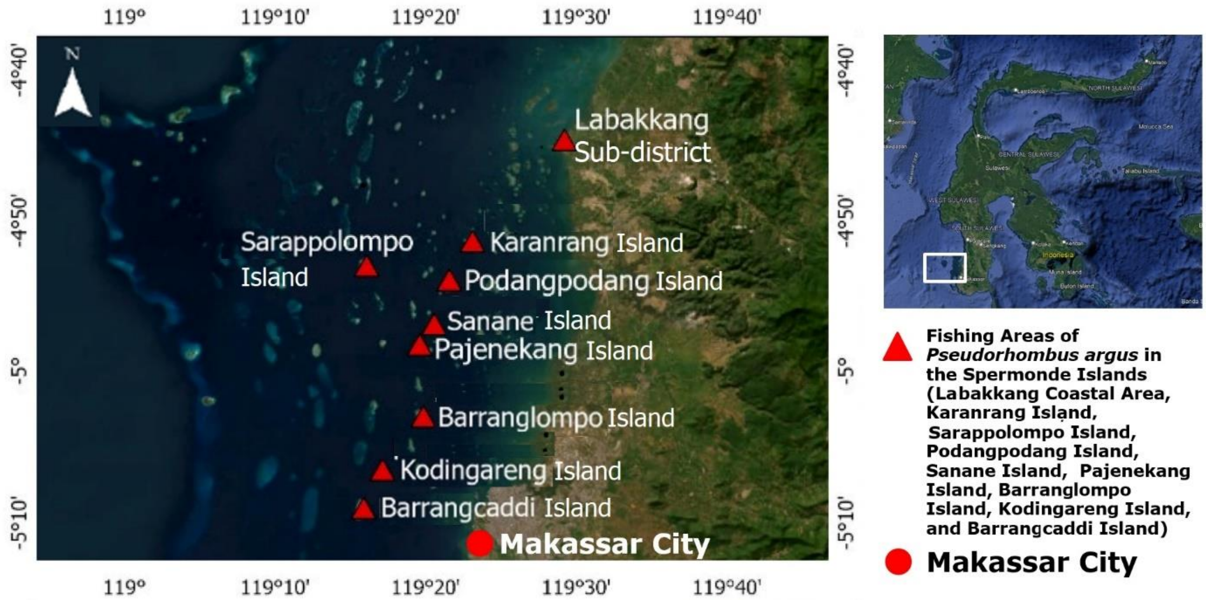

Figure 2. Map of distribution of fishing areas of rivulated peacock flounder *Pseudorhombus argus* in the Wallace Line, Spermonde Islands water.

The reproductive parameters calculated were sex ratio, gonadosomatic index (GSI), and length at first maturity (LFM). The sex ratio (SR) was calculated by referring to previous studies in Spermonde waters (Tresnati et al 2020b; Tuwo et al 2020a, b) using the equation:

$$SR = \frac{\sum M}{\sum F} x\ 100$$

where: ∑M was the number of males and ∑F was the number of females. Chi-square was used to test whether the difference in the sex ratio of males and females was significantly different (Scherrer 1984).

The GSI was calculated by referring to Tuwo & Conand (1992) using the equation:

$$GSI = \frac{GW}{TBW} x\ 100$$

where: GW is gonad weight, and TBW is total body weight.

The LFM was estimated using male and female individuals at stages III, IV, and V. All male and female individuals in stages III, IV, and V were considered mature. Males and females were grouped by length class and plotted based on length class (X-axis) and percentage of gonad-mature (Y-axis). The regression curve was created using a polynomial trend line. On the curve, LFM was determined by drawing a horizontal line from the Y-axis at the 50% point towards the curve and then drawing a vertical point from the curve at the 50% position to the X-axis indicating the LFM value (Tuwo et al 2021).

Results

Sex-ratio. The number of samples observed during the study was 645, of which 392 (60.78) were females and 253 (39.22) were males (Figure 3a). The chi-square test results showed that the SR of *P. argus* based on sampling time was significantly different ($p > 0.05$) between males and females. The SR based on maturity stage (Figure 3b) and based on length class (Figure 3c) also showed that females were more numerous than males and were significantly different ($p > 0.05$) between males and females.

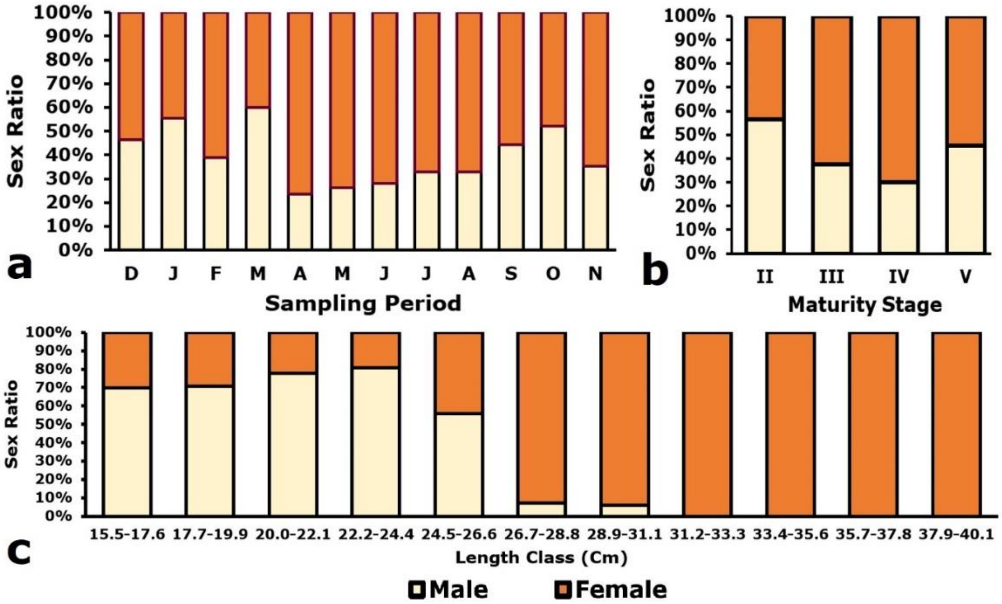

Figure 3. Sex ratio of rivulated peacock flounder *Pseudorhombus argus* in the Wallace Line, Spermonde Islands water: a - based on sampling period, b - based on maturity stage, c - based on length class.

Morphologic characteristics of gonads. Morphologically, the gonads of *P. argus* can be distinguished based on their color and size (Figure 4). Female and male gonads have different sizes at each stage of gonadal maturity from MS II to V. The weight and length of the ovaries and testes increased progressively along with the development of maturity stage (Table 1).

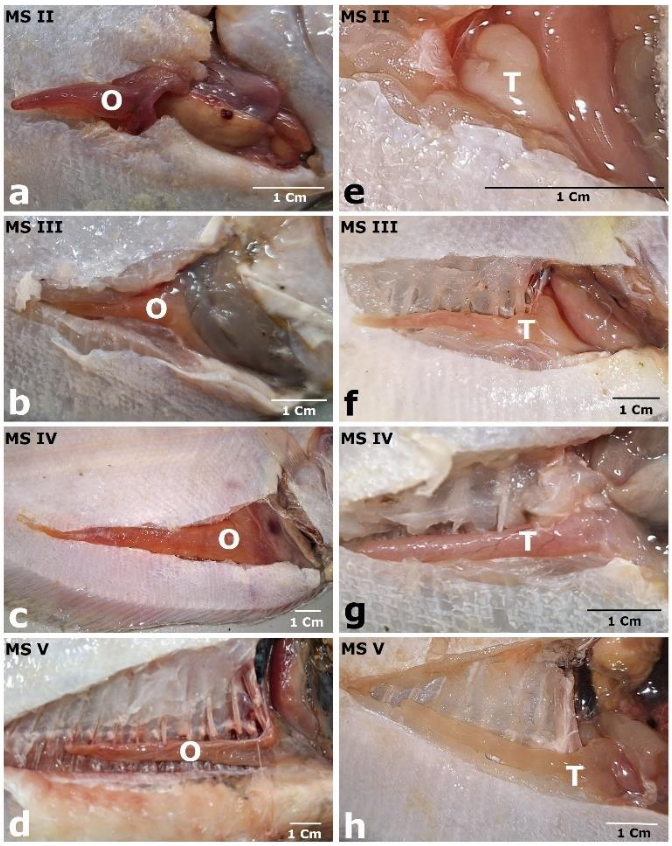

Figure 3. Macroscopic characteristics of gonads of female (a-d) and male (e-h) rivulated peacock flounder *Pseudorhombus argus*, T: testis, O: ovary.

Table 1

Morphological parameters and gonad sizes of rivulated peacock flounder *Pseudorhombus argus*

MS	*Female*	*Male*
II	Ovaries are reddish brown and full; oocytes are not yet visible. Ovarian weight is between 0.02 and 1.84 g (average 0.77±0.60 g), and ovary length is between 0.7 and 6.2 mm (average 3.8±1.8 mm).	Testes are clear, brownish, whitish, and quite full. Testes weight between 0.01 and 0.18 g (average 0.06±0.04 g), testes length between 0.7 and 2.9 mm (average 1.5±0.6 mm).
III	Ovaries are reddish brown, and oocytes have begun to appear. Ovarian weight varies between 0.22 and 28.92 g (average 7.58±4.91 g), and ovary length between 7.6 and 15.3 mm (average 11.0±1.9 mm).	Testes are clear, brownish, and denser. Testes weight varies from 0.09 to 3.58 g (average 0.42±0.63 g), and testis length between 0.2 and 3.0 mm (average 1.6±0.7 mm).
IV	Ovaries are reddish brown, and oocytes are visible. Ovarian weight varies between 2.17 and 26.02 g (average 8.56±4.66 g), and ovary length between 3.3 and 15-5 mm (average (10.2±2.3 mm).	The testis is more prominent and whitish brown. Testis weight varies between 0.14 and 3.00 g (average 0.55±0.51 g, and testis length between 0.8 and 5.3 mm (average 2.7±1.2 mm).
V	Ovaries are brown and flat. Oocytes are visible. Ovarian weight between 0.01 and 2.78 g (average 0.57±0.61 g), length between 0.8 and 9.4 mm (average 1.9±3.3 mm).	The testis is deflated and whitish brown. Testis weight between 0.01 and 0.13 g (average 0.07±0.04 g), testis length between 0.3 and 3.2 mm (average 1.4±0.7 mm).

Maturity stage distribution. The distribution of MS III and MS IV is entirely synchronous between females (Figure 4a) and males (Figure 4b) with varying percentages; the presence of MS IV between females and males is almost the same or synchronous during the sampling period. MS synchrony, especially during the spawning period, is essential because it will determine the success of reproduction. The increase in MS V from September to December indicates that *P. argus* spawned in those four months. The MS related to the length class shows that MS IV can be found in all of the sample length classes in female fish, from 15.5-17.6 cm to 37.9-40.1 cm (Figure 4c) and from 15.5-17.6 cm to 28.9-31.1 cm in male fish (Figure 4d).

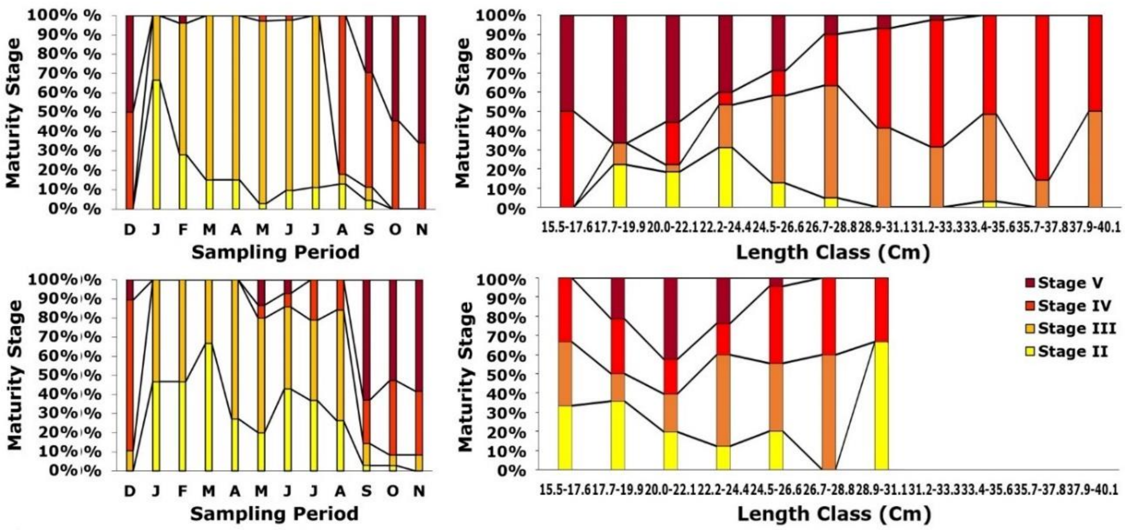

Figure 4. Stage of maturation of rivulated peacock flounder *Pseudorhombus argus* in the Wallace Line, Spermonde Islands water based on sampling period (a and b) and based on length class (c and d).

Gonadosomatic index. The GSI, based on sampling time, has more varied values in females than males. In males, the highest GSI was found in April (Figure 5a), while in females, the highest GSI was found in March (Figure 5b).

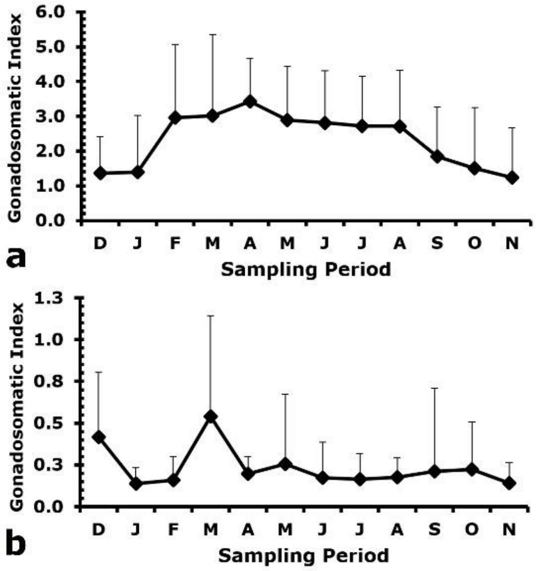

Figure 5. Gonadosomatic index of rivulated peacock flounder *Pseudorhombus argus* in the Wallace Line, Spermonde Islands water based on sampling period in males (a) and females (b).

First maturity size. Males reach first maturity earlier than females. Males reach first maturity at 22.0 cm (Figure 6a), while females reach first maturity at 26.9 cm (Figure 6b).

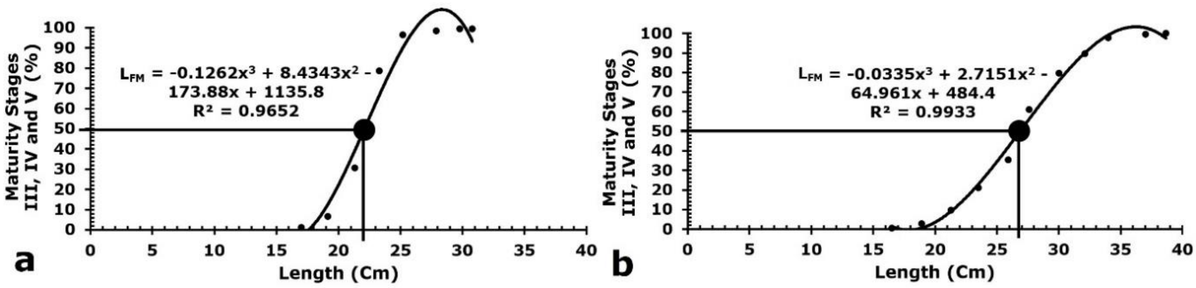

Figure 6. Size of first maturity of rivulated peacock flounder *Pseudorhombus argus* in the Wallace Line, Spermonde Islands eater in males (a) and females (b).

Discussion

Sex-ratio. The results of this study indicate several important components in the reproductive biology of *P. argus* in Spermonde Waters. Sex ratio imbalance, where the population of *P. argus* has fewer males than females, can have significant biological and ecological consequences, especially if the species is intensively exploited. Biologically, sex ratio imbalance can lead to reduced reproductive potential, reduced genetic diversity, and changes in reproductive behavior. The number of males present can reduce reproductive success. Although a single male can fertilize many females, the lack of males can produce fewer fertilized eggs and fewer larvae. This can impact recruitment and reduce the population (Sadovy & Domeier 2005). An imbalanced sex ratio can cause decreased genetic diversity in a population. Sex ratio imbalance can lead to changes in reproductive behavior, such as competition between females for mates, changes in the size and age of gonad maturity, and an increased likelihood of hybridization with other species (Hamilton 1980).

Ecologically, sex ratio imbalance can negatively impact the food chain, biodiversity, and economy. *P. argus* is part of the demersal ecosystem food chain. The balance of the ecosystem, both as predator and prey, can be disturbed by population decline due to sex ratio imbalance. This can cause changes in the structure and number of communities (Duffy 2002). Sex ratio imbalance can cause a decline in the population of *P. argus* and local extinction. Losing one species can endanger the Spermonde waters and other species' biodiversity (Worm et al 2006). Small scale fishermen who depend on *P. argus* as a source of income will suffer losses if the *P. argus* population decreases. According to Pauly et al (1998), sex ratio imbalance can negatively impact the catch and sustainability of fishermen's livelihoods.

The dominance of female individuals in various analyses (sex ratio based on time, gonad maturity, and size class) is an interesting phenomenon and requires further research. Several previous studies on other species in Spermonde Island showed the same thing, where females were always more dominant, such as blackeye thicklip wrasse *Hemigymnus melapterus* (Tresnati et al 2020b), yellowfin parrotfish *Scarus flavipectoralis* (Tresnati et al 2020a), Indian parrotfish *Chlorurus capistratoides* (Tuwo et al 2020a).

Several hypotheses can be used to explain this phenomenon. First, fish mortality rates may be influenced by variations in predation, disease, fishing activity (Jennings et al 2009). Female will dominate the population when the mortality rate of males is higher than that of females. Second, there will be differences between male and female migration patterns. Females have a greater tendency to migrate to spawning areas in the Spermonde waters, meaning that females dominate the sex ratio in the area. Third, fish sex determination may be influenced by environmental and genetic factors (Devlin & Nagahama 2002; Villarreal et al 2024).

Different reproductive strategies and energy allocation between males and females may be related to sexual dimorphism in growth (Policansky 1982). Females require more energy for gonadal development and egg production, resulting in faster somatic growth (Wootton & Smith 2014).

In many species, females outnumber males. In Soleidae, females are 56.46%, and males are 43.54% (Bouain et al 2018), as well as in *Solea aegyptiaca*, females are 53.4%, and males are 46.6% (Ahmed et al 2010). In reef fish, female dominance is even greater; parrotfish and wrasse species can reach a ratio of 1 male to 3 females (Tresnati et al 2019; Yanti et al 2019). Female dominance does not always occur in flatfish. In *Paralichthys*, males can be more dominant with a ratio of 1-2 males to 1 female (Kumagai 1999; Smith et al 1999; Bambill et al 2006). A balanced ratio of males and females in nature will open up opportunities for fertilization of eggs by a large number of spermatozoa. Existence of a fish species can be maintained if the sex ratio is balanced or there are more females in a population because one male can produce enough spermatozoa to fertilize the many eggs produced by several females (Smith et al 1999).

Maturity stage. Morphologically, there are differences in color of gonad at each stage of maturity. Generally, females have color variations from pink to dark red, while males are whitish brown to milky white. At each stage of gonad maturity of *P. argus*, morphological observations show apparent differences in color and size. This is due to the characteristics of gonad development in fish, where gonad morphology changes with maturity (Brown-Peterson et al 2011). The synchronization of gonad maturity stages between males and females in *P. argus* is an adaptation to maximize reproductive success. Environmental factors such as temperature, salinity, and food availability simultaneously affect the reproductive regulatory mechanism (Munro et al 1990). This pattern is often found in tropical fish with a short spawning season, such as *P. erumei* (Tresnati 2001) in Spermonde waters. However, in flatfish species living in subtropical and warm climates with a more extended spawning season, such as *Pleuronectes platessa* (Horwood et al 1986), the synchronization of gonadal maturity stages between males and females may not be so tight. At the peak of the spawning season, there are always males and females of *P. erumei* that spawn simultaneously or synchronously spawning between males and females; however, not all males and females spawn at the peak of the spawning season because there are always individuals that are at maturity stage III that will spawn later or outside the peak of the spawning season (Adela et al 2016; Tresnati 2001).

The increase in the percentage of MS V from September to December indicates that *P. argus* spawns during these four months. The spawning season of *P. argus* in Inhaca Island, Mozambique, occurs from September to December (Van Schie & De Boer 2003). This season is similar to the spawning season of *P. argus* in the Spermonde Islands. *Tanakius kitaharai*, another species of flatfish, spawns during this period, with its peak spawning in December (Narimatsu et al 2007). Various factors influence the gonad maturation process. Gonadotropin-releasing hormone, estrogen, and testosterone are the main factors contributing to gonad development (Wootton & Smith 2014; Grieshaber et al 2016; Zou et al 2022). In addition, ideal food availability affects gonad development and maturity (Smith et al 1999; Furuita et al 2000; Narimatsu et al 2007). The differences in gonad development are influenced by food availability, temperature, and salinity during a single reproductive season (Ji et al 1997; Furuita et al 2000; Lowerre-Barbieri 2009; Barnes 2015).

Male flatfish, such as halibut and flounder, are usually larger than females. Differences in reproductive strategies, food availability, competition with males, and growth hormones can cause the variations in he number of eggs produced by females per spawning. Females produce many eggs, thus requiring more energy and resources, and females have larger bodies, which increases the chances of reproductive success. In addition, females spend more energy on reproduction compared to males. Males often compete for access to females; therefore, with a smaller body size, males can compete better and fertilize more eggs. Females require a more significant amount of food to support their growth and reproduction. Smaller males may benefit from natural selection when food resources are limited. Different levels of growth hormones between males and females may influence the growth rate and final body size (Ricker 1975; Fairbairn 1997; Rijnsdorp et al 2014; Freitas et al 2024). Differences in growth rate, age at first gonadal maturity, and metabolism may also contribute to size differences between males and females (Bouain et al 2018).

Gonadosomatic index*.* With higher fluctuations in the GSI in females compared to males, female gonadal development is more dynamic. The high GSI in March indicates a period of active gonadal development leading up to spawning. The hypothesis that *P. argus* spawns under the best environmental conditions to support the survival of larvae and juveniles is supported by the decrease in GSI in May as the dry season begins. This reproductive strategy is common in tropical fish, where spawning occurs during seasons with high food availability and stable environmental conditions (Blaber 2000). Fluctuations in the GSI and correlation with the dry season in *P. argus* indicate that this species spawns under ideal environmental conditions. This pattern is similar to that found in Indian parrotfish *Chlorurus capistratoides* in Spermonde Islands (Tuwo et al 2020a).

P. argus has a high GSI from February to August, similar to that found in *P. erumei* and *P. arsius*, which have high GSI from April to September (Ramanathan & Natarajan 1979). Spatial differences can cause high differences in the period (temporal) of GSI (Midway et al 2024). These two ecological scales, spatio-temporal, greatly influence the life history of aquatic organisms (Tuwo et al 2025).

The increase in GSI of *P. argus* when water temperatures increase or during the summer in tropical areas is a sign of gonadal maturation. Similar that occurs in Pleuronectidae, which live in warm areas in the northeastern Bering Sea (TenBrink & Wilderbuer 2015). This increase in GSI may be related to temperature because temperature can increase metabolism and hormones, both of which are responsible for the role of gonadal maturation (Wang et al 2019; Zou et al 2022).

The decrease in GSI from September to December suggests that *P. argus* breeds from the end of the dry season to the beginning of the rainy season when sea surface temperatures decrease and rainfall increases. The prolonged decrease in GSI in females suggests that the reproductive strategy of females is more complex and may involve multiple spawnings in a year or spawning period. *P. erumei*, another flatfish species, also uses this reproductive method (Hussain 1990).

Length at first maturity. Males and females have almost the same growth rate and gonadal development in the early stages of life, as they reach first sexual maturity. Several other flatfish species, such as *Scophthalmus maximus*, have this pattern (Imsland et al 1995). However, the size of first sexual maturity between males and females can be very different in some species, especially those that show apparent sexual dimorphism (Devlaming et al 1982). In fisheries management, data on the size of first sexual maturity is critical because it can be used as a reference for determining the allowable catch size (Austin et al 2008; Saputra et al 2009; Lappalainen et al 2016; Koh et al 2024).

At first maturity, *P. argus* is more prolonged (25.2 cm in males and 27.6 cm in females) than *P. erumei* (20-20.6 cm) (Adela et al 2016). However, the length of *P. argus* at first maturity is smaller than other flatfish species, *P. arsius* (35 cm) (Bawazeer & AL-Baz 1990) and female *P. elevatus* (42 cm) (Hashemi et al 2013).

The length of male *P. argus* at first maturity is shorter than *P. erumei* (male 30 cm, female 38.2 cm) and *Paralichthys orbignyanus* (male 30 cm, female 38.5 cm) (Coulson & Poad 2021; Ghanbarzadeh et al 2021). This difference in length may be due to the more significant energy requirements of females to reach gonad maturity and produce high-quality eggs (Alam et al 2013; Gibson et al 2014). Factors such as variations in temperature and salinity levels in the environment may also cause differences in the LFM (Ahmed et al 2010; Ali Ben Smida et al 2014).

Conclusions. Based on time, gonad maturity level, and size class, *Pseudorhombus argus* in Spermonde waters has a female-dominated sex ratio. Females have larger body sizes than males. Morphologically, the gonad maturity stage of *P. argus* shows differences between females and males. The highest gonadosomatic index was recorded in April for males and in March for females. For females, the length at the first maturity occurred at 20.70 cm, and for males, 20.25 cm. This study is an important first step in understanding the ecology and economic value of this flatfish species. It will inform more effective conservation efforts and, ultimately, ensure the survival of *P. argus* for current and future generations in the Spermonde habitat. Utilizing the results of this study in the most effective way possible will be essential to achieving the stated goals.

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Conflict of interest. The authors declare that there is no conflict of interest.

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