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SRP proposal:
Estimating size/age at maturity of southern bluefin tuna

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1 Abstract

The 2013 Extended Scientific Committee (ESC) adopted a new maturity schedule based on the available biological information (CCSBT-ESC/0108/20) for use in the 2014 stock assessment. Previous assessments had used “knife edged” maturity for age 10+. 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.

A draft proposal to estimate an unbiased maturity schedule for SBT was presented to the ESC in 2013 and the methods were supported in the Scientific Research Program (2014-18). Sampling in 2014 (and ongoing) was classified as a high priority and the processing of samples over the subsequent 2-3 years as medium priority.

The maturity proposal recommended that ovaries and otoliths were collected across the full range of SBT in the southern latitudes from fish ≥110 cm fork length during the non-spawning months of April to August. The presence of ‘maturity markers’ in histological sections of the ovaries would be used distinguish mature-resting from immature females. The presence of these maturity markers in SBT ovaries has now been demonstrated in fish sampled in Australia in June 2014, confirming that the methods proposed are appropriate for SBT.

Here we update some of the information given in the 2013 proposal, including the collaborative sampling program and estimate costs for the proposed research, for further development with member scientists at this year’s ESC.

2 Introduction

There remains uncertainty about the size and age that southern bluefin tuna (SBT) mature and the functional form of the maturity schedule. Previous estimates of the length/age at 50% maturity for female SBT converged at between 152-162 cm and between 10-12 years old. Up until 2013, the SBT operating model (OM) used a “knife-edge” maturity relationship, which specified that 0-9 yr olds made no contribution to the spawning biomass or reproductive output of the population and 10+ yr olds all contribute in proportion to their weight. This definition, i.e. SSB10+is still reported in the OM outputs for comparisons with stock status estimates from previous years. In 2013 the method was updated to use the currently available estimates of maturity (CCSBT-ESC/0108/20) to give a spawning potential by age. The maturity component of this is still largely uncertain.

The 2013 CCSBT Operating Model and Management Procedure Technical Meeting (Anon. 2013a) recognised the uncertainty in the size and age at maturity. The 2013 Extended Scientific Committee (ESC) noted that although the CCSBT OM was not sensitive to different assumptions regarding the maturity schedule, “a robust estimate of size at maturity would be important for estimating MSY” and related quantities.

A proposal to estimate an unbiased maturity schedule for SBT was presented at the 2013 ESC meeting (Farley et al. 2013a; CCSBT-ESC/1409/41). The method was based on recent work on albacore tuna (Farley et al., 2014) where ‘maturity markers’ were identified in histological sections of ovaries that could be used to identify mature-resting female SBT during the non-spawning season. This potentially provides a basis to be able to obtain an estimate of maturity for SBT from samples collected off the spawning ground. It was proposed that ovaries and otoliths be collected across the full range of SBT in the southern latitudes from fish ≥110 cm fork length (just below the minimum size suggested as mature in previous studies). It was also suggested that this could be achieved through the national fisheries observer programs, which have ongoing otolith sampling responsibilities. Such a sampling program would maximise the potential to collect ovaries from the largest spatial area, allowing for spatial variation in maturity-at-length/age to be accounted for in the maturity estimation models, and providing a representative estimate of size/age at maturity for use in future updates of the OM.

The proposed methods to estimate length/age at maturity were supported in the Scientific Research Program (Anon. 2013b). Sampling in 2014 (and ongoing) was classified as a high priority and the processing of samples over the subsequent 2-3 years as medium priority. The ESC recommended the collection and preservation of ovaries following the protocols in Farley et al. (2013a) across fisheries and size classes potentially via member observer programs. The ESC recommended histological processing and analyses after sufficient numbers of samples were collected (preferably before 2016) and an approximate cost of $50,000 to $100,000 was noted.

In this paper we update the information given in Farley et al. (2013) and include recent proof of concept work to demonstrate the presence of appropriate maturity markers in SBT ovaries sampled during the non-spawning season.

3 Methods

As detailed in Farley *et al.* (2013a), there are three main requirements for estimating size/age-at-maturity for a fish population (see Schaefer 2001):

1) Precise criteria to identify mature and immature fish.
2) Unbiased sampling of ovaries from fish in the appropriate size range, which includes both immature and mature females, and at the time of year when it is possible to distinguish between the two reproductive states.
3) Fitting an appropriate statistical model to the maturity at length (or age) data to estimate the maturity schedule (or 'ogive'). The maturity ogive is the relationship between proportion mature and size or age. This estimated relationship can then be used to predict the proportion sexually mature at specific lengths and/or ages (e.g. length/age at 50% maturity) and in stock assessment models as a basis for the stock recruitment relationship.

Stage 1: Criteria to identify mature and immature SBT – proof of concept

Histological analysis of ovaries is the most accurate method to determine the reproductive state of tuna. Farley *et al.* (2013b; 2014) developed a histological classification scheme for female albacore which identified 'maturity markers' that could be used to identify mature-resting female SBT during the non-spawning season (winter months of April to August).

To determine if similar maturity markers are present in SBT ovaries, an Australian Fisheries Management Authority (AFMA) observer started collecting ovaries from SBT > 110 cm fork length (FL) from Australia's east coast longline fishery in June 2014. Initially, 19 ovaries from SBT ranging from 134-190 cm were sampled. An additional ovary was sampled in February 2014 in South Australia from an SBT measuring 120 cm FL.

A cross-section sample was removed from the middle of one ovary lobe from each fish and fixed in 10% buffered formalin. The samples were then embedded in paraffin and standard histological sections prepared (cut to 3 µm and stained with H&E). The histological sections were read to assess the ovarian development phase following Schaefer (1998), Brown Peterson *et al*. (2011) and Farley *et al.* (2013b).

The results show that it is possible to distinguish immature from mature-resting SBT by the presence of maturity markers in the ovary. Figure 1a (Appendix 1) shows an immature ovary with tightly packed unyolked oocytes as the most advance present, and no maturity markers. Figure 1b-d shows mature-resting ovaries with maturity markers (late stage atresia and muscle bundles) and unyolked oocytes as the most advanced present. The presence of these maturity markers in SBT ovaries confirms that the methods proposed to distinguish immature from mature SBT caught during the non-spawning season are appropriate for SBT. However, the temporal window when they are present still needs to be determined. This will be possible from analysis of further ovary samples collected in July and August.

Stage 2: Collaborative sampling

A well designed, length stratified, sampling program is central to the success of this project. As noted in Farley *et al* (2013), the best time and place to sample SBT ovaries would be during the non-spawning season (April to August) in the southern temperate oceans as both immature and mature females are present. The aim of the project is to collect ovaries and otoliths from female SBT ≥110 cm FL from the main tuna longline fisheries in the southern oceans (Table 1). As noted previously, it may be possible for ovaries and otoliths to be collected via fisheries observer programs as part of the otoliths sampling programs but sampling would be dependent on fishing locations, months, size range of SBT caught and observer deployment.

We recommend that sampling is stratified by length with the aim of collecting ovaries and otoliths from a minimum of 10 fish per 5-cm length class. If the size range of females is 110 – 220 cm FL, the total number of fish sampled per statistical area is 220. Sampling can be started immediately at relatively little cost. Sampling can continue annually. Ovaries can be stored frozen &/or fixed until required for analysis (see methods in Farley *et al.* 2013a).

Table 1. Draft design for collaborative ovary and otolith sampling program by CCSBT statistical area. Sampling of females only from April to August.

STASTICAL AREA	CCSBT MEMBER	NO. OF FEMALES TO SAMPLE
Area 4	Australia, Japan	220
Area 5	Japan	220
Area 6	New Zealand	220
Area 7	Japan	220
Area 8/14	Japan, Taiwan, Korea	220
Area 9	Japan, Korea	220
Total		1320

In addition to the above sampling program, the collection of ovaries from small SBT on the spawning ground (Statistical Area 1) by Indonesia would provide the opportunity to confirm if all SBT migrating to the spawning ground are mature (and actively spawning) or not. Farley *et al.* (2014) noted that in 2012/13 and 2013/14, the size frequencies of SBT in the Indonesian longline catch had a new mode of relatively small fish (<155 cm FL) that had not been previously observed. It is not known if these fish were caught on, or south of, the spawning ground. If they were caught on the spawning ground, then information on their maturity status would be important in relation to the current project.

Stage 3: Laboratory analysis

The preparation of standard histological sections of ovaries is straightforward. Similarly, sectioning and reading of SBT otoliths can be undertaken by CCSBT member countries using standard agreed methods (Anon. 2002). Alternatively, all samples could be sent to specific members for preparation and reading. One option could be that the histological sections of ovaries are prepared at the Indonesian Research Institute for Tuna Fisheries[3] in Benoa, Bali. Tissue samples fixed in "10% formalin" are only 3.7% formaldehyde and

[3] Research Institute for Tuna Fisheries is a sub-institute of Research Centre For Fisheries Management and Conservation, Indonesia. The new building of RITF has been operational since February 2014.

are unregulated for transport (i.e., can be sent through the post). To increase the quality/precision of age estimates, all otoliths could be read by one laboratory, e.g. Fish Ageing Services in Queenscliff, Australia.

Preliminary work has been undertaken in stage 1 (above) to develop an ovary histological classification scheme which distinguishes between immature and mature-resting females. A short reproductive biology workshop could be held to finalise this classification scheme and develop a manual with the classification scheme for future maturity work by members. It is suggested that an appropriate venue for the workshop is the Indonesian Research Institute for Tuna Fisheries in Bali as this is central for most members and would also provide the opportunity for capacity building activities.

Stage 4: Data analysis

Standard logistic regressions are commonly used to model maturity at length/age, but other models can also be applied. There is an opportunity to develop models to fully include the spatial structure of the data. The modelling will determine if the proportion of female SBT mature-at-length varies spatially and seasonally in the southern oceans. If such variation does not exist, a single maturity ogive could be estimated by pooling samples across space and time. If variation does exist, then methods would be developed to account for this variation. Recently, models were developed to estimate a maturity ogive for albacore tuna (Farley *et al.,* 2014) that may also be applicable to SBT, given their migratory nature. The method weighted the maturity ogive by the relative abundance of females by area, accounting for the widespread migratory behaviour and spatial variation in relative abundance of albacore.

4 Approximate costs

The anticipated costs to analyse 1320 ovary and otolith samples in the laboratory and undertake data analysis are shown in Table 2. Additional costs associated with the sampling programs, project management, report writing, additional travel are not included.

Table 2. Approximate costs for proposed maturity study.

STAGE	COMPONENTS	TIMING	COSTS
Stage 1	Develop criteria to identify mature & immature SBT (currently underway)	Year 1	$0
Stage 2	Ovary and otolith sampling	Year 1	$0 - if this could be undertaken by observers as part of the each Member's national observer program
Stage 3	Lab analysis: Prepare and read ovary histology and otolith sections	Year 2	~$70,000: prepare and read otoliths ($25 ea), prepare histology sections of ovaries ($25 ea). Additional cost for experienced tuna reproductive biologist to read histology is not included (0.2 FTE)
	CCSBT reproductive biology workshop (possibly in Bali, Indonesia)		~$40,000: travel for 1 scientist from each member, workshop expenses
Stage 4	Data analysis: modelling to estimate an unbiased maturity schedule	Year 2	~0.1 FTE experienced statistician

Appendix 1

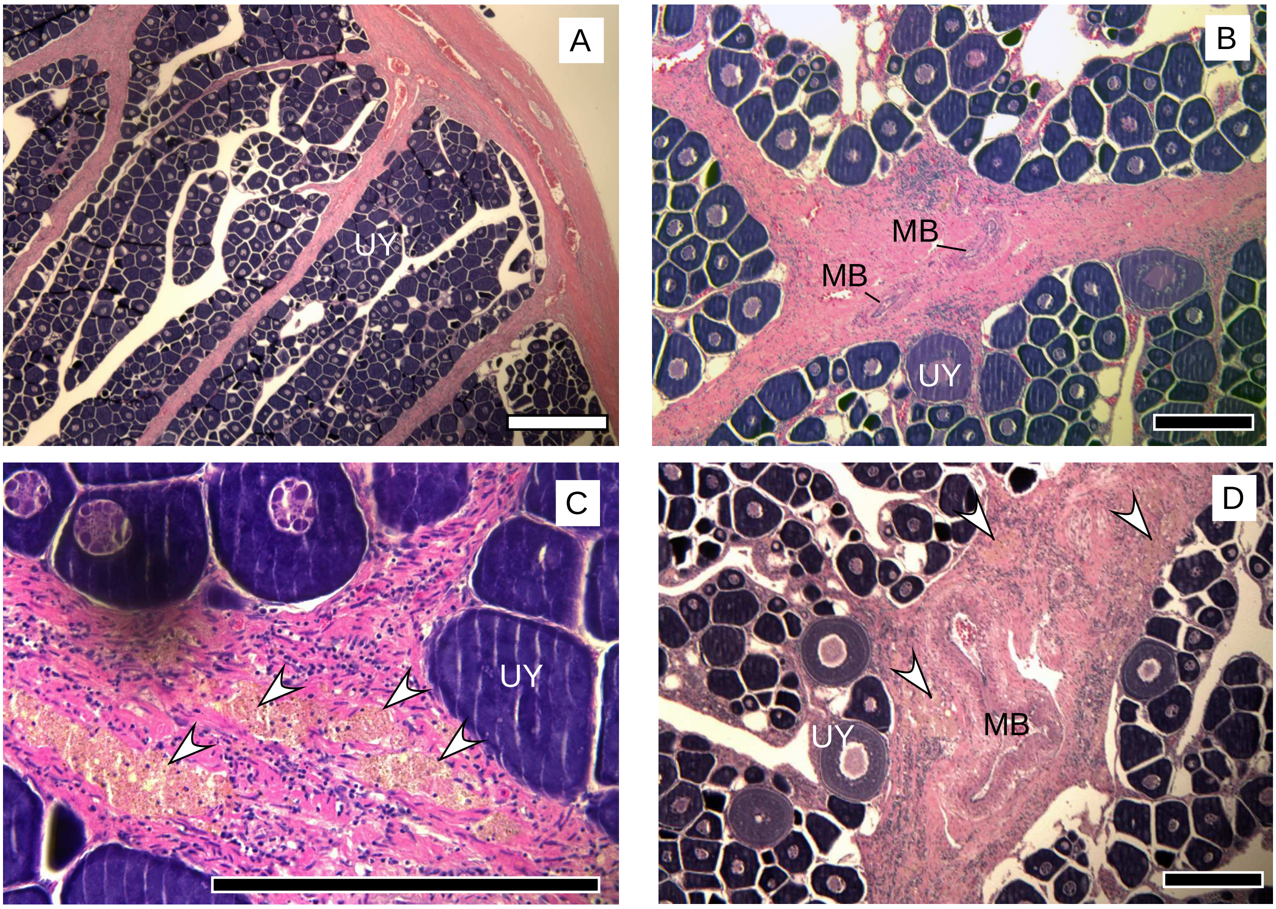

Figure 1. Histological sections of ovaries showing examples of immature (A) and mature-resting (B-D) development classes in SBT.

(A) unyolked oocytes in an immature ovary (120 cm FL)

(B) unyolked but developing oocytes in an mature-resting ovary. Two muscle bundles are indicated. 147 cm fork length (FL)

(C) unyolked oocytes in an mature-resting ovary. Several late-stage atresia (orange bodies) indicated. 162 cm FL

(D) unyolked but developing oocytes in an mature-resting ovary. One large muscle bundle and late-stage atresia indicated (190 cm FL)

UY = unyolked, MB = muscle bundle, arrow = late-stage atresia. The scale bars are 500 µm (white) and 200 µm (black).

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