Report of the second CCSBT workshop on otolith-based ageing of southern bluefin tuna (*Thunnus maccoyii*)

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Abstract

In June 2025, the 'Second CCSBT workshop on otolith-based ageing of southern bluefin tuna (*Thunnus maccoyii*; SBT)' was conducted online. A total of 12 fisheries scientists and researchers from four CCSBT Members participated. The core aims of the workshop were to update standardised age protocols, compare age estimates of a reference data set, and discuss emerging approaches to improve the provision of age data for SBT. To understand precision and sources of difference in age estimates between readers, an image collection of 41 thin sectioned SBT otoliths of mostly known age fish were shared with workshop participants one month prior to the workshop. At least one participant from each CCSBT Member read and annotated the images with their age estimates. Resulting CVs between participant assigned ages and known ages ranged from 5.1% to 22.4%. The key issues discussed included: (1) the identification of the first 1–2 opaque zones; (2) the identification of subsequent zones; (3) the determination of edge type; (4) the timing of annuli formation, particularly the first opaque zone; (5) intra- and inter-read quality assurance and ageing precision; (6) potential approaches for estimating decimal age; (7) the use of emerging technology for age estimation, in particular artificial intelligence/machine learning; and (8) recommendations for future research. Workshop participants also made several recommendations for updating the current SBT ageing protocol.

Introduction

Southern bluefin tuna are one of the world's largest tuna species, reaching over 2.5 m in length and 260 kg in weight. They can live for over 40 years, with the oldest individual recorded reaching 42 years (Shimose and Farley 2015). Although there is some uncertainty regarding the age at which SBT reach maturity, available data suggest they do not mature younger than

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8 years old, and possibly not until as late as 15 years old (CCSBT 2023). The species forms a single stock that ranges from the south-eastern Atlantic Ocean eastwards to east of New Zealand in the Pacific Ocean, with adults returning to spawn in a single spawning ground in the north-eastern Indian Ocean between Australia and Indonesia. Spawning occurs between September and April (Farley et al. 2015). After a short larval phase, young-of-the-year (YOY) migrate south down the west coast of Australia, reaching the south coast when they are approximately one year old. Young juveniles (1–3-year-olds) spend their first few years in the Great Australian Bight (GAB), whist older juveniles, sub-adults, and adult disperse widely across the southern oceans (Hobday et al. 2015).

To standardise age estimation of SBT by its Members, the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) held an inaugural workshop on SBT age estimation in Queenscliff, Australia, in June 2002. The main aims of this workshop were to facilitate skills exchange between scientists regarding the collection, preparation and reading of SBT otoliths, and to develop a common standard in estimating age of SBT. Participating scientists form CCSBT Members developed an ageing manual entitled 'A manual for age determination of southern bluefin tuna *Thunnus maccoyii*' (Anon 2002) (hereafter termed the 'SBT Age Determination Manual'). The manual covered the sampling, preparation and interpretation of both whole and sectioned SBT otoliths.

With more than 20 years having passed since the inaugural SBT ageing workshop and the development of the SBT ageing manual, and significant advancements occurring in the ageing of tunas and other pelagic fishes in the intervening years, a second ageing workshop was proposed to estimate precision and bias among otolith readers and standardise age estimation processes amongst CCSBT Members (Farley et al. 2014, Farley 2022). In June 2025, the 'Second international workshop on southern bluefin tuna age estimation' was held online. The workshop brought together fisheries scientists, biologists, and researchers involved with ageing SBT from CCSBT Members to update standardised age protocols, compare age estimates from a reference data set, and discuss emerging approaches to improve the provision of age data for SBT. The specific objectives of the workshop were to:

1) Understand approaches used by different laboratories to estimate SBT age.
2) Understand precision and potential sources of difference between SBT otolith readers.
3) Improve age estimation protocols and quality control procedures amongst CCSBT Members, including on the identification of the first annual zones, reading of subsequent zones, and determination of edge type.
4) Discuss methods to calculate decimal age.
5) Update the CCSBT SBT age determination manual, including approaches related to the identification of the first opaque zone and reading otolith margins, and
6) Foster collaboration and knowledge sharing amongst Members involved in SBT ageing.

The workshop discussions and conclusions are synthesised herein. A list of workshop participants is provided as Appendix 1 of this report, and the workshop agenda is provided as Appendix 2. Terminology used in this report follows that of the SBT Age Determination Manual (Anon 2002).

Member updates of significance

Australia

Australia started collecting SBT otoliths in the 1960s. In recent years, approximately 200–300 SBT are sampled for otoliths annually from the purse seine fishery operating in the GAB, with around 100 of these aged. Australia also used to receive around 1500 otoliths from the Indonesian longline fishery that operates on the spawning ground, with 500 of these aged each year. There has been a slight hiatus on this in recent years but otolith collections have now resumed. Over 50,000 SBT otoliths have been archived since collections began.

Ageing is performed by Fish Ageing Services (FAS) based in Queenscliff, Australia, using protocols developed by CSIRO and implemented in the SBT Age Determination Manual. For each prepared otolith, 4–5 serial sections (~300–350 µm thick) are taken from the nucleus of each otolith. Sectioned otoliths are mounted on slides, covered with glass coverslips and read using research grade Leica stereo microscopes (M125 or M80) illuminated with transmitted light. A calibration read from a reference set is conducted before reading any new material. Historically, all otoliths were read by a single reader at least twice, with a subset (around 10%) read by a second reader (and particularly for the samples from the spawning ground provided by Indonesia). More recently, FAS has moved to a two-reader process, with both readers performing a blind read of each sample, and the more experienced reader of the pair doing a final read for those samples where the age estimates from the first two reads did not agree. Two images are captured from each section read, one annotated with the counted increments and another without. The distance between the primordium to the distal edge of each opaque zone, and to the edge of the otolith, is recorded (Farley 2022).

Fishing Entity of Taiwan

Taiwan has been ageing SBT since 2002. Initially, SBT otoliths were sampled by scientific observers onboard commercial longline vessels. In recent years however, the numbers of otoliths collected by observers has decreased. Accordingly, since 2018, otoliths have been sampled from fish processing companies. Heads are purchased for sampling and are accompanied by the CCSBT Catch Documentation Scheme (CDS) tag number, which allows linking of the head to the length, weight, and catch date of the fish, and the CCSBT management area where it was caught.

Otoliths are prepared for ageing as thin transverse sections and mounted onto glass slides using a permanent mounting medium. Images of sectioned otoliths are captured using a compound light microscope at 40 x magnification (4 x objective lens) and ages are estimated from the images using the protocol described in Anon (2002). Each otolith is read by a single reader 2–3 times before a final age is assigned.

New Zealand

New Zealand started ageing SBT in 2001. Between 2001 and 2017, otoliths were collected by scientific observers onboard surface longline vessels. Observers would measure SBT and sample a subset of these for otoliths. Around 250 otoliths were aged in each year, with ageing following the protocols described in Anon (2002). However, since 2018, longline observer coverage has declined, with few otoliths collected in 2018–2022. According, since 2022, otoliths have been collected from heads provided by Licensed Fish Receivers (LFRs, i.e., fish processors). The LFRs provide the head accompanied by the CCSBT CDS tag, to enable linking of the sample back to the length, weight, and collection information. Around 150 age estimates are generated annually (Moore et al. 2025).

Current ageing protocols for SBT by New Zealand follow those outlined in the SBT Age Determination Manual (Anon 2002). Otoliths are prepared as thin transverse sections, with two sections cut from each fish, including one through the primordium. Otoliths are mounted onto glass slides and read under a stereo microscope at variable magnification.

Before reading any new material, the primary and secondary readers will recalibrate by reading a subset of 30 otoliths from the reference collection, with knowledge of the previously assigned age. Readers then read approximately 100 otoliths from previously aged material without knowledge of the previous age, and, if precision metrics are appropriate, qualify to reading new material. The primary reader reads all otoliths at least twice. Where ages from the two reads agree, the age is accepted as the assigned age. Where ages from the two reads differ, the otolith will be read a third time, this time with knowledge of the previous age estimates, to derive a final age. A minimum of 10% of new material is read by the secondary reader, and precision amongst the readers evaluated using the Index of Average Percent Error (IAPE) and Coefficient of Variation (CV), as well as visually using the age-bias plots of Campana et al. (1995) (Moore et al. 2025). An unmarked and marked image, the latter showing the count and placement of annual bands, is captured for each aged otolith.

Republic of Korea

Korea collects around 80–120 SBT otoliths annually. Otoliths are collected by scientific observers working on Korean longline vessels. Otoliths are prepared as thin sections and read using the protocols described in the SBT Age Determination Manual (Anon 2002).

Daily ageing of SBT

FAS presented preliminary results from a daily ageing exercise conducted on small (42–79 cm fork length [FL]) SBT. The intention of this work was to document the relationship between daily age and otolith size. An understanding of this relationship is the first part of the 'Jesstimation' approach of Farley et al. (2025) for converting annual zones counts into decimal age (see 'Decimal age calculation' section). Otoliths were hand ground to approximately 100 µm to expose the primordium. Most otoliths (n=74) were prepared on the transverse plane, while 37 were prepared on the longitudinal plane along the primordium to the postrostral axis, using the approach described in Schaefer and Fuller (2006) and Williams et al. (2013). Where both otoliths from the pair were available, one was prepared longitudinally and the other was prepared transversely. Otoliths were then read using a compound microscope at 400 to 1000 x magnification with transmitted light. The total count of micro- (presumed daily) increments from the nucleus to the edge was completed for all samples. Longitudinal sections were considered to provide a better section for ageing, in that they 1) provided more consistent counts, and 2) were easier to interpret, which collectively resulted in higher confidence in the daily age estimates.

Results were compared against the daily age estimates of Itoh and Tsuji (1996; based on otoliths examined using Scanning Electron Microscopy [SEM]) and Rees et al. (1996; based on otoliths examined using light microscopy and SEM). Fish examined by FAS were found to have a substantially larger FL for a given daily age estimate than those of Itoh and Tsuji (1996) or Rees et al. (1996) for either transversely or longitudinally prepared otoliths, and this difference became progressively larger with fish size. Moreover, back-calculated birth months of the FAS-examined fish indicated that based on the daily counts, these fish would have been born between March-July, outside of the known SBT spawning season. Combined, these two lines of evidence suggest an underestimation of age in the recent samples compared to Itoh and Tsuji (1996) or Rees et al. (1996). It may be that the SEM used by both Itoh and Tsuji (1996) and Rees et al. (1996) provides a more accurate estimate of daily age, particularly for samples > 35 cm FL. To provide the data required for 'Jesstimation', the workshop recommended sourcing and measuring the second otolith of the pair used in the Itoh and Tsuji (1996) and Rees et al. (1996) studies and aligning these with the daily count data, and/or undertaking SEM work on small SBT to improve age estimates. For the latter, the workshop recommended that this should include examination of historical samples as well as recently collected individuals, given changes in growth observed in recent years.

Otolith image exchange exercise

A key objective of the workshop was to estimate the precision of age estimates assigned by different readers and determine sources of potential bias in reader age estimates. To facilitate this, an image collection of 41 thin sectioned SBT otoliths were shared with workshop participants one month prior to the workshop. The objectives of this exchange exercise were to: 1) determine the precision among different age readers or institution involved with SBT ageing; 2) identify any sources of bias, where present; and 3) use this information to improve current SBT ageing procedures and the CCSBT age determination manual. All otoliths were prepared as thinly cut (~300–350 µm) transverse sections, with care taken to ensure that at least one section from each cut otolith included the primordium.

Of the 41 images shared, 35 were of otoliths from fish that had been tagged at lengths corresponding to 1, 2, or 3 years old, and recaptured between 0.9 and 22.2 years later. 'True' age estimates of these fish were calculated based on time between their estimated birth date (assuming a birth date of 1 January) and tagging, and the time between tagging and recapture, assuming one opaque zone forms in each full year of life (Figure 1). Two individuals were not tagged, and four individuals were tagged at lengths where ages could not be reliably estimated or had incomplete tagging data (e.g., missing release/recapture information). 'True' age estimates for these otoliths were derived from opaque zone counts of an experienced reader.

Each reader was asked to read the otoliths without knowledge of the fish's length or collection date using their current protocols, and provide their opaque zone count, along with their determination of edge type. Three edge type categories were specified: narrow translucent

(NT), whereby there is translucent material past the last opaque zone that is less than 1/3 of the previously completed zone; wide translucent (WT), whereby there is translucent material past the last opaque zone that is greater than 1/3 of the previously completed zone; and opaque (O), whereby opaque material is visible on the otolith edge. Readers were also asked to provide a measure of the readability of each section, consistent with the criteria for sectioned otoliths in Anon (2002), and an annotated image, with their zone counts clearly marked on each otolith. Each image was then annotated by an experienced reader who had knowledge of the 'true' age of the fish. Precision and bias of the reads against the 'true' age was estimated using the IAPE and CV, the age-bias plots of Campana et al. (1995), and by annotating a single image for each sample with the annotations of the individual readers.

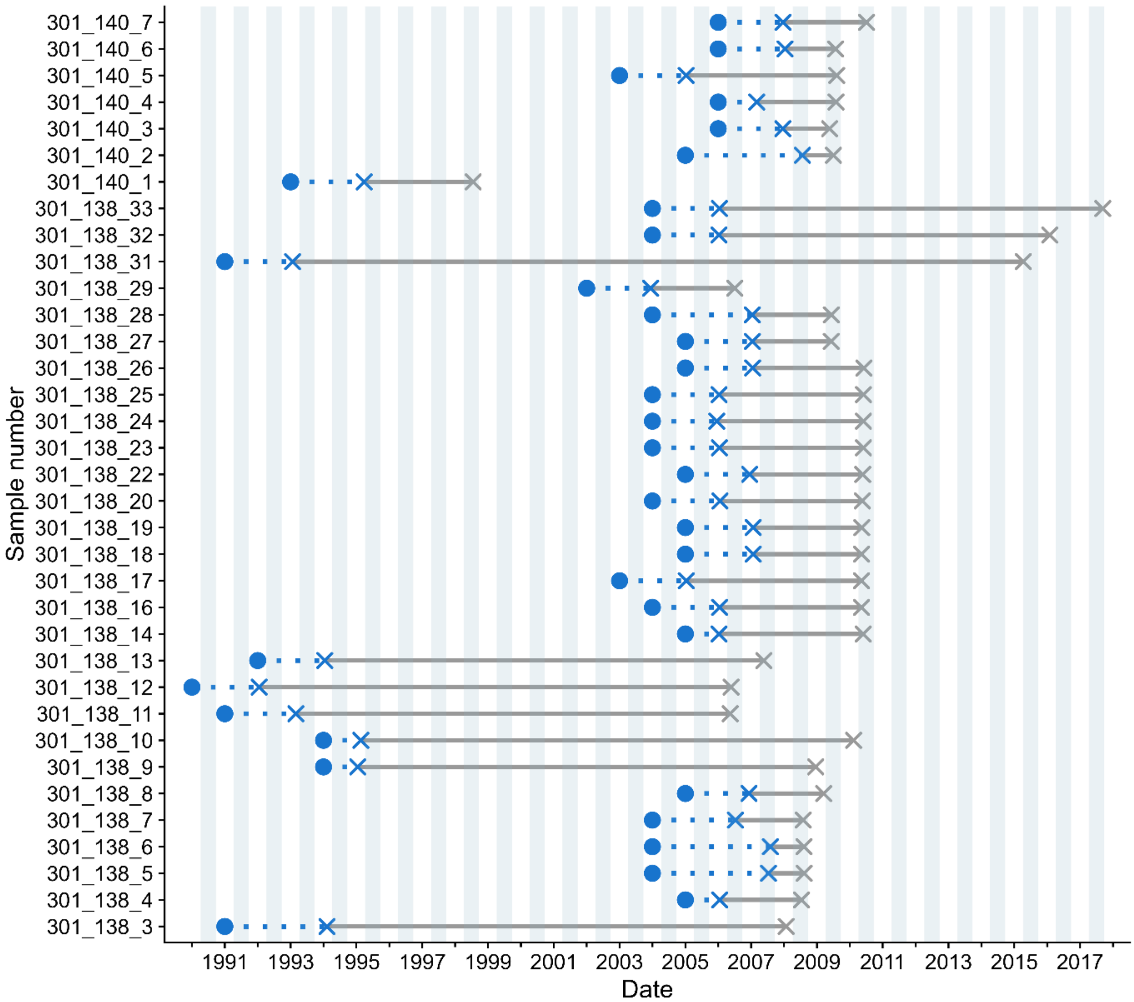

Figure 1. Timelines of the 35 tagged fish of known age used in the otolith image exchange. Blue circles represent estimated birthdates (considered to be 1 January), blue crosses indicate when a fish was tagged, and grey crosses indicate recapture dates. Dotted blue horizontal lines indicate the estimated time from birth to the date of tagging, and solid grey horizontal lines represent the time between tagging and recapture. Light blue bars represent the winter period in the southern hemisphere.

At least one participant per CCSBT Member that attended the workshop provided their reading results (Figure 2), and 4 of the 5 readers provided annotated images. Mean CV and mean IAPE ranged from 5.07–22.42% and 3.59–15.85%, respectively. Age bias plots revealed biases in two of the readers, including age-related bias (e.g., underestimation of the age of fish after around 15 years by one reader) and overall bias (e.g., consistent overestimation of age by another reader). Mean CV at age values were generally lower in older individuals for most readers, likely because the opaque zones are more distinct in older fish, which helps in pattern recognition improving the delineation of opaque zones in the earlier years of a fish's life. The main sources of variation amongst readers identified by the group were (1) the identification of the first 1–2 opaque zones; (2) the identification of outermost zones, particularly where the otolith matrix becomes more diffuse towards the marginal edge, (3) the determination of edge type; (4) readers missing annual zones (possibly interpretating annual zones as sub-annual or split zones); and (5) readers counting sub-annual zones as annual zones (Figure *3*).

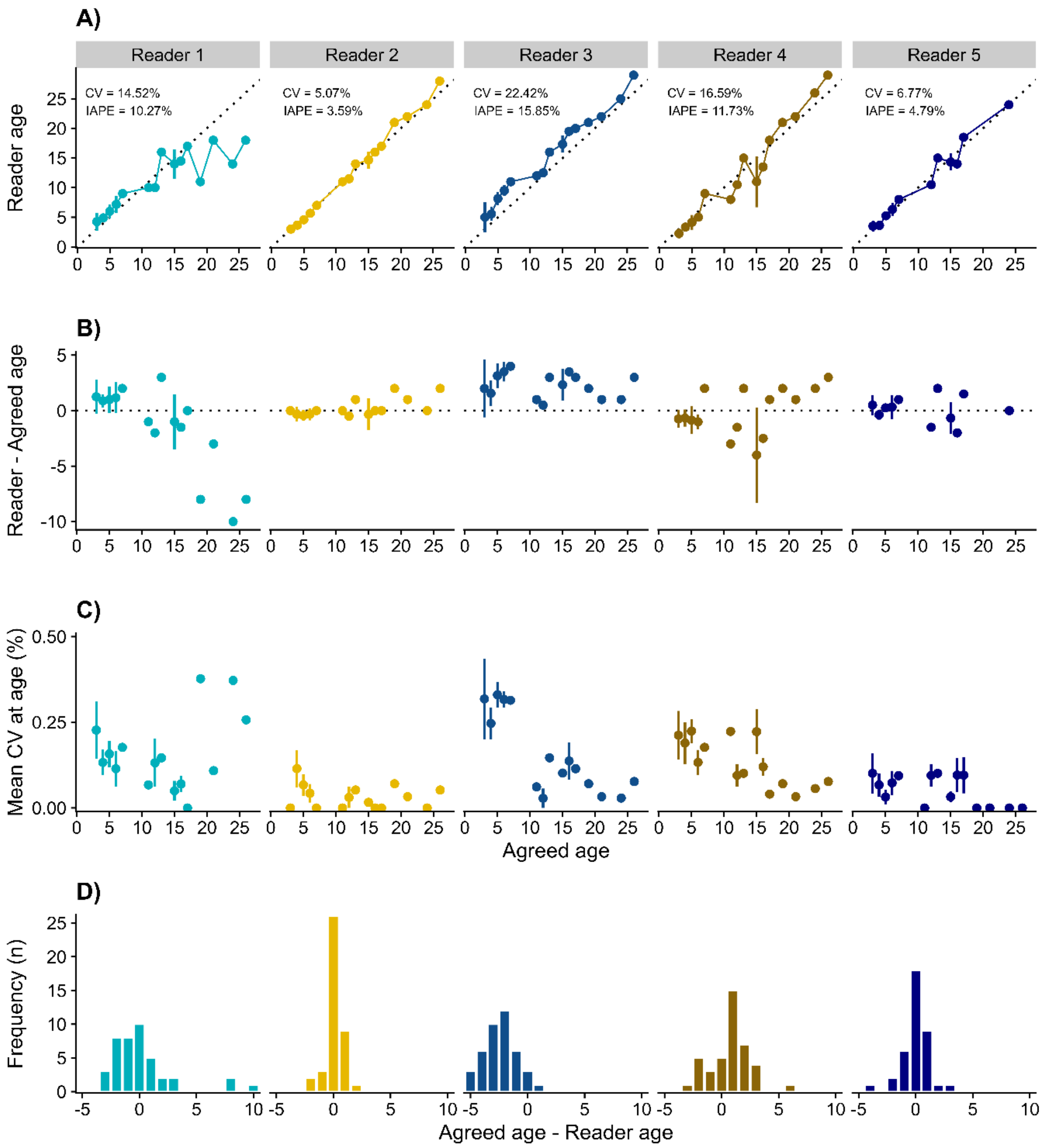

Figure 2. Results from the otolith image exchange exercise between different readers of southern bluefin tuna (*Thunnus maccoyii*) otoliths. A) Age-bias plot, B) plot of ageing differences (reader age minus agreed age) between readers and agreed age, C) Mean Coefficient of Variation at age, D) histogram of ageing differences (agreed age minus reader age).

Figure 3. Examples of otolith images from the image exchange, and the annotations made by individual readers (dots). Dot colours match those used in Figure 2. White dots are the annotations made by an experienced reader with knowledge of the 'true' age of the fish. Squares indicate were a reader counted five opaque zones.

Ageing protocols

Discussion regarding the improvement of standardised ageing protocols progressed from those first developed during the 2002 SBT ageing workshop described in Anon (2002). The key focal areas discussed were: 1) the identification of the first opaque zone, and timing of its formation, 2) the identification of subsequent (middle and outer) opaque zones, 3) otolith edge interpretation, 4) quality assurance and ageing precision, and 5) otolith image capture, marking, and measuring.

Identification of the first opaque zone

Opaque zone formation has been directly and indirectly validated as occurring on an annual basis in both young and old SBT. Southern bluefin tuna injected with strontium chloride that were released in the GAB and successfully recaptured (*n* = 59) show that opaque zones are formed annually in fish ranging from at least 1 to 6 years (Clear et al. 2000). Bomb radiocarbon dating analysis showed that otolith core $\delta^{14}C$ values in old individuals were aligned with the known values in seawater in the years when they were estimated to have hatched, as back-calculated from their opaque zone counts (Kalish et al., 1996).

Discussion was held on approaches to delineate the first opaque zone. Four approaches were considered. First, it was recommended that the reference samples be augmented with samples where the first opaque zone is reasonably prominent and clear to interpret. This would assist with identification in samples where the first zone is less clear.

The workshop then discussed the use of external features or landmarks to help locate the first opaque zone. It was noted that the first opaque zone can often coincide with a change in the growth axis or a 'bump' that may be visible on the antisulcal or sulcal surfaces (Figure 4).

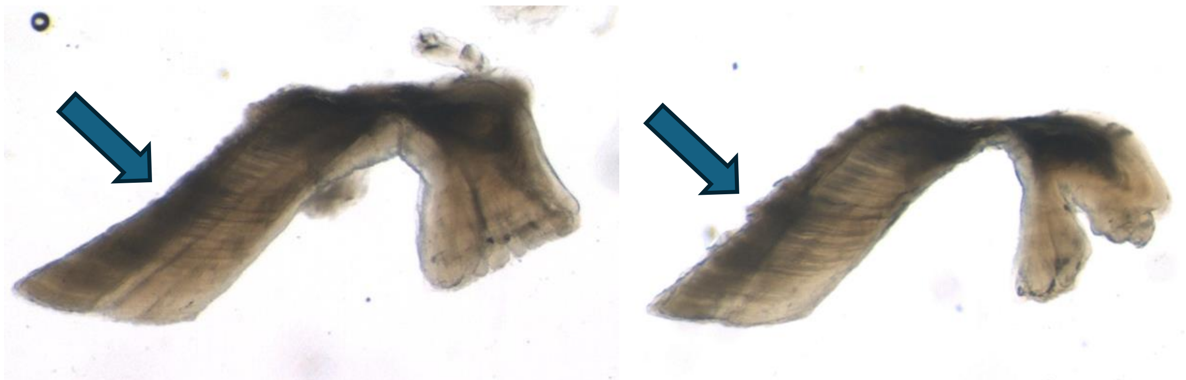

Figure 4. Examples of otoliths demonstrating a change in growth axis (left) or a 'bump' (right) on the antisulcal surface coinciding with the formation of the first opaque zone.

Measuring the distance from the otolith core and putative first opaque zone was also discussed as a guide to identifying the first increment. A similar approach has been developed for locating the first few opaque zones in both Atlantic bluefin tuna (*Thunnus thynnus*, ABT; Rodríguez-Marín et al. 2019) and Pacific bluefin tuna (*Thunnus orientalis*, PBT; Shimose and Ishihara 2015). For ABT, two thin transverse sections are commonly taken from each processed otolith, one that incorporates the primordium and a second section slightly further out. The section that contains the primordium is typically used for isotopic work, and the second section is used for ageing. A reference scale of 1 mm is used, measured from the bottom centre of the bridge between the two arms and extending up the inner ventral arm at the sulcus margin. The first opaque zone occurs within this scale (Rodríguez-Marín et al. 2019).

For PBT, Shimose and Ishihara (2015) documented that measurements from the primordium to the first opaque zone ranged from 0.61 to 1.02 mm (n = 90, mean=0.79) but noted that this will varying depending on the timing of hatching, with early hatched fish (those hatching between May and June) expected to have a longer distance from the primordium to the first opaque zone than those hatched late in the spawning season (July-August). The authors further documented the distance from the primordium to the middle of the second opaque zone, which ranged from 1.07 to 1.9 mm (n = 21, mean = 1.18).

To provide a measurement proxy to assist with the identification of the first opaque zone for SBT, FAS presented results of an analysis of the distance between the otolith primordium and first and second opaque zones of the ventral arm, using otolith sections from 20 young SBT individuals that had exceptionally clear first opaque zones and high reader-assigned readability. For each otolith section, two measurements were taken: one from the primordium to the end of the first and second opaque zones along the inner edge of the ventral arm (hereafter termed the inner measurement), and a second from the first apex to the end of the first and second opaque zones close to the outer (distal) edge of the ventral arm (hereafter termed the outer measurement) (Figure 5). Measurements from the primordium to the end of the first opaque zone ranged from 0.85–1.16 mm for the inner measurement (n = 20, mean = 0.96 mm), and 0.75–1.05 mm for the outer measurement (n = 20, mean = 0.89 mm). Measurements from the primordium to the end of the second opaque zone ranged from 1.18–1.61 mm for the inner measurement (n = 19, mean = 1.31 mm), and 1.15–1.39 mm for the outer measurement (n = 19, mean = 0.89 mm) (Figure 6). As with PBT, variations amongst individuals likely relate to differences in hatching time, with fish hatched in October-December, towards the start of the spawning season, expected to have a longer distance between the core and first opaque zone than late (March-April) hatched fish. Prevailing environmental conditions at the time of hatching could also drive further variation.

The workshop noted that in addition to the ventral arm, the dorsal arm can often be quite useful for ageing, particularly to verify patterns observed in the ventral arm. As such, the workshop recommended developing a similar measurement proxy for the dorsal arm.

Identification of subsequent zones

The workshop then discussed the identification of subsequent opaque zones, including 'middle' zones (zone 2 to the second inflection) and 'outer' zones (zones beyond the second inflection. The first few annual zones after the first opaque zone (i.e., opaque zones 2 to ~5) can often be quite diffuse, particularly in young fish, and often contain multiple finer, irregular sub-annuals bands that merge on the sulcal margin (Figure 7). Marginal crenulations may be present to aid in the identification of growth zones (Figure 7) and opaque zones are generally easier to interpret close to the outer edge than the inner ventral edge closer to the sulcus. Closer to the second inflection (i.e., opaque zones 6 to ~10), opaque zones are usually less diffuse and become increasingly closer together (Figure 7).

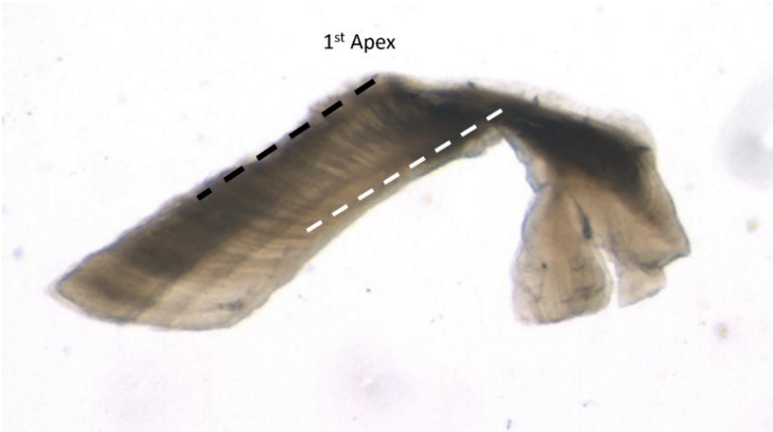

Figure 5. Examples of the measurements taken to develop a proxy measurement for the identification for the first opaque zone. White dashed lime = measurement taken from the primordium to the end of the first opaque zone along the inner edge of the ventral arm; black dashed line = measurement taken from the first apex to the end of the first opaque zone close to the outer (distal) edge of the ventral arm.

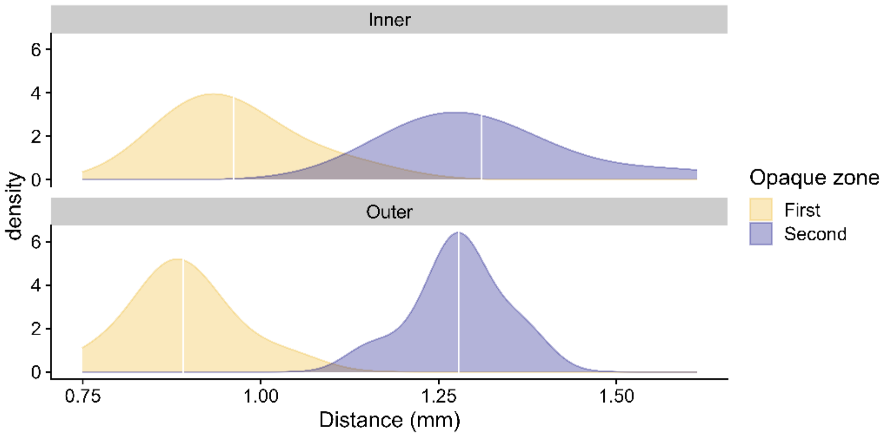

Figure 6. Distribution of distances from the otolith primordium to the first and second opaque zones of young SBT otoliths, Inner = measurement taken from the primordium to the end of the first and second opaque zones along the inner edge of the ventral arm, outer = measurement taken from the first apex to the end of the first and second opaque zones close to the outer (distal) edge of the ventral arm. The white line indicates the mean distance.

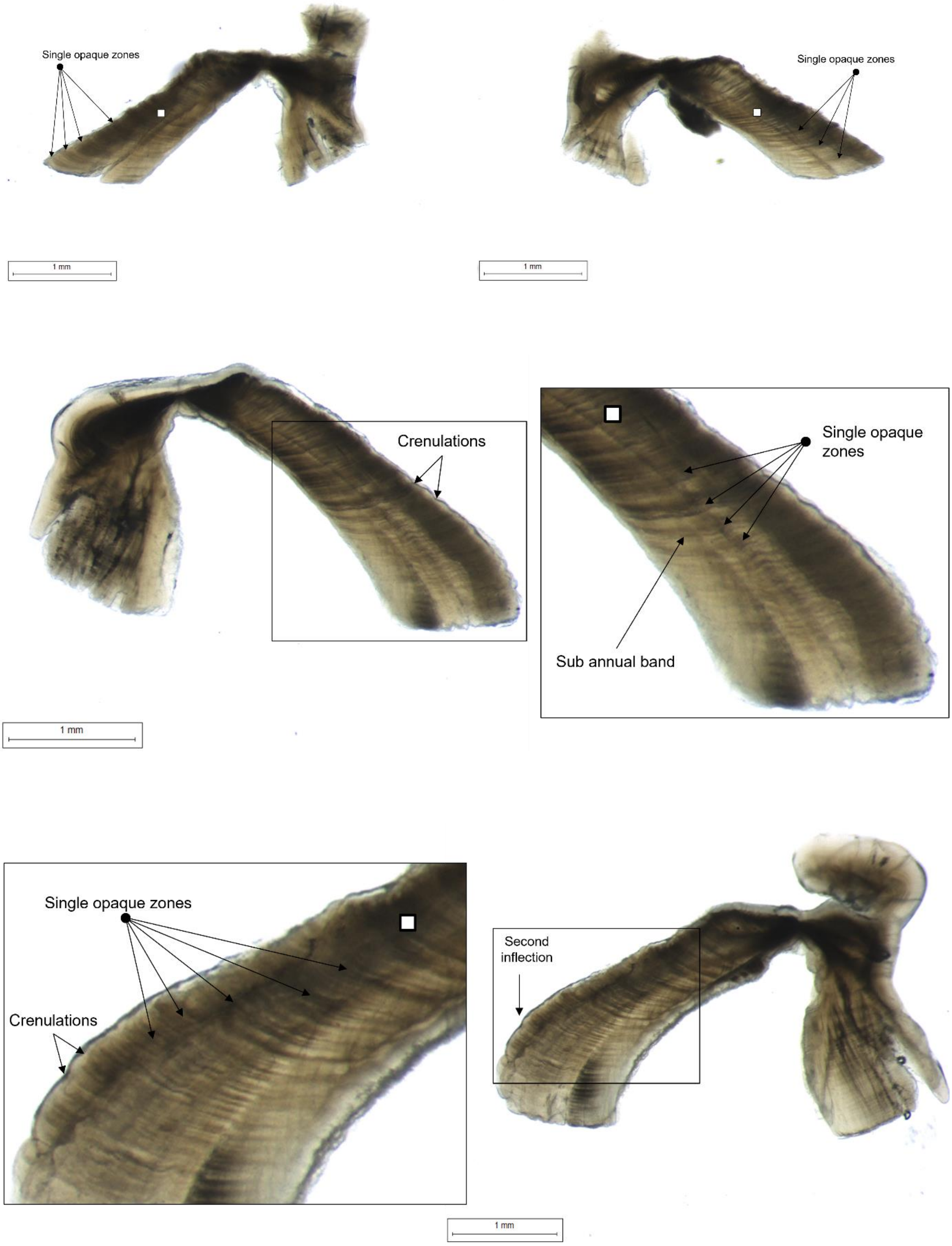

Figure 7. Examples of middle opaque zones. White squares indicate the first opaque zone for each otolith.

After the second inflection, opaque zones become much more uniformly spaced, and darker and clearer than preceding opaque zones (Figure 8). Opaque zones are often more easily counted on the sulcal side of the ventral arm rather than the anti-sulcal side, although care should be taken to find an appropriate ‘crossing-over’ point when transitioning from the antisulcal side, such as an opaque zone that shows good continuity from the sulcal to the antisulcal sides (Figure 8). A similar approach is often used for ABT (Rodríguez-Marín et al. 2019) and PBT (Shimose and Ishihara 2015).

It was noted that some outer zones appear as ‘doubles’ (Figure 9). These can be interpreted by using a higher magnification to determine whether the two structures merge at the groove and/or sulcal margin (in which case they should be counted as one zone) or are distinct throughout their length (and in which case might be considered as two separate opaque zones).

Key criteria and recommendations discussed for identifying and enumerating outer opaque zones in SBT included:

- That outer zones are usually more regular in width and appearance, and darker and clearer than inner or middle zones.
- Spacing between outer opaque zones is narrower than that of middle zones.
- That outer zones usually consist of a single (not split) translucent and single opaque zone.
- Higher magnification may be required for counting the closely spaced outermost zones.
- Physically tilting the sectioned otolith can help to get clearer/sharper views of the annuli and the edge (Figure 10).

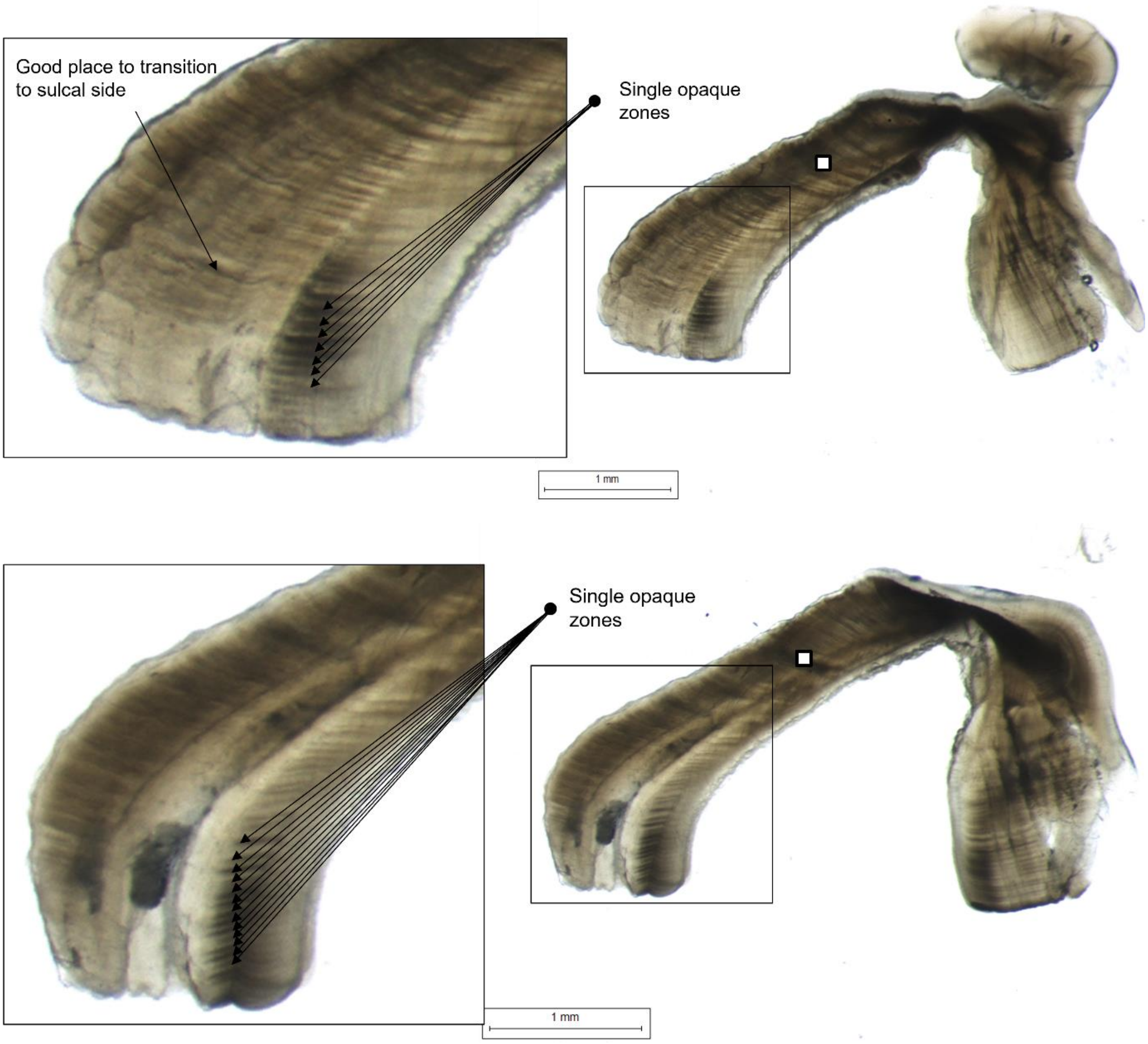

Figure 8. Examples of clear outer opaque zones. White squares indicate the first opaque zone for each otolith.

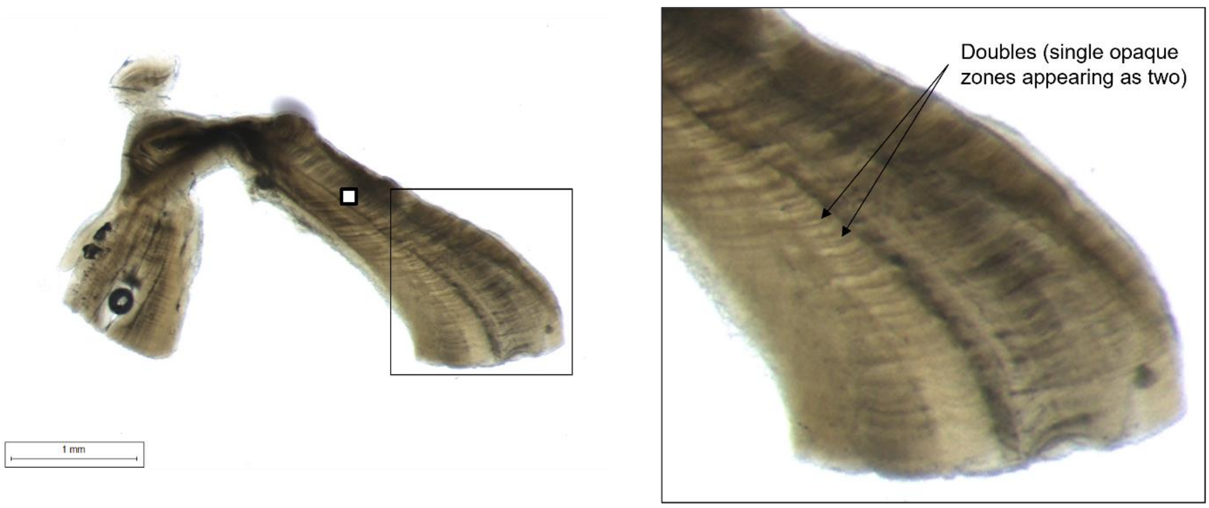

Figure 9. Examples of double outer opaque zones. In these instances, the zones merge into a single opaque zone of the antisulcal side and should thus be counted as a single zone. The white square indicates the first opaque zone.

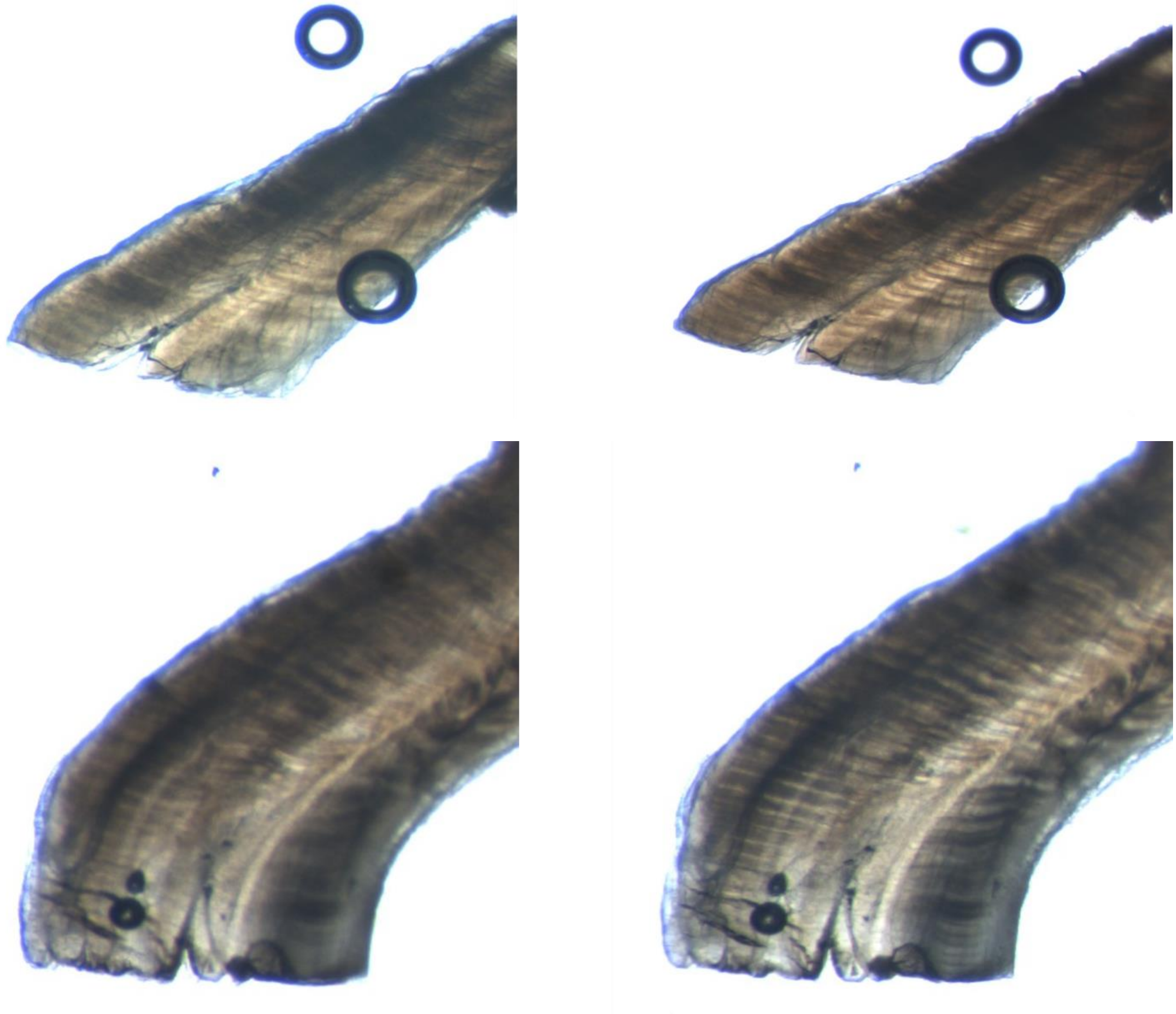

Figure 10. Examples of ‘untilted’ otolith sections (left column) and the same sections when ‘tilted’ (right column). Note the improved opaque zone clarity in the tilted sections.

Otolith edge interpretation

The workshop discussed including a description of marginal edge type when reading otoliths in the updated age protocols as it was not included in the 2002 SBT Age Determination manual. Documenting edge type is useful for a range of purposes, including when undertaking an edge type analysis to try to validate age classes, or when converting opaque zone counts to decimal age or fractional age. Moreover, documenting edge types is currently considered ‘best-practice’ for standardising age estimates between readers.

Edge type assignment was identified as a source of ageing error in the otolith image exchange due to variation between readers (see ‘Otolith image exchange exercise’ section above). To help ensure standardisation in the way edge types were interpreted, CSIRO presented a schematic (Figure 11) of the edge type interpretation initially developed for swordfish by Farley et al. (2016) and now used by both Australia and New Zealand when ageing SBT. Under this approach, three edge types are recorded: NT, WT, and O (see ‘Otolith image exchange exercise' section for definitions of each category). Opaque zones at the terminal edge of the otolith are counted only if some translucent material was evident after the opaque zone. Australia has also begun recording a readability score of the edge type using a 1–3 scoring system, whereby 1 = not confident, 2 = confident in completeness and not with the type, and 3 = confident. A similar protocol has been adopted for ABT (Rodríguez-Marín et al. 2019).

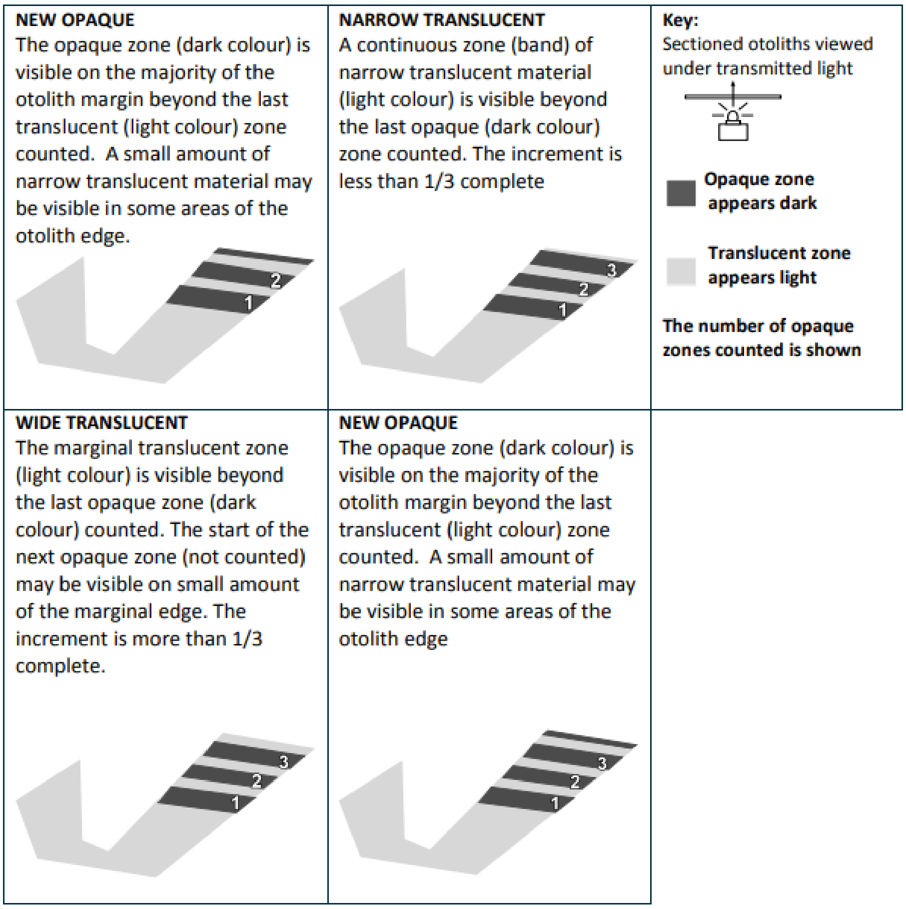

Figure 11. Schematic of the cycle of each marginal edge classifications used by Australia and New Zealand to categorise SBT otolith edge types. From Farley et al. (2016).

Timing of annuli formation

The timing of annuli formation in tunas is variable between species and locations. For Atlantic bluefin tuna, opaque zone formation begins in early (northern hemisphere) summer (i.e., June), towards the end of the spawning season, and is completed by late spring (i.e., November) (Rodríguez-Marín et al. 2022). For Pacific bluefin tuna, opaque zone formation occurs during April to July, coinciding with the peak spawning season (Shimose et al. 2009, Shimose and Ishihara 2015). For albacore tuna in the North Pacific Ocean, opaque zone formation occurs over winter (Chen and Holmes 2015), while opaque zone formation in albacore tuna in the South Pacific Ocean occurs over austral summer and is typically completed by autumn to winter (Farley et al. 2013). In SBT, opaque zone formation is typically completed in ~winter, with a greater proportion of fish collected in August showing narrow marginal increments and narrow translucent edges relative to those collected in the preceding months (Gunn et al. 2008, Moore et al. 2025).

With SBT spawning occurring between approximately September and April (Farley et al. 2015), and opaque zone deposition occurring in ~winter (Gunn et al. 2008), there was an open question of whether the opaque zone typically annotated as the first opaque zone was forming in the first winter or second winter of a fish's life. CSIRO presented preliminary work undertaken to explore this question. Here, images of sectioned otoliths were taken for seven young SBT collected off Western Australia in December-April. These fish were assumed to be one year old based on length data and thus considered to have resulted from the previous spawning season. The outlines of the otolith sections were extracted and overlaid on the otolith images used for the image exchange exercise, with care taken to ensure the two images were kept at the same scale. Logically, if the opaque zone assumed to be the first in the otolith image exchange otoliths formed in the first year of a fish's life, it should then appear inside the otolith outline of the young fish, while the second opaque zone should appear outside of the outline.

Results from this preliminary exercise revealed that the opaque zone considered as being the first opaque zone generally fell within the outlines of the one-year-old fish (Figure 12), providing a preliminary indication that the opaque zone considered to be the first annual zone was indeed forming in the first year of life. Combined daily ageing and annual ageing of paired otoliths of 2-year-olds (i.e., young fish for which the first zone should be evident in thin transverse sections) is required to further test the hypothesis that the zone considered as being the first opaque zone is deposited in the first year of a fish's life.

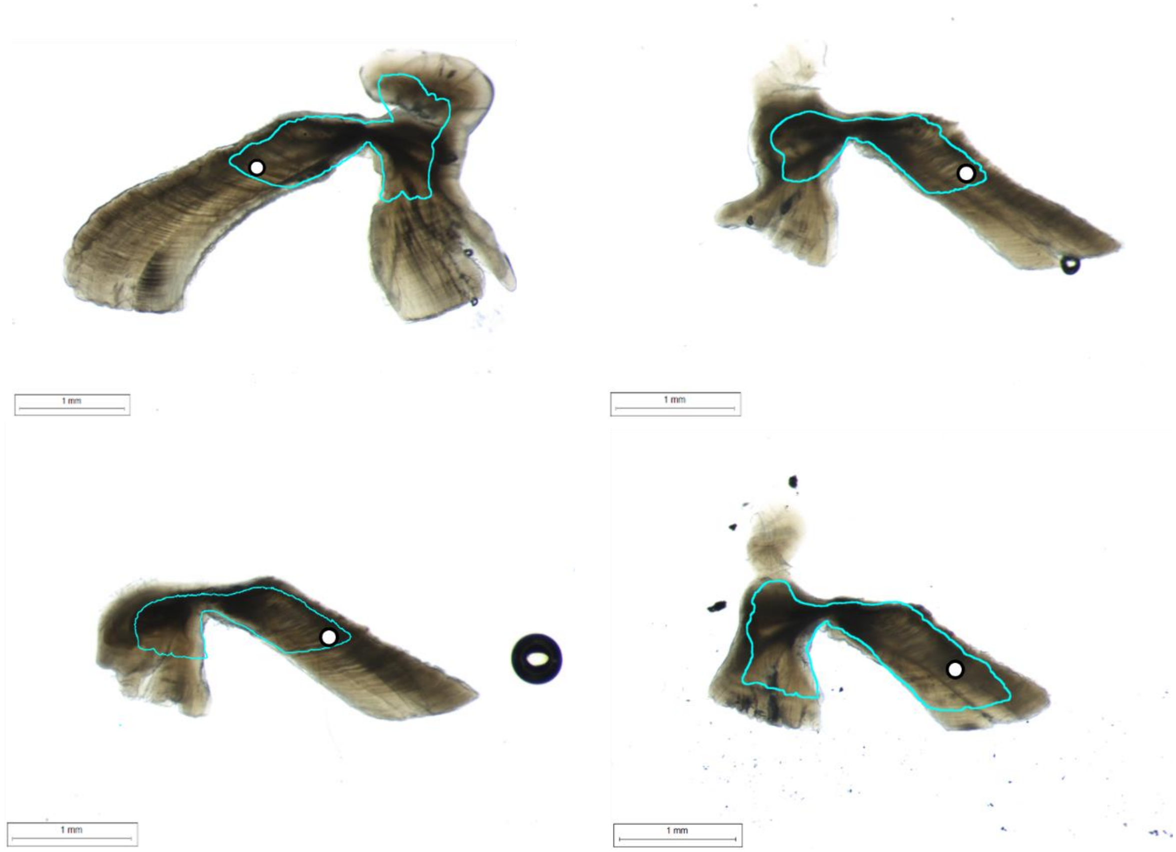

Figure 12. Examples of otolith outlines from one year old SBT overlaid onto samples from the otolith image exchange. The white circle indicates the opaque zone considered to be the first opaque zone.

Quality assurance and ageing precision

Ageing precision of tunas, including SBT, is typically quantified through calculation of the IAPE (Beamish and Fournier 1981) or CV (Chang 1982). The workshop reviewed level of precision reported from ageing studies of temperate tunas (Table 1). Where reported, studies into ageing of temperate tunas have reported levels of precision of around 5% mean IAPE. The workshop acknowledged that the number of repeated reads is often subject to the contract conditions a research provider is working under but generally recommended both intra- and inter-reader checks be undertaken. The workshop agreed that for repeated reads a mean IAPE of less than 5% was a feasible target.

The workshop then discussed otolith imaging and annotating, including measurement of opaque zones. Capturing both unannotated and annotated images is typically regraded as 'best practice' in fish age estimation, providing a useful means of quality assurance and reference material for future training. Unmarked images are also key inputs for the development of AI-based age estimation algorithms. There was general agreement amongst participants that both unannotated and annotated images should be collected for each fish aged, and most participant laboratories were already capturing both an unannotated and an annotated image of each otolith.

NIWA in New Zealand gave a presentation on the SmartDots age estimation platform that they are integrating into their routine commercial ageing work. SmartDots was originally developed by Flanders Research Institute for Agriculture, Fisheries, and Food (ILVO) to facilitate age estimations based on otolith images and management of resulting data. The platform is now used within the International Council for the Exploration of the Sea (ICES). SmartDots is open-source software available at https://www.ices.dk/data/tools/Pages/smartdots.aspx. The platform allows a user to annotate otolith images with their age estimations and collect measurement data from these annotations. Multiple users can annotate a single image with their readings, making it particularly suited to training and for quality assurance of age estimates from routine ageing. The workshop agreed that SmartDots could be a good tool moving forward for future otolith exchanges, calibration or testing exercises.

Table 1. Reported levels of ageing precision in ageing studies of temperate tunas. IAPE = Index of Average Percent Error, CV = Coefficient of Variation.

Species	Metric used	Value	Comparison	Reference
Southern bluefin tuna	Mean IAPE	2.59	Within reader – experienced	Gunn et al. (2008)
	Mean IAPE	4.21	Within reader – moderate experience	
	Mean IAPE	8.47	Within reader - novice	
	Mean IAPE	3.92	Between readers – experienced vs. mod. exp.	
	Mean IAPE	9.44	Between readers – experienced. vs. novice	
Atlantic bluefin tuna	Mean IAPE	1.52	Between readers	Rodríguez-Marín et al. (2014)
	Mean CV	1.90	Between readers	
Pacific bluefin tuna	Mean IAPE	4.51	Within reader	Shimose et al. (2009)
	Mean CV	6.38	Within reader	
Pacific bluefin tuna	Mean IAPE	4.88	Between two readers	Shimose and Ishihara (2015)
	Mean CV	6.90	Between two readers	
North Pacific albacore	Mean IAPE	4.75	Between two readers	Wells et al. (2013)
	Mean CV	6.72	Between two readers	
South Pacific albacore	Mean IAPE	4.77	Within reader	Farley et al. (2013)
	Mean IAPE	6.82	Between two readers	

Decimal age calculation

In the current SBT Age Determination Manual (Anon 2002), opaque zone counts are considered as the final age of the fish. However, for many research questions, it is desirable to have a more precise fractional age. This is particularly relevant for SBT, as national fisheries can operate at different times of the year.

The approach of using a specified birthdate, the counts of opaque zones, and the date of capture to derive a decimal or fractional age was considered. The approach is arguably the most commonly used method of assigning a decimal age when estimating fish age, particularly for species with clearly defined, temporally restricted spawning seasons. Using a birthdate corresponding to the middle or peak of the spawning season is common in tunas. For example, Wells et al. (2013) assigned decimal ages to albacore tuna (*Thunnus alalunga*) in the North Pacific Ocean based on a birthdate of May, which corresponds to peak spawning in the North Pacific (Chen et al. 2010). Farley et al. (2013) assumed a birth date of 1 December for South Pacific albacore, which corresponds to the middle of the spawning season in the South Pacific.

CSIRO presented an approach developed by Eveson et al. (2004) to convert counts of opaque zones to decimal age, using the principle described above. A decimal age can be assigned to each aged fish using:

$$\text{Equation 1: } a = n + r/365 \qquad \text{if } r < d$$

or

$$\text{Equation 2: } a = n - 1 + r/365 \text{ if } r > d$$

where a = decimal age, n = the opaque zone count, r = the recapture date (days elapsed since last birthday) and d = day of opaque zone formation. If a fish has already formed a new growth zone in the time between its last birthday and its day of capture, then its integer age is $n - 1$; otherwise, its integer age is n (assuming that the opaque zone count is correct). Thus, the age calculated is simply the fish's integer age plus the fraction of the year between its birthday and its day of capture (Eveson et al. 2004).

The workshop also considered the 'Jesstimation' approach developed by Farley et al. (2025). This approach was developed for tropical tunas which spawn throughout the year (and thus for which a common birthdate cannot be assumed) and for which increments can form over a much longer period of time than in SBT. The method converts counts of annual growth increments into fractional ages using daily ageing of young-of-the-year (YOY) fish and otolith measurements using a four-step process (Figure 13). In the first step, the age of the fish is estimated by fitting the distance between the 1st apex and the outer edge of the first opaque zone to a curve (power or similar) representing the relationship between otolith distance and daily age. In step two, the number of complete annual increments (i.e., opaque + translucent zones) is counted, with the first opaque zone excluded from the count. In step 3, the time elapsed between the deposition of the last counted opaque zone and the fish's date of capture is estimated by expressing the distance from the outer edge of the last completed opaque zone to the otolith edge as a proportion of the full increment observed in all other fish. Finally, the values derived in each step are summed to provide a decimal age estimate (Figure 13). A key advantage of the approach is that it does not need knowledge of birthdate or timing of annual increment formation (Farley et al. 2025). While the data for step 3 are available, in order to implement the Jesstimation approach of Farley et al. (2025) to SBT, work is required to obtain more accurate daily age estimates and a better understanding of the relationship between daily age and otolith size.

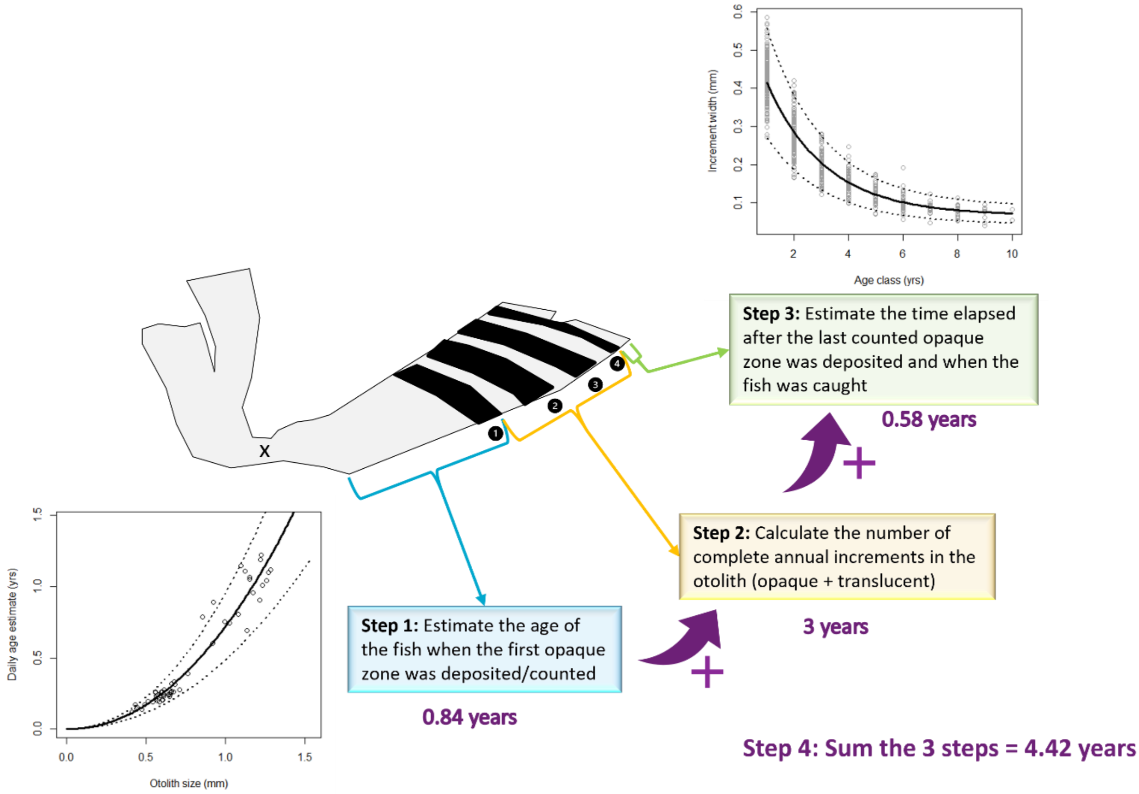

Figure 13. The 'Jesstimation' process developed to estimate decimal ages by Farley et al. (2025).

Use of artificial intelligence / machine learning

The use of artificial intelligence (AI) and machine learning (ML) to estimate age from images of otoliths or other hard parts is a rapidly growing area of research. Several studies have successfully applied AI to images of otoliths to estimate fish age, often with results comparable to human readers (e.g., Moore et al. 2021, Cayetano et al. 2024). In these approaches, a deep learning model (e.g., Convolutional Neural Network (CNN)) is typically trained on images of otoliths of 'known' age individuals and tested against an unseen subset of images. In one particularly pertinent example, Ma et al. (2024) used CNNs to estimate age of Pacific bluefin tuna (*Thunnus orientalis*) from otolith images. The authors trained three separate CNN models: a baseline model, which was trained on otolith images only, a model that was trained on otolith images as well as otolith mass, and a model that was trained on otolith images and otolith mass, with images subjected to a range of image augmentation procedures. Of the three models developed, the latter model achieved the highest accuracy (72.81% of age estimates being ±1 of the 'true' age) and lowest CV (7.38%). The workshop was presented with a summary of a similar development for SBT being undertaken by National Taiwan University and colleagues, with initial results showing promise, with the best model reaching an accuracy of 79.11% (within ± 1 year of the 'true' age; Jen-Chieh Shiao *unpublished data*).

Recommendations

The workshop made the following specific recommendations when revising the SBT Age Determination Manual:

1. That the otolith terminal edge type be recorded following the three category approach currently used by Australia and New Zealand i.e., narrow translucent (NT), whereby there is translucent material past the last opaque zone that is less than 1/3 of the previously completed zone; wide translucent (WT), whereby there is translucent material past the last opaque zone that is greater than 1/3 of the previously completed zone; and opaque (O), whereby opaque material is visible on the otolith edge.
2. That the readability of the edge type be recorded using the 3-level approach currently used by FAS, i.e., whereby 1 = not confident, 2 = confident in completeness and not with the type, and 3 = confident.
3. That a mean IAPE of 5% be the target level of precision for all intra- and inter-reader age estimates.

The workshop made the following recommendations for future research:

1. Improving daily age estimates to provide a better understanding of the relationship between daily age and otolith size. Two specific recommendations were made:
    a. Undertake SEM analysis of small SBT to establish the relationship between daily age and otolith size. Ideally, this should include young fish from both historical collections and from recent years, given observed changes in growth in recent years. It was noted that there might be some samples of age 1+ fish available through CSIRO's recent gene tagging activities and archived otolith collections that could be used for this purpose.
    b. Source and measure the second otolith of the pair used in the studies by Itoh and Tsuji (1996) and Rees et al. (1996).
2. Undertake further measurements of the distance between the primordium and first opaque zone on the ventral arm to refine the yardstick measurement approach. As with Item 1 above, ideally this should be done on young fish from both historical collections and from recent years.
3. Develop a similar measurement proxy for identifying the first opaque zone on the dorsal arm.
4. Conduct otolith exchanges and inter-laboratory otolith reading comparisons on a regular basis (e.g., every few years) to improve the quality and reliability of age data provided to CCSBT.
5. Continue to investigate the use of AI for estimating the age of individual SBT from otolith images.

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Appendix 1. List of workshop participants.

First name	Last name	Title	Position	Organisation
CHAIR				
Brad	MOORE	Dr	Senior Research Scientist	CSIRO Environment
AUSTRALIA				
Jessica	FARLEY	Ms	Research Group Leader	CSIRO Environment
Naomi	CLEAR	Ms	Senior Scientist	CSIRO Environment
Kyne	KRUSIC-GOLUB	Mr	Senior Scientist	Fish Ageing Services (FAS)
FISHING ENTITY OF TAIWAN				
Ching Ping	LU	Dr	Assistant Professor	National Taiwan Ocean University
Jen-Chieh	SHIAO	Dr	Professor	National Taiwan University
NEW ZEALAND				
Caoimhghin	Ó MAOLAGÁIN	Mr	Senior Technician	National Institute of Water & Atmospheric Research Ltd (NIWA)
Niki	DAVEY	Ms	Marine Ecologist	National Institute of Water & Atmospheric Research Ltd (NIWA)
Tom	BARNES	Dr	Fisheries Scientist	National Institute of Water & Atmospheric Research Ltd (NIWA)
REPUBLIC OF KOREA				
Junghyun	LIM	Dr	Scientist	National Institute of Fisheries Science (NIFS)
Miran	KIM	Ms	Technician	National Institute of Fisheries Science (NIFS)
Sanggyu	SHIN	Mr	Advisor	National Institute of Fisheries Science (NIFS)

Appendix 2. Workshop agenda

Time (approx.; AEST)	Agenda item	Presenter
08:00–08:30	Welcome and introductions Overview of agenda Goals of this workshop	Chair
08:30–08:45	Overview of the 2002 SBT ageing workshop	Naomi Clear (CSIRO)
08:45–09:40	CCSBT Member updates: overview of SBT ageing methods by each Member	All
09:40–10:00	Morning tea break	
10:00–10:15	Results of daily ageing exercise by FAS	Kyne Krusic-Golub (FAS)
10:15-10:25	Estimating age from tagged fish	Naomi Clear (CSIRO)
10:25–11:00	Results of the otolith exchange exercise	Chair
11:00–12:00	Protocols for age estimation – identification of first opaque zone(s)	Chair/FAS
12:00–12:45	Lunch break	
12:45–13:15	Protocols for age estimation – identification of subsequent opaque zones	Chair
13:15–13:45	Protocols for age estimation – otolith edge interpretation	Jessica Farley (CSIRO)
13:45–14:15	Protocols for age estimation – quality assurance and ageing precision, otolith imaging, marking, and measuring	Chair
14:15–14:35	Afternoon tea break	
14:35–15:00	Decimal age calculation – what is possible with current information?	Jessica Farley (CSIRO)
15:00–15:30	Research advances and recommendations	Chair
15:30–16:00	Next steps	Chair
16:00	Close of workshop	