



# Update on Scientific Research Program activities

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# Executive Summary

In 2013, the Extended Scientific Committee (ESC) developed a new Scientific Research Plan for southern bluefin tuna (SBT). The SRP was updated in 2014 and several items were identified as high priority in the 2015 work plan including the continued collection and archiving of tissue samples for close-kin genetics, and the collection of ovaries and otoliths for an independently estimated maturity ogive. In addition, the work plan for 2016 included a workshop on otolith-based ageing to be held in conjunction with a workshop on maturity estimation.

Here we provide an update on progress in the sampling program for close-kin tissue and ovaries and otoliths for maturity estimation, and requirements for the ageing and maturity workshops. The dates for these workshops have not yet been set hence it would be useful if the ESC could advise on the most appropriate timing for these workshops, if they are to proceed in 2016.

# Introduction

In 2013, the ESC developed a new Scientific Research Plan (SRP) for SBT. The SRP was updated in 2014, with a range of activities identified as essential and several items identified as high priority for 2015 (Anon 2014). The high priority items included:

- 1) Design study for a gene-tagging program,
- 2) Design study and expert review of future close-kin mark-recapture approaches,
- 3) Ageing of Indonesian SBT otoliths,
- 4) Continued collection and archiving of tissue samples for close-kin genetics (in Australia and Indonesia), and
- 5) Sampling ovaries and otoliths for an independently estimated maturity ogive.

In addition, the ESC work plan identified a second workshop on otolith-based ageing in conjunction with a maturity estimation workshop as high priorities for 2016 (Anon 2014). The ESC will also reconsider potential research using otolith microchemistry and electronic tagging to understand within season spawning behaviour and skipped spawning behaviour in adult SBT (Table 2B in Attachment 10 of Anon 2014).

The gene-tagging design study, review of future close-kin mark-recapture approaches, and ageing of Indonesian otoliths were funded by CCSBT in 2015, and reports are provided for ESC consideration (see CCSBT-ESC/1509/14; 18; 19).

In this paper we provide an update on progress in the sampling program for close-kin tissue and ovaries and otoliths for maturity estimation, and requirements for the ageing and maturity workshops.

## 1 Tissue sampling for close kin

In 2013, the project to obtain a fishery-independent estimate of spawning biomass of SBT using close-kin genetic techniques was successfully completed (Bravington et al. 2014). The project analysed tissue samples collected from juvenile and adult SBT in 2006 to 2010. Since then, tissue sampling has been ongoing with approximately 3200 (1600 juveniles and 1600 adults) samples collected and archived annually.

In 2015, the CCSBT allocated funding to continue tissue sampling in Port Lincoln and Benoa during the 2014/15 fishing season.

Sampling of adult SBT occurred between September 2014 and April 2015 at the Port of Benoa, Bali, using the existing Indonesia-CSIRO SBT catch monitoring system for the longline fishery (see Proctor et al., 2006). Muscle tissue was collected and frozen by a trained sampler using protocols provided by CSIRO. Tissue was obtained from 1609 fish ranging from 117 to 201 cm FL. Sex was identified for all fish based on residual gonad tissue. The frozen tissue samples are stored at RIMF in Muara Baru (North Jakarta) and will be transported to Hobart for archiving as soon as possible.

Sampling of juvenile SBT began in July 2015 and is still in progress at tuna processors in Port Lincoln. Muscle tissue was collected and frozen by Protec Marine Pty Ltd according to protocols provided by CSIRO. Tissue has been obtained from 1600 SBT ranging from 103 to 109 cm fork length (FL) (age 3 fish). Once the sampling is complete, the tissue will be transported to the CSIRO in Hobart.

The samples are stored in consecutively labelled boxes with 100 positions (10 by 10) in each box (A01 through J10). Individual sample are given a unique identification label (e.g., SbPL2014\_Bx01\_A01) and placed in either -80 or -20°C for long term storage. Where possible, boxes are stored together as a year class.

## 2 Ovary and otolith sampling for maturity

There remains uncertainty about the size and age that SBT mature and the functional form of the maturity schedule. 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. In 2013, the method was updated to use the currently available estimates of maturity and additional information provided by the close-kin estimate to give a spawning potential by age (Anon 2013a). It was acknowledged, however, that there was no independent estimate of a maturity schedule for SBT (Anon 2013b). In 2014, a costed proposal for developing one (Farley et al., 2014) was supported by the ESC, and sample collection for maturity was listed as a high priority in the work plan for 2015 and ongoing. Table 1 shows the draft sampling plan proposed by Farley et al. (2014)

**Table 1. Design for collaborative ovary/otolith sampling program by CCSBT statistical area. Sampling of females only from April to August.**

STATICAL 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
<b>Total</b>		1320

In addition to the above sampling program, it was proposed that the collection of ovaries from small SBT by Indonesia would provide the opportunity to confirm if all SBT caught in the Indonesian fishery are mature (and actively spawning) or not. Farley et al. (2015) shows that in 2012/13 to 2014/15 the size frequencies of SBT in the Indonesian longline catch has a new mode of relatively small/young fish (<155 cm FL, <10 years) 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.

In 2014, Australian observers collected 19 ovary samples preserved in 10% formalin (134-190 cm FL). It is anticipated that additional samples will be collected over the next month if observers are deployed onto longline fishing vessels on Australia's southeast coast. Where possible, otoliths will be collected from sampled fish in port using the "drill and hole-saw" method (Anon 2002) which does not affect the external condition or quality of the fish. In 2015, New Zealand collected 122 ovary samples, preserved in formalin, from Area 6.

### **3 Age estimation workshop – 2016**

In 2002, the CCSBT led the development of standardised direct ageing methods for SBT among member nations (see Anon 2002). It is recognised, however, that there is a need to regularly examine the precision and bias of age estimates between readers and among laboratories to maintain a consistent level of precision and minimise the potential for systematic biases in ageing estimates. In 2015, the ESC work plan listed a 3-day workshop to review otolith sampling design and age estimation/calibration as a priority for 2016. Indonesia's Research Institute for Tuna Fisheries was identified to host the workshop.

Potential issues for consideration are:

- Ageing errors and difficulties identified during the calibration exercise,
- Standardising otolith margin interpretation and converting counts to age estimates,
- The level of acceptable ageing error,
- Future research requirements.

Proposed aims are to:

- Review otolith extraction, sectioning and reading methods including any recent age validation work,
- Provide capacity building training for members who have not been involved in SBT age estimation,
- Improve age estimation protocols and quality control procedures (checking precision and drift),
- Update otolith reference set for among laboratory comparisons and determine a future quality control agenda,
- Revise the age determination manual with respect to methods related to reading otolith margins.

Prior to the workshop, it is recommended that an inter-laboratory otolith exchange exercise be undertaken. The main objective of the calibration exercise would be to estimate precision and relative bias in readings from scientists at CCSBT member laboratories, and to ensure that the precision/bias levels are within acceptable limits. This is an area for potential collaboration among tuna RFMOs given the interest in bluefin tunas in particular, but also the tropical tunas.

## 4 Maturity estimation workshop – 2016

In 2015, the ESC work plan listed a 3-day workshop to discuss and finalise maturity criteria for SBT as a priority for 2016. It was suggested that this workshop be held in conjunction with the ageing workshop (above) at Indonesia's Research Institute for Tuna Fisheries to reduce costs by some participants.

Proposed aims are to:

- Develop standardised histology classification criteria for ovaries,
- Provide capacity building training for members who have not been involved in maturity estimation,
- Finalise maturity classification for SBT,
- Initiate analysis of the results to estimate the maturity schedule of SBT,
- Discuss future research requirements,
- Develop a manual with the classification scheme for future maturity work by members; this manual may be applicable to other tuna species and the respective tuna RFMOs.

Prior to the workshop, it is recommended that histological sections are prepared for all ovary material collected, and that an inter-laboratory histology interpretation exchange exercise be undertaken. It is recognised that ovary classification may vary between researchers due to the semi-quantitative approaches used, so the purpose of the exchange exercise would be to allow all laboratories to examine and classify the ovaries prior to discussion at the workshop.

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