The reproductive potential of yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788) caught in the eastern part of the Indian Ocean

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Abstract. Knowledge of reproduction biology provides valuable information to better understanding population dynamics, including a population's resilience to fishing. The yellowfin tuna reproductive biology in the eastern Indian Ocean was studied by analyzing some essential characteristics: size at maturity, the spawning season, frequency of spawning, egg diameter, and batch fecundity. From 2018 to 2020, 206 female yellowfin tuna were sampled (23-170 cmFL). They were using histological and morphological analysis, resulting in the size at maturity (Lm_{50}) of yellowfin tuna being 92.73 cmFL. The spawning season of yellowfin tuna occurred between September and May, spawning every 1.55 days within the spawning period. The oocytes size ranged between 27.1 – 570.5 μm, and the mean size of oocytes (mean ± standard deviation) was 189.5±129.4 μm. The mean batch fecundity was 3.1±1.7 million oocytes, and the mean relative batch was 82.4±38.4 oocytes gram^{-1} of GGT weight. The uncertainties in current yellowfin stock assessment models can be reduced by providing complete information on the reproductive traits in the region to achieve a sustainable fishery.

1 Introduction

Yellowfin tuna (*Thunnus albacares* Bonnaterre, 1878) is a large epipelagic species distributed in tropical and subtropical waters [1]. It is the primary target species for tuna fishery in the Indian Ocean [2, 3]. Yellowfin tuna is harvested by different fishing gears worldwide due to its high demand [4]. Longlines, handlines, pole and lines, purse seines, gillnets, and troll lines are mainly used to catch this fish [5-7]. Since the early 1980s, in the Indian Ocean, the total annual catch of yellowfin tuna has increased significantly. The yearly average catch reached 424,103 metric tons in 2015-2019. The Indian Ocean Tuna Commission (IOTC) determined that yellowfin tuna stock remains overfished and subject to overfishing. Due to various uncertainties, the decline in stock status is not well understood [8]. A proper estimation of the stock's reproductive traits would better manage this species in the area.

Yellowfin tuna is multiple spawners species with an asynchronous development and indeterminate fecundity type [4, 9]. The spawning frequency of yellowfin tuna is around 1.5

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days [10, 11] throughout the year in a large area in the tropical zone [12-14]. In the Indian Ocean, spawning occurs from December to March in the equatorial region [15]. Histological analysis is required to obtain an accurate classification of gonadal development. The size of the gonads females of yellowfin tuna can be similar between the immature and the post-spawning phase, so there is often a misclassification among them [11] [9].

Few studies have focused on reproductive biology because of the ecological importance of yellowfin tuna and the overfished stock status in recent years [3, 4, 16]. Research on the reproduction of yellowfin tuna has also been conducted in Indonesia [14, 17-19]. This study aims to determine the reproductive potential of yellowfin tuna in the eastern part of the Indian Ocean, including size at maturity (Lm_{50}), the spawning season, spawning frequency, egg diameter, and fecundity.

2 Materials and methods

2.1 Sample collection

Samples of Yellowfin tuna ovaries were collected from port sampling (n=158) and surveys onboard on commercial longline tuna operated in the eastern Indian Ocean (n=48). The port sampling collection was conducted in two ports in Bali, which were Benoa and Kedonganan ports. A total of 206 ovaries length ranged between 23-170 cmFL. Samples were collected from March to August 2018, January to December 2019, and February to May 2020.

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. Samples from Kedonganan were caught by handline tuna based on FADs fisheries. Meanwhile, from Benoa port and observer onboard were caught by longline tuna.

2.2 Sample analysis

Histological analysis used criteria for the south Pacific albacore tuna [20] (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 [20, 21].

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 presently classed as immature. Based on the spawning activity, the mature-active includes spawning and spawning capable stage. Regressing, regressed 1, reverted 2, and regenerating stage classed as mature-inactive. In contrast, all immature phases (immature and developing) are organized as immature-inactive (see Appendix 1). Small fish length range between 23-68 cmFL staged visually using yellowfin tuna sexual development criteria [22].

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) [23]. 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) [24, 25]. Binomial logistic regression was used [26, 27], 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 adult 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 [28, 29]. Advanced yolked was used as the threshold of mature fish. The migratory nucleus/hydrated oocytes 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.

The spawning interval is the total number of females with POFs divided by the number of spawning females. The spawning frequency is estimated as the inverse of the spawning fraction [30, 31]. Assuming that POFs are still visible for 24 hours in the ovary [20].

3 Result

3.1 Length at 50% maturity

Most of the samples (79%) analyzed in this study were classed as mature fish. The smallest mature fish was 99 cmFL, while the largest immature was 108 cmFL. The Lm_{50} was estimated at 92.73 cmFL (R^2=0.95, N=206) as a maturity threshold at the advanced yolked stage.

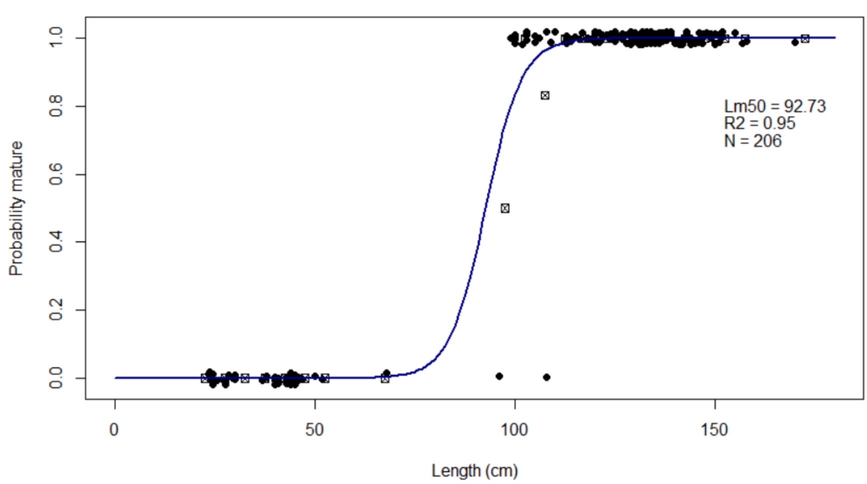

Fig. 1. The estimated proportion of mature female yellowfin tuna.

3.2 Spawning season and spawning frequency

The spawning activity (Appendix 1) showed that mature-active fish were found nearly throughout the years except for June-July (Fig.2). Spawning and spawning capable (mature-active) females occurred between September and May. Mature-inactive females occurred in January and March to July. Seventy-six spawning female ovaries were observed for the presence of the POFs from January to May, October, and December (Table 1). The fraction of spawning females with POF was 0.64, equal to the spawning interval of 1.55 days.

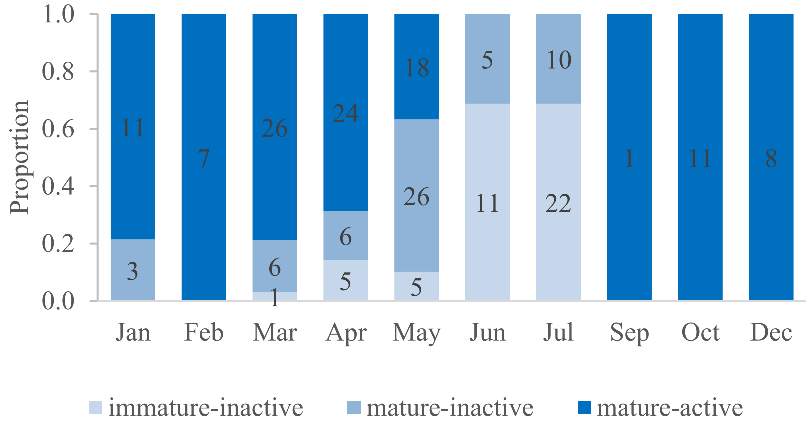

Fig. 2. The proportion of spawning activity of female yellowfin tuna by month.

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

Month	Count of POFs	Count of Mature Females
January	9	11
February	3	7
March	11	17
April	17	19
May	7	9
October	1	9
December	1	4
Total	**49**	**76**

3.3 Oocyte size and fecundity

The oocytes size ranged between 27.1 – 570.5 µm (Table 2), and the mean size of oocytes (mean±standard deviation) was 189.5±129.4 µm. 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.

29 female fish ranging between 109-144 cmFL were analyzed to estimate the batch fecundity. Twenty-two samples had advanced yolked, and seven had hydrated oocytes as the MAGO. The batch fecundity was between 0.65 – 7.24 million oocytes. The mean batch

fecundity was 3.1±1.7 million oocytes, and the mean relative batch was 82.4±38.4 oocytes gram-1 of GGT weight.

Table 2. Oocyte size of female yellowfin tuna observed

No.	Oocyte development stage	Minimum size (μm)	Maximum size (μm)
1	Unyolked	27.12	121.09
2	Early yolked	83.04	329.92
3	Advenaced yolked	169.42	465.69
4	Migratory nucleus	239.13	570.47
5	Hydrated oocytes	308.67	554.00

4 Discussion

The size at maturity was estimated at 92.73 cmFL. The previous study in the western Indian Ocean applying the same threshold (advanced yolked) has shown a higher value of 102 cm FL [3]. In the west of the Pacific Ocean, [32] estimated that Lm_{50} of yellowfin tuna was 107.77 cmFL. [19] reported a slightly different result of Lm_{50} (94.6 cmFL). While [12] found that Lm_{50} of yellowfin tuna in the Molucca Sea was 98.1 cm. The difference in latitude and marine environment may be the cause of the different results. The area nearer the equator may cause the yellowfin tuna to reach sexual maturity more quickly [32]. [12] suggested that yellowfin tuna at higher latitudes may have a delay in maturity.

Figure 1 also shows two lengths groups, sizes 23-68 cm FL and 96-170 cmFL. The lack of samples sized between 70-100 cmFL is because they are challenging to obtain. It is presumably due to the north-south movement where the fish at that size moved to the southern waters (>20 ° S) where the Indonesian tuna fishing fleets do not reach the area. The same movement activity showed in some temperate tuna species such as albacore, where immature fish spread in the southern region (30 °S) while mature fish concentrated in the 10-25 °S [31], [33].

Based on the spawning activity classes, the spawning season occurred almost yearly (September to May). In the western Indian Ocean, yellowfin tuna has two main reproductive periods based on the GSI values (November to February and June) [3]. While [6] identified a single period from November to April in the eastern part. The spawning period estimated in this study was found to be longer than in previous studies. In the tropical waters, yellowfin tuna spawns all year round [12, 14, 32]. 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 °C [31], [34].

The spawning frequency was estimated at an interval of 1.55 days. Using the same method [32], the spawning interval in the west of the Pacific Ocean was 1.33 days. In comparison, [12] reported the spawning interval was 1.99 days using hydrated oocytes presence as a method. The result in this study assumed that yellowfin tuna spawned almost daily during the spawning period. A study in the western Pacific Ocean [32] reported that in the peak spawning season (February to June), the mature yellowfin has a spawning interval of 1.4 days. At the same time, the mean spawning interval in the whole spawning period (January to December) was 1.97 days which was lower than the peak season.

Histological analysis showed that yellowfin tuna in the eastern Indian Ocean is classified as capable spawning or higher (spawning and regression stage), containing oocytes at various stages (Appendix 2), assuring an asynchronous development. The oocyte size ranged between 27.1 – 570.5 µm (Table 2), and the mean size of oocytes (mean±standard deviation) was 189.5±129.4 µm. The continuous oocyte size without any gap in the diameter has been considered a sign of indeterminate fecundity [35].

The mean batch fecundity estimated in this study was 3.1±1.7 million oocytes. This result is still within the range of yellowfin tuna studied in the western Indian Ocean [4], [36] (3.07 and 3.27 million oocytes, respectively). At the same time, the mean relative batch was 82.4±38.4 oocytes per gram of GGT weight. This result was higher than the previous studies [3, 12] that estimated 74.4 and 63.5 oocytes gram-1 of the gonad-free weight. The higher result is due to the divisor parameter used, which was the gilled and gutted (processed) fish weight. While previous studies generally use the whole fish weight (gonad-free weight). The yellowfin processed weight was 14% smaller than the entire weight [37], [38]. However, other factors that could cause the discrepancies between studies were geographic differences and intra-population variability in fecundity [3].

5 Conclusion

The size at maturity (Lm_{50}) was estimated at 92.73 cmFL, with the maturity threshold set at the advanced yolked stage of the oocyte development. Spawning season occurred from September to May and spawned every 1.55 days. It has asynchronous oocyte development and indeterminate fecundity. The reproductive aspects described in this study may provide several parameters for improving the yellowfin tuna stock assessment in the eastern Indian Ocean.

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References

1. B.B. Collette, C.E. Nauen, *FAO Species Catalogue. 2 Scombrids of the world. An Annotated and Illustrated catalogue of tunas, mackerels, bonitos, and related species known to date* (FAO, Rome, 1983)
2. V.S. Somvanshi, S. Varghese, A.K. Bhargava, S.P. Varghese, WPTT-04-24 IOTC Proceedings **7**, 8 (2004)
3. I. Zudaire, H. Murua, M. Grande, N. Bodin, Fish.Bull **111**, 252 (2013)
4. I. Zudaire *et al*., Fish Res. **138,** 80 (2013)
5. N. Muhammad, A. Barata, BAWAL **4**, 161 (2012)
6. P. Nootmorn, A. Yakoh, K. Kawises, *Report of the Seventh Session of the IOTC Working Party on Tropical Tunas* (IOTC, Phuket, 2005)
7. R.K. Sulistyaningsih, A. Barata, K. Siregar, JPPI **17**, 185 (2011)
8. IOTC-2020-SC23-R[E]-Rev1, *Report of the 23rd Session of the IOTC Scientific Committee* (IOTC, Seychelles, 2020)
9. K.M. Schaefer, Inter-Amer. Trop. Tuna Comm., Bull. **21,** 5 (1998)
10. G.R. McPherson, Mar. Freshwater Res. **42**, 5 (1991)

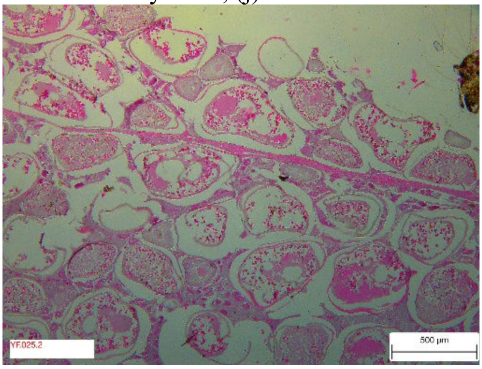
11. K.M. Schaefer, Fish Phy. **19**, 225 (2001)
12. D.G. Itano, *The reproductive biology of yellowfin tuna (Thunnus albacares) in Hawaiian waters and the western tropical Pacific Ocean: project summary* (University of Hawaii, Joint Institute for Marine and Atmospheric Research Hawaii, Hawaii, 2000)
13. B. Stéquert, J.N. Rodriguez, B. Cuisset, F. Le Menn, Aquat. Living Resour. **14**, 5 (2001)
14. R. Andamari, J.H. Hutapea, B.I. Prisantoso, JITKT. **4**, 1 (2012)
15. IOTC-2012-SC15-R[E], *Report of the Fifteenth Session of the IOTC Scientific Committee* (IOTC, Seychelles, 2012)
16. G. Zhu, L. Xu, Y. Zhou, L. Song, J. Ocean Univ. China **7**, 3 (2008)
17. K. Wagiyo, A. Suman, M.P. Patria, BAWAL. **7**, 3 (2015)
18. S. Mardlijah, M.P. Patria, BAWAL. **4**, 1 (2012)
19. G.L. Arnenda, I. Jatmiko, A. Kusdinar, JKPT. **1**, 2 (2019)
20. J.H. Farley, A.J. Williams, S.D. Hoyle, C.R. Davies, S. J. Nicol, PLoS ONE **8**, 4 (2013)
21. M.T. Zischke, J.H. Farley, S.P. Griffiths, I.R. Tibbetts, Rev. Fish Biol. Fish **23**, 4 (2013)
22. M.B. Schaefer, C.J. Orange, IATTC Bull. **1**, 6 (1956)
23. M.A. Williams, *Quantitative Methods in Biology, in Practical Methods in Electron Microscopy,* (Elsevier/North Holland Biomedical Press, Amsterdam and New York, 1997)
24. N.F. Fontoura, A.S. Braun, P.C.C. Milani, Neotrop Ichthyol. **7**, 2 (2009)
25. D.A. Somerton, Can. J. Fish. Aquat. Sci. **37**, 10 (1980)
26. I. Girault, P. S. Sabarros, E. V. Romanov, P. Bach, *17th Working Party on Billfish* (IOTC, La Reunion, 2019)
27. A. Zuur, E.N. Ieno, G.M. Smith, *Analysing Ecological Data. Statistics for Biology and Health* (Springer, New York, 2007)
28. J.R. Hunter, N.C.H. Lo, R.J.H. Leong, NOAA Technical Report NMFS **36**, 67 (1985)
29. T.B. Bagenal, J. Mar. Biol. Assoc. U. K **36**, 2 (1957)
30. J.R. Hunter, B.J. Macewicz, J. R. Sibert, Fish. Bull. **84**, 4 (1986)
31. Z. Dhurmeea *et al*., PLoS ONE **11**, 12 (2016)
32. C.L. Sun, W.R. Wang, S. Yeh, *1st Meeting of the Scientific Committee of the Western and Central Pacific Fisheries Commission* (WCPFC-SC1. BI WP-1, Noumea, 2005)
33. I.C. Chen, P.F. Lee, W.N. Tzeng, Fish Oceanogr. **14**, 71 (2005)
34. H. Ashida, M. Horie, Fish. Sci. **81**, 861 (2015)
35. G. West, Mar. Freshw. Res. **41,** 2 (1990)
36. O.I. Timohina, E.V. Romanov, IPTP Collective **9**, 247 (1996)
37. I. Jatmiko, H. Hartaty, B. Nugraha, IFRJ **22**, 77 (2017)
38. WCPFC-SC2-2006/ME IP 3, *Scientific Committee Second Regular Session* (WCPFC, Manilla, 2006)

7 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. Histological section of yellowfin tuna ovaries. (a) Spawning, (b) Spawning capable, (c) Regressing, (d) Regressed 1, (e) Regressed 2, (f) Regenerating, (g) uy= unyolked, ey=early yolked, mn=migratory nucleus, (h) POFs, (i) Beta atresia, alpha atresia, AY=advanced yolked, (j) BB/bb=brown bodies.



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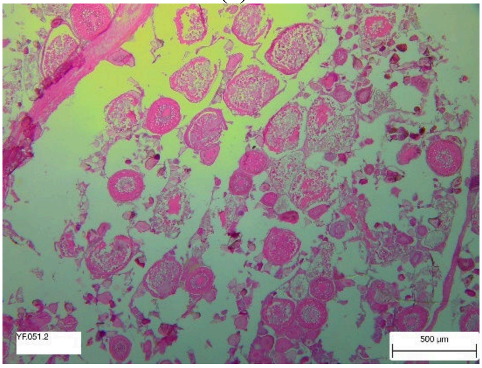

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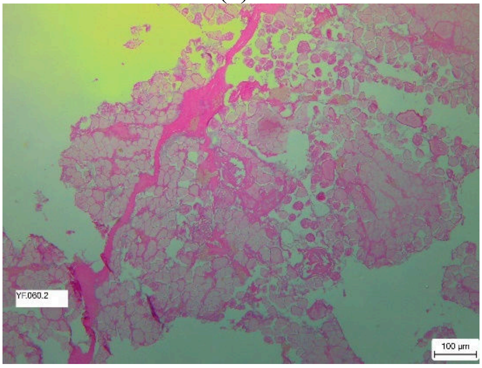

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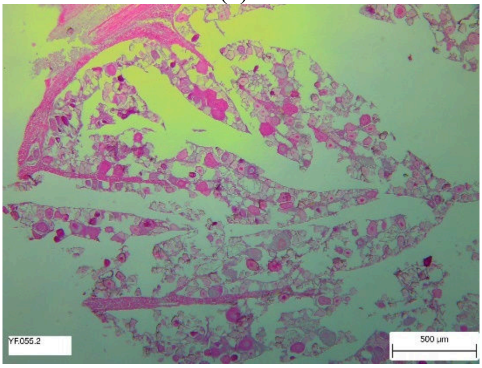

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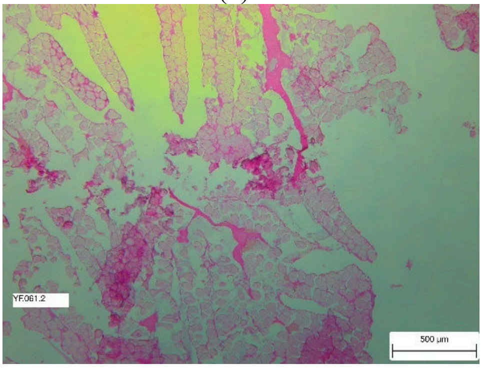

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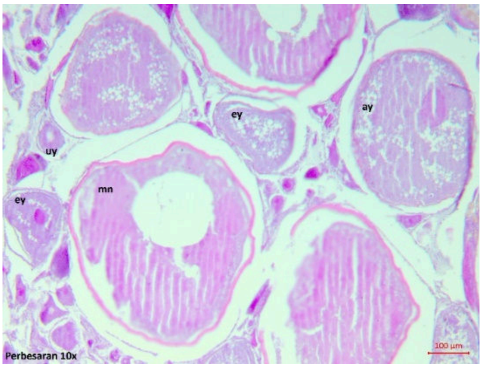
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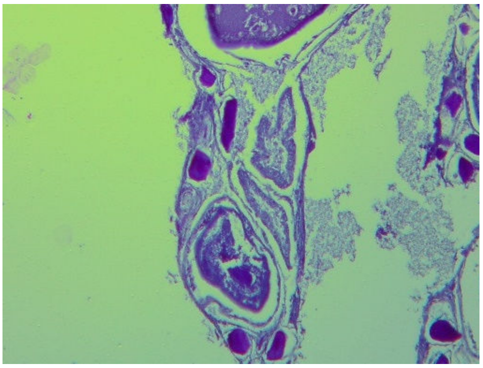

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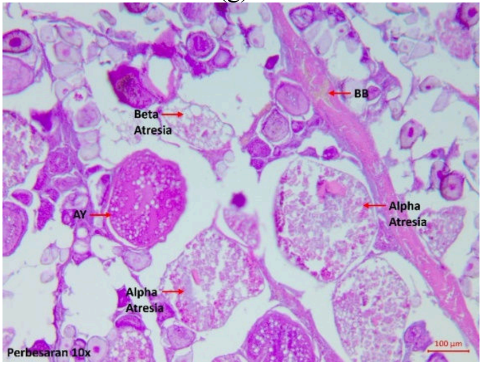
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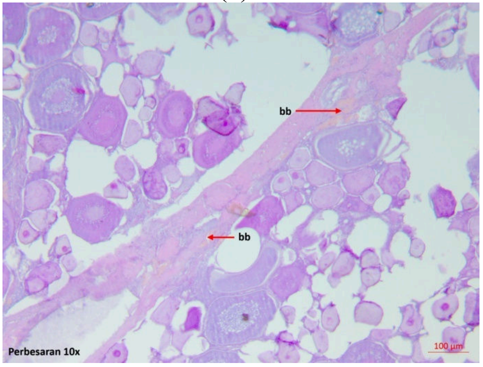
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