CCSBT-ESC/2108/Info 02
(ESC Agenda item 4.1)

SBT REPRODUCTIVE – **Indonesia**

Reproductive activity of Southern Bluefin Tuna (*Thunnus maccoyii*) caught in Indonesian tuna fisheries

Hety Hartaty, Bram Setyadji, Ririk Kartika Sulistyaningsih

Research Institute for Tuna Fisheries

Prepared for the 26th Meeting of the Extended Scientific Committee Meeting (ESC26) of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT)
23 – 31 August 2021 (Online)

Research Institute for Tuna Fisheries
Ministry of Marine Affairs and Fisheries – Indonesia

Summary

This paper provides updated information about reproductive studies of southern bluefin tuna (SBT) being undertaken in Indonesia. The standard reproductive classification was used to assess the ovaries of 254 females collected by the Indonesian scientific observer and the port landing monitoring program in Benoa, Bali. Samples were collected in 2017-2020 from the scientific observer and port landing monitoring program. All samples were by Indonesian tuna longline vessels. The length of SBT caught ranged between 134 and 194 cm fork length (cm FL). Gonad samples were fixed in 10% buffered formalin and then embedded in paraffin and standard histological sections were prepared (cut to 5 µm and stained with H&E). Histological sections were classified using criteria of southern bluefin tuna and south Pacific albacore tuna. All samples were classified as mature fish. The development class were identified as spawning, spawning capable, regressing-potentially reproductive, regressed 1, regressed 2 and regenerating. Based on its reproductive activity, from 122 fish that were at spawning stage, 44% of them were small fish (<155 cm FL). Further ovary samples are required (and are currently being collected) from statistical areas 1 and 2 to further examine the reproductive activity of SBT.

Introduction

Tuna is an oviparous species that have asynchronous oocyte development and are multiple spawners (Schaefer, 1998, 2001). Accurate information of 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, incorrect assignment can have implications for 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 (Schaefer, 1998).

To collect scientific information on SBT, biological samples including gonads were collected by the Indonesian scientific observer and port landing monitoring program. The Indonesian scientific observer program started in 2005 but SBT gonads have only been collected since 2017. This paper provides updated information on the analysis of SBT gonads collected from Indonesian tuna fisheries.

Materials and Methods

Sample Collection

Ovaries were collected in 2017-2020 from the Indonesia scientific observer and port landing monitoring program in Benoa, Bali. Samples were collected during bluefin tuna fishing season (September to April) from the scientific observer and port landing monitoring program in Benoa Port, Bali. All samples were caught by Indonesian tuna longline vessels.

Fork length (FL) and body weight (gutted) were recorded for each fish from the port landing samples. Scientific observer samples do not have body weight data due to the limited equipment onboard the vessels. The length of SBT sampled ranged between 134 and 194 cm FL and weight ranged between 45 and 161 kilograms. Straight fork length was measured using caliper that has a precision of 1 cm.

For the observer sampling, a cross-section was removed from the anterior, middle and posterior of one ovary lobe from each fish, which was then frozen through the fishing trip. For the port sampling, the whole ovary cannot be sampled as the gonads are removed at sea, along with the gills and guts, leaving only the posterior part of the gonad available for sampling (Appendix 1). Samples from the landing port were in fresh condition when collected.

Samples were then transferred to the laboratory and immediately fixed in 10% buffered formalin before being placed into cassettes. Samples were embedded in paraffin and standard histological sections prepared (cut to 5 µm and stained with Harris H&E).

Histological classification

Histological sections were classified using criteria for southern bluefin tuna and South Pacific albacore tuna (Davis et al., 2003; Farley et al., 2013). Females were classified into development stages based on the most advanced group of oocytes (MAGO), postovulatory follicles (POFs), alpha and beta atresia, and maturity markers present in the ovary (seeAppendix 2 for classification used). The most advanced group of oocytes (MAGO) was staged into one of 5 classes: unyolked, early yolked, advanced yolked, migratory nucleus or hydrated oocytes (Appendix 3). Each ovary was also scored based on the presence or absence of postovulatory follicles (POFs) (Appendix 4). The maturity markers considered were well defined muscle bundles, numerous brown bodies, residual hydrated oocytes and the thickness of the ovary wall (Appendix 5). The maturity markers are considered as signs of prior reproductive activity (Farley et al., 2016; 2013; Zischke et al., 2013).

Ovaries containing advanced yolked, migratory nucleus or hydrated oocytes and/or POFs were classed as mature, and ovaries with unyolked or early yolked oocytes as the MAGO but with maturity markers present were also classed as mature as well. Ovaries containing unyolked and early yolked oocytes as the MAGO but no POFs, atresia or maturity markers were classed as immature (Farley et al., 2013).

Results

Based on the study, all 254 ovaries examined were classed as mature; 122 were classed as spawning (48.0%), 66 as spawning capable (26.0%), 41 as regressing-potentially reproductive (16.1%), 12 as regressed 1 (4.7%), 11 as regressed 2 (4.3%) and 2 as regenerating (0.8%). Brown bodies were found in 70% from the total samples but only one sample had residual hydrated oocytes (Appendix 5f). Numerous brown bodies also found in the ovaries at regenerating stage. The brown bodies were the very late stages of atresia (gamma/delta) and were yellow-orange-brown in colour (Appendix 5a, b); these are linked to post-spawning activity in the gonad (Farley et al., 2013; Hunter & Macewicz, 1985). The thickness of the ovary wall was not observed in the remaining ovaries, possibly due to the limitation of the samples being too small (i.e., the remaining posterior part of the gonad) or not enough tissue was to cut for the histology section.

The size of the female SBT samples ranged between 134-194 cm FL (Fig. 1). Fish classed as spawning ranged between 135-188 cm FL, spawning capable 134-194 cm FL, regressing 136-185 cm FL, regressed 1 ranged between 143-172 cm FL, regressed 2 ranged between 140-185 cm FL and regenerating ranged between 153-169 cm FL. Fish <155 cm FL in the Indonesian catch are considered to be small/young fish (Farley et al., 2007; Sulistyaningsih et al., 2018). In this study 47% of fish sampled would be categorized as small/young individual. From 122 fish that were at spawning stage, 44% of them were small fish (<155 cm FL). This result could be useful to generate advice and development of management strategies to maintain the sustainability of bluefin tuna stock in Indian Ocean. However, further ovary samples are required (and are currently being collected) from statistical areas 1 and 2 to further examine the reproductive activity of SBT.

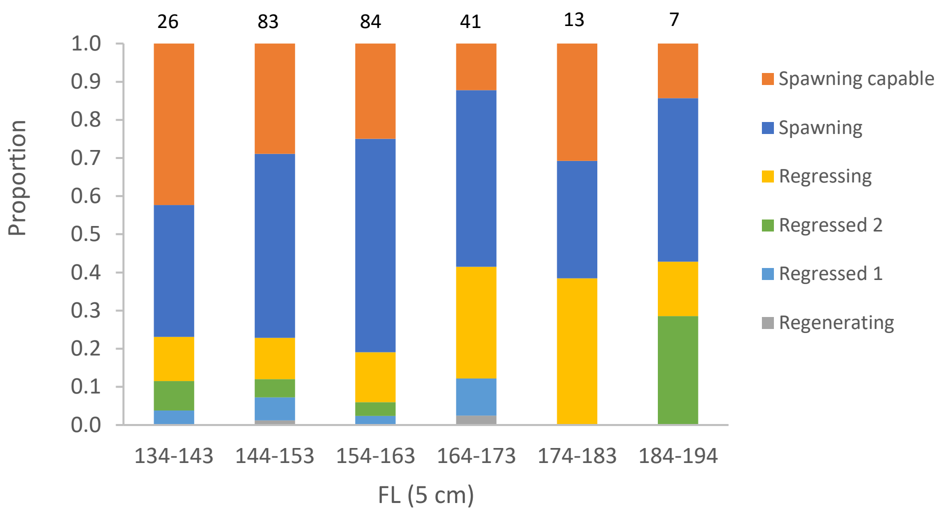


Figure 1. Length frequency of female SBT in each development class (the number at the top of the column is the number of samples for each length class)

Acknowledgement

The success of the SBT monitoring program in Indonesia has only been possible due to the dedicated efforts of all participating scientists at the Research Institute for Tuna Fisheries (Bali), in particular that of the observers and the enumerators and the laboratory assistants Ms Indrastiwi Pramulati and Ms Desy Shintya Irene in collecting and preparing the gonad samples. We also thank Ms Jessica Farley for the assistance and the contribution to the development of the reproductive biology of tuna studies.

References

Davis, T. L. O., Farley, J., Bravington, M., & Andamari, R. (2003). *Size at first maturity and recruitment into egg production of southern bluefin tuna: Final report*. CSIRO Marine Research.

Farley, J., Andamari, R., & Proctor, C. (2007). Update on the length and age distribution of SBT in the Indonesian longline catch. *CCSBT 8th Meeting of the Stock Assessment Group and the 12th Meeting of the Extended Scientific Committee*, 4–8.

Farley, J., Clear, N., Kolody, D., Krusic-Golub, K., Eveson, P., & Young, J. (2016). *Determination of swordfish growth and maturity relevant to the southwest Pacific stock. CSIRO Oceans & Atmosphere, Hobart*. CSIRO Oceans & Atmosphere, Hobart.

Farley, J. H., Williams, A. J., Hoyle, S. D., Davies, C. R., & Nicol, S. J. (2013). Reproductive Dynamics and Potential Annual Fecundity of South Pacific Albacore Tuna (Thunnus alalunga). *PLoS ONE*, *8*(4), e60577. https://doi.org/10.1371/journal.pone.0060577

Hunter, J. R., & Macewicz, B. J. (1985). Rates of atresia in the ovary of captive and wild northern anchovy, Engraulis mordax. *Fishery Bulletin*, *83*(2), 119–136.

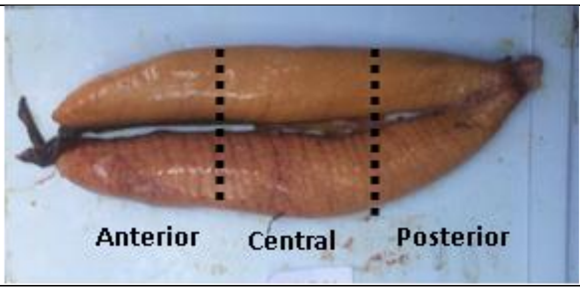
Schaefer, K. M. (1998). Reproductive biology of yellowfin tuna Thunnus albacares in the eastern Pacific Ocean. *IATTC*, *21*(5), 05–269.

Schaefer, K. M. (2001). Reproductive biology of tunas. *Fish Physiology*, *19*, 225–270.

Sulistyaningsih, R., Farley, J., & Proctor, C. (2018). *Update on the length and age distribution of southern bluefin tuna (SBT) in the Indonesian longline catch*. CCSBT-ESC/1809/09.

Zischke, M. T., Farley, J. H., Griffiths, S. P., & Tibbetts, I. R. (2013). Reproductive biology of wahoo, Acanthocybium solandri, off eastern Australia. *Reviews in Fish Biology and Fisheries*, *23*(4), 491–50

Appendix 1. SBT gonad samples, (a) cross-section of 3 parts of the gonad; (b-c) port landing samples, mostly get remainder of the posterior part only; (d-e) observer samples, mostly get complete gonad pair



(a)

(b)

(c)

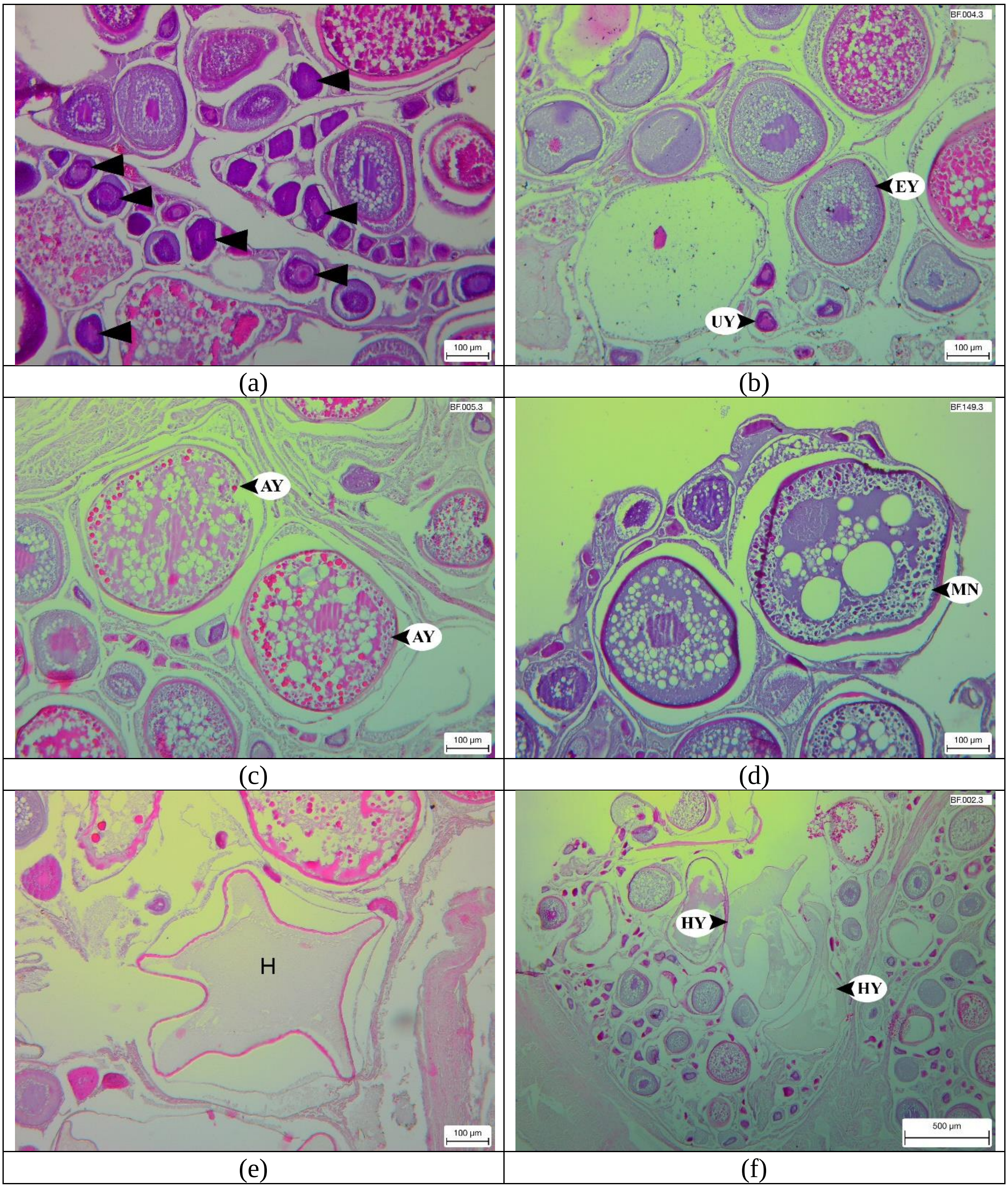
(d)

(e)

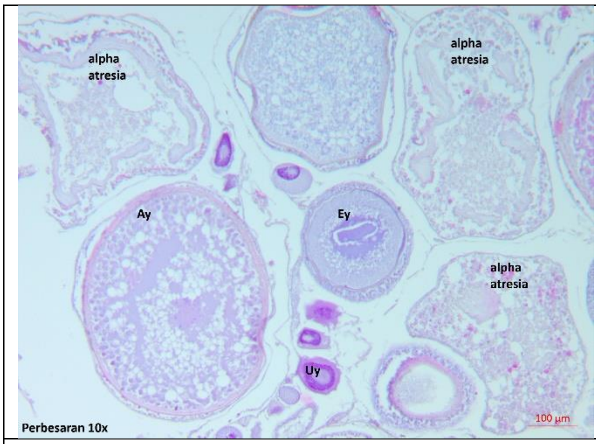
Appendix 2. Histological classification criteria based on the most advanced group of oocytes (MAGO), postovulatory follicles (POFs), atresia and maturity markers. UY = unyolked, EY = early yolked, AY = advanced yolked, Mn = migratory nucleus, H = hydrated

Class	Maturity Status	Activity	Development Class	MAGO	POFs	Atresia		Maturity Marker						
						α	β	Orange/brown Bodies	Ovary cross-section	Ovary wall	Cords	Muscle Bundles	Residual Hydrated	Oocytes structure
1	Immature	Inactive	Immature	UY	No	No	No	Absent or verysmall	Small	Thin	Thin	Absent or very small	Absent	Usually compact
2	Immature	Inactive	Developing	EY	No	No	No	Absent or very small	Small	Thin	Thin	Absent or very small	Absent	Usually compact
3	Mature	Active	Spawning Capable	AY	No	< 50%	May be present	Maybe present	Large	Thick	Thick	Maybe present	Maybe present	Compact or disorganised
4a	Mature	Active	Spawning	AY	Yes	< 50%	May be present	Maybe present.	Large	Thick	Thick	Maybe present	Maybe present	Usually disorganized with space between oocytes
4b	Mature	Active	Spawning	Mn, or H	Possibly	< 50%	May be present	Maybe present	Large	Thick	Thick	Maybe present	Maybe present	Usually disorganized with space between oocytes
5	Mature	Inactive	Regressing - Potentially Reproductive	AY	No	>50 %	May be present	Many, often large or in clumps	Large	Thick	Thick	Many, often large and "folded"	Maybe present	Usually disorganized with space between oocytes
6a	Mature	Inactive	Regressed 1	UY/ EY	No	100 %	May be Present	Many, often large or in clumps	Medium	Thick	Thick	Many, often large and "folded"	Maybe present	Usually disorganized with space between oocytes
6b	Mature	Inactive	Regressed 2	UY/ EY	No	No	Yes	Many, smaller than class 6a	Small	Thick	Thinner than class 6a	Many, smaller than class 6a	Maybe present	Disorganized but usually more compact than 6ba
7	Mature	Inactive	Regenerating	UY/ EY	No	No	No	Many, smaller than class 6b	Small	Often thicker than class 1 or 2	Thinner than class 6b	Many, smaller than class 6a	Maybe present	Disorganized but usually more compact than 6b

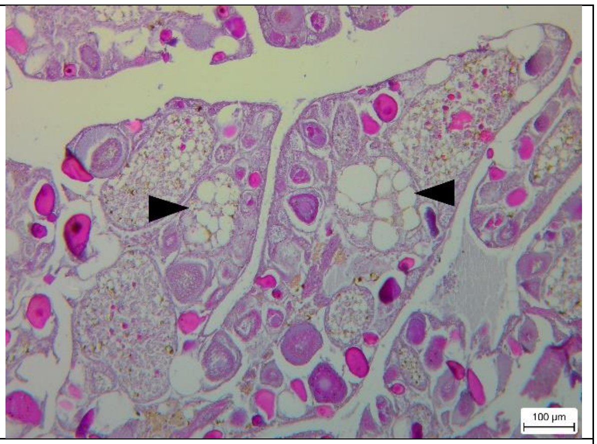
Appendix 2. Oocyte development classes in histological section of SBT ovaries: (a) Unyolked (UY, black arrows); (b) early yolked (EY); (c) advanced yolked (AY); (d) migrated nucleus (MN); (e-f) hydrated oocytes (H/HY).



Appendix 3. Atresia of advanced yolked oocytes and postovulatory follicles in SBT ovaries: (a) alpha atresia; (b) beta atresia (black arrows); (c-d) postovulatory follicles (POF).

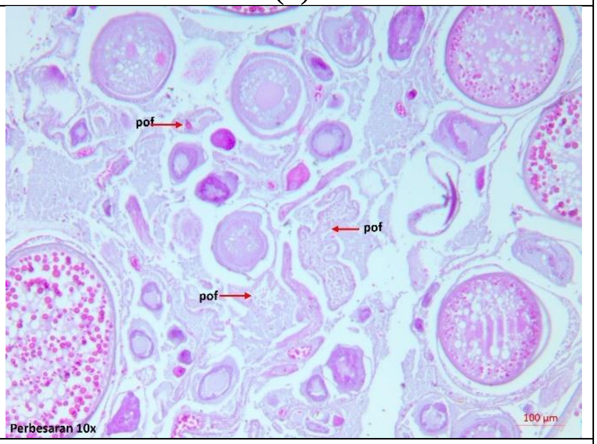


(a)



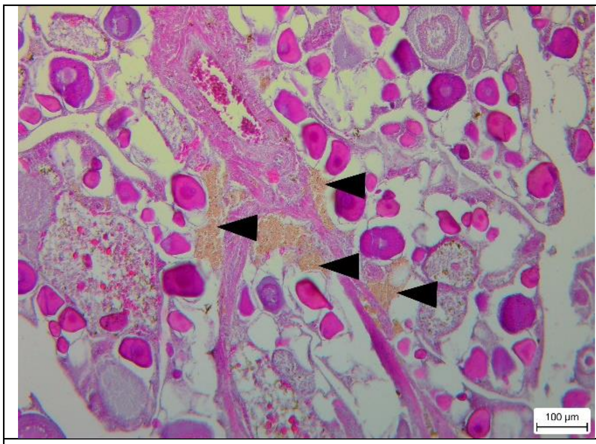
(b)

(c)

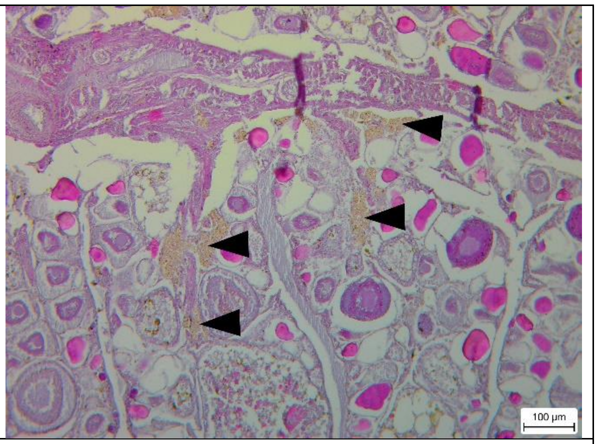


(d)

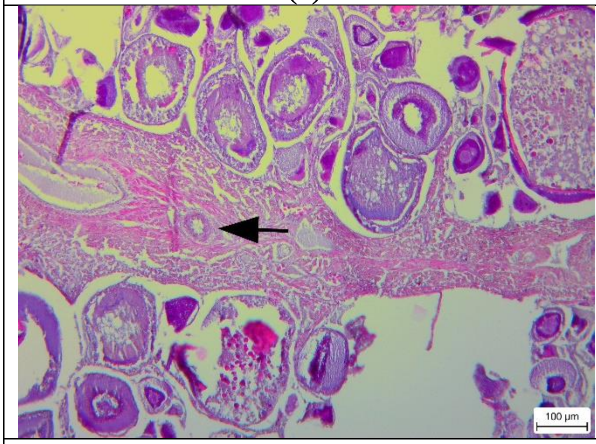
Appendix 4. Histological section of SBT ovaries showing examples of maturity markers: (a-b) brown bodies (black arrows); (c-e) muscle bundles (black arrow, MB); (f) residual hydrated oocytes (RH).



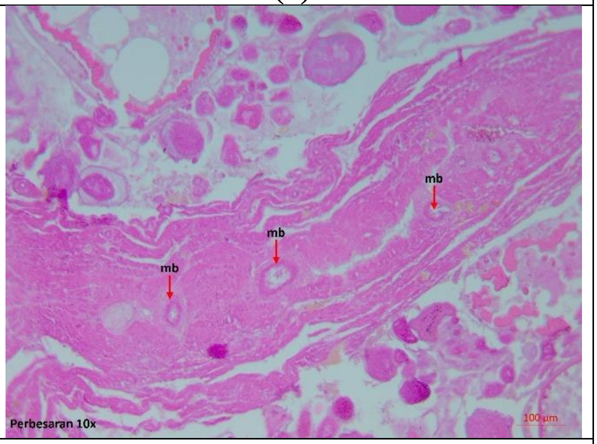
(a)



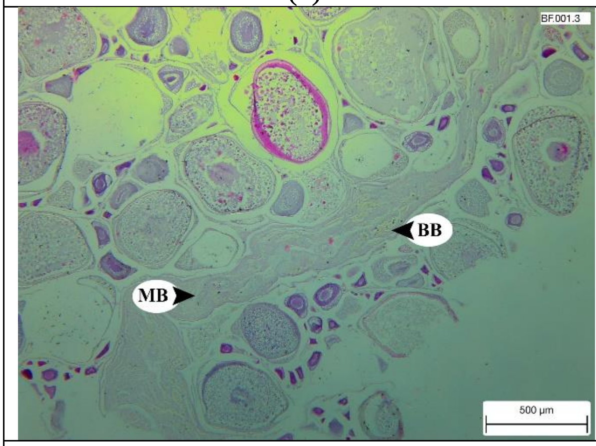
(b)



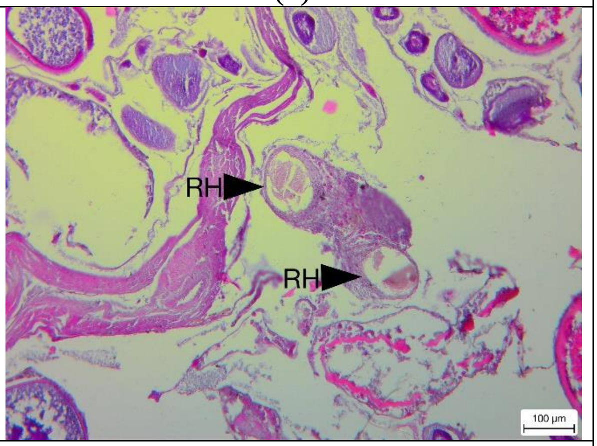
(c)



(d)



(e)



(f)