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Research Article

Reproductive Cycle, Size at Maturity and Fecundity of *Giuris margaritaceus* in Limboto Lake

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Abstract

Understanding fish reproduction biology, particularly sexual maturity, the reproductive cycle, and fecundity, is useful for managing fisheries. The reproduction of *Giuris margaritaceus* in Limboto Lake is regrettably unknown. Over a year, fish specimens (N = 662) were gathered. They ranged in length from 6.5 to 20.9 cm (mean SD 10.9±2.5). Fecundity varied from 30,057 to 61,920. The first sexual maturity of male and female fish occurred at different sizes, 11.2 cm and 10.3 cm, respectively. This information is needed to determine this fish's lowest acceptable harvest length under the management aim to enter all-female fish for reproduction at least once. We estimated the reproductive of *G. margaritaceus* cycle using the size of the oocytes, histological analysis of the ovaries, and monthly mean GSI. These techniques produced the same reliable conclusion: *G. margaritaceus* can spawn yearly, with maximal between September and October. Based on this finding, spawners of *G. margaritaceus* can be kept from entering the reproductive stock between September and October. Future efforts to fine-tune fishing efforts for the effective management of *G. margaritaceus* may be aided by the study's findings.

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1. Introduction

The *Giuris margaritaceus* is one of the original fish in the waters of Lake Limboto, a member of the Eleotridae family (Lamadi *et al.,* 2023), which is known to consist of 40 genera and 150 species (Larson, 2019). Local people use this fish for consumption, and some make it ornamental. This fish has a high selling price, the price in the traditional Gorontalo market ranges from 0.3-1.0 US dollars per head. The population of this natural species is declining rapidly due to habitat degradation. But so far, there has been no significant progress towards bringing these species into the culture in Gorontalo or restoring their natural habitat within a conservation framework. Several studies on the biological aspects of this fish have been carried out both in Limboto Lake and in other locations in Indonesia, including the relationship between length and weigh, biological aspects, DNA Barcoding, species description and sexual dimorophism (Makmur *et al.,* 2019; Auliyah, 2019; Keith *et al.*, 2021; Ndobe *et al.,* 2023; Lamadi *et al.,* 2024). However, there is still minimal information about the reproduction of *G. margaritaceus*.

Fish conservation is an effort to protect, preserve, and utilize fish resources, including ecosystems, species, and genetics, to ensure the existence, availability, and continuity of fish species for future generations. Several conservation strategies are commonly implemented, including sustainable management and fishing, fish habitat management, and fish cultivation. Studying fish reproductive biology can be a conservation strategy. Studying fish reproductive behaviour is crucial for understanding population dynamics and developing effective breeding strategies. By investigating reproductive behaviour, researchers can gain insights into various aspects that impact fish populations, such as spawning patterns, mate selection, breeding success, and parental care (Gebremedhin *et al.,* 2021). Reproductive knowledge can produce fisheries management decisions and stock recovery projects (Stoner and Appeldoorn, 2021).

Understanding fish reproduction is indeed crucial for effective fisheries management and conservation efforts. Data on size at maturity, spawning season, fecundity, and sex ratio are essential for assessing the reproductive potential of fish populations and determining the status of exploited stocks (Dinh *et al.,* 2022). These parameters provide valuable information for setting management measures such as minimum size limits, fishing seasons, and protected areas to ensure sustainable exploitation and conservation of fish stocks. By studying these reproductive characteristics, researchers and fisheries managers can make informed decisions to maintain healthy fish populations and ecosystems (Hasan *et al.,* 2020).

The first maturity is the stage at which an individual organism reaches sexual maturity for the first time and is capable of reproduction. It is an important biological parameter used in fisheries management to understand the reproductive potential of a population and to inform sustainable harvesting practices (Prince *et al.,* 2020). The size at first maturity is a key metric in fisheries science and conservation biology, providing insights into reproductive potential, guiding management decisions, assessing population health, and supporting conservation efforts for sustainable fish populations (Gebremedhin *et al.,* 2021). Knowledge of the various stages of fish gonad synthesis provides essential information needed to stop fishing during the reproductive period to improve the recovery of fish stocks in nature (Rahman and Samat, 2021).

Developed a simulation model to convey how reproductive trait variations impact fish populations' capacity to survive and recover from high fishing pressure (Brosset *et al.*, 2021). Knowing the reproduction of a species, it will be easier to design the breeding of a species and to interpret data in environmental monitoring programs (Barrett *et al.*, 2020). Therefore, efforts should be made to replenish lying fish stocks and increase industrial cultivation of economically important fish species such as *G. margaritaceus*. Knowledge of reproductive biology is the key to intensive maintenance of this species (Nunes *et al.*, 2021). Further research focusing on the biological aspects of reproduction is essential to enhance our understanding of how reproductive performance influences population dynamics in fish species. By conducting more studies on fecundity, size at maturity, spawning seasons, and sex ratios, researchers can gather valuable data to improve the accuracy of stock assessments and management strategies. Additionally, advancing the methods used in reproductive studies can lead to more precise estimations of reproductive parameters, which are crucial for sustainable fisheries management and conservation efforts. By expanding our knowledge of fish reproduction through continued research, we can better protect fish populations and ensure the long-term health of aquatic ecosystems (Hasan *et al.*, 2020; Gebremedhin *et al.*, 2021).

This lack of information has resulted in several areas in Indonesia, often leading to overfishing and management failure. Regulations and legislation need to be implemented to prohibit fishing of size and spawning season to prevent a cessation of fish production. Unfortunately, in Limboto Lake, there is still minimal information about the reproduction of *G. margaritaceus* fish. This study seeks to contribute to overcoming deficiencies by investigating sex ratio, reproductive cycle, sexual maturity, fecundity, and the correlation of fecundity with the length and weight of *G. maragaritaceus* over 12 months. The information from this paper can be presented as an important reference for decision-making in *G. maragaritaceus* management.

2. Materials and Methods

2.1 Materials

The materials for this research are Gilson reagent, neutral buffer formalin 10% (NBF), paraffin, Hematoxylin-Eosin (HE), alcohol, and Xylol. while the tools are Automatic Tissue Processor (LEICA TP1020), analytic scales (Kern – Jerman, Model: ADB 200-4), Automated Rotary Microtome (Médicas Myr M-250), Digital Microscope (Leica DM750), Micropipette, Micrometer, section set, tissue cassette.

2.1.1 Ethical approval

This research was conducted with the approval of the research ethics committee for the use of laboratory animals at the Faculty of Marine and Fisheries Technology, State University of Gorontalo, Indonesia. The approval is recorded under reference number 4472/UN47.B10/PT.00/2024.

2.1.2 Sample sites

This research was conducted from June 2022 to May 2023 at Lake Limboto, Gorontalo Province, Indonesia, at coordinates 0°35’2” N 122°58’48”E (Figure 1) with an average temperature of 21.6 to 34.4,

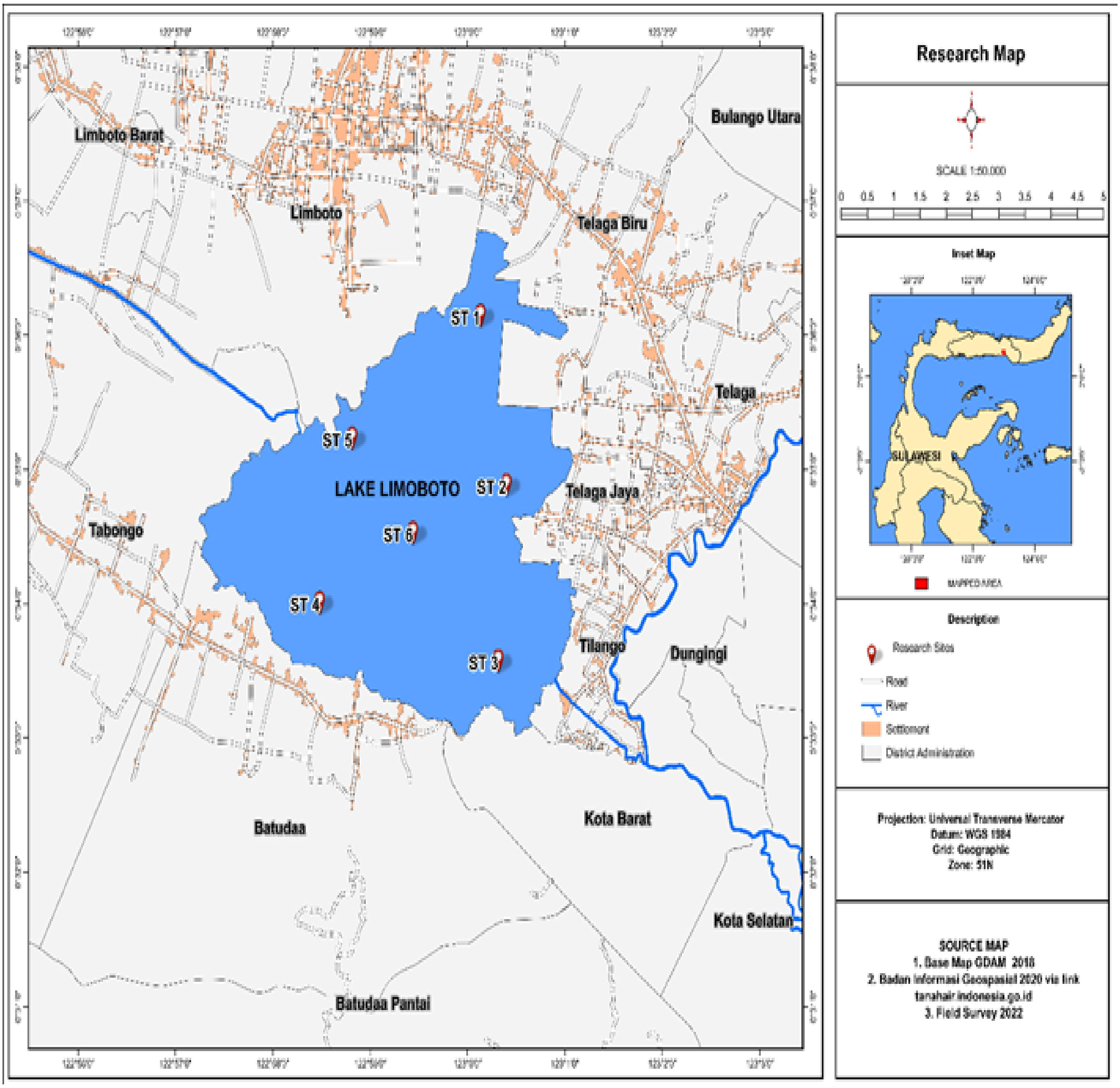

Figure 1. Observation sites surround Lake Limboto, consisting of 6 stations

rainfall of 80 to 204 mm. Fish samples were taken from 6 observation stations using gill nets with a length of 10 m, a height of 3 m and a mesh size of 1 cm.

2.2 Methods

2.2.1 Fish collection

This research has met the standards for handling living creatures, especially fish, in the euthanasia process. The captured specimens are placed in a plastic bag filled with water and oxygen and labeled to indicate the location and time. The specimen is taken to the laboratory, and then ice cubes are placed outside the plastic to reduce the temperature gradually. When the specimen is fixed, marked by a nonmoving oper culum cover, the specimen is taken to be measured in length and weight and dissected. When the fish even tually perished, the gill covers stopped moving, which was a sign of its demise. A digital caliper with an accuracy of 0.5 mm was used to measure the specimen's length, and a digital scale with an accuracy of 0.01 g was used to determine its weight (Wiadnya *et al.*, 2023).

Confirmation of species was done using an identification key by Lamadi *et al.* (2023). The gonads of each fish were collected by dissection and weighed using the same digital scale. Sex was determined via the observation of the gonads, naked eye, or microscope. The naked eye checked sexual dimorphism. Each month, ten percent of the total ovary was randomly selected in 10% phosphate-buffered formalin for histological examination, and other ovaries were preserved in Gilson's fluid to estimate oocyte diameter. Gonadosomatic index (GSI), size at first sexual maturity (SM), fecundity, oocyte size, and histological examination of the ovary were estimated as female reproductive parameters, while GSI and SM were estimated as male reproductive parameters.

2.2.2 Histological examination of ovaries

The steps in making histological preparations followed the protocol modified by Longenecker *et al.* (2020), 1) Fixation Stage: Fish organs were cut into small pieces and fixed in boudin solution (75 ml picric acid, 25 ml formaldehyde, 5 ml acetic acid) for 24 hours. 2) Dehydration and Clearing Stage: Organs that have been fixed are put into a cassette and dehydrated using graded alcohol: 70%, 80%, 85%, 85%, 100%, and 100% or 2 hours each. Clearing using xylene I, II, and III for 30 minutes each. 3) Impregnation Stage: The organs are placed in liquid paraffin I and II for 2 hours each. 4) Embedding Stage: The organ is placed in an iron mold (base mold) that has been heated on a hot plate and then filled with liquid paraffin and left until the paraffin solidifies (hardens). 5) Sectioning stage: The hardened paraffin block is sliced using a microtome with a thickness of 3-5 pm; the resulting slices are dipped in warm water at a temperature of 42-45°C until the tissue expands then placed on a glass object and dried. 6) Staining Stage: The tissue attached to the glass object is put into xylene I and II for 5 minutes each, 100% alcohol I and II for 1 minute, 95% alcohol I and II for 1 minute. The tissue was then stained with Hematoxylin for 10 minutes. The stained tissue was then put into 4 dips of distilled water, 4 dips of acid alcohol, and running water for 10 minutes. Furthermore, the organs were stained with Eosin for 2 minutes, put in 95% alcohol I and II for 2 each, 100% alcohol I and II for 1 minute each, and xylene I, II, and III for 2 minutes each. 7) After the histology preparation is ready, the development of the ovaries is observed according to the method. Histologically determining the gonad maturity levels of male and female fish using a modified method from (Burow *et al.*, 2019; Longenecker *et al.*, 2020)

2.2.3 Estimation of gonadosomatic index, size at first sexual maturity, fecundity and oocyte diameter

GSI was determined applying the formula
GSI (%)=GW/BW x 100

Where :
GW = gonad weight (g)
BW = body weight (g)

Estimation of the average first size of gonad mature fish can be estimated by separating the immature gonad groups (TKG I and II) and the gonad mature groups (TKG III, IV, and V). The method used to determine the size of the first mature gonads is the Sperman-Karber:

$$m=\left(xk+\frac{x}{2}\right)-(x\sum pi), \text{ and } antolog\ m = m \pm 1{,}96\sqrt{x^2\sum\left(\frac{(pi\ x\ qi)}{(ni-1)}\right)},$$

with *m*= the log length of fish at maturity first gonad, *xk* = the log mean the last long class of fish has been gonad maturity, *x* = the log increase length at the middle value, pi = the proportion gonadal mature fish at length class *i* with the number of fish in the *i* length interval, ni = the number of fish in the long class to *i*, *qi = 1 – pi*, and *M* = length the first time the fish matures the gonads are as big asantilogue m.

To calculate oocyte diameter, all ovaries that had been maintained in Gilson fluid were used. Ovaries that had been preserved (in Gilson fluid) and had a GSI of 2.2% were deemed mature and utilized to calculate fecundity. One hundred four ovaries in all were used to estimate fecundity. The number of eggs was counted under a stereomicroscope after a sub-sample of 0.05 g was obtained from six parts (the anterior, middle, and posterior of both lobes). Using the formula F = (Nss Wo) /Wss, the fecundity (F) was determined. Nss stands for the number of oocytes in each of the six subsamples, Wo for the overall weight of the ovary, and Wss for the combined weight of the six subsamples. The formulae F = aLb and F = aWb were used to calculate the associations between fecundity (F) body length (L), and weight (W), respectively. By applying linear regression to the logarithmically converted equations LogF = log a + b logL and LogF = log a + b logW, respectively, the constants b and R2 were found. Using a microscope attached to a digital camera installed on a computer, the size of oocytes was measured.

2.2.4 Analysis data

The significant difference between the male and female ratio was determined through the χ2 test. To determine the temporal change of the GSI, a one-way ANOVA was conducted (significant at P 0.05). If the effect was significant, the Tukey test came after the ANOVA. Before running the ANOVA, all of the data were checked to see if the parametric test would be appropriate.

3. Results and Discussion

3.1 Sex Ratio

Sex ratio values from all stations from June 2022 to May 2023 ranged from 0.1-1.9, with an average of 0.7. The highest total ratio occurred in October 2022, with a ratio of 24:17 or a sex ratio of 1.4, and the lowest was in September 2022, with a ratio of 3:38 or a sex ratio of only 0.1 (Table 1).

Table 1. Ratio of *G. margeritaceus* females by size and month from June 2022 to May 2023

	Male	Female	Total	Rasio
Month				
Jun ‘22	21	20	41	1.0
Jul ‘22	24	15	39	0.6
Aug ‘22	29	10	39	0.3
Sep ‘22	38	3	41	0.1
Okt ‘22	17	24	41	1.4
Nov ‘22	39	20	59	0.5
Des ‘22	45	17	62	0.4
Jan ‘23	53	15	68	0.3
Feb ‘23	33	26	59	0.8
Mar’23	27	21	48	0.8
Apr ‘23	56	11	67	0.2
Mei ‘23	50	33	83	0.7
Size class (cm)				
6.1-7.0	4	4	8	1.0
7.1-8.0	39	11	50	0.3
8.1-9.0	73	15	88	0.2
9.1-10.0	87	31	118	0.4
10.1-11.0	89	42	131	0.5
11.1-12.0	62	33	95	0.5
12.1-13.0	25	14	39	0.6
13.1-14.0	17	14	31	0.8
14.1-15.0	10	13	23	1.3
15.1-16.0	12	16	28	1.3
16.1-17.0	6	9	15	1.5
17.1-18.0	6	8	14	1.3
18.1-19.0	0	3	3	-
19.1-20.0	0	1	1	-
20.1-21.0	1	1	2	1.0

Sex ratio based on class intervals showed the same results as calculations based on month and observation station; namely, the number of male *G. margaritaceus* fish was more than that of female *G. margaritaceus* fish at class intervals between 7.1-14.0 cm with a sex ratio value ranged from 0.2-0.8. Different results were obtained at an interval of 6.1-7.0 cm with a sex ratio value of 1.0. The sex ratio of female *G. margaritaceus* fish gradually increases with increasing size between classes; in sizes with a class interval of 14.1-18.0, the number of female *G. margaritaceus* fish is more than the male *G. margaritaceus* fish with a sex ratio value between 1.3-1.5.

Statistical analysis of two samples T-test of male and female sex ratios based on the month of observation (June 2022-May 2023) showed a significant difference (P <0.05), meaning that the number of male *G. margaritaceus* fish every month was more than female. Meanwhile, the sex ratio of males and females based on the length class interval did not show a significant difference (P>0.05), meaning that the number of male *G. margaritaceus* fish in the long class interval was relatively the same as the number of females.

G. margaritaceus caught in Lake Limboto was dominated by fish in the 10.1-11.0 cm size class with a total of 131 individuals. Meanwhile, the number of *G. margaritaceus* fish that was the least was in the size class 19.1-20.0 with only one fish. *G. margaritaceus* caught in Lake Limboto has a smaller size than that caught in Lake Bolano Sau, based on research by Putera *et al.* (2022) *Giuris laglaizei* fish in Lake Bolano Sau are dominated by the 13.1-14.0 cm size class. *G. laglaizei* males, were found more often than females. This is thought to be caused by the activity of male fish so that they can be widely distributed in waters (Putera *et al.,* 2022). Information related to sex ratio is needed to know the sex ratio, which can predict the balance of the *G. margaritaceus* population, which is one of the factors in maintaining the natural survival of fish (Mamangkey *et al.,* 2019). This follows the statement (Gebremedhin *et al.,* 2021) identifying and addressing sex ratio imbalances in endangered populations is critical for mitigating the risk of extinction, maintaining genetic diversity, ensuring reproductive success, and supporting the long-term viability of threatened species. By prioritizing the monitoring and management of sex ratios, conservation efforts can play a vital role in safeguarding endangered populations and promoting their survival in the wild.

Male *G. margaritaceus* has more active and agile movements compared to females, so it can be well distributed in Lake Limboto, in line with the results of research by Putera *et al.* (2022) which states that male fish have higher activity than females so they can be distributed widely in waters. Furthermore, *G. margaritaceus* have the characteristic that they tend to flock together with others of their kind and of the same size. This is thought to cause variations in the size of *G. margaritaceus* caught due to the biological characteristics of the fish which are related to schooling behaviour, in accordance Kasmi *et al.* (2018) which states that generally fish will gather in groups of the same size and the same type. Because male fish are more active and like to gather in groups, they are easier to catch than female fish. Mon *et al.* (2020) stated that the dominance of one sex in a group of fish is influenced by factors that make it easier to catch one sex.

3.2 Fecundity

The fecundity varied from 30,057 to 61,921 with an overall mean of 43,756 ± 8,284 (standard deviation). Although not reported here, monthly mean fecundity of *G. margaritaceus* was statistically similar ($P > 0.05$). Fecundity (F) of fish increased with total length (TL) and total body weight (W): $F = 32.98 \times TL^{0.97}$ ($R2 = 0.64$, $P < 0.05$, $n = 37$) and $F = 62.76WG^{0.34}$ ($R2 = 0.64$, $P < 0.05$, $n = 37$) (Figure 2).

The fecundity of *G. margaritaceus*in Lake Limboto is currently higher than in the previous year, in research Auliyah and Oli'i (2018) which obtained a fecundity range of 1,266-16,866 eggs. However, based on research Makmur *et al.* (2019) in other locations, *G. margaritaceus* from Lake Tondano had a fecundity ranging from 36,892-90,102 grains with an average of 58,888. Based on the graph (Figure 1), it can be seen that the relationship between fecundity and body length and body weight of *G. margaritaceus*fish has a strong correlation because the value of r (correlation coefficient) is 0.64 which indicates that the body length and weight of *G. margaritaceusfish* affects fecundity by 64%.

Different fecundity values can reflect their reproductive strategy, where varying fecundity results from differences in adaptation to their environment. Fish spawning for the first time have low quality and quantity of eggs, which can affect their recruitment. Differences in fecundity values for each fish can be influenced by brood size. Generally, the larger the brood size, the higher the fecundity value will be (Fasya and Mufidah, 2022). This is supported by a statement Kant *et al.* (2016) stating that larger fish can produce more eggs in absolute and relative to body mass. The key pa rameter to accurately assess fecundity in a fish population is to look at the condition and size of the fish. In addition, fecundity can also be affected by factors such as fertility, spawning frequency, parental protection, egg size, environmental conditions, and population density (Rochmatin *et al.,* 2019). Fish habitats play a crucial role in the reproductive aspects of fish, as they provide the necessary environment for spawning, development of eggs, and growth of juveniles.

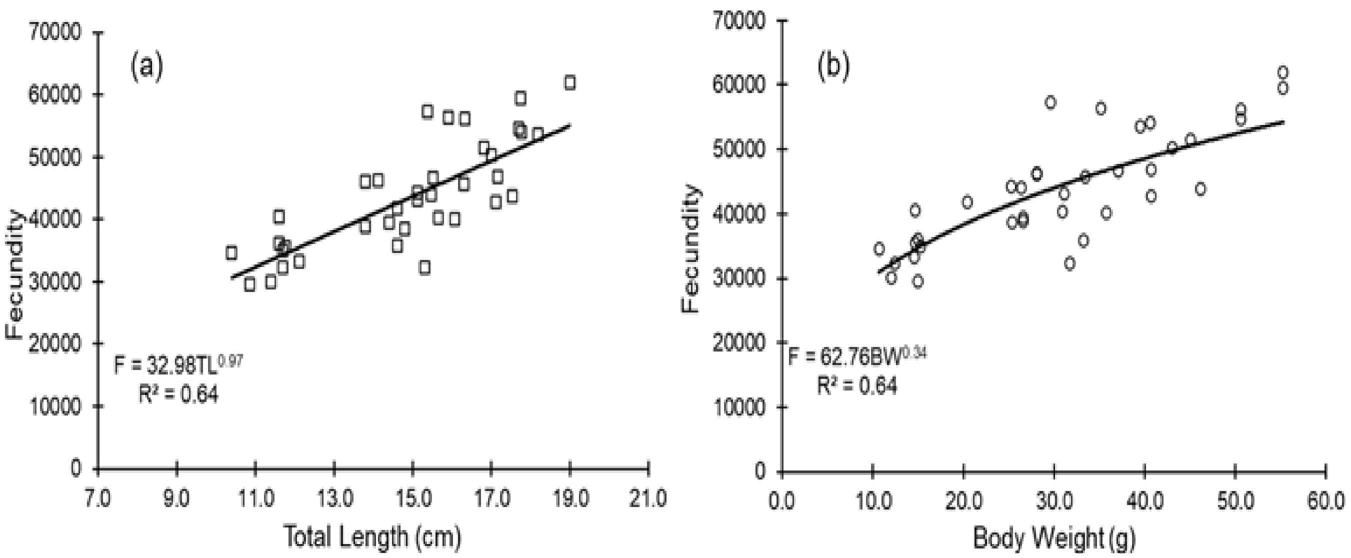

Figure 2. Relationship of fecundity with total length (a) and body weight (b) of *G. margaritaceus.*

Syarifudin *et al.* (2023) stated the quality of water, which is a significant component of fish habitats, directly influences the health and reproductive success of fish populations. Parameters such as temperature, dissolved oxygen, and the presence of toxic substances like ammonia can significantly affect the reproductive processes of fish. For instance, specific temperature ranges are essential for the optimal reproductive performance of certain fish species, as temperatures outside this range can reduce fish appetite and affect their overall health, indirectly impacting their reproductive capabilities.

3.3 Oocyte Diameter

The average monthly gonadosomatic index of male and female *G. margaritaceus* is presented in (Figure 3). Male fish had the lowest average gonadosomatic index in April 2023 and the highest average in October 2022. Male fish had the lowest average gonadosomatic index in April 2023 and the highest average in October 2022. The gonadosomatic index in male fish did not experience a significant decrease or increase. gonadosomatic index of female fish did not change significantly, and there was a constant increase from February to May 2023.

3.4 Development of Oocytes

Based on observations, the average gonadosomatic index of male *G. margaritaceus* was 1.85% lower than the average gonadosomatic index of female fish, which was 3.71%. Generally, female fish had a higher gonadosomatic index than male fish, caused by the increase in the weight of the ovaries, which is greater than the weight of the testes. Ovarian weight gain is related to gonadal development at the vitellogenesis stage, while testicular weight gain is related to spermatogenesis stages and increased semen volume in the seminiferous tubules (Kant *et al.* 2016). In addition, the low gonadosomatic index value in male fish can be caused by meager energy investment during gamete production (Rahman *et al.* 2018; Tagarao *et al.* 2020). The gonadosomatic index (GSI) value will increase with the maturation of the fish, GSI will be at its maximum during peak maturity, and it will decline suddenly (Borthakur, 2018).

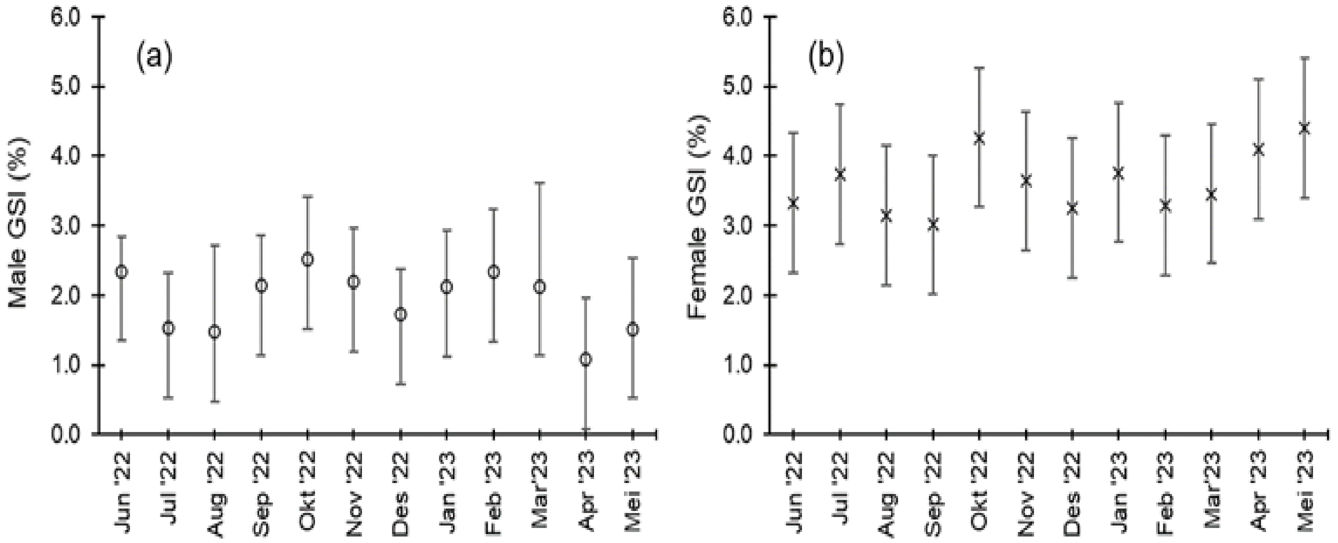

Figure 3. Monthly variation of mean GSI, gonadosomatic index (%) in male (a) and female (b) population during Juny 2022–May 2023

The increase in the male gonadosomatic index began in September 2022, and after reaching its peak in October 2022, there was a decrease in November and December 2022. After that, there was an increase in February 2023, but in March and April 2023, the male gonadosomatic index decreased again and increased in May 2023. For female fish, the lowest average gonadosomatic index was in June 2022, August 2022, December 2022, and January 2023. The highest average gonadosomatic index for female fish occurred in October 2022 and March 2023. Like male fish, the

The male *G. margaritaceus* fish has an average gonadosomatic index of 1.85% at an average length of 10.5 cm (Figure 4). Female *G. margaritaceus* fish have an average gonadosomatic index of 3.71% at an average length of 11.8 cm (Figure 4). The results of the comparison of the gonadosomatic index with the total length of the fish show that some individuals are larger and have a low gonadosomatic index value. Conversely, some individuals are smaller in size but have a high gonadosomatic index value. The relationship between these two variables is thought to be influenced by oocyte development, sampling period, and individual age because in general, fecundity will increase with increasing body size of fish (Rahman *et al.*, 2018). level III the highest frequency was in September 2022 (100%), the highest frequency of level IV in October (33%), and November (35%), and the highest frequency of level V in November 2022 (15%).

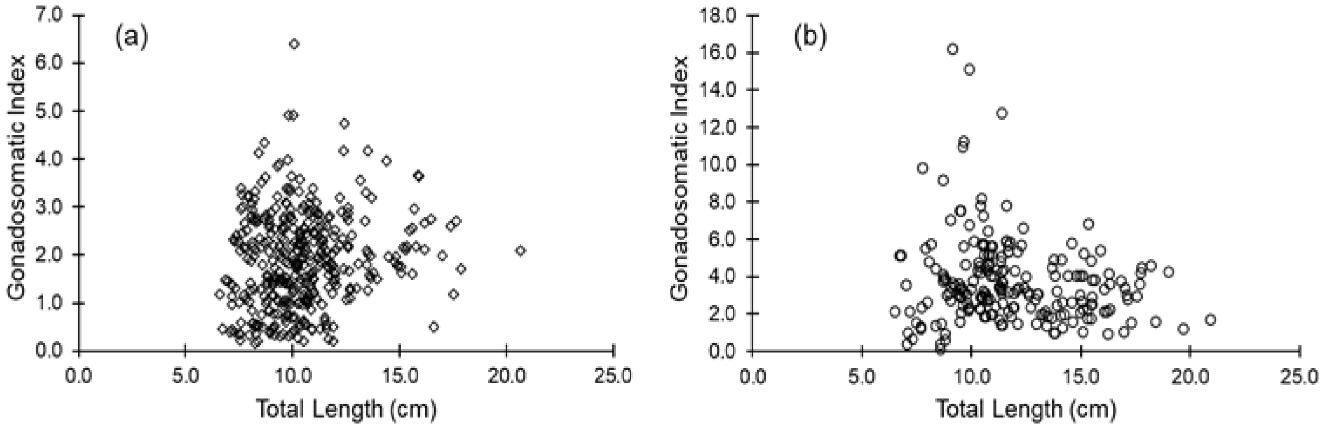

Figure 4. Relationship of gonadosomatic index (GSI) with total length of (a) male(n = 432) and (b) female (n = 215)

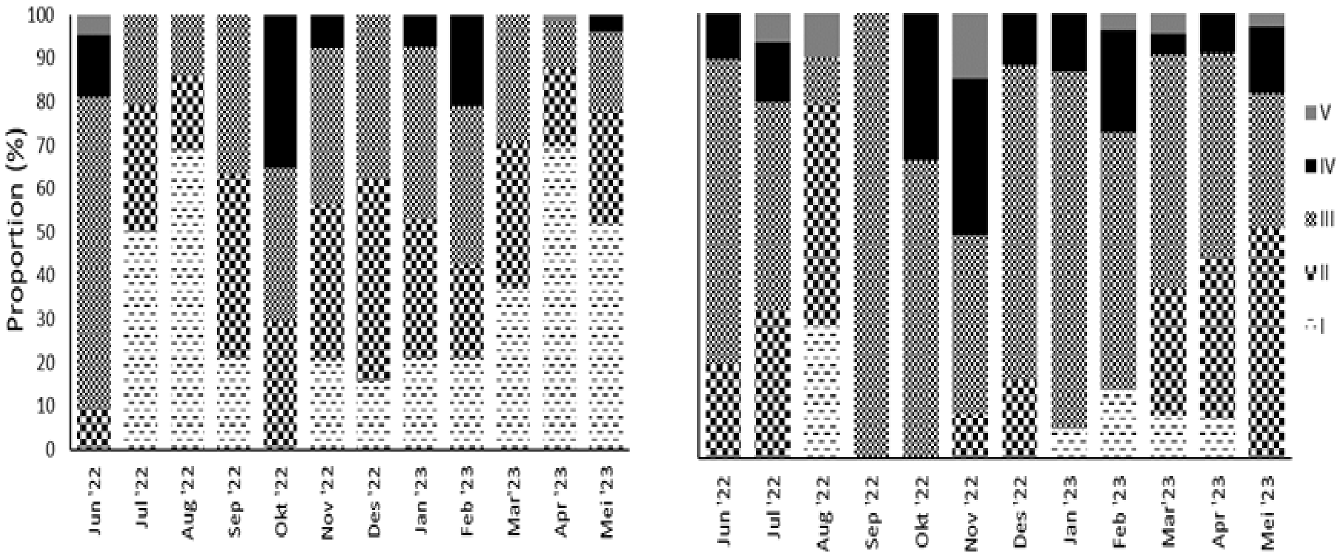

Figure 5. Percentage of composition of various maturity stages observed in (a) male and (b) female during June 2022–May 2023 (I:immature, II: maturing, III: ripening, IV ripe, V: spent).

Percent composition of various gonad maturity level can be seen that from June 2022-May 2023 (Figure 5), the dominant male *G. margaritaceus* was at level II, while the dominant female fish was at level III. The highest frequency gonad maturity level I in August 2022 (65%) and April 2023 (70%), level II had the highest frequency in December 2022 (47%), level III had the highest frequency was in June 2022 (71%), level IV highest frequency in October 2022 (35%), and level V highest frequency in June 2022 (5%). The highest frequency of female level I was in August 2022 (30%), level II the highest frequency in August 2022 (50%),

The size at first maturity (Figure 6) of the male *G. margaritaceus* fish was 11.2 cm, while that of the female was 10.3 cm. This means that female fish reach the adult phase faster than male fish. The gonad maturity size of the female fish in Lake Limboto is not much different from the size of the female fish in Lake Tondano, which is 10.7 cm (Makmur *et al.*, 2019). As for Lake Bolano Sau, the size at first maturity for the payangka fish gonads was 11.9 cm (Putera et al. 2022). One indicator of the fish spawning season is an increase in the value of the gonadosomatic index (Tagarao *et al.*, 2020), based on this, the spawning season for *G. margaraceus* is thought to occur in September to Oktober. Changes in the gonadosomatic index every month can indicate the spawning season, ovarian activity, and the reproductive cycle of a species (Sang *et al.,* 2019; Tagarao *et al.,* 2020). Differences in gonadosomatic index values can be influenced by environmental factors such as the availability of food as an energy source for fish which can support their somatic and reproductive development (Hasim *et al.,* 2021).

The average rainfall at the Gorontalo Climatology Station Rain Station around Lake Limboto (Mongolato Village and Talumelito Village) from June 2022 to May 2023 is 130.4 ± 45.2 mm. The average monthly rainfall is in the moderate category (101-300 mm), except for July 2022 (86.5 mm), December 2022 (80.5 mm), and May 2023 (98.0 mm) which are in the low category (0-100mm). The highest average rainfall is in July 2022 (204.1 mm), October 2022 (154.4 mm), and November 2022 (164.4 mm). The average air temperature in the Gorontalo area throughout the year (June 2022-May 2023) monitored at the Gorontalo Climatology Station is 27.1±1.1°C. The air temperature is relatively stable in the range of 26-27°C throughout the year, except for December 2022, which rose dramatically to 30.6°C.

Throughout the year (June 2022-May 2023), the proportion of mature female *G. maragaritaceus* fish averaged above 50%; the highest yields were in September 2022 and October 2022, which each reached a proportion of 100%. Different results were found for males; the proportion of maturity per month averaged less than 50%; only in June 2022 it reaches 90% and in October 2022, which reached 71%.

Partial regression analysis between rainfall (X1) had no significant effect (P>0.05) on the proportion of gonad maturity (Y), and the partial analysis between air temperature (X2) on the proportion of gonad maturity (Y) the results obtained had no effect real (P>0.05). Simultaneous regression analysis also showed results that had no significant effect (P>0.05) between rainfall (X1) and temperature (X2) on the proportion of gonadal maturity (Y) (Figure 7).

The maturity level of the gonads is affected by the increase in the body weight of the fish, so the greater the body weight of the fish, the greater the level of gonad maturity, and it is a sign that the fish is also growing (Tresnati, 2019). Differences in the level of gonad maturity in fish can be caused by several factors such as density, competition for food, and water quality

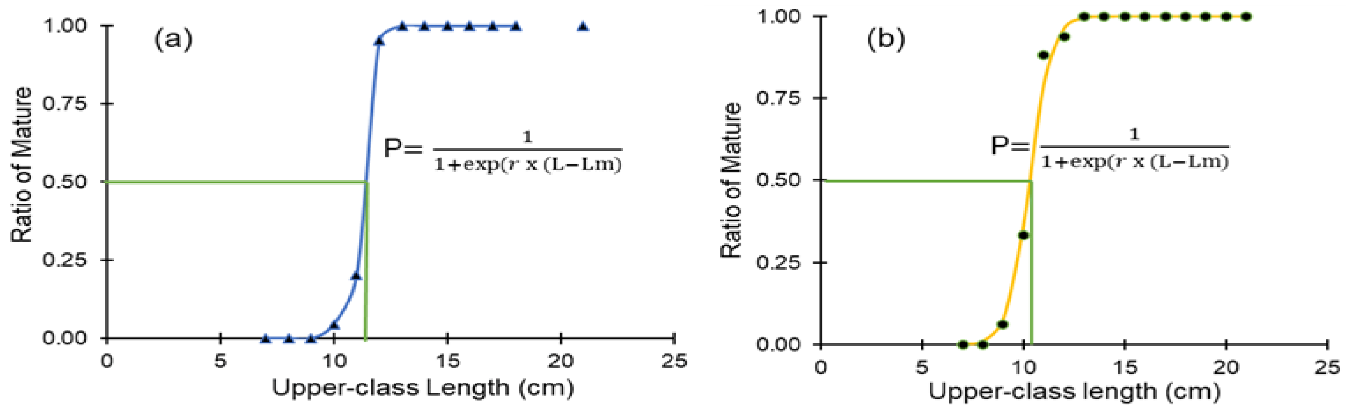

Figure 6. Length at maturity for (a) male, and (b) female specimens of *G. margaritaceus*

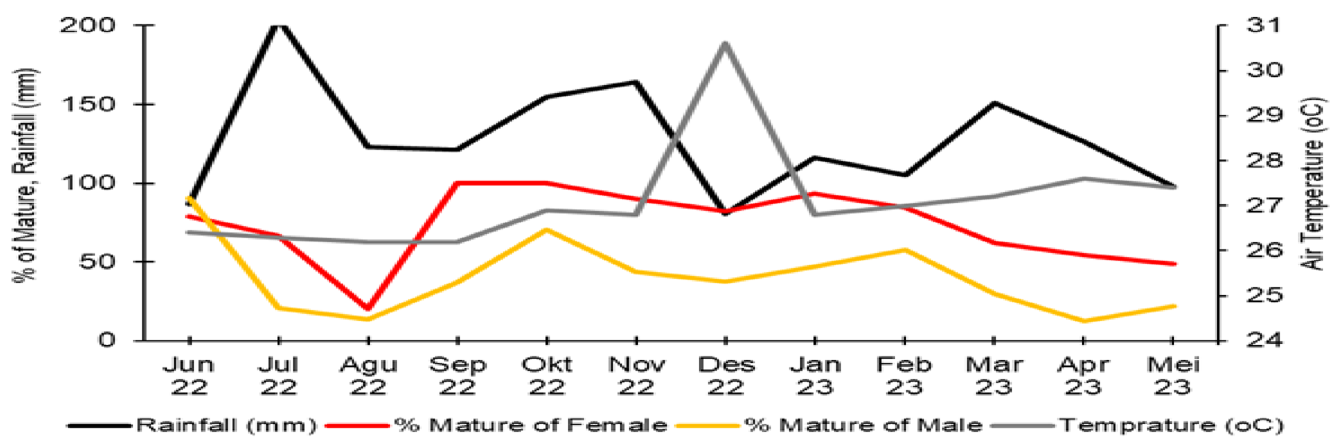

Figure 7. Relationship between Rainfall and Temperature on the Gonad Maturity Level of Male and Female *G. margaritaceus*

consisting of temperature, brightness, salinity, and others that affect fish growth (Wu *et al.,* 2008) Other factors that affect the level of gonad maturity include the genetic nature of the population, differences in area location, water quality, and the amount of fishing pressure (Hasim et al. 2021). In addition, the maturity of the gonads of fish can also be affected by differences in species, age, and size of the fish (Hossen *et al.,* 2020).

4. Conclusion

G. margaritaceus reaches its first mature gonad size at 11.2 cm for males and 10.3 cm for females. *G. margaritaceus* can spawn throughout the year, with the average monthly gonad maturity for males being 40 ± 23% and females 73 ± 23%. The peak of spawning is from September to November, which is the peak of the rainy season in the Limboto Lake area. Ovaries ready to spawn fill 70% of the abdominal cavity, and the eggs are yellow, arranged tightly and easily separated. Mature ovaries are dominated by secondary vitellogenesis oocytes measuring 190-220 µm and post vitellogenesis oocytes measuring 210-300 µm. Testicles ready to spawn fill 25% of the abdominal cavity, the ridges are wider, thicker and stronger. Spermatozoa are clearly visible filling the sacs of the seminiferous tubules.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follow, Arafik Lamadi; collected the data, drafted the manuscript, and designed the figures. Feni Iranawati, Maheno Sri Widodo and Dewa Gede Raka Wiadnya devised the main conceptual ideas and critical revision of the article. All authors discussed the results and contributed to the final manuscript

Conflict of Interest

The authors declare that they have no competing interests.

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