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UPDATE STUDY OF THE REPRODUCTIVE ACTIVITY OF SBT CAUGHT IN INDONESIAN TUNA FISHERIES

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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 54 females collected by the Indonesian scientific observer and the port landing monitoring program in Benoa, Bali. Samples were collected in March from the scientific observer program (n=5 samples) and in September to December (n=49 samples) from the port landing monitoring program. All samples were caught in area 1 of CCSBT statistical area by Indonesian tuna longline vessels. The length of SBT caught ranged between 136 and 186 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, and regressed 1. Based on its reproductive activity, 30% of the small fish (<150 cm FL) were spawning. 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 2019 from the Indonesia scientific observer and port landing monitoring program in Benoa, Bali. Samples were collected in March from the scientific observer program (n=5 samples) and in September to December (n=49 samples) from the port landing monitoring program. 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 138 and 186 cm FL and weight ranged between 54 and 146 kilograms. Straight fork length was measured using caliper that has a precision of 1 cm. All samples were captured in area 1 of CCSBT statistical areas. The captured area was obtained from the Catch Documentation Scheme (CDS) data.

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 in a cassette. Samples were embedded in paraffin and standard histological sections prepared (cut to 5 µm and stained with 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 (see Appendix 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 54 ovaries examined were classed as mature; 14 were classed as spawning (25.9%), 29 as spawning capable (53.7%), 10 as regressing-potentially reproductive (18.5%), and 1 as regressed 1 (1.9%). Brown bodies were found in all samples but only one sample had residual hydrated oocytes (Appendix 5e). The brown bodies were in 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). Approximately 83% of fish had well defined muscle bundles that indicated previous spawning. Muscle bundle were 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. Muscle bundles are distinct structures of remnant muscle and connective tissue surrounding blood vessels and are indicator of previous spawning (Brown-Peterson et al., 2011; Lowerre-Barbieri et al., 2011).

The size of the female SBT samples ranged between 138-186 cm FL. Fish classed as spawning ranged between 142-186 cm Fl, spawning capable 138-170 cm FL, regressing 146-182 cm FL and there was only one fish classed as regressed 1, which was 154 cm FL (Fig. 1). The same length range of fish was analysed in the previous year samples; they ranged between 136-185 cm FL (Appendix 6). Fish <155 cm FL in the Indonesian catch are considered to be small/young fish (Farley et al., 2007; Sulistyaningsih et al., 2018). In our study 55% of fish sampled would be categorized as small/young individual, and 30% of those were classed as spawning based on our histological analysis. 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.

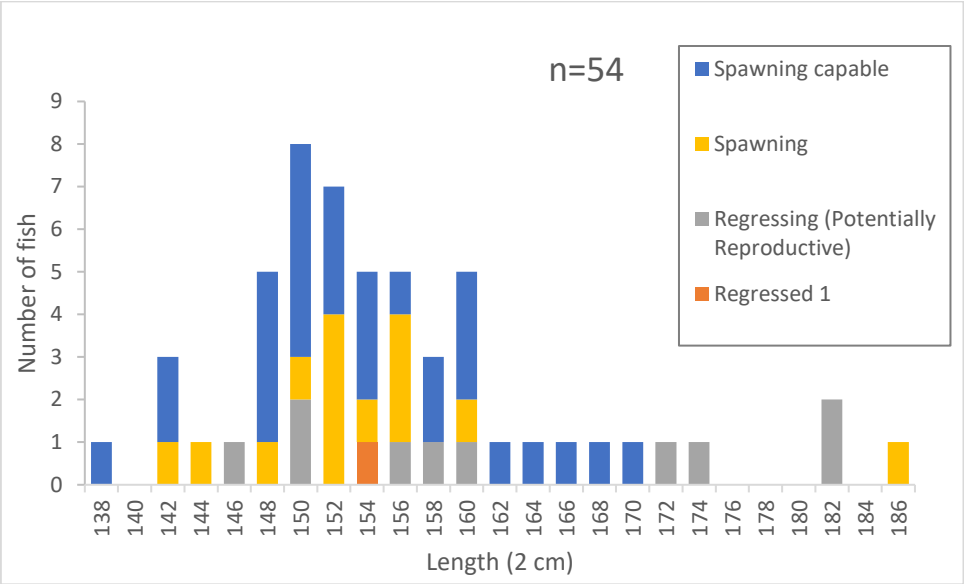

Figure 1. Length frequency of female SBT in each development class caught in area 1 (n=54)

Acknowledgement

The success of the SBT monitoring program in Indonesia has only been possible due to the dedicated efforts of all participating scientists at 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.

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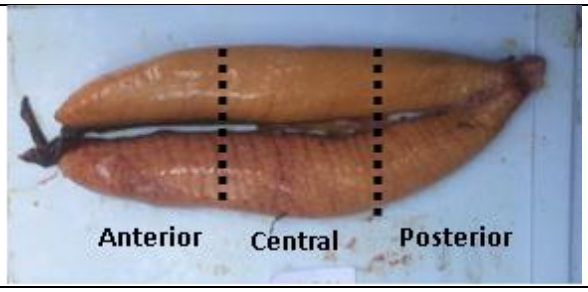
Appendix 1. SBT gonad samples, (a) cross-section of 3 parts of the gonad; (b-c) port landing samples, mostly get remainder of the posterior part only; (d-e) observer samples, mostly get complete gonad pair



(a)

(b)

(c)

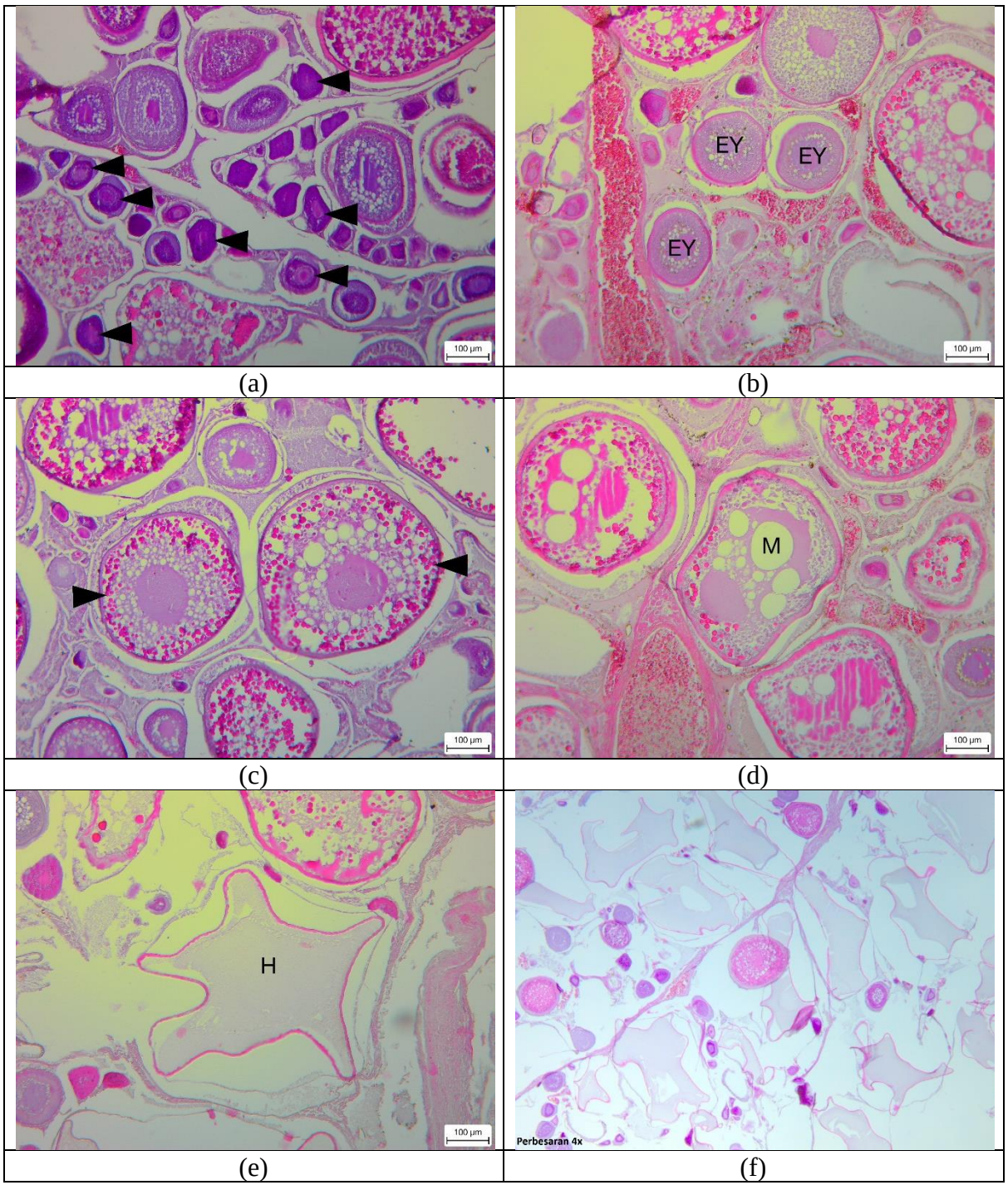
(d)

(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	inner than class	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	icker than cla	inner than class	Many, smaller than class 6a	Maybe present	Disorganized but usually more compact than 6b

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

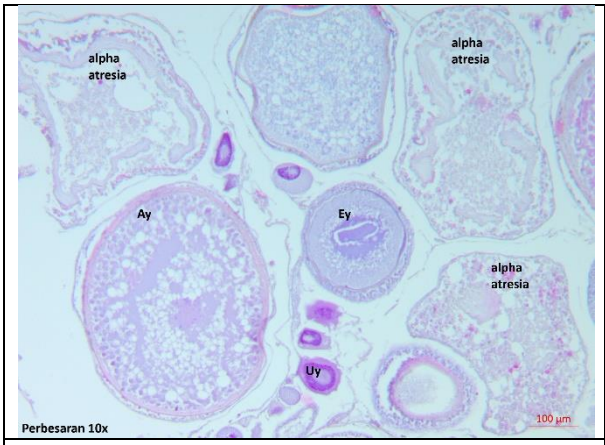


(a) (b)

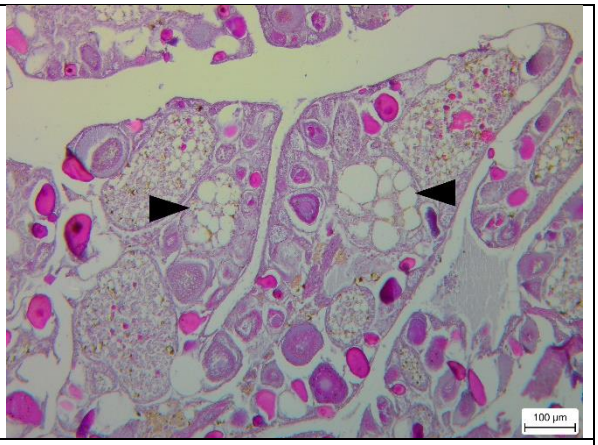
(c) (d)

(e) (f)

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).

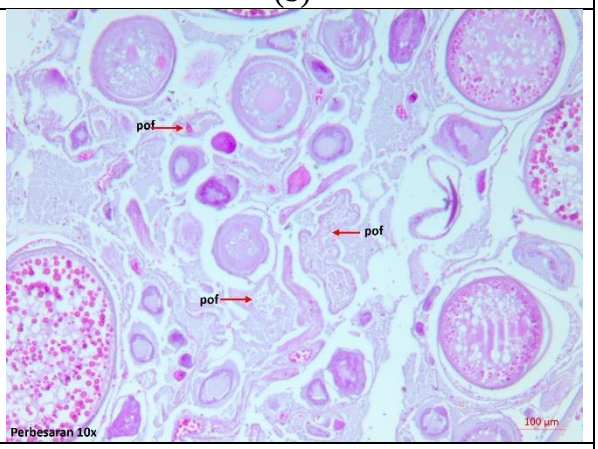


(a)



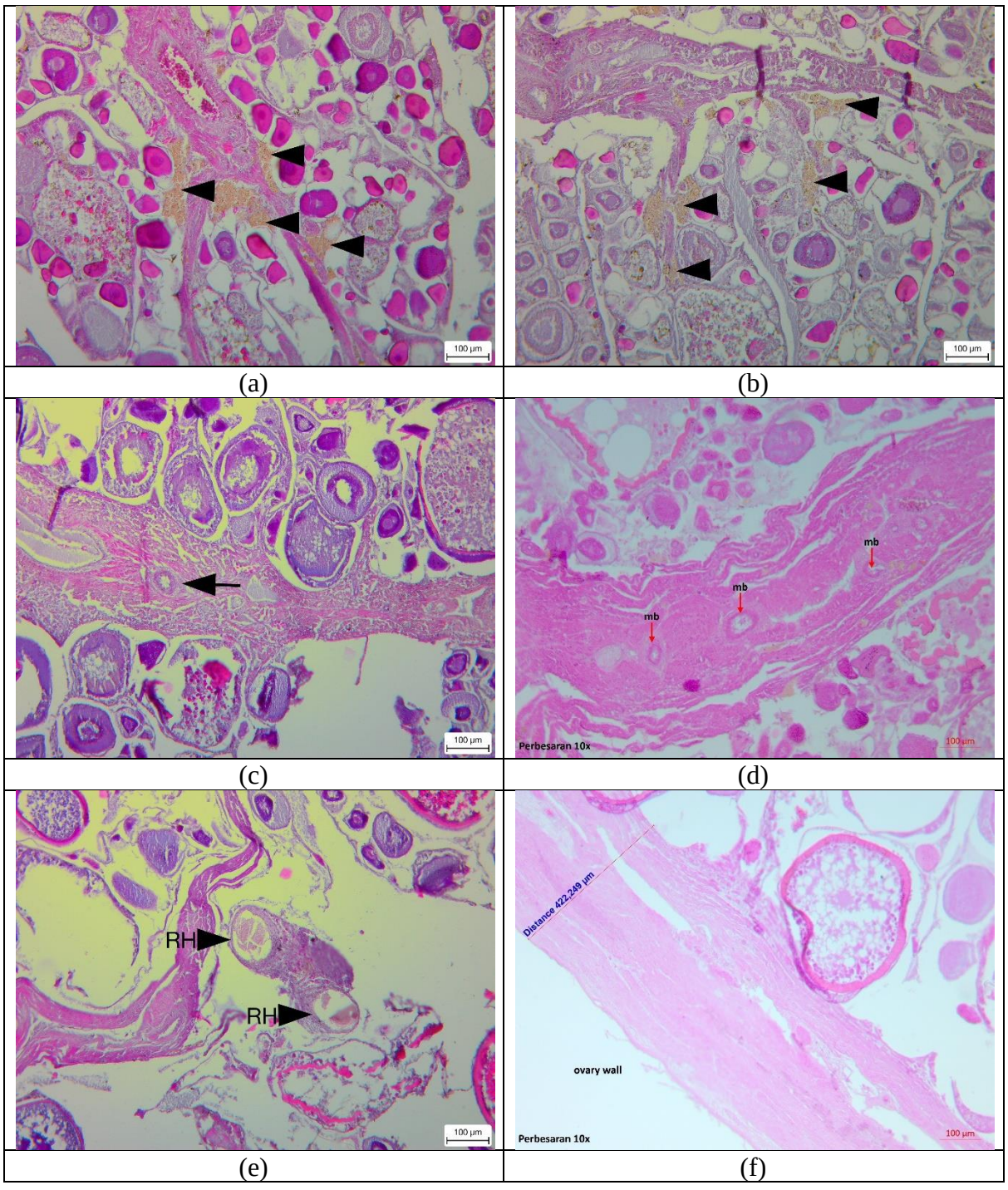
(b)

(c)



(d)

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



Appendix 6. Summary of the samples and reproductive activity of SBT from 2017-2019

Year/study	Length (cmFL)	Captured Area	Number of samples	Maturity	Gonad Classification	Data source
2017-2018 (Hartaty et al., 2019) CCSBT-ESC_1909_42 rev.1	136 – 185	1	3	All mature	Regressed 1 Regressed 2	Observer program
	157 - 185	2	27	All mature	Spawning capable Regressing Regressed 1 Regressed 2	Observer program
2019 (this study)	138 - 186	1	54	All mature	Spawning Spawning capable Regressing Regressed 1	Observer and port landing program