Updated Reproductive Biology of Skipjack Tuna (*Katsuwonus pelamis*) in Northeastern Indian Ocean

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Abstract. Skipjack tuna (*Katsuwonus pelamis*) is a tropical tuna species and has been historically exploited in the south and western parts of Indonesia waters. The objective of this study was to determine the biological reproductive parameters of female skipjack obtained from the Indonesia Fisheries Management Area 572 and 573 (Northeastern Indian Ocean). Samples were collected from the landing site in southern Java and Bali (Cilacap, Kedonganan, and Benoa), and western Sumatra (Lampulo, Sibolga, and Padang). Catches come from various gears such as handline, purse seine, lift net, and longline. Samples were collected during 2018-2021 and a total of 400 ovaries were obtained from fish with the length ranged between 28.3 - 72 cm FL. The size at first maturity (Lm50) of female skipjack in the northeastern Indian Ocean was 38.2 cm FL. The estimated mean batch fecundity was 0.29±0.18 million oocytes (n=11), and the mean relative batch fecundity was 107.11±29.26 oocytes/gr. The peak spawning season of skipjack tuna occurred between September and February, spawning every 1.82 days within the spawning period.

Keywords: maturity, histology, skipjack tuna, Indonesian wate

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1 Introduction

According to Hunter et al., (1986), skipjack tuna is a species that can spawn many times in a single season. To ascertain the frequency and duration of spawning events, as well as the fish's initial gonad maturity, research on fish reproductive biology is essential. This includes investigating gonad maturity levels, egg diameter, and fecundity. A key factor in assessing a fish population's capacity for regeneration is its reproductive traits. This knowledge can be applied to fisheries management to preserve spawning populations and protect stocks from overfishing.

Accurate information on the reproductive characteristics of tuna is an important factor in determining the regeneration capacity of a population. While macroscopic analysis of ovaries is useful for rapid field-based assessment of reproductive stage and maturity, an incorrect assignment can have implications for the precision and accuracy of the parameter estimates derived from these data. Microscopic/histological analysis is the most appropriate method to accurately assess maturity status and estimate reproductive parameters for tuna (Farley et al., 2013; Schaefer, 1998; Zudaire et al., 2010).

Several studies regarding the gonad maturity level and biology reproductive of skipjack tuna using microscopic (histological) and macroscopic (visual morphology) observation methods have been carried out in Indonesian waters (Hartaty & Arnenda, 2019; Jatmiko et al., 2015; Mallawa et al., 2012, 2014; Nugraha & Mardlijah, 2008) and in the western Indian Ocean (Ashida et al., 2010; Grande et al., 2012, 2014; Hunter et al., 1986; Stequert & Ramcharrum, 1996). The objective of this study was to provide information on the reproductive biology, in particular, length at first maturity (Lm_{50}, size at which 50% of the individuals are mature), the spawning season, spawning frequency, egg diameter, and fecundity of female skipjack in the northeastern Indian Ocean.

2 Material and methods

2.1 Sample collection

Samples of skipjack tuna ovaries were collected from port sampling and surveys onboard commercial longline tuna operated in the eastern Indian Ocean. The sampling collection was conducted in five ports in Kedonganan and Cilacap (FMA 573), and Padang, Sibolga, and Lampulo (FMA 572). A total of 400 ovaries length ranged between 28.3-72 cm FL. Samples were collected from 2018 – 2021. Samples were obtained from catches of various gears such as handline, purse seine, lift net, and longline.

A cross-section was taken from the middle of one of the ovarian lobes and fixed in 10% buffered formalin. Samples were embedded in paraffin and prepared using standard histology. Cut in 5 µm and stained with Harris-Haemotoxylin and Eosin.

2.2 Sample analysis

Histological analysis using criteria for the South Pacific albacore tuna Farley et al., (2013) (Appendix 1). The most advanced group of oocytes (MAGO) was classed into unyolked, early yolked, advanced yolked, migratory nucleus, or hydrated oocytes stage. The presence or absence of postovulatory follicles (POFs) in each ovary was also an assessing factor. Markers of maturity considered are numerous brown bodies, well-defined muscle bundles, and residual hydrated oocytes. Maturity markers were regarded as signs of previous reproductive activity (Farley et al., 2013; Ziscke et al., 2013).

Mature ovaries contained oocytes at advanced yolked, migratory nucleus, hydrated oocytes stage and POFs, and ovaries with unyolked or early yolked oocytes as the MAGO but with maturity markers present. Meanwhile, ovaries contained unyolked and early yolked oocytes as the MAGO, but no POFs, atresia, or maturity markers were present classed as immature. Based on the spawning activity, the mature-active includes spawning and spawning capable stage. Regressing, regressed 1, regressed 2, and regenerating stage classed as mature-inactive. In contrast, all immature phases (immature and developing) are classed as immature-inactive (see Appendix 1).

2.3 Data analysis

All slides from histology samples were measured under a microscope Zeiss Axiocam with imaging software Zen2000. The diameter of the oocyte (Dn) was the average of the horizontal and vertical length of the oocyte (1) (Williams, 1997). The actual diameter (d) is the average of the five oocytes diameter measured (2).

$$D_n = \frac{D_x + D_y}{2} \tag{1}$$

$$d = \frac{D_1 + D_2 + D_3 + D_4 + D_5}{5} \tag{2}$$

When 50% of the sampled fish were sexually mature, it was considered the length at 50% maturity (Lm50) (Fontoura et al., 2009; Somerton, 1980). Binomial logistic regression was use (Girault et al., 2019; Zuur et al., 2007), where X is regarded as the explanatory variable, and the random variable is the maturity stage of female yellowfin tuna (immature: 0; mature: 1). The regression parameters a and b from the fitted maturity curves used to calculate the mean length at which 50% of mature females, with Y = 0.5, following:

$$L_{50} = \frac{\left(\log\left(\frac{Y}{1-Y}\right) - a\right)}{b} \tag{3}$$

The gravimetric method is used to estimate the total number of oocytes released per batch (Hunter and Macewicz 1985; Bagenal, 1957). Ovaries with hydrared oocytes as the MAGO was used for the fecundity analysis. The eggs were counted under a Zeiss stereomicroscope. The mean number of oocytes per gram was multiplied by the weight of the gonad, then dividing it with the bodyweight of the fish to estimate the relative batch fecundity.

One day or less following ovulation, postovulatory follicles can be seen in the ovary (Schaefer and Fuller, 2019). The inverse of the spawning fraction was used to estimate the frequency of spawning females. Using the postovulatory follicles approach (Hunter and Macewicz, 1985), the spawning fraction was calculated as the ratio of active spawning females (those having postovulatory follicles) to the total number of spawning females.

3 Result

3.1 Length at 50% maturity

Most of the samples (89%) analyzed in this study were classed as mature fish. The smallest mature fish was 35 cm FL, while the largest immature was 72 cm FL. The Lm_{50} was estimated at 38.2 cm FL at the advanced yolked stage as a maturity threshold.

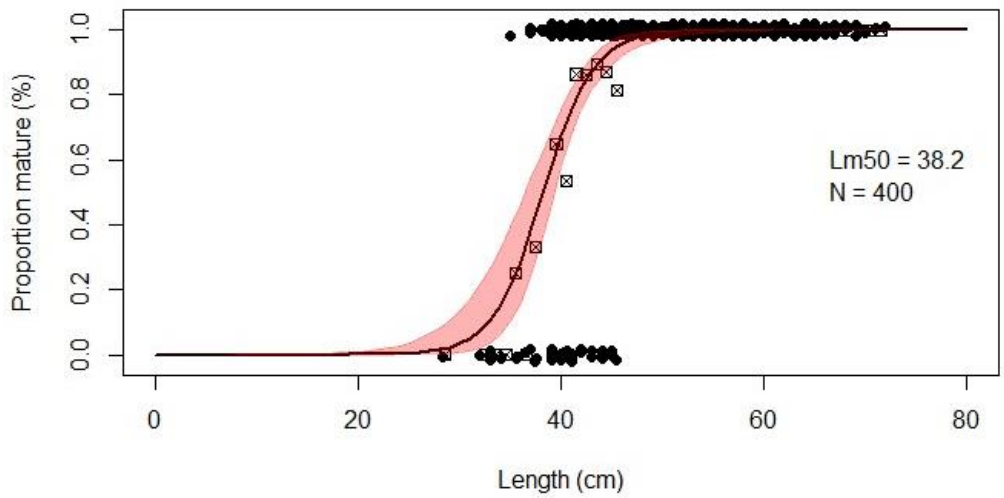

Fig. 1. The estimated proportion of mature female skipjack tuna.

3.2 Spawning frequency and spawning season

Based on the spawning activities, fish in the phase of mature-active, mature-inactive and immature were found nearly throughout the years (no samples collected in January, March, and December) (Fig.2). Eighty-nine spawning female ovaries were observed for the presence of the POFs from February to November (Table 1). The fraction of spawning females with POF was 0.55, equal to the spawning frequency of 1.82 days. The peak spawning season of skipjack in northeastern Indian Ocean estimated to be happened between September and February based on the highest monthly spawning fraction (Table 1).

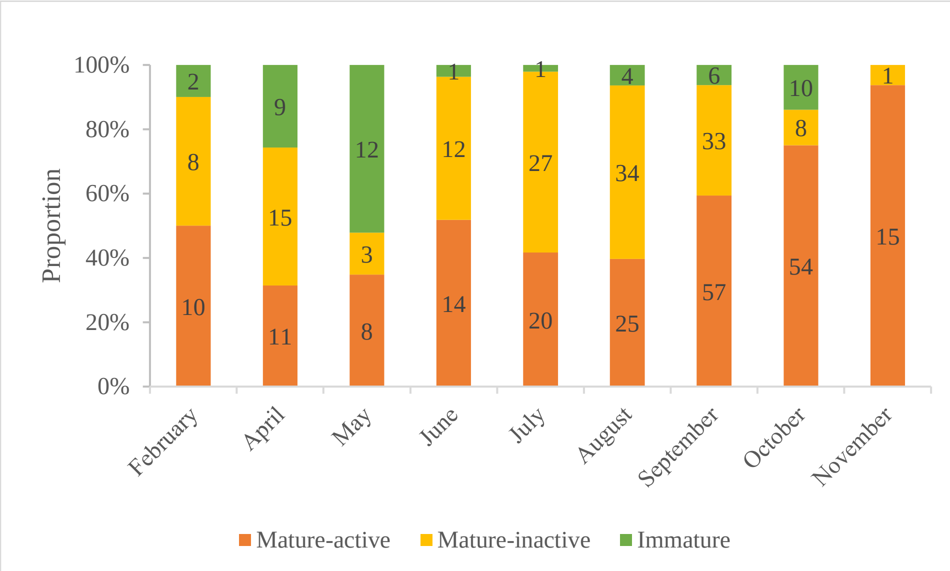

Fig.2. The proportion of spawning activity of female skipjack tuna by month. The number on the bars shows the number of fish.

Table 1. Number of spawning females with POFs presence on months observation

Month	Count of spawning females with POFs	Count of spawning females	Spawning fraction
February	7	7	1,00
April		1	
May		3	
June	1	2	0,50
July	3	11	0,27
August	1	3	0,33
September	15	20	0,75
October	11	27	0,41
November	11	15	0,73
Total	**49**	**89**	

3.3 Oocyte size and fecundity

The oocytes size ranged between 16.7 – 620.1 μm (Table 2). The oocyte size at different oocyte development stages (unyolked to hydrated) was continuous without any gap in diameter. The highest stage of the oocyte (hydrated) has a smaller maximum size than the migratory nucleus's maximum size. It occurs because the shape of the oocyte at the hydrated stage has a form that is not ideally round due to the histological preparation process.

A total of 11 female fish ranging between 40 – 61.5 cm FL were analyzed to estimate the batch fecundity. The batch fecundity was between 0.12 – 5.45 million oocytes. The mean batch fecundity was 0.29±0.18 million oocytes, and the mean relative batch was 107.11±29.26 oocytes/gr.

Table 2. Oocyte size of female skipjack tuna observed.

No.	Oocyte development stage	Minimum size (µm)	Maximum size (µm)
1	Unyolked	16.7	133.6
2	Early yolked	68.6	307.4
3	Advenaced yolked	153.7	503.2
4	Migratory nucleus	377.1	528.9
5	Hydrated oocytes	339.2	620.1

4 Discussion

The size at maturity was estimated at 38.2 cm FL. Using advanced yolked (AY) or Vitellogenic 3 (Vtg3) as indicator of maturity, the result of this study is smaller than the previous studies in the western Indian Ocean by Stequert & Ramcharrum (1996), Grande et al., (2014); Timohina & Romanov (1996) which proposed Lm50 of female skipjack tuna of 42, 43.5 and 43 cm FL respectively. Studies on yellowfin tuna reported that fish in the area nearer the equator may reach sexual maturity more quickly, while tuna in higher latitudes may have a delay (Sun et al., 2005; Itano, 2000).

Based on the spawning activity classes, the spawning season occurred almost throughout the year (February to November), with the peak season between September and February. Schaefer (2001) indicates that skipjack tuna spawn throughout the year in equatorial waters when the water surface temperature is above 24 ºC. The sea surface temperature is one of the main factors in fish spawning activity. The tropical waters are the ideal temperature for gonad development and spawning of tuna above 24 ºC (Dhurmeea et al, 2016; Ashida and Horie, 2015). During the spawning season, skipjack can spawn at an interval of 1.82 days. Therefore, skipjack from the Northeastern Indian Ocean are capable of spawning every day. A study by Ashida and Horie (2015) in the Northwestern Pacific Ocean showed that skipjack spawning fraction was 1.88 days, while in the Mediterranean Sea the spawning fraction was 1.03 days (Puerto et al., 2022).

The continuous oocyte size without any gap in the diameter has been considered a sign of indeterminate fecundity. The batch fecundity and relative fecundity estimated in this study was higher than the previous studies in the western equator of Indian Ocean (Timohina and Romanov, 1996) that estimated 0.9 – 2.8 million oocytes and 56.8±14.3 oocytes/gr respectively. While Grande et al., (2014) reported a higher relative batch fecundity for skipjack in the western Indian Ocean that was 140±64 oocytes/gr. However, besides the geographic differences, the intra-population variability could cause discrepancies between studies (Schaefer, 1996).

5 Conclusion

The size at maturity (Lm_{50}) was estimated at 38.2 cm FL with the maturity threshold set at the advanced yolked stage of the oocyte development. Spawning season occurred from September to February and spawned every 1.82 days. The reproductive aspects described in this study may provide several parameters for improving the skipjack tuna stock assessment in the Notheastern Indian Ocean.

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8 Appendixes

Appendix 1. The histological classification criteria were used in this study. Un = unyolked, Ey = early yolked, Ay = advanced yolked, Mn = migratory nucleus, Hy = hydrated, x = not present, √ = present.

Class	Maturity Status	Activities	Class of Development	MAGO	POFs	Atresia		Maturity Marker		
						Alpha	Beta	Brown Bodies	Muscle Bundles	Residual Hydrated
1	Immature	Inactive	Immature	Un	x	x	x	Absent or very small	Absent or very small	x
2	Immature	Inactive	Developing	Ey	x	x	x	Absent or very small	Absent or very small	x
3	Mature	Active	Spawning Capable	Ay	x	< 50 %	Possibly	Possibly	Possibly	Possibly
4.1	Mature	Active	Spawning	Ay	√	< 50 %	Possibly	Possibly	Possibly	Possibly
4.2	Mature	Active	Spawning	Mn/Hy	Possibly	< 50 %	Possibly	Possibly	Possibly	Possibly
5	Mature	Inactive	Regressing	Ay	x	≥50 %	√	Many, often large or clumped	Many, often large and folded	Possibly
6.1	Mature	Inactive	Regressed 1	Un/Ey	x	100 %	Possibly	Many, often large or clumped	Many, often large and folded	Possibly
6.2	Mature	Inactive	Regressed 2	Un/Ey	x	x	√	Many, smaller than class 6.1	Many, smaller than class 6.1	Possibly
7	Mature	Inactive	Regenerating	Un/Ey	x	x	x	Many, smaller than class 6.2	Many, smaller than class 6.2	Possibly

Appendix 2. Ovaries histology cross-section of skipjack tuna in Indian Ocean Indonesian EEZ Un=unyolked; EY=early yolked; AY=advanced yolked; MN=migratory nucleus; Hy=hydrated; POF=post-ovulatory follicles; α=alpha atresia; β= beta atresia BB=brown bodies; MB=muscles bundle.