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First Report on the Reproductive Biology of *Saurida tumbil* in Jizan Waters, Saudi Arabia Based on Histological Analysis

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Abstract

A scientific study on *Saurida tumbil* is essential, particularly concerning fishing activities. The current study aimed to investigate the reproductive biology of *S. tumbil* through monthly sampling. Fish were dissected to determine sex and gonadal maturity stages, with gonads furthermore preserved in a 10% formalin solution. Reproductive parameters, including sex ratio, gonad maturity, length at first sexual maturity, gonadosomatic index (GSI), and fecundity, were analysed, followed by length at first capture analysis. Histological techniques were applied to assess gonadal maturity at a microscopic level. The peak reproductive period of *S. tumbil* in Jizan waters was identified between February and April (winter–spring), with the highest GSI values recorded for females (3.6) and males (0.96). Reproductive activity declined between October and December (autumn–winter). The estimated length at first sexual maturity was 23.49 cm for females and 23.94 cm for males. Both values were lower compared to the length at first capture (22.75 cm). Absolute fecundity averaged 42,528 eggs, ranging from 4,485 to 225,549 eggs. Identifying optimal fishing areas and seasons simultaneously in Jizan waters is crucial for maximising sustainable fisheries.

INTRODUCTION

The exploitation of *Saurida tumbil* (Bloch, 1795) or the greater lizardfish in Jizan waters is considerably active, indicated by the production of Saurida tumbil through time. It produced around 28 tonnes of *S. tumbil* in 2020. The value even reached 58 tonnes in 2016 and 57 tonnes (2018). Although the production was low with 19 tonnes in 2017 and 15 tonnes (2019) (FAO, 2021). However, the utilisation of its

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resources causes issues in the fisheries biology aspect, such as the reproductive biology of the species.

Active fishing reduces fish populations, impacting their biology. Population size affects behaviour, physiology, and food availability. Variations in age, size, and abundance influence population dynamics and hormone production, including reproductive hormones. Exploitation also alters feeding behaviour by changing food accessibility, which can affect reproductive potential (Hart and Reynolds, 2002). These factors highlight the need to study the reproductive biology of *S. tumbil*.

Studying the reproductive biology of *S. tumbil* helps assess its response to fishing pressure. Key findings, such as length at first maturity and capture, reveal if fishing practices allow reproduction and spawning before fish are caught. If many are caught prematurely, regulations may need adjusting to prevent depletion. Understanding spawning, fecundity, and reproductive cycles aids in sustainable fisheries management, ensuring the species can recover despite environmental and human impacts (Cochrane and Garcia, 2009).

Scientific studies on *S. tumbil* have been conducted by many authors (Fofandi, 2011; Kalhoro *et al*., 2015; Najmudeen *et al*., 2015). Nevertheless, there are only a few studies on greater lizardfish in the Red Sea. The first one studied the reproductive biology of the lizardfish *S. tumbil* in Jizan fisheries (Bakhsh, 1996). The second one was investigated on the fish growth and stock assessment of the lizardfish *S. tumbil* in Jizan fisheries (Gabr and Mal, 2017). The third one studied on growth and feeding habits of *S. tumbil* (Hartono *et al*., 2024).

The present study aimed to further analyse the reproductive biology of *S. tumbil*, building upon the findings of Hartono (2024). The development of reproductive organs and their cellular structures was examined and described histologically. Notably, the last comprehensive study on the species' reproduction was conducted over 30 years ago (Bakhsh, 1996), leading to a significant knowledge gap. The temporal disparity has resulted in differing insights into the reproductive biology of *S. tumbil*, particularly in Jizan waters.

METHODOLOGY

Ethical Approval

The study did not involve the use of live animals. All samples were obtained from dead specimens collected by local fishermen. Therefore, ethical approval for animal experimentation was not required.

Place and Time

The research consisted of samples and data collection, fish dissection, specimen collection, and specimen analysis. Samples of *S. tumbil* were collected from the main landing site of Jeddah (central fish market) between September 2021 and April 2022. The samples were the trawl catch shipped from Jizan waters (Figure 1). The samples were transported directly to the laboratory of the Marine Biology Department, King Abdulaziz University, for reproductive biology analysis, morphologically. The histological analysis was conducted in the King Fahd Medical Research Centre, King Abdulaziz University.

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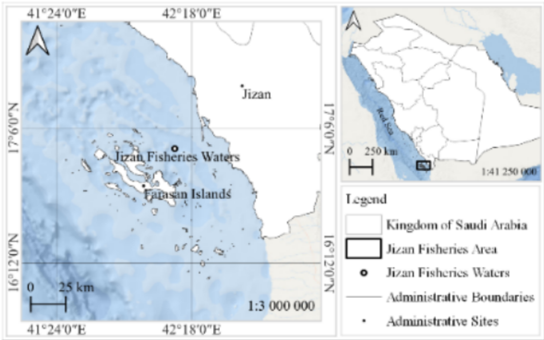

Figure 1. Location of Jizan fisheries in the Red Sea.

Research Materials

Fish were caught using 2-3 m diameter cast-nets and 25 m long seines, with 01 cm and 2.5 cm mesh sizes, respectively. The length and weight data of fish were collected using a measuring scale and an electrical balance (KERN KB 1000 – 2, Germany). Fish were anesthetized using MS 222 (Tricaine methanesulfonate; Sigma-Aldrich Chemicals Private Limited, India). Histological sections of the gills of infected fish were taken using a microtome (Leica microtome RM2235, China). Microscopical studies were conducted using a dissecting microscope (Olympus CX21FS1, Philippines). The chemicals and reagents used were Giemsa's solution, hematoxylin and eosin stains, Bouin's fixative, and ethanol (Sigma-Aldrich Chemicals Private Limited, India).

Research Design

Fish were collected and examined monthly with 8 months in total, covering four different seasons in Saudi Arabia. Several reproductive biology analyses were performed, namely morphological maturity stage analysis, reproductive potential analysis, histological analysis, first sexual maturity, and fecundity.

Work Procedure

Environmental Variable

Sea surface temperature data, as additional data was collected from Aqua-MODIS satellite, which was retrieved from NASA (2023) and extracted on SeaDAS software. The data was used to classify the seasons in Saudi Arabia into two classes, namely the hot season and the cold season.

Morphological Maturity Stage and Sex Ratio

Reproduction aspects were analysed first by determining the sex and its gonad maturity stage for each fish. The morphological maturity stage used in the study was based on the maturity stage by Mostarda *et al*. (2016) with modifications as listed in Table 1.

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Table 1. The maturity stages description and gonad status.

Stage	Description	Status
I	Ovary is small and has elongated shape. It fills a small area of the body cavity. Ova cannot be seen by naked eyes. Testis is transparent and thin.	Immature
II	Ova is still undetected by naked eyes. Testis is milky and whitish, but still flat. Both ovary and testis fill about half of body-cavity due to increasing in weight and volume.	Maturing
III	Ovary distended more or less two-third of the body-cavity with reddish to pinkish ovum. Testis get increasing in weight and volume, whitish, and considerably has more vascular.	Ripening
IV	Ovary and testis fill most of body-cavity. Ovum get bigger, have round shape, and pinkish. Testis is still whitish and fattier. Blood capillaries are clearer than before for both of ovary and testis.	Ripe
V	Ripe ovary and testis start to release ovum and sperm respectively which result in decreasing the mass of gonad.	Spawning
VI	Ovary becomes soft and shrunken. It contains residual ovum. Testis is still a little bit flaccid, whitish, and shrunken.	Post-spawning

Gonadosomatic Index (GSI)

Gonadosomatic index was estimated to determine the reproductive potential. The estimations of GSI for each specimen were calculated by the following equation.

$$GSI = \left(\frac{WG}{W}\right) x100$$

Where WG is the gonad weight (g) and W is the total weight (g).

Histological Analysis

Six samples of each ovary and testis were examined histologically. The common procedure of histological analysis was used, which consisted of six major sequential steps, namely tissue fixation, dehydration and clearing, embedding, sectioning, staining, and microscopy observation (Ramadan *et al.*, 2017). The results were examined under AmScope Zoom Stereomicroscope integrated with a digital video camera and connected to a computer system.

Length at First Sexual Maturity (L_{50})

The length at first sexual maturity, where 50% of fish are sexually mature, was presented in a logistic curve (Gunderson *et al.*, 1980). The length class (L) and number of mature fish were established to produce the proportion of mature fish (P_m) at each length class (L). Furthermore, the calculation of $log_e(1/P_m–1)$ was conducted, which was followed by the estimation of linear regression on mid-length and $log_e(1/P_m–1)$ to determine x and y, which are respectively the slope and intercept of the equation. Then, L50 was calculated from the estimated values of x and y by the following formula:

$$L_m = -\frac{y}{x}$$

The logistic curve was produced by regressing the mid-length of the length class and adjusted proportion of mature fish AP_m at length (L). The adjusted proportion of mature fish was estimated by the following equation.

$$AP_m = \frac{1}{1 + e^{(yL+x)}}$$

Length at First Capture (L_c)

The length at first capture (L_c), defined as the length at which 50% of the fish are caught, was determined using a logistic curve. The mathematical approach used was similar to that for estimating the length at first sexual maturity. The length class (L) and number of fish were established to produce the proportion of fish (P_c) at each length class (L). The analysis provides an estimate of the fish length at which there is a 50% probability of capture. The length at first sexual maturity is when 50% of the fish are sexually mature. Meanwhile, the length at first capture is defined as the length at which 50% of the fish are caught. Both lengths were determined using a logistic

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curve, as described by Gunderson *et al.* (1980).

Fecundity

Absolute fecundity was then calculated using the following equation:

$$F_{abs} = \frac{\left(\sum_{i=1}^{3} \frac{N_i}{S_{wi}}\right)}{n} \times G_w$$

Where F_{abs} is the absolute fecundity. G_w is the total weight of the female's ovary. N_i is the total number of ova from the sub-sample i, S_{wi} is the weight of the sub-sample i, and n is the total number of sub-samples (King, 2007).

The relative fecundity (F_{rel}) was estimated by dividing the absolute fecundity by total length (cm) and body weight (g). The relationships between fecundity (either absolute or relative fecundity) and both total length and total weight were investigated and determined by using a power equation.

Data Analysis

Normality and homogeneity tests were conducted on environmental and biological data. A parametric statistical test was performed when these assumptions were met. Otherwise, a non-parametric statistical test was used (Mendenhall *et al.*, 2013). Normality of the data was tested using the Shapiro-Wilk test. Meanwhile, Levene's test was applied to assess the homogeneity of variances between the two groups. For the parametric test, an independent t-test was also conducted to assess differences between groups. Alternatively, two non-parametric tests used in the study were the Mann-Whitney U test and the Kruskal-Wallis test. All statistical analyses were conducted using standard procedures in Python (Wang *et al.*, 2022).

RESULTS AND DISCUSSIONS

Environmental Variable

The monthly mean temperature in Jizan waters averaged 28.7°C, with an average monthly maximum of 31.0°C and an average monthly minimum of 26.9°C (Table 2). The highest recorded temperature was 34.3°C in September. From October 2021 to March 2022, temperatures gradually declined, reaching the lowest value of 24.3°C in March. However, this trend reversed in April, when temperatures increased again, with a minimum of 26.8°C and a maximum of 32.1°C.

The study period was classified into hot season and cold season based on mean temperatures, and then further tested using statistical analysis. The results showed that September, October, November, and April were classified as the hot season. Meanwhile, December, January, February, and March were classified into the cold season ($t=4.460$, $p<0.05$).

Table 2. The descriptive statistics of temperature data in the habitat waters of *Saurida tumbil*.

Details	Temperature in habitat waters of *Saurida tumbil* (C°)							
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Mean	31.7	31.3	29.9	28.0	27.3	26.5	26.5	28.7
Mode	32.0	31.0	30.5	28.6	27.6	26.3	26.4	28.9
Minimum	29.9	30.4	27.0	25.8	26.0	24.9	24.3	26.8
Maximum	34.3	33.6	31.6	30.1	28.9	28.8	28.8	32.1
Data number (n)	6808	6243	6987	6002	5142	6544	6953	6592

Sex Ratio

The number of fish by sex and their monthly ratios are shown in Table 3. Females were consistently more abundant than males each month. The total number of males was 126 (37%), significantly lower than the 217 females (63%), resulting in an overall sex ratio of 1:1.70. The greatest difference occurred in April (1:4), while the closest ratio was observed in October (1:1.15). The number of males and females differs significantly statistically (Mann-Whitney test, $U=7$; $p<0.05$). Significant variation across months (Kruskal-Wallis test,

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$H=6.83$; $p<0.05$), indicating monthly fluctuations in male and female distributions.

Table 3. Sex ratio of *Saurida tumbil* in Jizan waters.

Month	Males	Females	Ratio
Sep	10	24	1:2.40
Oct	20	23	1:1.15
Nov	21	32	1:1.52
Dec	24	43	1:1.79
Jan	10	19	1:1.90
Feb	21	32	1:1.52
Mar	15	24	1:1.60
Apr	5	20	1:4.00
Total	126	217	1:1.72

The recent study of *S. tumbil* regarding sex ratio in Jizan waters showed the same pattern as the study conducted by Bakhsh (1996). The ratio between males and females was 1:1.72 (recent study), while it was 1:1.79 in 1986. The result was different compared to the study by Gabr and Mal (2017) (1:1.17). In some Iranian waters, females were dominant over males (Motlagh *et al*., 2012; Abaszadeh *et al*., 2013; Mirzaei *et al*., 2015). In contrast, other studies from Indian waters showed the fluctuations during monthly sampling (Metar *et al*., 2009; Mali *et al*., 2017).

The differentiation between studies can be explained by some reasons related to fishing activities. It has impacts on the exploited population, which consists of the reduction of its abundance, spawning potential, and possibly, the disadvantageous changes in population parameters (growth, maturation, etc.). They lead to fluctuations of age and size structure, sex ratio, genetics, and species composition of the target resources, as well as of the associated and dependent species (Garcia, 2003).

Gonad Maturity and Gonadosomatic Index

Fish were categorised into immature and mature based on their maturity stage. Immature fish are in stages I and II; meanwhile, mature fish are in stages III, IV, V, and VI. The number of fish according to their sex and maturity stage is presented in Figure 2. The monthly distribution of males and females based on gonad maturity stage, respectively, is displayed in Figure 3. The GSI mean value then described the pattern of fish reproduction activity throughout the study period (Figure 4). Statistical test showed that there is no significant difference between the GSI means of the hot and cool seasons on males and females (female, $t=-1.234$, $p>0.05$; male, $t=-1.234$, $p>0.05$).

Descriptive statistics indicated higher gonadosomatic index (GSI) values during October–December (autumn–winter) and February–April (winter–spring), suggesting peak reproductive activity. This finding aligns with Bakhsh's (1996) study, which reported that *S. tumbil* in Jizan waters spawns almost year-round, from October to May. However, statistical analysis did not show a significant difference in GSI values between the cold and hot seasons. Although reproductive activity appeared more frequent during the cold season, the variation was not statistically significant. According to Miller and Kendall (2009), tropical fishes often spawn multiple times per year or even continuously. The frequent reproductive pattern may contribute to relatively low per-cycle reproductive output, as their reproductive cycles are rapid.

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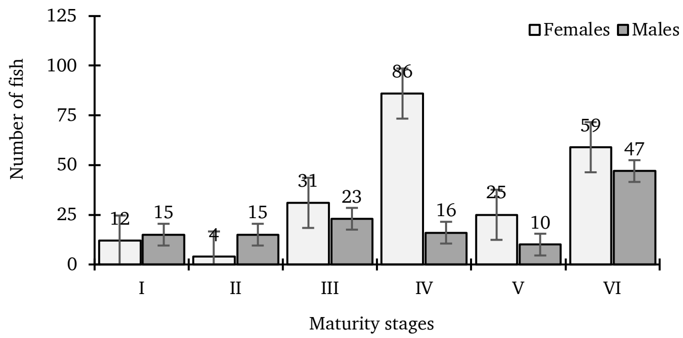

Figure 2. Sex distribution at different maturity stage.

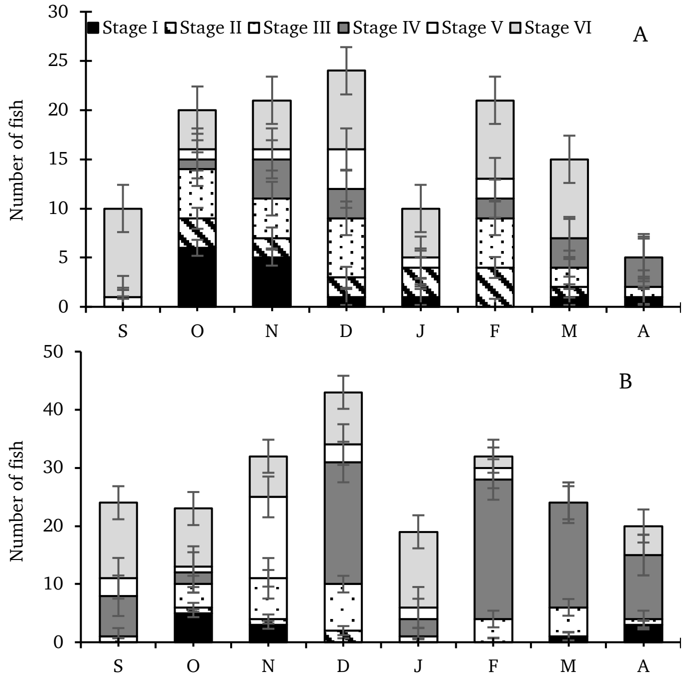

Figure 3. Monthly distribution of *Saurida tumbil* maturity stage: Male (A), Female (B).

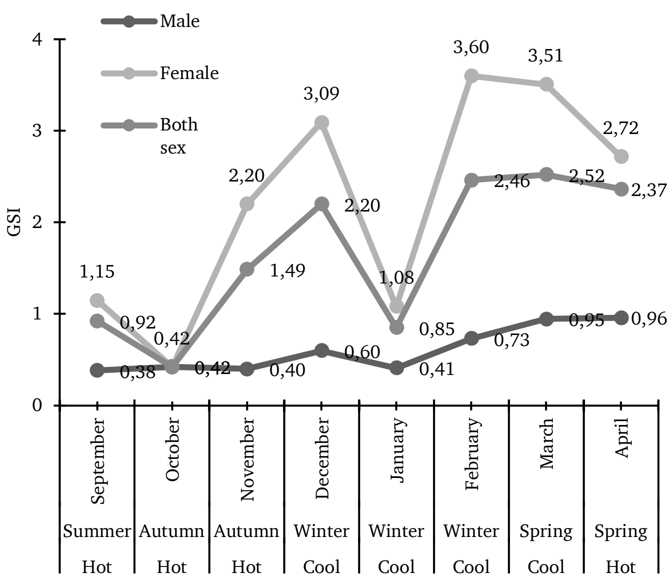

Figure 4. Monthly distribution of GSI according to total fish, males, and females.

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Histological Analysis of Female

The examination of the ovaries showed the development of oogonium to ovum (Figure 5). Oogonium and immature oocyte stages were observed by examining the ovaries from the fish at stage I of the maturity stage (Figure 5A). An oogonium, which has been developed into an immature oocyte, was spotted with a nucleus that existed in the middle area surrounded by cytoplasm and contained within the follicle. As the cells developed further, they reached the perinucleolar and cortical alveoli stage, which was observed from the maturing ovaries (stage II). It was indicated by the decrease of cytoplasm and nucleus, followed by the existence of cortical alveoli (Figure 5B). Furthermore, the oocyte was developed to the vitellogenic stage when yolk granules had been formed, and most of them could be observed from the ripening ovaries (stage III) (Figure 5C). The histological section of ripe ovaries (stage IV) showed the post-vitellogenic stage, where zona radiata, granulosa (follicle epithelium), and theca cells could be observed clearly (Figure 5D).

At the mature oocyte stage, yolk granules started to increase in size while the nucleus size decreased, followed by the occurrence of yolk vesicles (Figure 5E). The development of the mature oocyte was continued by the disappearance of the nucleus, which was seen from the histological section of the ovaries at the spawning stage (stage V) (Figure 5F). At the post-spawning stage (stage VI), the examined ovaries showed the signs of the post-ovulatory stage, where atretic follicles and post-ovulatory follicles were found, which are presented in Figure 5G and Figure 5H consecutively. Atretic follicle was indicated by the existence of degenerated yolk, meanwhile, post-ovulatory follicle by the germinal vesicle breakdown (GVBD).

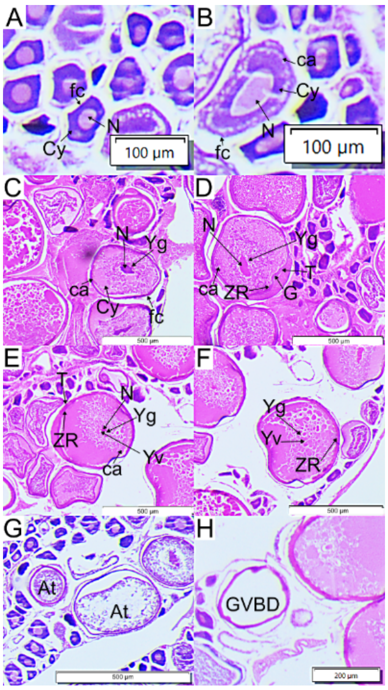

Figure 5. Histological structure of the oocyte at each developmental stage in female *Saurida tumbil* from Jizan fisheries, Red Sea, Saudi Arabia.

Description: (A) immature oocyte stage, (B) perinucleolar and cortical alveoli stage, (C) vitellogenic stage, (D) post-vitellogenic stage, (E) mature oocyte stage with nucleus, (F) mature oocyte stage without nucleus, (G) post-ovulatory stage, (H) post-ovulatory stage. N represents nucleus, Cy (cytoplasm), fc (follicle), ca (cortical alveoli), Yg (yolk granules), ZR (zona radiata), G (granulosa or follicle epithelium), T (theca cells), Yv (yolk vesicles), At (atretic follicle), and GVBD (germinal vesicle breakdown).

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Six stages of oocyte growth were identified, consistent with Genten *et al*. (2009), who stated that oocytes can be divided into five or six stages based on morphological features. At the oogonium and immature oocyte stage, oocytes undergo mitosis but are arrested in prophase I, with no significant meiosis progression, as described by McMillan (2007) and Mokhtar (2021). The stages reflect the strong basophilia in the cytoplasm and prominent nucleoli, as noted by Genten *et al*. (2009). The study also observed the vitellogenic stage, where yolk granules form and oocytes accumulate nutrients, halting meiosis during this process (McMillan, 2007; Mokhtar, 2021). Post-vitellogenic changes, including nuclear migration and yolk accumulation, mark the transition to secondary oocytes, which arrest at metaphase II (Kirschbaum and Formicki, 2020). The mature oocyte stage occurs upon ovulation and fertilisation, completing meiosis II and producing a haploid egg ready for fertilisation (McMillan, 2007; Kirschbaum and Formicki, 2020). At post-spawning, remaining oocytes degenerate or are reabsorbed, resetting the reproductive cycle (Genten *et al*., 2009; Mokhtar, 2021).

Histological Analysis of Male

The examination of the testes showed the development of spermatogonia to spermatozoa. The results were presented in Figure 6. The primary spermatogonia stage was recorded in Figure 6A. The existence of spermatogonia is followed by the finding of lumina and Sertoli cells. The same tissue contents were found at the secondary spermatogonia stage, which was discovered in testicular histology from testes at the maturing stage, except that its spermatogonia size was smaller than the spermatogonia size at the primary spermatogonia stage (Figure 6B). Moreover, secondary spermatogonia developed to the next stage and turned into primary spermatocytes (Figure 6C). The development was discovered by examining the ripe testes (stage IV). The secondary spermatocytes were found alongside a small number of spermatids (Figure 6D). Afterwards, testes at the spawning stage (stage V) produced the histological section, which indicated the cells were at the spermatid stage alongside a small number of spermatozoa (Figure 6E), before the domination of spermatozoa was examined at the spermatozoa stage (Figure 6F).

Spermatogenesis progresses from spermatogonia to spermatozoa, as described by Kirschbaum and Formicki (2020). Immature testis showed primary spermatogonia with Sertoli cells providing support. Secondary spermatogonia, smaller and more basophilic, were observed in maturing testes (Mokhtar, 2021). Primary spermatocytes undergo meiosis I, producing haploid secondary spermatocytes, followed by meiosis II, forming spermatids (Kirschbaum and Formicki, 2020; Mokhtar, 2021). The spermatids develop into spermatozoa, and after spawning, the testis enters a resting phase, where spermatogenesis slows or stops until the next reproductive cycle (Kirschbaum and Formicki, 2020).

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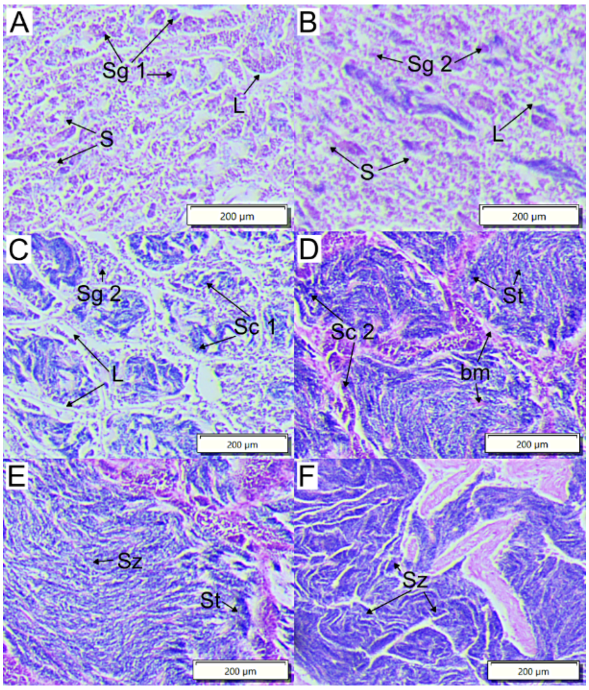

Figure 6. Histological structure of the spermatogonia at each developmental stage in male *Saurida tumbil* from Jizan fisheries, Red Sea, Saudi Arabia.

Description: (A) primary spermatogonia stage, (B) secondary spermatogonia stage, (C) primary spermatocytes stage, (D) secondary spermatocytes stage, (E) spermatids stage, and (F) spermatozoa stage. Sg 1 represents primary spermatogonia, L (lumina), S (Sertoli cells), Sg 2 (secondary spermatogonia), Sc 1 (primary spermatocyte), Sc 2 (secondary spermatocyte), St (spermatids), Sz (spermatozoa), bm (basement membrane), and Ap (apoptotic cells).

Length at First Maturity (L_{50}) and Length at First Capture (L_c)

The logistic curve function generated the length of fish at first maturity for both sexes (Figure 7). Male fish were estimated to have a 50% probability of reaching sexual maturity at a length of 23.94 cm. Meanwhile, it was estimated to be 23.49 cm for females. For length at first catch (L_c), the fish was estimated to have a 50% probability to be captured at a length of 22.75 cm, lower than L_{50}.

One study at Jizan waters in 1986 found that 50% of female fish were mature at 19 cm in length (Bakhsh, 1996). Furthermore, a study on length at first maturity from different regions resulted in various values in length. Mirzaei *et al.* (2015) discovered that males and females became mature at a total length of 26.2 cm and 27.4 cm, respectively. Motlagh *et al.* (2012) assessed that female fish of *S. tumbil* reached first maturity at 29.5 cm in length. Both studies were conducted in Iran waters. Meanwhile, Metar *et al.* (2009) found length at first maturity was 29.6 cm in samples from Indian waters.

Fisheries exploitation in Jizan waters still caught *S. tumbil* that was immature according to the concept of length at maturity. For the insurance of sustainable fisheries, the evaluation of fishing activity needs to be implemented. According to guidelines by Cochrane and Garcia (2009), fisheries utilisation is allowed when the resources have attained a size whereby they can spawn at least once before capture.

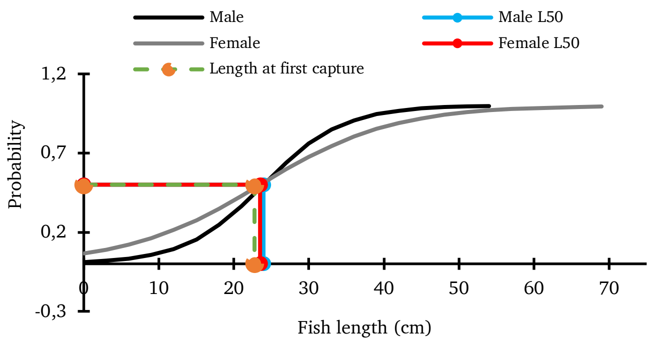

Figure 7. Length at first maturity (L50) of *Saurida tumbil* in the logistic curve.

Fecundity

The reproductive potential of *S. tumbil* in Jizan fisheries was described by the result of the fish's absolute fecundity analysis. The fish could produce 42,528 eggs in a year. The minimum number of ripe eggs produced was 4,485 eggs, and the highest number was 225,549 eggs.

The average of absolute fecundity according to the mean of total length was explained by $F_{abs}=0.0217L^{4.33}$ ($R^2=0.9279$). Meanwhile, the average of relative fecundity according to the mean of total length was explained by $F_{rel}=0.0166L^{3.4083}$ ($R^2=0.9097$). Moreover, the average of absolute fecundity according to the mean of total weight was explained by $F_{abs}=9.4777W^{1.5596}$ ($R^2=0.9756$). Meanwhile, the average of relative fecundity according to the mean of total weight was explained by $F_{rel}=12.902W^{0.5298}$ ($R^2=0.9655$).

Fecundity of *S. tumbil* in Jizan waters was first determined in the present study. The result of fecundity estimation was crucial to describe the potential of fish recruitment or population dynamic analysis (Tadena, 2021). The normal fecundity value among coastal fishes is 10^5 to 10^6 eggs (Miller and Kendall, 2009). The fish fecundity of the current study was 42,528 eggs, with means from data of fecundity, which ranged from 4,485 to 225,549 eggs. Additionally, most fish produced around 17,776 eggs.

Several studies from different locations estimated the fecundity of *S. tumbil*. In Iranian waters, the fecundity was from 24,160 to 172,000 eggs (Mirzaei *et al*., 2015). A study by Soofiani *et al*. (2006) estimated that the fecundity varied between 74,444 and 250,452 eggs. Meanwhile, an old reference by Rao (1983) stated that the fecundity of *S. tumbil* in Bangladesh waters was 37,569 to 214,981. The fecundity of the present study and several studies from different locations tended to be comparable in the fecundity range value. The maximum value is not exceeded by more than 3×10^5 eggs.

CONCLUSION

The study on *Saurida tumbil* in Jizan waters revealed a sex ratio (1:1.72) consistent with past findings but differing from other regions, likely due to fishing impacts. Peak spawning occurs two times, with continuous reproductive cycles typical of tropical species. Histological analysis confirmed six oocyte development stages and typical spermatogenesis patterns. Length at first maturity (L_{50}) estimates suggest some fish are caught before spawning, raising sustainability concerns. Fecundity averaged 42,528 eggs, aligning with findings from other regions. Fisheries managers should regulate fishing activities to safeguard the reproductive cycles of *S. tumbil* and conduct continuous research to provide data-driven recommendations.

CONFLICT OF INTEREST

We declare that there is no conflict of interest among all authors upon writing and publishing the manuscript.

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AUTHOR CONTRIBUTION

SH: Conceptualization, Methodology, Verification, Analysis and Investigation, Visualization, Resources, Writing–Original Draft, Writing–Review and Editing. MBS: Conceptualization, Methodology, Verification, Analysis and Investigation, Visualization, Resources, Writing–Original Draft, Supervision. GP: Verification, Analysis and Investigation, Supervision. SS: Methodology, Visualization, Resources, Writing–Review and Editing. MHA: Conceptualization, Methodology, Verification, Analysis and Investigation, Visualization, Resources, Writing–Original Draft, Supervision.

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