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Estimating size/age at maturity of southern bluefin tuna

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1 Abstract

The recent CCSBT Operating Model and Management Procedure Technical Meeting (Anon, 2013) noted the uncertainty in the size and age at maturity for southern bluefin tuna (SBT). Previous estimates of the length/age at 50% maturity for female SBT have converged at between 152–162 cm and between 10-12 years old. The current implementation of maturity in the SBT operating model uses knife-edge maturity at the age of 10 years which is lower than the currently available estimates. The OMMP Working Group recognised that importance of obtaining an updated and unbiased estimate proportion of the population that is sexually mature given that the studies informing the current estimates are all largely based on samples collected from the spawning ground.

Estimating a maturity ogive is complicated for a species, such as SBT, where the mature fish migrate to a particular area to spawn, as this can result in a bias towards mature or immature (virgin) fish (and/or larger, faster growing individuals) in sampling programs depending on the area and time sampled. For SBT, the most appropriate time and region to sample ovaries for maturity estimation is in the southern temperate oceans during the non-spawning season, as both immature and mature females are present. At this time of year it is difficult to differentiate histologically between immature (virgin) and mature-resting (post-spawning/regenerating) females. In most reproductive studies, the only method used to identify mature, but post-spawning, females is by the presence of yolked oocytes (eggs) undergoing the process of resorption (known as atresia) or residual hydrated oocytes. Such studies only identify the early stages of atresia of yolked oocytes (i.e. alpha and beta), which do not persist in the ovary for extended periods. Once these stages have been reabsorbed, an ovary will resemble an immature ovary and, therefore, it has generally been considered it is not possible to separate immature females from females that were mature but are resting during the non-spawning season.

Recent work (Farley et al 2013; CCSBT-ESC/1309/Info-1) has identified additional 'maturity markers' (or post-spawning markers) in histological sections of ovaries that could be used to identify mature-resting female SBT during the non-spawning season. This development potentially provides a basis to be able to obtain an unbiased estimate of maturity for SBT from samples collected off the spawning ground. To do so, it would be essential that ovaries and otoliths were collected from across the full range of SBT in the southern oceans from fish ≥110 cm fork length (just below the minimum size suggested as mature in previous studies). A well designed, length stratified, sampling program would be central to the success of the project. It is suggested that this could be achieved through the national fisheries observer programs, which already have ongoing otolith sampling responsibilities (to minimise the cost of sampling). 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 models, and providing a representative estimate of size/age at maturity for future assessments.

2 Introduction

In fish, sexual maturity generally occurs over a range of sizes/ages. While many studies report the size at first maturity as being equal to the smallest mature fish sampled, the standard method used in fisheries science is to use the length at 50% maturity (L_{50}) (also termed mean size at first maturity). This is the average size at which 50% of the individuals examined are sexually mature and is the standard biological parameters used for modelling population dynamics of wild stocks. The proportion of the population at each size or age class capable of reproduction is of interest in a population dynamics context, not the extremes of the distribution (i.e. the earliest or latest age of first reproduction). For many species, L_{50} is estimated for females only as it is assumed that male sperm production is not limiting.

Estimating a maturity ogive is complicated for any species, such as SBT, where the mature fish migrate to discrete areas to spawn and immature fish do not. This can lead to a bias towards mature or immature (virgin) fish in the sampling program depending on the area and time from which fish are sampled. A maturity ogive based on samples collected only from spawning areas will be biased towards mature

females, while a maturity ogive based on samples collected off the spawning ground during the spawning season would be biased towards immature females (as mature females will be on the spawning ground).

For SBT, the most appropriate time and region to sample ovaries for maturity estimation would be during the non-spawning season in the southern oceans as both immature and mature females are present. However, a difficulty arises when trying to differentiate between immature (virgin) and mature-resting (post-spawning/regenerating) females during the non-spawning season. This is because after spawning, females absorb all remaining yolked oocytes (eggs) leaving only unyolked (previtellogenic) oocytes. The ovaries of these mature-resting females were thought to be histologically identical to the ovaries of immature females, with the same oocyte size frequency distributions. Occasionally, residual hydrated oocytes may be present in the lumen of the ovary but these are relatively rare.

Recent work (Farley et al., 2013; CCSBT-ESC/1309/Info-1) has identified additional 'maturity markers' (post-spawning markers) in histological sections of tuna ovaries that could be used to identify mature-resting female SBT during the non-spawning season. We propose that the ESC consider using histological 'maturity markers' to distinguish immature from mature- resting female SBT sampled from the southern oceans during the non-spawning season. If successful, these data could be used these to provide and unbiased estimate of the maturity ogive for the SBT population.

3 Need

An unbiased estimate of the proportion of the population that is sexually mature is an important reproductive parameter required for the assessment of fish stocks and estimating references points, such as MSY. The current MP has been tuned to meet an interim rebuilding target of 20% of unfished SSB, not MSY-based reference points. However, the Commission has requested the ESC to provide advice on MSY related quantities in the context of potential target reference points once the interim rebuilding reference point has been achieved. MSY related quantities are sensitive to uncertainty in size at maturity, and other quantities. For example, for the same steepness, selectivity and growth dynamics the F_{MSY} will always be smaller if the true age-at-maturity is older than assumed. Hence, obtaining an unbiased estimate of size at maturity for SBT should be considered a longer-term priority.

There remains some uncertainty about the size and age that SBT mature and the form of this relationship. Previous estimates of the length/age at 50% maturity for female SBT converged at between 152 – 162 cm and between 10-12 years old. The current version of the SBT operating model (*basesqrt*) uses a "knife-edge" maturity relationship, which specifies that 0-9 yr olds make no contribution to the spawning biomass or reproductive output of the population and 10+ yr olds all contribute in proportion to their weight.

The recent incorporation of the close-kin Parent-Offspring-Pair data into the CCSBT operating model (OM, *baseCK, Hillary et al., 2012, 2013*) required a revision to the form of the maturity ogive and, consequently, the definition of "effective reproductive output" as a currency within the OM, in addition to spawning stock biomass 10+.

The recent CCSBT Operating Model and Management Procedure Technical Meeting (Anon, 2013) noted that the specification of effective reproductive output made a number of assumptions relating to:

- the spawning migration, behaviour and vulnerability on the spawning grounds, and:
- size/age at maturity of SBT.

It was noted that there were no directed observations of spawning behaviour and that the majority of the work on size at maturity has used samples taken from fish on the spawning grounds and, as a result were likely to be toward larger size and younger ages relative to the population as a whole. In the absence of data, the Working Group examined a range of scenarios to bound what we considered to be extreme forms of the maturity ogive and noted the value in obtaining more representative samples of SBT off the spawning ground as the basis for a less biased estimate of the maturity ogive for us in future assessments.

4 Previous SBT maturity estimates

Davis et al. (2001) reviewed earlier studies of maturity for SBT. In brief, initial studies reported that SBT mature at around 130 cm FL based on gonad index data and the smallest lean (post-spawning) fish caught on the spawning ground (Shingu, 1970; Warashina and Hisada, 1970). Using length-at-age relationships available at the time (Robins, 1963), fish of this size were estimated to be age 8 years old. This estimate was the minimum length that SBT can mature rather than the length at 50% maturity. Campbell (1994) used Warashina and Hisada's (1970) data to demonstrate that the length at 50% maturity (L_{50}) was 146 cm in the late 1960s, and then estimated that L_{50} had increased to 154 cm FL in the 1980s and 157 cm FL by the 1990s. In 1994, the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) Scientific Committee agreed to use age 8 as the age-at-50% maturity (Anon, 1994).

Thorogood (1986) sampled ovaries from SBT caught around the coast of Australia and suggested that the minimum length-at-maturity was 110-125 cm FL (aged 5-7 years). The study used partially yolked (yolk vesicle) oocytes or more advanced as the criteria for determining mature fish. Although this maturity classification has been used for other fish species (see Brown-Peterson, 2011), in tunas the presence of fully yolked ococytes is considered the minimum needed to identify sexually mature fish (Schaefer, 2001). It is unknown if the fish with partially yolked oocytes would have gone on to develop fully yolked oocytes and spawn in the following spawning season.

Davis (1995) used measurements of egg diameter and gonad index for females caught between August and December in a pre-spawning area south of both the "Oka" (Spawning ground) and "Oki" (Staging ground) fishing ground (~35-45°S; 90-120°E). L_{50} was estimated at between 152 and 162 cm FL. An estimated mean of 157cm was recommended, which is consistent with that estimated by Campbell (1994). Using direct ageing data, Gunn et al. (2008) estimate SBT of this size to be 10-12 years old. These estimates of L_{50} were considered preliminary given that it was not known if all SBT classed as mature in the pre-spawning area would have gone to the spawning ground and spawned in that year.

Gunn et al. (1996) showed that fish aged 9-11 were almost absent in the Indonesian catch on the spawning ground, and suggested that an estimate of mean age at 50% maturity of "12-13 yrs seemed more appropriate". Davis et al. (2001) estimated the L_{50} by comparing the length distribution of SBT caught in the Indonesian longline fishery on the spawning ground (assumed unbiased size distribution of the spawning population) with the length distribution from the Japanese longline catch in the southern oceans (assumed unbiased size distribution of SBT off the spawning ground). They estimated the L_{50} of the population that had recruited to the spawning ground as between 158.4 – 163.1 cm (~11-12 years) for the six years of data examined at the time. These estimates are similar to the earlier estimates of L_{50}, and they concluded that for stock assessment purposes, the age at 50% maturity in SBT is around 11-12 years old.

Farley et al. (2012) show that there has been considerable change in the size (and age) distribution of SBT caught by Indonesia on the spawning ground since monitoring began in the early 1990s. Changes were also observed in the size distribution of SBT caught by Japan between the 1960 and 1990s (Davis et al. 2001). Given these temporal changes, there is uncertainty when using spawning ground data to infer maturity. Furthermore, the observed changes in growth rate of SBT between decades raises the question of whether this is likely to have influence the size/age at maturity over the same period.

5 Estimating a maturity ogive for SBT

There are three main requirements for estimating size- or 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.

Histological analysis of ovaries is the most accurate method to determine the reproductive state of tuna. A histological classification scheme for females was developed for yellowfin tuna by Schaefer (1998) and later adapted to other tuna species including SBT (e.g., Farley and Davis 1998; Schaefer et al. 2005; Chen et al. 2010; Farley et al. 2013; CCSBT-ESC/1309/Info-1).

To estimate a maturity ogive for females, it is often recommended that ovaries are sampled just prior to and/or during the spawning season as this is the time of year when it is assumed that all mature females will display histological evidence of maturity in their ovaries (Hunter and Macewicz, 2003). This recommendation is for species where there is no spatial segregation in the distribution of mature and immature females during the spawning season (e.g., no spawning migration). For SBT, which undertake seasonal migrations to spawning grounds from ~September to April, it would be difficult to obtain representative samples of ovaries from mature and immature females in proportion to their abundance in the population during the spawning season.

As noted above, the best time and place to sample SBT ovaries would be during the non-spawning season in the southern temperate oceans as both immature and mature females are present. At this time of year, however, it is difficult to differentiate histologically between immature (virgin) and mature-resting (post-spawning) females. In most reproductive studies, the only method used to identify mature but post-spawning females is by the presence of yolked oocytes undergoing the process of resorption (known as atresia) or residual hydrated oocytes in the ovary. Such studies only identify the early stages of atresia of yolked oocytes (i.e. alpha and beta), which do not remain in the ovary for extended periods. Once these stages are no longer present, an ovary will resembles an immature ovary and often no attempt is made to separate immature females from females that were mature but are resting during the non-spawning season. Note that postovulatory follicles only remain visible for ~24 hours after spawning in tunas (see Farley et al., 2013; CCSBT-ESC/1309/Info-1) and are not useful for identify mature-resting females.

It may now be possible to differentiate immature from mature-resting SBT during the non-spawning season using 'maturity markers' in ovary histology. Recent work on albacore tuna (*Thunnus alalunga*) (Farley et al., 2013; CCSBT-ESC/1309/Info-1) identified two maturity markers: (i) very late stages of atresia (gamma/delta) which are small yellow/brown granular structures (called melano-macrophage centres or brown bodies) and (ii) residual encapsulated hydrated oocytes that remained in the ovary after spawning. These maturity markers were quite common in mature-resting albacore throughout the non-spawning season allowing for the estimation of a maturity ogive using females sampled year-round (Farley et al., submitted PLoS ONE). Additional work on broadbill swordfish (*Xiphias gladius*) (J. Farley, unpublished data) identified the same histological markers in mature-resting females. These, along with other maturity markers such as muscle bundles, thick ovary walls, circumnuclear oil droplets, and interstitial tissue, are considered to be signs of prior spawning activity, and are used in other fish species to identify mature-resting females (Adams et al 2001; Brown-Peterson, 2011).

It is unknown if maturity markers will be visible in SBT ovaries throughout the non-spawning months, as was the case for albacore. If these structures are persistent, however, then the temporal window for sampling out of the spawning/migratory season may be relatively long.

Standard logistic regressions are commonly used to model maturity at length/age, but other models can also be applied. There is opportunity to develop models to fully include the spatial structure of the data.

6 Aims and time-frame of a collaborative study

Stage	Aim	Time-frame
1.	Collect ovaries and otoliths from female SBT ≥110 cm FL from the main tuna longline fisheries in the southern oceans during the non-spawning season (Apr to Aug).	Sampling of ovaries and otoliths can be started immediately at relatively little cost. Sampling can continue annually. Store ovaries (frozen &/or fixed) until required for analysis.
2.	Determine if maturity marker are present in SBT ovaries and the temporal window when they are present.	When funding is available
3.	Develop a standardised SBT ovary histological classification scheme (including histological distinction between immature and mature-resting females).	When funding is available
4.	Develop appropriate statistical models to estimate maturity ogvie for female SBT.	When funding is available
5.	Continue to collect and analyse ovaries to estimate an annual (or less frequent) maturity ogive. This would provide a low-cost basis for monitoring potential changes in size/age at maturity as the stock rebuilds.	When funding is available

7 Proposed Methods

7.1 Sampling by trained fisheries observers

The project would focus on sampling ovaries and otoliths from females ≥ 110 cm FL which is just below the minimum size suggested as mature in previous studies. Ideally, samples would be collected from across the southern oceans during the non-spawning season April until August.

It may be possible for ovaries (and otoliths) to be collected via fisheries observer programs as part of the otoliths sampling programs.

If possible, weigh the ovary (with any fat removed) immediately after sampling for calculating gonad index. Immediately fix a small cross-section of the ovary in 10% neutral buffered formalin. Immediately freeze the remainder of the ovary. Measure the fork length (FL) of all females sampled.

7.2 Minimum sample size

The proposed sampling program could be (depending on fishing locations, months, size range of SBT caught and observer deployment):

- Stratified by 5 cm length class.
- If size range of females is 110 – 220 cm FL = 22 length classes. Although large females may be relatively rare in catches.
- Aim for a minimum of 10 fish per 5-cm length class (n=220) for each CCSBT statistical area and fishery.

7.3 Laboratory analysis

Weigh the frozen ovary if whole. If ovary material has not been fixed in 10% neutral buffered formalin, then remove a cross section of tissue and fix in the laboratory. The tissue should be removed while the ovary is still frozen to reduce damage to the tissue. i.e., do not defrost the ovary before preservation. Standard ovarian histological slides should be prepared of the full cross section if possible; cut to 6-8 µm and stained with Harris' haematoxylin and eosin.

7.4 SBT ovary histology workshop

Hold a short workshop to agree on a classification scheme for SBT ovary histology. Specifically, histological criteria will be identified to distinguish between immature and mature-resting females. Develop a manual with the classification scheme and digital images of maturity markers.

Ovaries should then be staged using the agreed classification scheme. Otoliths should be prepared and aged according to Anon (2002).

7.5 Data analysis

Modelling would be required to 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., in revision PLoS ONE) 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.

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