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New maturity ogive estimates for southern bluefin tuna

Jessica Farley[1], Paige Eveson[1], Ching-Ping Lu[2], Sung Il Lee[3], Hung-Hung Hsu[4], Doo Nam Kim[3], Campbell Davies[1]

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[1] CSIRO Oceans and Atmosphere, Australia

[2] Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, Taiwan

[3] National Institute of Fisheries Science, Republic of Korea

[4] Eastern Marine Biology Research Center, Fisheries Research Institute, Taiwan

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Abstract

This report provides results of combined analyses of SBT ovaries collected across the southern oceans by CCSBT Members to estimate a maturity ogive. A total of 861 ovaries were collected from fishing grounds south of 30°S between 2010 and 2019, generally between the designated non-spawning months of April to August. Females ranged in size from 66 to 190 cm fork length (FL), although most were between 110 and 160 cm FL. An agreed histological classification scheme was used by two readers to differentiate mature from immature females. Immature and regenerating females were differentiated by the presence/absence of maturity markers in sectioned ovaries. The proportion of mature females was modelled as a function of length and age using logistic regression. Maturity ogives were similar across the four areas examined suggesting that spatial differences in maturity do not exist or are not large enough to be detected with the current data. The data suggests, however, that differences among readers may exist that result in different forms of the maturity ogive. This may be due to differences in classification methods, sample sizes and/or the size range of fish analysed. We recommend that all samples are read by multiple readers with consensus results before drawing any conclusions.

Preliminary results of fitting a maturity ogive to data by Reader 1 (75% of slides) predicted length at 50% maturity of 145.1 cm FL (8.1 years). In addition to the traditional maturity ogive, based on the established criteria, Reader 1 scored the histological sections by the relative abundance and size of the maturity markers present. Ovaries with larger numbers/size of maturity markers were assumed to be highly fecund. Based on this new 'fecundity index', the predicted length at which 50% of females were highly fecund was much higher at 158.8 cm FL (11.2 years). Although qualitative, the fecundity ogive may be a more direct measure of reproductive potential than the traditional maturity ogive as it combines maturity status and annual egg production.

Background

The 2013 Extended Scientific Committee (ESC) adopted a new maturity schedule based on the available biological information and additional information provided by the close-kin maturity estimate to give a spawning potential by age for use in the 2014 stock assessment (Anon 2013). Previous assessments had used "knife-edged" maturity for age 10+ years. The ESC noted that there was no independent estimate of maturity and recognised this uncertainty and the importance of obtaining an updated and unbiased estimate of the proportion of the population that is sexually mature by age and length.

Estimating an unbiased maturity ogive for SBT is complicated because the mature individuals migrate from various winter feeding grounds in the southern ocean to a separate, single spawning area south of Indonesia during the austral summer. As a result of this annual spawning migration, bias towards mature or immature fish can occur depending on the time and area from which fish are sampled.

In 2013 and 2014, a costed proposal for developing an independently estimated maturity ogive (Farley et al., 2013, 2014) was supported by the ESC, and sample collection for maturity was listed as a high priority in the Scientific Research Plan (SRP) work plan for 2015 and ongoing. The

proposal suggested that sampling occur in areas and times when immature and mature fish were mixed on their feeding grounds between April and August. At that time of year females that are mature but resting (after spawning) may be misidentified as immature females. To avoid this misidentification, it was proposed that the histological classification scheme developed for albacore tuna (Farley et al., 2014) be used, where 'maturity markers' in histological sections of ovaries were used to differentiate immature from mature-resting females. It was proposed that ovaries from fish ≥110 cm fork length (FL) be collected, which is just below the suggested minimum size at maturity from previous studies.

Ovaries were subsequently collected by the CCSBT members including Australia, Korea, New Zealand and The Fishing Entity of Taiwan. Histological sections of the ovaries were prepared by Members and read using the criteria provided in Farley et al. (2014). In 2019, a 2-day workshop to review the ovary sampling programs and develop a standard ovary histology classification scheme for SBT was hosted at the Research Institute for Tuna Fisheries in Bali, Indonesia (Anon, 2019). Preliminary maturity ogives were calculated at the workshop, but it was not possible to finalise the histological classification of all samples available at the workshop. Discussions at the workshop suggested that the histological classification (and, thus, maturity status) may be incorrect in some cases. Thus, these samples needed to be revisited before any conclusions could be drawn.

Since that time, additional work has been undertaken to finalise the maturity criteria and classification scheme used for SBT so that consistent methods can be used to classify reproductive state/maturity status. Although no samples have been read by multiple readers yet, this report provides the preliminary results of the combined analyses by Members involved in the study.

Methods

Sample collection and preparation

A total of 861 ovaries were collected by four CCSBT Member countries from SBT caught across the southern oceans between 2010 and 2019. Fish were caught from the southeast Atlantic (CCSBT statistical area 9), across the Indian Ocean (areas 2, 8 and the east part of 14), and into the southwest Pacific (areas 4, 5 and 7) (see map in Appendix 1). All fish were caught south of 30°S and the majority (96.7%) were collected in the designated non-spawning months of April to August. Of the samples collected outside these months, three were collected in March and 27 samples in September.

A subsample was taken from all ovaries and fixed in 10% buffered formalin for histological processing by member countries. Tissue samples were embedded in paraffin and standard histological sections were prepared and stained with Harris' haematoxylin and eosin.

Histology classification

Seventy five percent of the histology slides were read by Reader 1 and 25% were read by Reader 2. The sections were read using standardised terminology (Brown-Peterson et al. 2011) and classified using agreed criteria similar to those developed for albacore tuna (*Thunnus albacares*) (Farley et

al. 2014). The most advanced group of oocytes (MAGO) was staged into one of 5 classes: unyolked (primary growth and cortical alveolar), early yolked (primary and secondary vitellogenic), advanced yolked (tertiary vitellogenic), migratory nucleus (germinal vesicle migration) or hydrated. Advanced yolked oocytes undergoing alpha (α) or beta (β) atresia and maturity markers were recorded. The maturity markers considered were "orange bodies", well defined muscle bundles, and residual hydrated (yolked) oocytes, indicating previous spawning activity (Figure 1). Orange bodies are aggregates of yellow-brown pigment that may be remnant atretic oocytes or melano-macrophage centres/aggregates (Blazer 2002), which are linked to post-spawning activity in the gonad. Muscle bundles are distinct structures of remnant muscle and connective tissue surrounding blood vessels and are an indicator of previous spawning (Shapiro et al. 1993; Brown-Peterson 2011; Lowerre-Barbieri et al. 2011).

In addition to the agreed classification criteria, Reader 1 also scored the sections by the relative abundance and size of the orange bodies and muscle bundles present. The score ranged from 0 (absent) to 3 (large and abundant) for both maturity markers. Ovaries with a larger number/size of maturity markers were assumed to be highly fecund. That is, they were more likely to have spawned over a longer time period, spawned more often during that time, and/or spawned larger batches of oocytes than females with smaller/fewer maturity markers in their ovaries. For each fish, the two maturity marker scores were summed to give a cumulative index of fecundity ranging from 0-6. Females with an index of 0-3 were classed as immature (i.e., maturity markers were absent or very rare) while females with an index of 4-5 were classed as low fecundity. Only females with a fecundity index of 6 were classed as having high fecundity (see Table 3 in the Results).

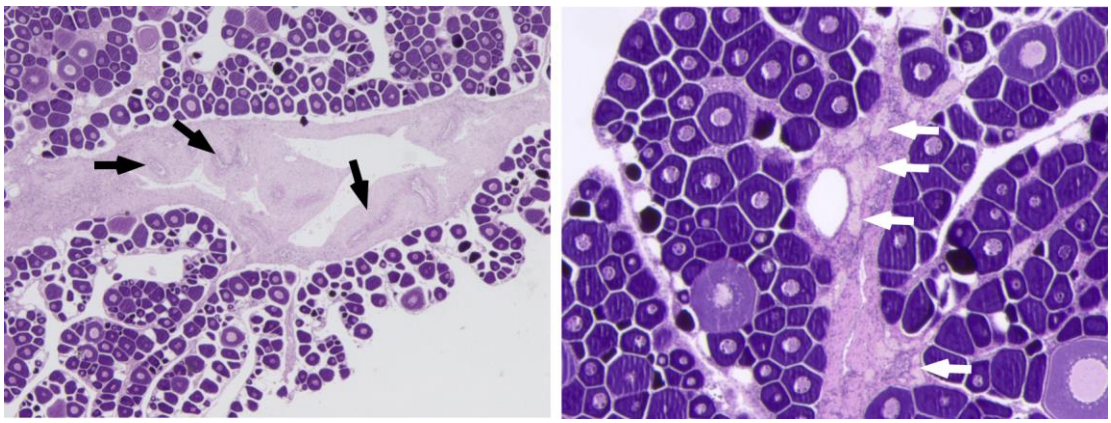

Figure 1. Histological sections of SBT ovaries containing maturity markers considered in this study including (left) well defined muscle bundles (black arrows) and (right) "orange bodies" (white arrows).

Modelling maturity and fecundity

The proportion of mature females, *p*, was modelled as a function of length using logistic regression:

$$log\left(\frac{p}{1-p}\right) = \alpha + \beta L$$

where L is fork length in cm. The models were fit using the glm function in R (R Core Team 2021) with maturity status as a binomial response variable with a logit link function. Once the models were fit, the length at 50% maturity could be calculated as L50 = $-\alpha/\beta$.

To investigate the potential for spatial differences in maturity, we fit maturity ogives to the data from four areas, as shown in Figure 2. For Area 2, for which 67% of the samples were read by Reader 2 (Table 1), we also fit maturity ogives to the data from each reader to see if differences existed.

Since the above investigations suggested no evidence of spatial differences in maturity but that reader differences may exist (see Results), maturity ogives were fit to all data (combined across areas) from Reader 1. In addition, ogives were fit to all data from Reader 1 using fecundity status (highly fecund or not) determined from the fecundity index as the response as a comparison to maturity status (mature or immature).

Finally, these last two ogives (i.e., which model maturity status and fecundity status using all data from Reader 1) were re-fit as a function of age by replacing length with age in the logistic regression model. Age was estimated for each fish from its length using the growth curve agreed by the CCSBT (referred to as VBLK2010). Once the models were fit, age at 50% maturity could be calculated as A50 = $-\alpha/\beta$.

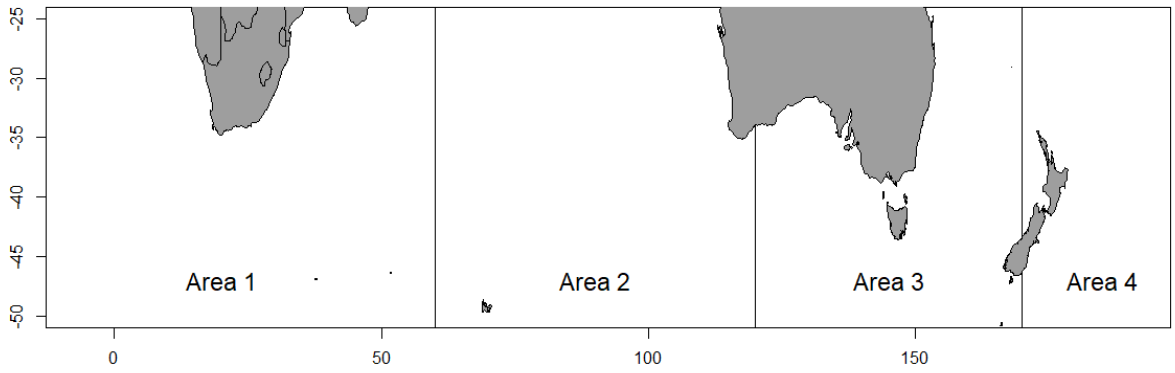

Figure 2. Areas defined for investigating spatial differences in maturity ogives. The east-west boundaries of each region align with the boundaries of CCSBT statistical areas (see Appendix 1).

Results and Discussion

Histology classification

Preparations of histological sections failed for seven samples due to the preservation or sectioning process. The number of ovaries successfully analysed by area is shown in Table 1. Females ranged in size from 66 to 190 cm fork length (FL), although the majority were between 110 and 160 cm FL (Figure 3). The size of fish was similar among the four areas, although slightly larger fish were sampled in Area 4. Based on the agreed classification system (Table 2), female SBT in our samples were classified as: immature (54.2%), developing (6.7%), spawning capable-non spawning (1.4%), regressing (0.8%) or regenerating (36.9%). In total, 520 SBT were classed as immature (66-168 cm FL) and 334 were classed as mature (97-190 cm FL). Figure 4 (top) shows the proportion of females

classed as immature and mature by 5-cm length class. The ovaries of females classed as developing or spawning capable sampled in Australia (CCSBT statistical area 4; see Appendix 1) contained atresia of yolked oocytes (Figure 5) suggesting that they were not about to spawn. Farley and Davis (1998) also found a high level of atresia in the ovaries of (assumed) pre-spawning SBT caught in the southeast Indian Ocean and off southeast Australia.

Based on the new method to estimate an index of fecundity, 105 mature females were classed as having high fecundity and 129 classed as having low fecundity (Table 3). The remaining females were classed as immature. Figure 4 (bottom) shows the proportion of females classed as immature or having low fecundity versus having high fecundity by 5-cm length class.

Table 1. Number of ovaries included in the analysis by histology Reader and sampling Area. The CCSBT member that collected the samples, and the sampling months are also shown.

Area	CCSBT Member	Months sampled	Histology reader	N
1	Korea	Apr – Aug	Reader 1	161
2	Korea and Fishing Entity of Taiwan	Mar - Sep	Reader 1 Reader 2	109 219
3	Australia	Apr - Aug	Reader 1	256
4	New Zealand	May - Jun	Reader 1	109
Total				854

Table 2. Number of southern bluefin tuna by histological classification.

Maturity status	Phase	Sub-phase	MAGO and POF stage	Atresia of advanced yolked oocytes	Maturity markers[2]	N
Immature	Immature		Unyolked, no POFs	Absent	Absent	463
Immature	Developing		Early yolked, no POFs	Absent	Absent	57
Mature	Spawning capable	Non-spawning	Advanced yolked, no POFs	α and β atresia may be present	Possible	12
Mature	Spawning capable	Actively spawning	Migratory nucleus or hydrated and/or POF's	α and β atresia may be present	Possible	0
Mature	Regressing		Unyolked or early yolked, no POFs	All yolked oocytes are in the α or β stages of atresia	Possible	7
Mature	Regenerating[1]		Unyolked or early yolked, no POFs	Absent	Present	315
Total						854

[1] Regenerating is equivalent to mature-resting.

[2] Maturity markers were orange bodies, well defined muscle bundles and residual hydrated (yolked) oocytes.

Table 3. Number of females classed by Reader 1 as having low or high fecundity based on the combination of maturity markers present in the ovary (see Methods). The score for orange bodies (OB) and muscle bundles (MB) ranged from 0 (absent) to 3 (large and abundant).

Orange bodies score (0-3)	Muscle bundle score (0-3)	Sum (OB+MB)	Fecundity index	N
0	0	0	(Immature)	172
0	1	1	(Immature)	24
1	0	1	(Immature)	33
1	1	2	(Immature)	122
1	2	3	(Immature)	15
2	1	3	(Immature)	25
2	2	4	Low	86
2	3	5	Low	18
3	2	5	Low	25
3	3	6	High	105

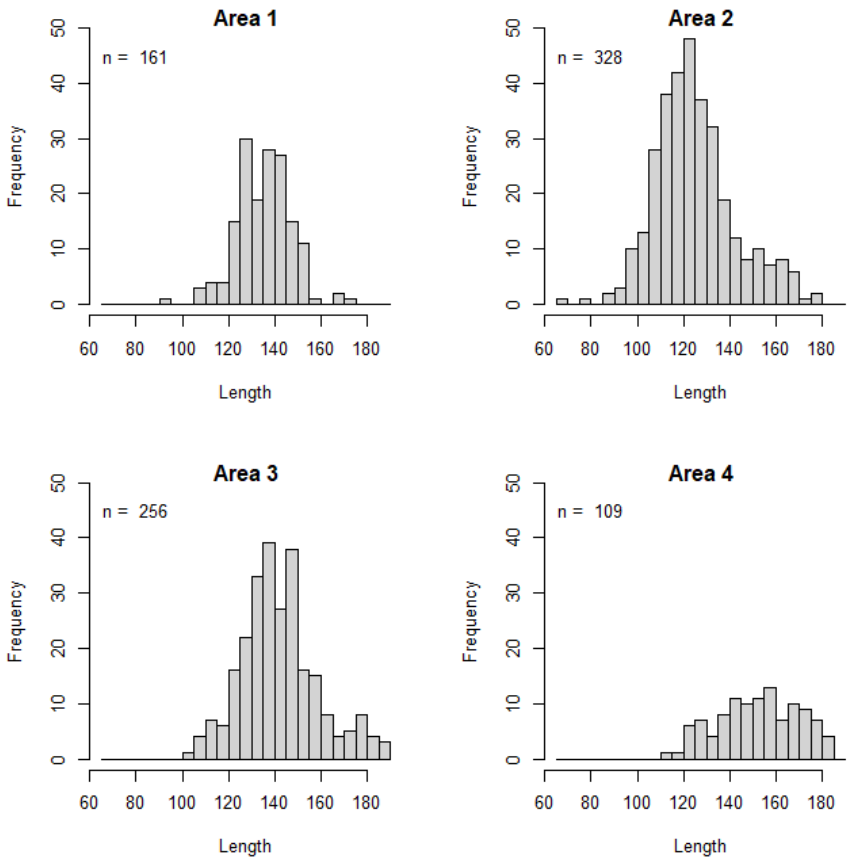

Figure 3. Length distribution of samples used in maturity modelling, broken down by areas shown in Figure 2.

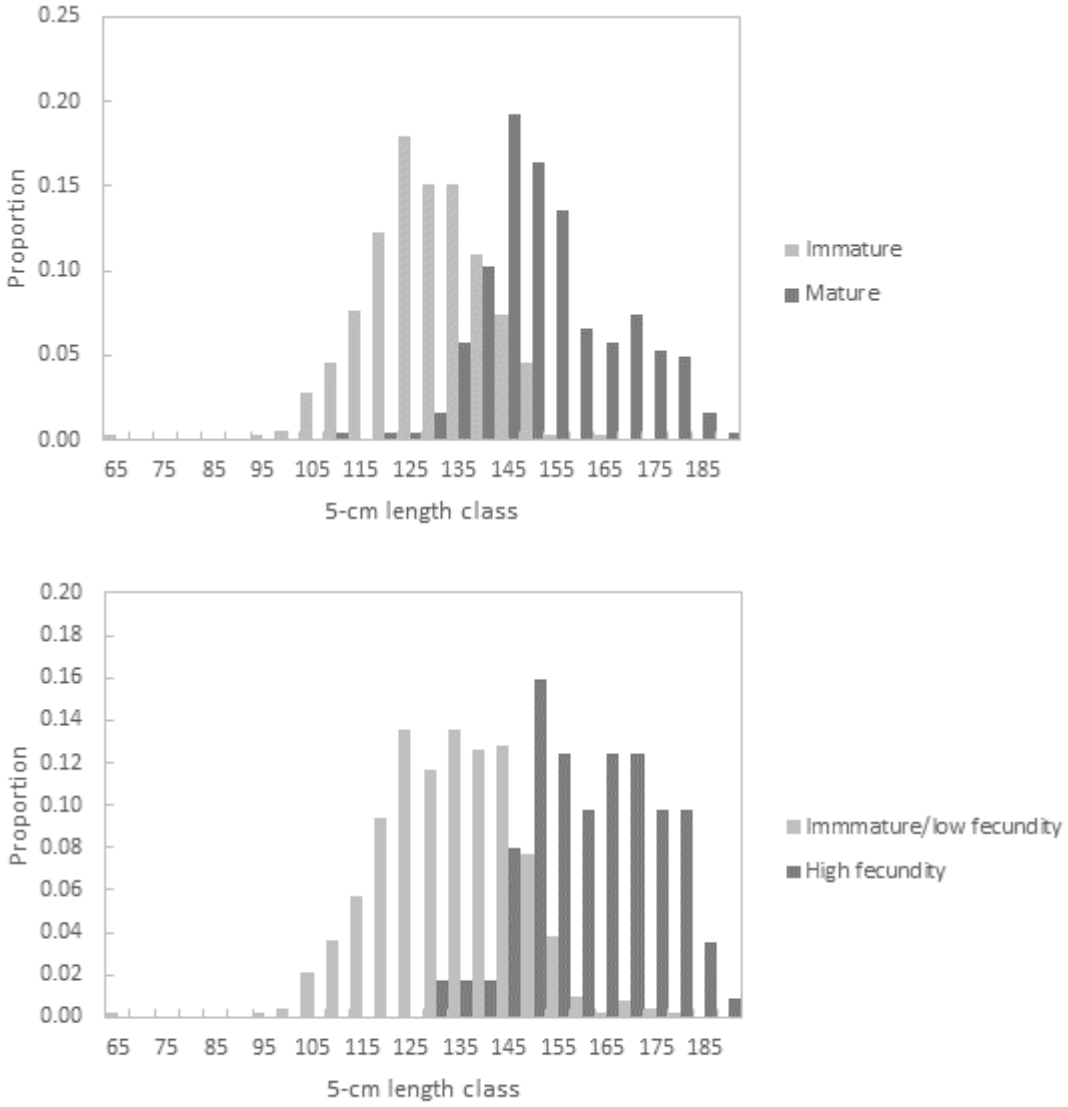

Figure 4. Proportion immature or mature (top) and immature/low fecund or highly fecund (bottom) by 5-cm length class.

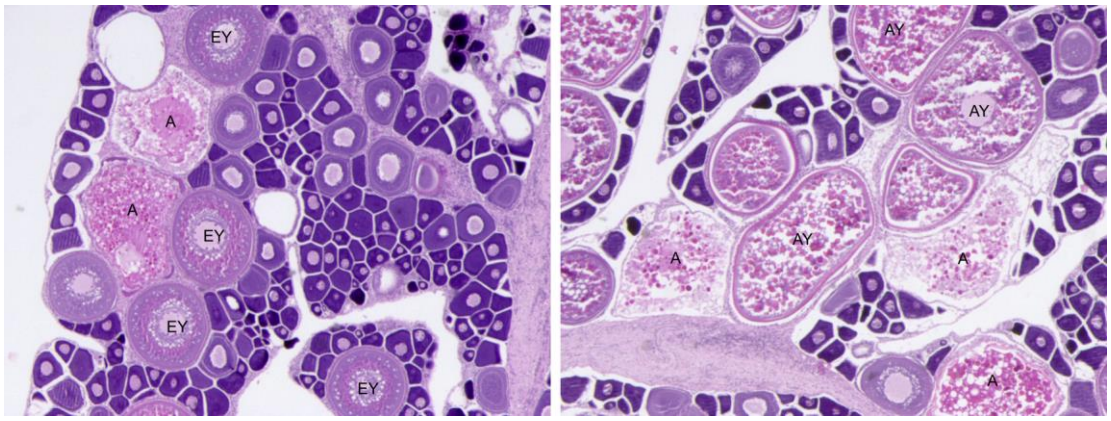

Figure 5. Histological sections of SBT ovaries containing (left) early yolked (EY) and (right) advanced yolked (AY) oocytes and atresia (A) of yolked oocytes.

Maturity and fecundity ogives

Length-based maturity ogives for each of the four areas are shown in Figure 6. The ogives in Areas 2-4 are very similar, with L50 estimates between 142 and 146 cm FL. The maturity ogive for Area 1 is less certain (higher standard error), which is most likely due to the near absence of fish >150 cm in the samples rather than an actual difference in proportion mature at length, although additional samples from large fish would be needed to confirm this. However, our current analysis suggests that spatial differences in maturity do not exist for SBT.

Figure 7 shows the maturity ogive for Area 2 separated by reader. Although the L50 estimates are similar (141.1 cm for Reader 1 and 143.4 cm for Reader 2), the form of the ogives is quite different and may indicate differences in classification methods, sample sizes, and/or the size range of fish analysed. Preferably, it is suggested that the reading of all the samples should be undertaken by multiple readers with consensus results before drawing any conclusions.

Figures 8 and 9 shows the length- and age-based maturity and fecundity ogives using all data from Reader 1. The predicted length at 50% maturity was 145.1 cm FL, while the predicted length at which 50% of females were highly fecund was much higher at 158.8 cm FL. Similarly, the predicted age at 50% maturity was 8.1 years, while the predicted age at which 50% of females were highly fecund was much higher at 11.6 years. It is important to note that age is very uncertain for large fish – e.g., a 170 cm SBT could be 12-25+ years old (Gunn et al., 2008).

A traditional maturity ogive gives the proportion of females at length/age that are mature and assumed to have spawned (i.e., contributed to egg production). A maturity ogive, however, does not account for individual variability in annual fecundity. Potential annual fecundity is difficult to estimate for multiple spawning species such as SBT because it requires estimates of spawning frequency, batch fecundity and spawning duration (Farley et al., 2015). The development of a ‘fecundity ogive’, using the abundance of maturity markers in the ovaries to develop a proxy for annual fecundity (a fecundity index), may help resolve this. Although qualitative, the fecundity ogive may be a more useful measure of reproductive potential than the traditional maturity ogive as it combines maturity and annual egg production.

Conclusion

The collaborative effort among CCSBT Members to collect ovaries, prepare histological slides and participate in the 2019 CCSBT maturity workshop have been extremely successful, and it culminated in the development of new maturity and fecundity ogives for SBT. The maturity estimates provided here, however, are preliminary and may need to be refined based on resolving any classification differences and the addition of samples from large fish >150 cm FL for Area 1. There was recognition by all participants about the importance and value of the collaborative research and there was a desire and willingness to continue further work in the future.

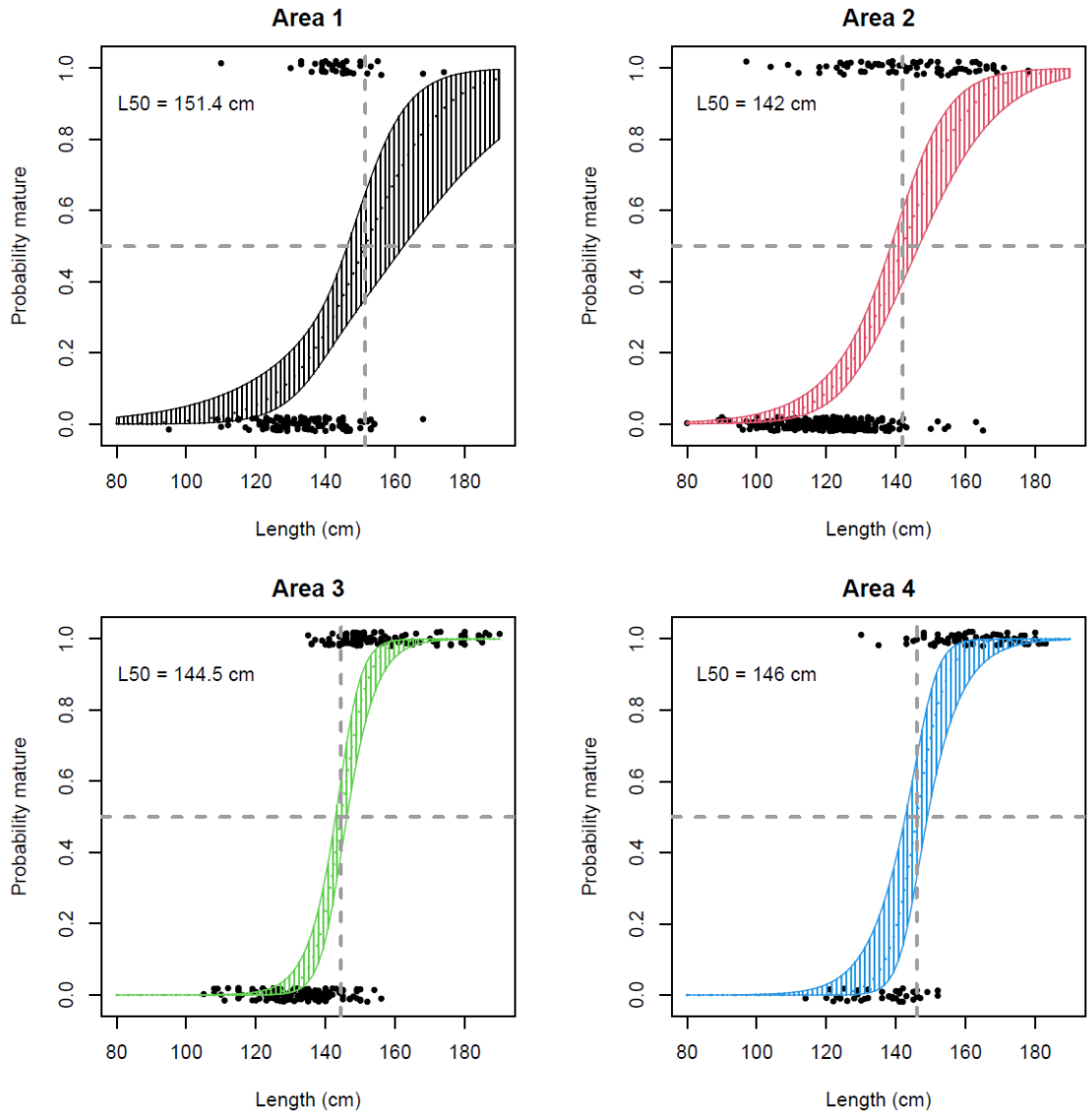

Figure 6. Maturity ogives fit to the data from each area defined in Figure 2. The mean proportion mature ± 2 standard errors is shown, as well as the estimated length at which 50% of females are mature (L50).

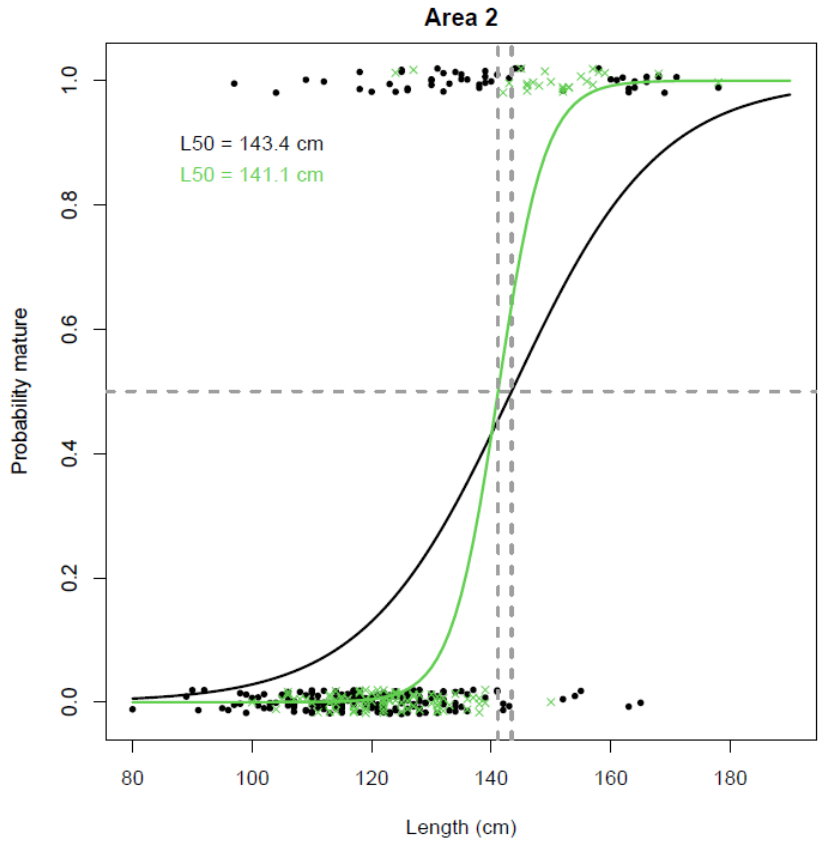

Figure 7. Maturity ogives fit to the data from Area 2 separated by reader (Reader 1 = green crosses; Reader 2 = black dots).

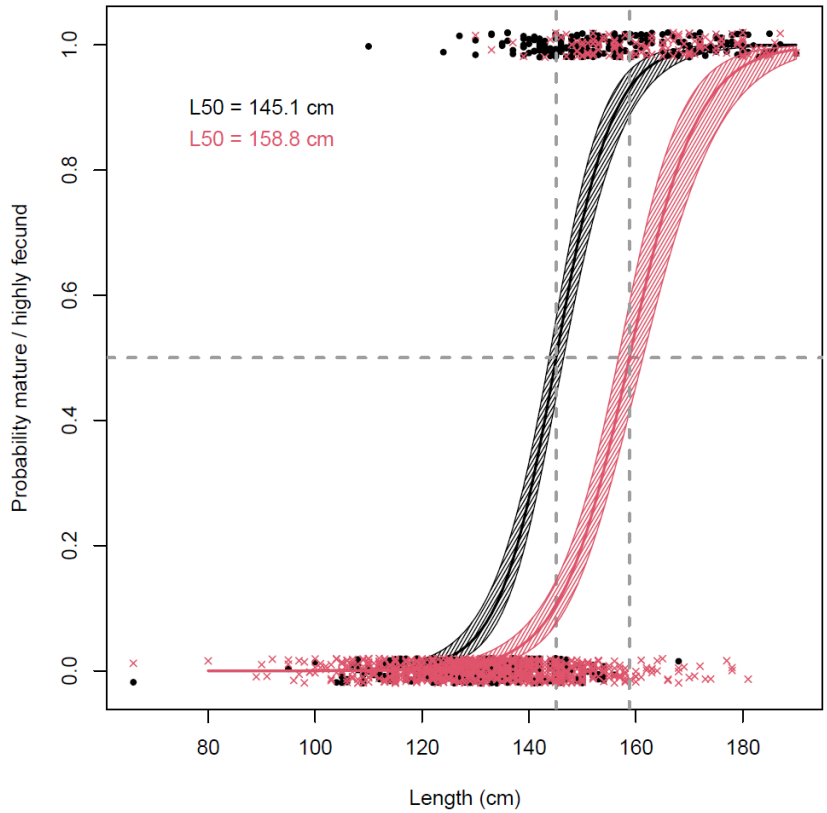

Figure 8. Length-based maturity ogives (black) and fecundity ogives (red) fit to all data from Reader 1. The mean proportion mature/highly fecund ± 2 standard errors is shown, as well as the estimated length at which 50% of females are mature/highly fecund (L50).

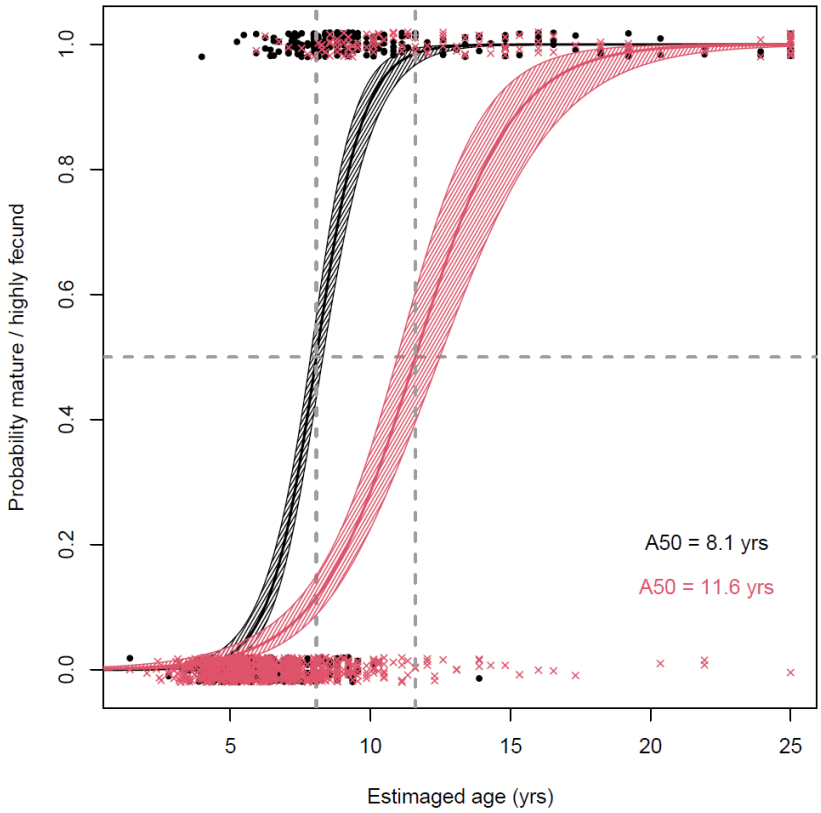

Figure 9. Aged-based maturity ogives (black) and fecundity ogives (red) fit to all data from Reader 1. The mean proportion mature/highly fecund ± 2 standard errors is shown, as well as the estimated age at which 50% of females are mature/highly fecund (A50). Note that age is truncated at 25 yrs.

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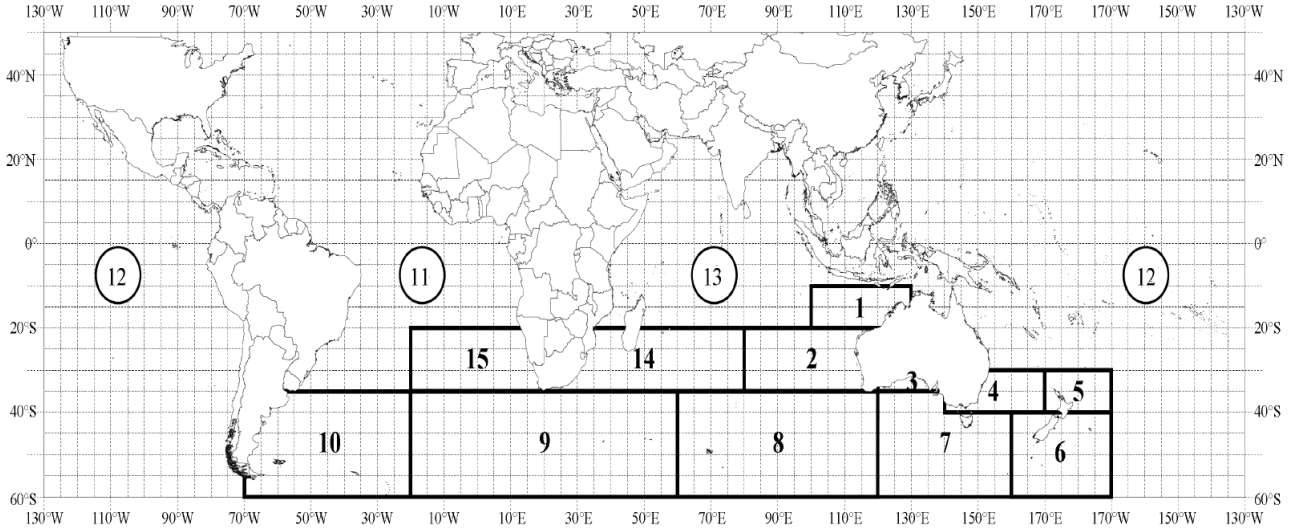
Appendix 1: Map showing CCSBT statistical areas.



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1300 363 400
+61 3 9545 2176
csiroenquiries@csiro.au
www.csiro.au

For further information

Oceans and Atmosphere
Jessica Farley
+61 6 6232 5189
Jessica.farley@csiro.au
csiro.au

Oceans & Atmosphere
Paige Eveson
t +61 3 6232 5015
e paige.eveson@csiro.au
w www.csiro.au