# Study of the Reproductive Activity of SBT Caught in Indonesian Tuna Fisheries

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## Summary

This paper provides information about reproductive studies of southern bluefin tuna (SBT) being undertaken in Indonesia. The standard reproductive classification was used to assess the ovaries of 30 females collected by the Indonesian scientific observer program. Samples were collected from two trips conducted in December 2017 (n=25 samples) and January 2018 (n=3 samples) from area 2 of CCSBT statistical areas, and one trip in April 2018 (n=3 samples) from area 1. The length of SBT caught ranged between 136 and 185 cm fork length (FL). All gonad samples were frozen during the fishing trip and were thawed in the laboratory before the fixation process. 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 (Farley & Davis, 1999) and south Pacific albacore tuna (Farley et al., 2013). The development class of SBT ovaries collected in area 2 were identified as spawning capable, regressing-potentially reproductive, regressed 1 and regressed 2, while ovaries from area 1 were identified as regressed 1 and regressed 2. 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, 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

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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 program. The Indonesian scientific observer program started in 2005 but SBT gonads have only been collected since 2017. This paper provides information on the analysis of gonads collected by these observers from Indonesian tuna fisheries.

#### Materials and methods

#### Material

Ovaries were collected in 2017 and 2018 from the Indonesia scientific observer program. Fork length (FL), capture date, and capture location were recorded for each fish. Samples were collected on two trips conducted in December 2017 to January 2018 (n=27 samples) from area 2 of CCSBT statistical areas, and one trip in April 2018 (n=3 samples) from area 1 (Fig. 1). The length of SBT caught ranged between 136 and 185 cm FL (Fig 2). Straight fork length was measured using a measuring tape that has a precision of 1 cm. Body weight and gonad weight were not recorded due to the limited equipment on board the vessels. A cross-section was removed from the middle of one ovary lobe from each fish and frozen through the fishing trip. 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  $\mu$ m and stained with H&E).



Figure 1. Map of the ovary sampling locations. The number of ovaries collected by area is indicated, n=30.



Figure 2. Length frequency of SBT sampled (area 1 n=3; area 2 n=27)

## Histological classification

Histological sections were classified using criteria for southern bluefin tuna (Farley & Davis, 1999) and south Pacific albacore tuna (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 (Appendix

1). The maturity markers used were orange/brown bodies, muscle bundles, and the thickness of the ovary wall.

## Results

All 30 ovaries examined were classed as mature; three were classed as spawning capable, 15 as regressing-potentially reproductive, 7 as regressed 1, and five as regressed 2. Ovaries were classed as mature because they contained advanced yolked oocytes and/or other "maturity marker", particularly the presence of many brown bodies and muscle bundles (Fig. 3). Ovaries collected in area 2 (December and January) were identified as spawning capable (n=3), regressing-potentially reproductive (n=15), regressed 1 (n=2) and regressed 2 (n=7) (Fig. 4). The three females classed as spawning capable had relatively low numbers of advanced yolked oocytes in their ovaries (see Fig. 3a) suggesting that they would not have spawned in the very near future. Ovaries collected from fish caught in area 1 (in April) were identified as regressed 1 (n=1) and regressed 2 (n=2) (Fig. 4).

#### Discussion

All fish sampled were classed as mature. Most fish were from area 2, which is acknowledged as a staging ground for SBT preparing to migrate to the spawning ground (Shingu, 1981; Farley & Davis, 1998; Farley et al, 2015). Post-spawning fish also migrate through this area on route to feeding grounds in the southern oceans. It is likely that the three females classed as spawning capable from area 2 were migrating north (to area 1) to spawn. It is assumed that females classed regressing 1 and regressing 2 had completed spawning and were migrating south to feeding grounds. It is unknown if females classed as 'regressing-potentially reproductive' were migrating to or from the spawning grounds. If these fish were migrating to the spawning ground, the high level of atresia of yolked oocytes in the ovaries of these fish may be a mechanism for the fish to regulate the supply of energy while migrating (Farley et al, 2015). It is unknown if the smallest fish sampled (<140 cm) would have spawned since fish of this size are rarely caught on the spawning ground (Sulistyaningsih et al. 2019). Further ovary samples are required (and are currently being collected) from statistical areas 1 and 2 to further examine the reproductive activity of SBT.

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Figure 3. Histological section of ovaries showing examples of development stages and maturity marker: (a) spawning capable, (b) regressing-potentially reproductive, (c) regressed 1, (d) regressed 2, (e) thickness of ovary wall, and (f) orange or brown bodies (BB) and muscle bundle (MB). Other annotations: unyolked (UY), early yolked (EY), advanced yolked (AY), alpha atresia ( $\alpha$ ), beta atresia ( $\beta$ )

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Figure 4. Length frequency of female SBT in each development class, (a) Area 1, and (b) Area 2

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Appendix 1. 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.

	Maturity Status	Activity	Development Class	MAG O	POFs	Atresia		Maturity Marker						
Class						α	β	Orange/brown Bodies	Ovary cross- section	Ovary wall	Cords	Muscle Bundles	Residual Hydrate d	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 Hy	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
ба	Mature	Inactive	Regressed 1	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	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	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