

## REPRODUCTIVE STATUS OF MUDSKIPPERS (FAMILY GOBIIDAE) IN THE KARANGTALUN MANGROVE FOREST, CILACAP

### Status Reproduksi Ikan Gelodok (Famili: Gobiidae) di Kawasan Hutan Mangrove Karangtalun Cilacap

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#### ABSTRACT

Mudskipper is a typical and unique fish in mangrove areas that is important for balancing mangrove ecosystems (keystone species) so it is necessary to have the availability of this fish in nature, namely by the process of reproduction. The purpose of this study was to determine the reproductive characteristics (TKG, IGS, sex ratio, and fecundity) and analyze the histology of the gonads of spadefish found in the Karangtalun Mangrove Forest Area. This research method includes purposive sampling method to determine research stations based on location characteristics. The mudskipper obtained were measured for length, weight, sex identification, dissection, gonad weighing and egg count, and Hemactoxilin-Eosin staining (Histology). The number of skinks found at both stations was 119 with a sex ratio of males and females categorized as balanced at 1.13:1. TKG of male and female mudskipper are I-IV with IGS values of 0-1.9% (males) and 0-4.7% (females). Fecundity values were categorized as medium to high with TKG III (4,088 ± 2,680 grains) and TKG IV (10,142 ± 2808 grains). Histology of TKG I (spermatogonium and primary spermatocytes), TKG II (primary and secondary spermatocytes), TKG III (secondary spermatocytes and spermatids), and TKG IV (spermatids and spermatozoa). Meanwhile, female octopus TKG I (primary oocytes), TKG II (secondary oocytes), TKG III (secondary oocytes and ootids), and TKG IV (ootids).

**Keywords:** Cilacap, Mangrove Forests, Mudskippers, Reproductive Histology

#### ABSTRAK

Ikan gelodok merupakan ikan khas dan unik di area mangrove yang penting bagi penyeimbang ekosistem mangrove (*keystone species*) sehingga perlu adanya ketersediaan ikan ini di alam yaitu dengan proses reproduksi. Tujuan penelitian ini yaitu mengetahui karakteristik reproduksi (TKG, IGS, rasio kelamin, dan fekunditas) serta menganalisis histologi gonad ikan gelodok yang ditemukan di Kawasan Hutan Mangrove Karangtalun. Metode penelitian ini meliputi metode *purposive sampling* untuk menentukan stasiun penelitian berdasarkan karakteristik lokasi. Ikan gelodok yang didapatkan akan dilakukan pengukuran panjang, berat,

identifikasi kelamin, pembedahan, penimbangan gonad, dan penghitungan jumlah telur, serta pewarnaan Hematoksin-Eosin (histologi). Jumlah ikan gelodok yang ditemukan di kedua stasiun sebanyak 119 ekor dengan rasio kelamin jantan dan betina dikategorikan seimbang yaitu 1,13:1. TKG ikan gelodok jantan dan betina yaitu I-IV dengan nilai IGS 0-1,9% (jantan) dan 0-4,7% (betina). Nilai fekunditas dikategorikan sedang sampai tinggi dengan TKG III ( $4.088 \pm 2.680$  butir) dan TKG IV ( $10.142 \pm 2808$  butir). Histologi ikan gelodok jantan TKG I (spermatogonium dan spermatis primer), TKG II (spermatis primer dan sekunder), TKG III (spermatis sekunder dan spermatid), dan TKG IV (spermatid dan spermatozoa). Sedangkan, ikan gelodok betina TKG I (oosit primer), TKG II (oosit sekunder), TKG III (oosit sekunder dan ootid), dan TKG IV (ootid).

**Kata Kunci:** Cilacap, Histologi Reproduksi, Hutan Mangrove, Ikan Gelodok

## INTRODUCTION

The Karangtalun Mangrove Forest Area is a forest area inhabited by various types of mangroves and has been managed since 1978, covering an area of 10 hectares (Suryadi *et al.*, 2022). The Karangtalun Mangrove Forest Area receives brackish water from the Lester River (Ikhtiangung and Utami, 2020) and is connected to the Segara Anakan estuary (Hastuti *et al.*, 2024). The Karangtalun Mangrove Forest Area is characterized by its proximity to residential areas, fish farming areas, fishing grounds, and the Holcim cement factory. The Karangtalun Mangrove Forest Area serves many important ecological functions, including as a habitat, spawning ground, nursery ground, and feeding ground for various species of fish, crustaceans, shrimp, and other biota (Saraswati and Rahmawati, 2023).

One of the fish species inhabiting the Karangtalun Mangrove Forest Area is the mudskipper. The mudskipper can live in both terrestrial and tidal waters, and is a species found near mangrove forests with muddy substrates (Sunarni and Maturbongs, 2017). Mudskippers adapt to their habitat by walking in mud, climbing mangrove roots, and breathing through their skin. From a mangrove forest perspective, mudskippers play a crucial role in maintaining the balance of mangrove ecosystem populations in the food chain, acting as primary consumers (predators) and secondary consumers. Therefore, maintaining a balance between mudskippers is crucial (Sujono and Muzaki, 2021).

Studies on mudskipper reproduction in the Karangtalun Mangrove Forest Area are still very limited. This is due to a lack of public awareness of the mudskipper's economic value, and the fish's lack of inclusion on the list of economically valuable fishery commodities in Indonesia. Studying mudskipper reproduction is important because it can provide an overview of the mudskipper population by understanding the spawning season. According to Nasution *et al.* (2016) important reproductive aspects of the mudskipper are sex ratio, gonad maturity, and fecundity. Furthermore, gonad maturity is carried out to determine the stages of gonad maturity so that it is possible to determine the status of whether the fish will or has spawned and the number of eggs is carried out for the management of fishery resources, namely population dynamics and in the production of cultivated seeds. Therefore, further research is needed on the reproduction of mudskipper fish, one of which is the mudskipper fish in the Karangtalun Mangrove Forest Area because reproduction is the process of producing new individuals in an area as a basic material for determining the mudskipper fishing season, conservation activities, fishing activities, and sustainable management.

## RESEARCH METHODS

This research was conducted from January to February 2025. Analysis of the reproductive characteristics of the mudskipper was conducted at the Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University, while histological

analysis of the mudskipper gonads was conducted at the Laboratory of the Faculty of Biology, Jenderal Soedirman University. Mudskipper samples were collected from local fishermen in the Karangtalun Mangrove Forest Area, Cilacap. The location was then divided into two stations using a purposive sampling method, observing the characteristics shown in Table 1.

Table 1. Location and Characteristics of the Research Site

Location	Characteristics	Coordinate
Station 1	Located near fish ponds, mangrove forests, and rice fields.	7°40'00.05"S; 109°1'19.18"E
Station 2	Located in brackish waters close to mangrove forests, residential areas and cement factories.	7°40'32.10"S; 109°0'46.85"E

The tools needed in this study are a digital scale with an accuracy of 0.01 g (fish body weight), a digital scale with an accuracy of 0.001 g (fish gonad weight), surgical scissors, gonad bottles, counters, electronic microscopes, embedding cassettes, slides, incubators, BOD bottles, rulers, and Erlenmeyer flasks. Meanwhile, the materials needed are mudskipper samples, water samples, 10% NBF, paraffin, entelan, 70% absolute alcohol, and chemicals for DO titration. The workflow in this study can be seen in Figure 1, which begins with determining the location and station, taking mudskipper samples, and taking water samples (temperature, pH, salinity, and DO) in situ.

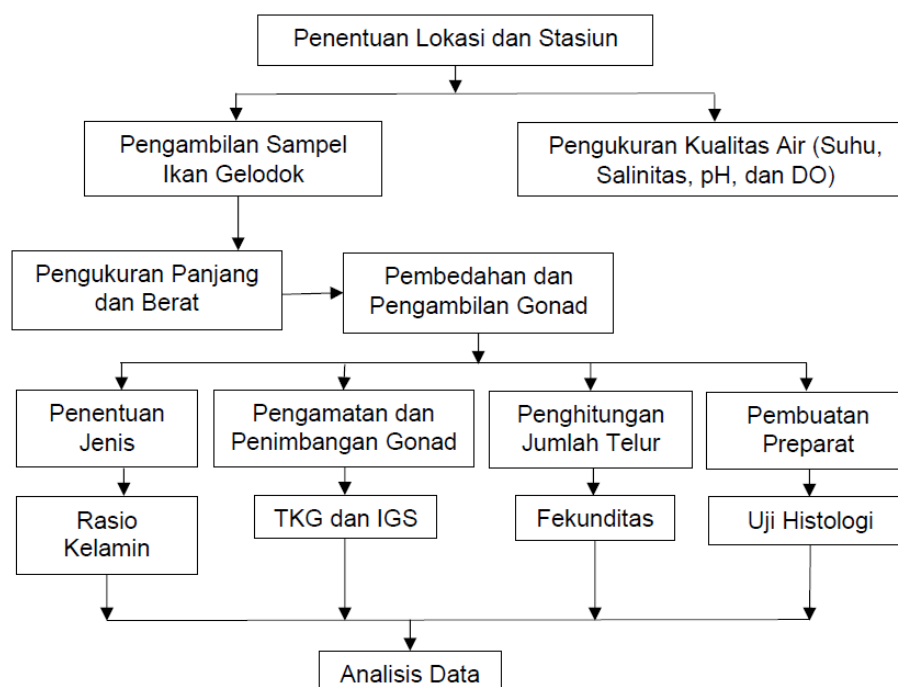


Figure 1. Research Flowchart

### Sex Determination, Surgery, and Gonad Measurement of Mudskipper Fish

The mudskippers will be weighed and their total length measured, then their sex will be identified based on the papilla. The fish will then be dissected to remove the gonads, re-identify the sex, and record the number of each sex. Fish gonad measurements consist of measuring the whole weight (Somatic Gonado Index analysis), the number of eggs in the female gonad, and weighing the partial weight (fecundity). The gonads will then be observed

for development to determine the level of gonad maturity macroscopically (Pradana *et al.*, 2021) and histologically (Sjafei *et al.*, 2017) and (Ray *et al.*, 2023) by making slides.

### Preparation of Histology Preparations

The gonad samples of mudskipper used for histological testing were one gonad from each male and female at each stage. The steps for preparing histological slides of mudskipper are shown in Figure 2.

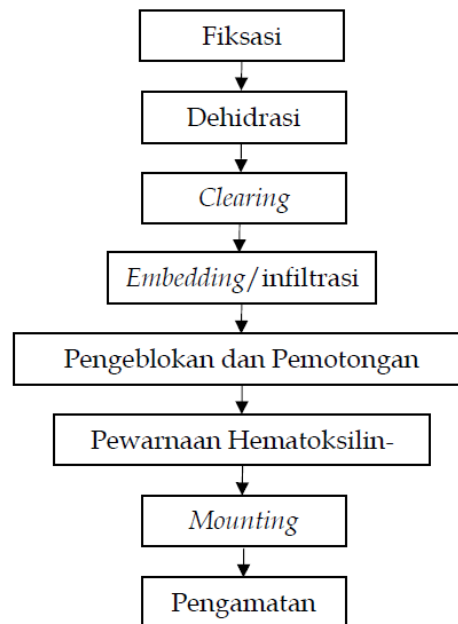


Figure 2. Flowchart for Making Histological Preparations of Mudskipper Fish

The preparation process is based on research by Hutagalung *et al.* (2015) and Zulfadhli *et al.* (2016) with the first step being fixation, which is intended to stabilize tissue elements and preserve gonads for testing. Fixation using 10% NBF, then fixation for 24 hours. Then, the dehydration process aims to remove the remaining fixative contained in the tissue. This process uses graded alcohol, namely 70%, 80%, 90% alcohol, and absolute I and II for 30 minutes. Then, clearing with a ratio of xylol: alcohol, namely 1:3, 1:1, and 3:1 for 20 minutes each and absolute xylol I and II for 30 minutes each. After that, the embedding/infiltration process (hardening the tissue) by infiltrating the tissue into liquid paraffin. This process is carried out in an incubator at 60°C using paraffin:xylol (1:3, 1:1, and 3:1 for 20 minutes), paraffin: absolute I (for 40 minutes), and paraffin: absolute II (for 60 minutes). Then, the paraffin block is dried and sectioned using a rotary microtome at a thickness of 6 microns.

The next step is the staining stage, which aims to clearly see the elements in the tissue so they can be identified. Staining using Hematoxylin-Eosin (HE) begins with a deparaffinization process with xylol II and I for 2 minutes to remove the paraffin. The next step is dipping in absolute alcohol II, I, 90%, 80%, 70% alcohol, and distilled water (solvent) for 30 dips each (rehydration) to incorporate water. Then, the stage of staining to Hematoxylin solution for 1.5 minutes, then wash with running water and Eosin for 0.5 minutes, then wash with water and soak with distilled water and alcohol graded from 70%, 80%, 90%, absolute I, and II (dehydration). Then, the final process is clearing by soaking in xylol I and II for 2 minutes and immediately given entelan on the slide so that the sample does not dry or blacken (mounting) and read the preparation with a microscope.

## Data Analysis

### Somatic Gonado Index (SGI)

The somatic gonado index (SGI) value was calculated using the Effendie (1997) formula in (Mahmudah et al., 2019) as follows:

$$IGS = \frac{BG}{BT} \times 100\%$$

#### Description:

BG = Gonad weight (grams)

BT = Body weight (grams)

### Sex Ratio

The sex ratio is used to determine the balance between male and female fish in an area and is considered balanced if the value is 1:1 (Sangadji et al., 2023). The sex ratio can be calculated using the following formula, based on Effendie (1997) in Sari et al., 2019:

$$SR = \frac{A}{B} \times 100\%$$

#### Description:

SR = Sex ratio (male to female)

A = Number of male/female fish species

B = Total number of fish found (tails)

### Fecundity

Fecundity is analyzed using gravimetry. Gravimetry is the calculation of gonad weight based on the difference between the weight of the female before and after spawning (example gonads). Furthermore, weight is measured and the number of partially laid eggs is calculated (Rozikin, 2022). A high fecundity value indicates high productivity in an area or a relatively high number of eggs produced by the female. Fecundity is calculated using the formula from Effendie (1997) as cited in Djumanto et al. (2012):

$$F = \frac{BG}{BS} \times Fs$$

#### Description:

F = Fekundity

BG = Whole gonad weight (grams)

BS = Partial gonad weight (grams)

Fs = The number of eggs in the gonads is partial

Fecundity indication based on Musick (2020) in (Jafar & Umar, 2021)

< 10 points: very low









10-100 grains: low

100-1000 grains: medium

> 1000 grains: high

## RESULT

Table 2. Visualization of Gonad Maturity Level (TKG) of Gelodok Fish

Gonad	TKG I	TKG II	TKG III	TKG IV	TKG V
Male					-
Female					-

Description:

- : Not found in the Karangtalun Mangrove Forest Area

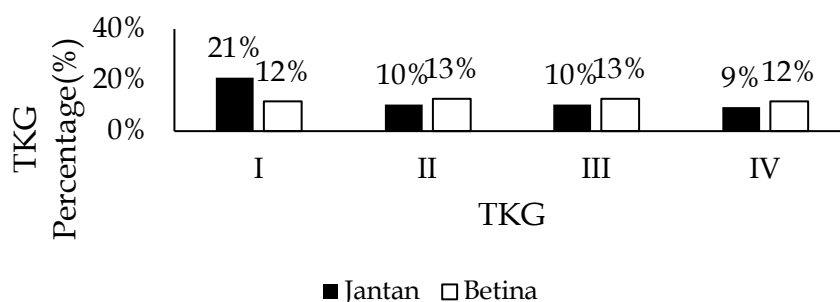


Figure 3. Percentage of the Number of Mudskippers per TKG at Station 1

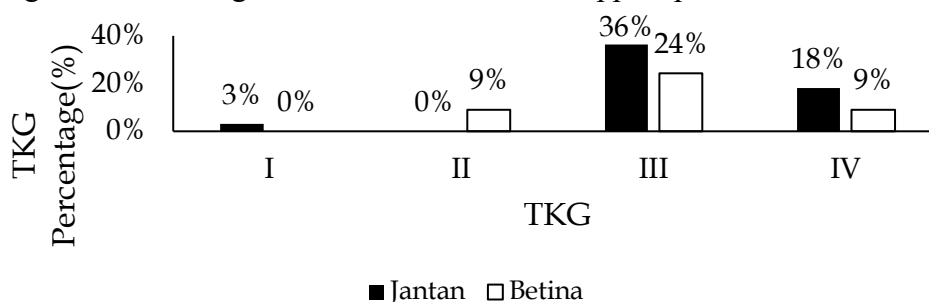


Figure 4. Percentage of the Number of Mudskippers per TKG at Station 2

Table 3. Size and Gonad Maturity Level (TKG) of Gelodok Fish

No.	Range of Body Length of Mudskipper Fish (cm)	TKG	Number (tail)			
			Station 1		Station 2	
			Male	Female	Male	Female
1.	5,8-8,7	I	9	9	0	0
		II	0	0	0	0
		III	6	3	0	0
		IV	0	0	0	0
		V	0	0	0	0
2.	8,8-11,7	I	8	1	1	0
		II	0	0	0	3
		III	2	1	5	7
		IV	2	2	1	2
		V	0	0	0	0
3.	11,8-14,7	I	1	0	0	0
		II	6	10	0	0
		III	0	5	7	1
		IV	5	7	2	1
		V	0	0	0	0
4.	14,8-17,7	I	0	0	0	0
		II	3	1	0	0
		III	1	2	0	0
		IV	1	1	3	0
		V	0	0	0	0

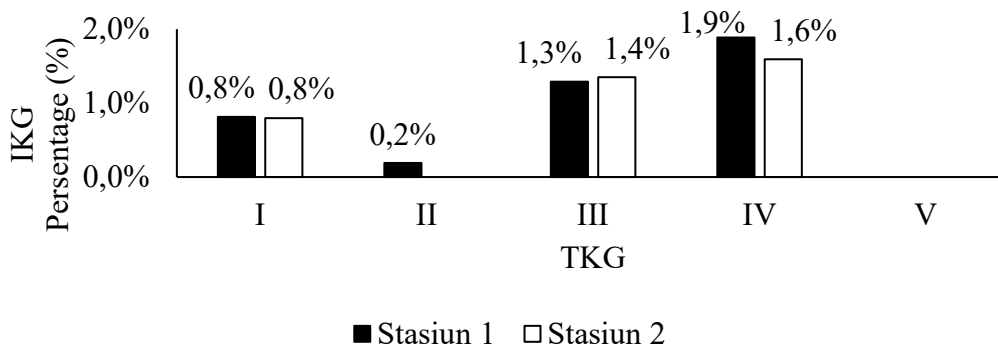


Figure 5. Average Percentage of IGS of Male Mudskippers

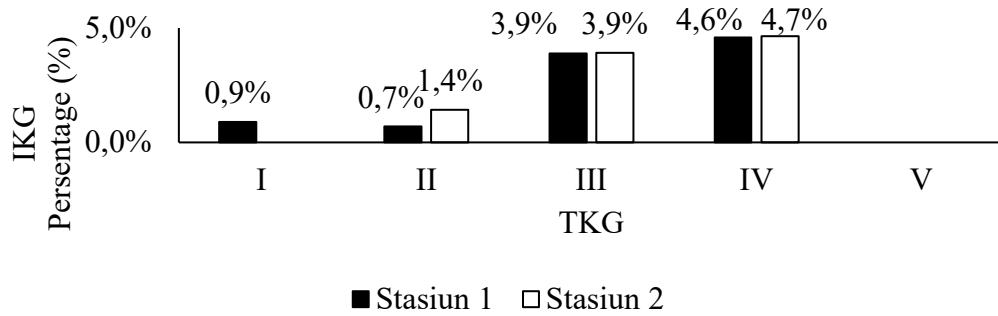


Figure 6. Average Percentage of IGS of Female Mudskippers

Table 4. Papillae of Male and Female Mudskippers in the Karangtalun Mangrove Forest Area

Gender	Genus <i>Oxyurichthys</i>	Genus <i>Boleophthalmus</i>
Male		
Female		

Caption: The arrow indicates the papilla of the mudskipper.

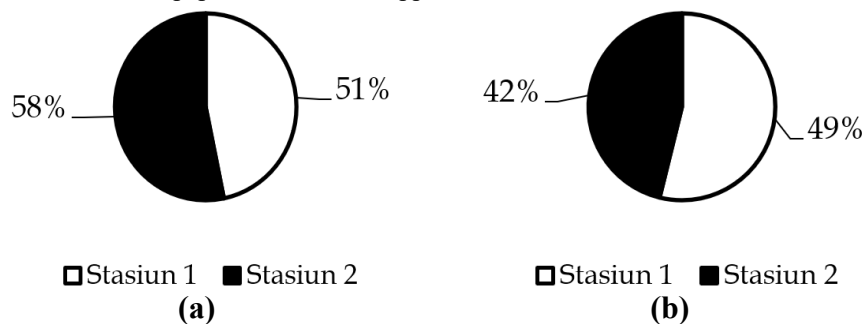


Figure 7. Percentage of the Sex of Mudskipper Fish (a) Male and (b) Female

Figure 7 shows that at station 1, the percentage of male mudskippers was 51% and female mudskippers 49%. Meanwhile, at station 2, the percentage of male mudskippers was 58% and female mudskippers 42%. The percentages at both stations are still considered balanced and are considered favorable for fertilization, although the percentage is dominated

by male mudskippers. The results of the sex ratio calculation and the Chi-Square test are presented in Table 5 below.

Table 5. Sex Ratio of Mudskippers

Station	Total (tail)	Number (tail)		Sex Ratio	X <sup>2</sup> dan X <sup>2</sup> tabel (SK 95%, dB=1)
		Male	Female		
1	86	44	42	1,05:1	0,05<3,84
2	33	19	14	1,36:1	0,76<3,84
Total	119	63	56	1,13:1	0,41<3,84

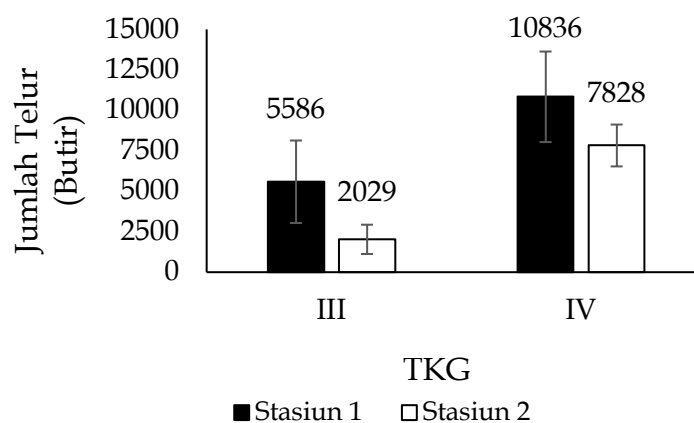


Figure 8. Average Fecundity of Female Mudskipper

## DISCUSSION

Based on the research results, it appears that the larger the body length of the mudskipper, the higher the gonad maturity level. The smallest size group (5.8-8.7 cm) was most frequently found at TKG I, indicating that the fish were still in the immature gonad stage. Meanwhile, fish measuring 8.8-11.7 cm and 11.8-14.7 cm began to be found at TKG II-IV, indicating that gonad development occurs concurrently with fish length growth. The most common size group (14.8-17.7 cm) was found at TKG IV (most mature) before spawning. According to Effendi (2002) in Senen and Deles (2017), there is a general positive correlation between fish body length and gonad maturity stages, which is expected to increase with increasing body length.

The IGS value (Figures 5 and 6) in this study was <20%, which is consistent with the research by Ridho *et al.* (2021) with the IGS values of male mudskippers in TKG I-V respectively 0.59-0.36%; 0.25-0.36%; 0.26-0.38%; 0.4-2%; 0.13-0.26%, while the female fish respectively 0.46-0.52%; 0.35-3.16%; 3.17-4%; 4-6%; 1.13-0.26%. An IGS value <20% can be interpreted as meaning that mudskippers spawn more than once per year (Mahmudah *et al.*, 2019). This statement is supported by Abdullah (2012) in Sari *et al.* (2021) found that mudskippers of the Gobiidae genus spawn twice a year, around January-May and August-December, with peak spawning occurring during the rainy season. Yustina and Arnentis (2001) in Wardani (2016) also stated that fish living in tropical areas generally spawn more than once per year, with relatively low IKG values (<20%).

Table 5 shows that the sex ratios of male and female mudskippers at stations 1, 2, and the total (male and female) were 1.05:1; 1.36:1; and 1.13:1, respectively. These differences are due to the greater number of male mudskippers caught than females. However, these values can still be categorized as balanced, or 1:1 (based on the sex ratio results), indicating an equal

number of male and female mudskippers in the Karangtalun Mangrove Forest Area. According to Effendie (1979) in Novandi *et al.* (2020), if the ratio between male and female mudskippers is still balanced, it means that the spawning process (fertilization of eggs by sperm) is also occurring in balance. This balanced spawning process is expected to increase the mudskipper population in an area and will also increase during the mating season (Damayanti *et al.* (2023). This balance also means that one male mudskipper will fertilize one female mudskipper.

Based on Figure 8, the average fecundity value at station 1 is higher than that at station 2, which is suspected because generally, mudskippers at station 1 have larger body size (length and weight) and IGS values (Figures 5 and 6) than at station 2. Effendi (2002) in Erni *et al.* (2018) stated that the number of eggs produced by female broodstock will vary due to the varying size of the female fish. Erni *et al.* (2018); Soekiswo *et al.* (2014) also added that there is a positive relationship between the size (length and weight) of female fish and the number of eggs produced. The heavier the fish, the more eggs they will produce. This is because larger fish usually have larger ovaries, and this is also reflected in the IGS value. Based on location characteristics, station 1 is close to ponds and mangrove forests, which provide a habitat rich in food and shelter, thus optimizing growth and reproduction (Rahmawati *et al.*, 2023). Furthermore, station 2 is adjacent to a cement factory, which may contribute to pollution or habitat degradation. Research conducted by Hapsari (2015) states that there are pollutants, namely heavy metals, such as iron (Fe) and manganese (Mn), which can disrupt the growth of mudskippers.

Figure 9. (a) shows that at maturity level I in male mudskippers, spermatogonia and primary spermatocytes can be seen. This statement is in accordance with the statement of Dinh *et al.* (2022) that TKG I male gonads are characterized by visible spermatogonia and those that have developed into primary spermatocytes. Figure 9. (b) is TKG II with dominant characteristics of primary spermatocytes and secondary spermatocytes, although they are still few in number. According to Dinh *et al.* (2022) and Yanti *et al.*, (2023) that TKG II male gonads are dominated by primary spermatocytes because spermatogonia develop into primary spermatocytes and begin to develop a few secondary spermatocytes. Figure 9. (c) shows many secondary spermatocytes and spermatids are seen which are suspected to be male gonads with TKG III. According to research by Dinh *et al.* (2022) TKG III male gonads are characterized by the presence of primary spermatocytes that develop into secondary spermatocytes and spermatids are found. Figure 9. (d) suspected TKG IV male gonads because there are many spermatozoa and spermatids that are suspected to be TKG IV. Research by Dinh *et al.*, (2022) states that male gonads with TKG IV can be identified by the presence of spermatids that fill the tubules.

## CONCLUSION

The number of mudskippers found in the Karangtalun Mangrove Forest Area, Cilacap was 86 (station 1) and 33 (station 2). The sex ratio of male and female mudskippers found was categorized as balanced, namely 1.13: 1. The TKG of male mudskippers found in the Karangtalun Mangrove Forest Area was level I-IV with an IGS value of 0-1.9% (male) with a body weight ranging from 14.65±8.57 g. The TKG of female mudskippers was found at level I-IV with an IGS value between 0-4.7% and a body weight ranging from 16.02±9.79 g. The average fecundity value of female mudskippers at station 1 with TKG III was 5,586 ± 2,548 eggs and TKG IV was 10,836 ± 2,797 eggs, while at station 2 with TKG III it was 2,029 ± 905 eggs and TKG IV was 7,828 ± 1,293 eggs. The histology of male mudskippers was seen in TKG I (spermatogonia and primary spermatocytes), TKG II (primary spermatocytes and secondary spermatocytes), TKG III (primary spermatocytes and spermatids), and TKG IV (spermatids and spermatozoa). Meanwhile, female mudskippers were seen in TKG I (primary

oocytes), TKG II (secondary oocytes), TKG III (secondary oocytes and ootid), and TKG IV (ootid).

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