SCIENTIFIC COMMITTEE
FIFTEENTH REGULAR SESSION

Pohnpei, Federated States of Micronesia
12-20 August 2019

Project 94: Workshop on yellowfin and bigeye age and growth

WCPFC-SC15-2019/SA-WP-02

Farley, J., Krusic-Golub, K., Clear, N., Eveson, P., Smith, N. and Hampton, P.

OCEANS AND ATMOSPHERE
www.csiro.au

Project 94: Workshop on yellowfin and bigeye age and growth

Jessica Farley[1], Kyne Krusic-Golub[2], Naomi Clear[1], Paige Eveson[1], Neville Smith[3], John Hampton[3]

[1] CSIRO Oceans and Atmosphere

[2] Fish Ageing Services Pty Ltd

[3] Pacific Community

Acknowledgements

We would like to thank Dan Fuller and Kurt Schaefer (IATTC) for collaborating on this project and hosting the technical meeting in July 2019. We particularly thank Dan for preparing and reading otoliths and for the amazing homemade hot sauce he gave us. We also thank the University of Tasmania (UTAS) for providing access to their scanning electron microscope facility, and Karsten Goemann for technical assistance in analysing the otoliths. We thank Allen Andrews (NOAA PIFSC) for useful discussions on bomb radiocarbon age validation methods. We thank Francois Roupsard his work in retrieving otoliths from the WCPFC Tuna Tissue Bank. We thank all the fisheries observers and port samplers involved in collecting the otoliths. Finally we thank Campbell Davies for his constructive comments on the report. This project was funded by the Western and Central Pacific Fisheries Commission and CSIRO Oceans and Atmosphere.

Contents

1 Executive summary 2

2 Introduction 3

3 Objectives 3

4 Results against objectives 4

4.1 Analysis of mark-recapture otoliths by CSIRO/FAS and IATTC 4

4.2 Comparison of daily ageing methods of WCPO bigeye and yellowfin from the western Pacific 6

4.3 Annual ageing of EPO yellowfin by FAS 9

4.4 Prepare and analyse 50 small bigeye otoliths. 11

4.5 Ageing methods workshops 11

5 Summary 13

6 References 14

Appendix A: Preparation of otoliths for SEM 15

Appendix B: Images of marked yellowfin and bigeye otoliths under SEM and light microscope.20

Appendix C: IATTC technical workshop agenda 28

1 Executive summary

This paper describes work undertaken by CSIRO, Fish Ageing Services (FAS) and the IATTC to assess and improve consistency in ageing methods using otoliths for bigeye and yellowfin. The objectives were to analyse otoliths from mark-recapture individuals of bigeye and yellowfin for age validation purposes; compare daily and annual age estimates from paired otoliths from the same fish; analyse otoliths from 50 very small bigeye from assessment area 7 using daily ageing methods; and participate in an inter-lab workshop to jointly read and examine otoliths and share ageing methods to improve skill and resolve differences in the approaches used.

The small technical workshop was scheduled to take place just prior to a larger IATTC workshop on age and growth of bigeye and yellowfin in the Pacific. However, the partial US Federal Government shutdown in late 2018-early 2019 prevented the required preparatory lab work being completed and access to the IATTC campus for the workshop. As a result the workshop was postponed until June 2019.

The main outcomes from the otolith analysis and subsequent discussion and microscope work during the workshop were:

- Preparation of otoliths for daily ageing is similar between IATTC and FAS; suggesting that this was not the cause of the higher estimates of daily age obtained by IATTC compared to FAS when reading otoliths form the same fish. The higher ages by IATTC is due to different interpretation methods through "problematic" areas of the otolith.
- There are differences in the micro-structure in bigeye and yellowfin otoliths from the western and eastern Pacific, which make counts of daily rings in otoliths of fish from the western Pacific more difficult to interpret.
- There were differences in age estimates from counting daily (IATTC) and annual (FAS) increments in sister otoliths from the same individuals. These differences were not able to be resolved in the workshop. They may only be resolved through large-scale direct age validation studies, such as mark-recapture experiments using chemical dyes to mark the otoliths.
- The results of available mark-recapture age validation studies provide initial evidence that daily growth increments are not a reliable source of age information for yellowfin >74 cm and bigeye > 82 cm in the western Pacific Ocean. It would be desirable to obtain additional mark-recapture samples to confirm this finding.
- The results of available mark-recapture age validation studies also provides initial evidence that counts of annual increments may be a reliable source of age information for yellowfin in the western Pacific Ocean. This is the first direct validation of annual ageing methods for yellowfin in the Pacific. It would be desirable to obtain additional mark-recapture samples to confirm this finding. The application of bomb radiocarbon methods to existing otolith collections may also provide an additional source of validation.

The workshop, while not long enough to fully discuss and resolve all aspects of our respective ageing methods, was very constructive and has provided a sound basis for further discussions and collaboration.

2 Introduction

Work has been conducted under several WCPFC projects to improve age and growth data used in stock assessments for yellowfin and bigeye tuna, with reports to SC13 and SC14 (Farley et al. (2017, 2018a, 2018b). In 2018, following a review of Project 81 (Update on age and growth of bigeye tuna in the WCPO: Farley et al. 2018a), and with consideration of a recommendation from the SPC Pre-Assessment Workshop (PAW), the Scientific Committee noted that the differences in ageing approaches between the WCPFC and the IATTC needed further investigation. The PAW recommendation that a workshop to be arranged to compare techniques and age estimates between otolith reading laboratories, to standardise the approaches used for counts of daily increment. It was proposed that, if possible, IATTC and FAS should read sister otoliths for daily counts, based upon otoliths marked with $SrCl_2$.

In late 2018, the WCPFC funded Project 94 with the intention to analyse otoliths marked with strontium chloride ($SrCl_2$) from bigeye and yellowfin in the WPO for daily and annual age validation, and to compare estimates of annual and daily age from sister otoliths of bigeye and yellowfin to resolve differences in ageing methods. The proposal was to then hold an inter-laboratory technical workshop in late January at IATTC to discuss and resolve ageing methods among readers. The results were then to be presented at an IATTC Workshop to Evaluate Bigeye and Yellowfin tuna ageing methodologies and growth models in the Pacific Ocean held on 23-25 January 2019 in La Jolla, California.

Unfortunately, the US Federal Government shutdown in late 2018 to early 2019 prevented the IATTC staff from preparing and analysing the otoliths from the WCPO. The technical workshop was, therefore, postponed and results could not be presented at the broader January IATTC workshop. However, annual increment counts of EPO otoliths were completed by FAS, which allowed for some comparisons of daily and annual increment counts to be presented and discussed (Anon 2019).

The preparatory laboratory work by IATTC was subsequently completed in March 2019 and the inter-laboratory technical workshop was held on 25-26 June 2019 at IATTC, La Jolla. A report of this workshop will be available on the IATTC website when finalised.

This paper describes the work undertaken by CSIRO, Fish Ageing Services (FAS) and IATTC prior to the technical workshop and a summary of the workshop outcomes to assess and improve consistency ageing methods using otoliths for bigeye and yellowfin.

3 Objectives

To further improve age estimates for bigeye and yellowfin tuna in the WCPO for use in stock assessments and related analyses through an inter-lab ageing workshop designed to specifically consider annual and daily ageing approaches for age estimation between WCPFC and IATTC.

The objectives of the project were to:

1. Prepare and analyse two $SrCl_2$ marked bigeye otoliths and two $SrCl_2$ marked yellowfin otoliths from the WCPO using the daily ageing method used by IATTC and the annual ageing methods used by FAS with the sister otoliths from the same fish.
2. Prepare and read three bigeye and three yellowfin tuna otoliths from the WCPO using the daily ageing method by IATTC and FAS to resolve differences in ageing methods (using sister otoliths from the same fish).
3. Prepare and read (annual age) sister otoliths from EPO yellowfin previously aged using daily ageing methods by IATTC.
4. Prepare and analyse 50 bigeye otoliths from small fish from region 7 using the daily increment method by FAS.
5. Participate in inter-lab ageing workshop and jointly examine WCPO and EPO otoliths and discuss and share ageing methods to improve skill and resolve differences in ageing methods.

4 Results against objectives

4.1 Analysis of mark-recapture otoliths by CSIRO/FAS and IATTC

Paired (left and right) otoliths were analysed from two bigeye and two yellowfin tuna tagged and released following injection with strontium chloride ($SrCl_2$) in the Coral Sea in the early 1990s, and recaptured 61 to 427 days later. The fish were tagged via SPC/CSIRO tagging programs. An additional pair of otoliths were analysed from a yellowfin tagged and injected with oxytetracycline (OTC) in the South Atlantic, and recaptured 375 days later. This fish was tagged through the ICCAT Atlantic Tropical tuna Tagging Program in the 2017. Given the sources of the release and recapture information for all $SrCl_2$-marked and OTC-marked fish, we are confident in the accuracy of the tagging and recapture data.

4.1.1 Daily age validation

Frontal sections, which included the primordium and the post rostral tip, were cut from each otolith. One otolith from each pair was prepared by CSIRO/FAS and the 'sister' otolith was prepared by IATTC. For the $SrCl_2$ marked otoliths, CSIRO used a scanning electron microscope (SEM) at the University of Tasmania to detect and locate the strontium mark in the otolith section, and obtain high resolution images. See Appendix A for a description of the otolith preparation methods used. The images were taken to accentuate the strontium mark in order to identify exactly where it was positioned on the otolith. For all otoliths, the $SrCl_2$ mark was clearly visible. For the OTC marked otolith, FAS used a light microscope with ultraviolet (UV) light to detect and locate the mark, obtain high resolution images and a measurement of the distance from the OTC mark to the otolith edge. IATTC used the SEM/UV images to locate the expected position of the $SrCl_2$/OTC mark on the sister otolith section, and counted the number of assumed daily increments from the location of the $SrCl_2$/OTC mark to the otolith tip. Two counts of increments were made for each otolith section and the mean was calculated. A comparison was made between the mean daily count and the known days at liberty (Table 1).

Yellowfin #1 was 74 cm at release and 77 cm at recapture, and was at liberty for 61 days. The mean increment count by IATTC was 35.5 days (difference of -41.8%) (Appendix Figure B1). Yellowfin #2 was 97 cm at release and an unknown length at recapture, and was at liberty for 261 days. The mean increment count by IATTC was 114.5 days (-54.1%) (Appendix Figure B2). Yellowfin #3 (from the eastern Atlantic Ocean) was 145 cm at release and 147 cm at recapture, and was at liberty for 375 days. The mean increment count by IATTC was 147.0 days (-60.8%) (Appendix Figure B3).

The OTC mark in yellowfin #3 was quite faint (Appendix Figure B3) and its position less certain in the sister otolith prepared by FAS. A microscope with UV burners was obtained for the technical meeting at IATTC (see section 4.5) and we were able to locate the OTC mark directly on the otolith section and make additional counts. The counts were ~70-79 days by IATTC and FAS, lower than IATTC obtained previously.

Bigeye #1 was 84 cm at release and 94 cm at recapture, and was at liberty for 245 days. The mean increment count was 185.0 days (-24.5%) (Appendix Figure B4). Bigeye #2 was 82 cm at release and 109 cm at recapture, and was at liberty for 427 days. The mean increment count was 287.5 days (-32.7%) (Appendix Figure B5).

The results provide initial evidence that daily growth increments are not a reliable source of age information for yellowfin >74 cm and bigeye > 82 cm in the western Pacific Ocean. Note that results for the Atlantic are less certain as only one otolith was examined (145 cm FL at release).

Additional SEM images of the frontal sections were taken to determine if accuracy increased when the growth increments after the strontium marks were accentuated. Increments were not visible in the two yellowfin otoliths (Appendix Figure B1 and B2) but were visible in the two bigeye otoliths (Appendix Figure B4 and B5). The reason for the difference in otolith structure is not known. The best image was for bigeye #1. The mean count of increments after the mark by an experienced reader at SPC was 212, and although this was still less than the known days at liberty (-13.5%), it suggests that a SEM may improve the accuracy of daily age estimation in otoliths.

Table 1. Results of blind micro-increment counts by IATTC on $SrCl_2$/OTC mark-recapture otoliths compared to known days at liberty.

Species	Ocean	Marked	Slide no.	Length at release / recapture (cm)	Days at liberty	Mean IATTC count (days)	% difference in age
YFT #1	Western Pacific	$SrCl_2$	1	74 / 77	61	35.5	-41.8
YFT #2	Western Pacific	$SrCl_2$	2	97 / NA	261	114.5	-56.1
YFT #3	Eastern Atlantic	OTC	83883	145 / 147	375	147.0	-60.8
BET #1	Western Pacific	$SrCl_2$	3	84 / 94	245	185.0	-24.5
BET #2	Western Pacific	$SrCl_2$	4	82 / 109	427	287.5	-32.7

4.1.2 Annual age validation

In addition to the daily age validation work, CSIRO also prepared a transverse section from each of the yellowfin otoliths to be used for annual age validation. As the otolith primordium was included in the frontal section for daily ageing, the transverse section did not include the primordium. This is acceptable as only the area after the $SrCl_2$ mark is required for purpose of validation. An image of each otolith section was obtained using light microscopy prior to the SEM imaging to locate the $SrCl_2$ mark. Yellowfin #1 was at liberty for only 61 days, so the subsequent growth was not enough to validate annual ageing (Appendix Figure B6). Yellowfin #2 was at liberty for 261 days and showed one opaque zone and one translucent zone after the $SrCl_2$ mark (Appendix Figure B7). Yellowfin tuna #3 was at liberty for 375 days and also showed one opaque zone and one translucent zone after the $SrCl_2$ mark (Appendix Figure B8). Although only two otoliths were suitable for analysis, the results are consistent with the counts obtained from the annual ageing method being equal to one year for yellowfin tuna.

4.2 Comparison of daily ageing methods of WCPO bigeye and yellowfin from the western Pacific

The aim of this component of the project was to examine and resolve differences in daily ageing methods between IATTC and FAS laboratories using sister otoliths from the same fish caught in the WPO.

The first comparison was between daily age estimates by IATTC and FAS from the same frontal section of three bigeye otoliths. The sections were prepared and read by FAS and then subsequently read by IATTC. The otoliths were from fish 39 cm, 107 cm and 119 cm fork length (Table 2). IATTC estimates of daily ages were higher than FAS estimates for both species. Given the results of the daily age validation (section 4.1.1), daily counts by IATTC and FAS for the two largest fish are likely to underestimate true age.

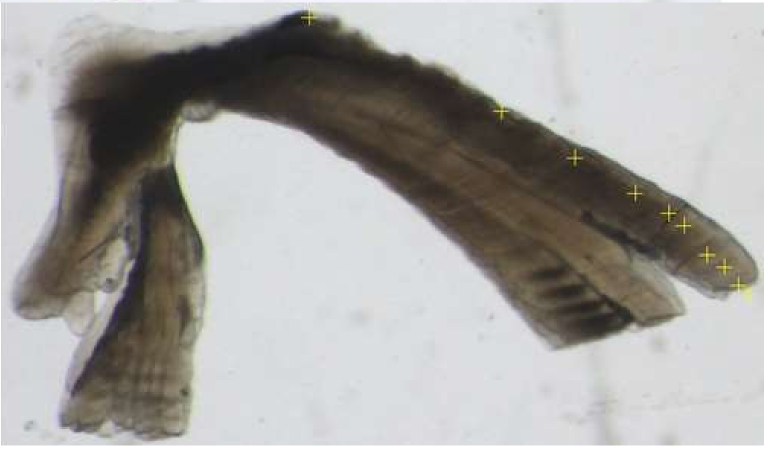
The second comparison was between estimates of daily age by IATTC and annual age estimates by FAS for three bigeye and three yellowfin using sister otoliths from the same fish. IATTC sectioned (frontal) and read one otolith from each pair (using frontal sections) and FAS sectioned and read the sister otoliths (using transverse sections). The otoliths were from bigeye 107 cm, 119 cm and 150 cm in fork length, while the yellowfin were 112 cm, 142 cm and 150 cm in fork length (Table 2). FAS annual ages were higher than IATTC daily ages for fish older than 2-3 years (Table 2). Figure 1 and Figure 2 show examples of yellowfin and bigeye otoliths prepared for annual ageing. The annual increments counted by FAS are marked, and IATTC and FAS ages indicated. Again, given the daily age validation work above, daily counts are likely to underestimate true age. The transverse sections of the largest bigeye and yellowfin show clear alternating opaque and translucent zones indicative of annuli. The size and weight of these otoliths are substantially larger than the otoliths from the smaller fish, providing further evidence that the daily ages underestimate true age of these fish. Further work is needed to compare ageing methods and better understand the basis for the difference in age estimates.

Table 2. Comparison of estimates of daily age by IATTC and annual age by FAS for yellowfin and bigeye tuna from the western Pacific. Estimates of annual age for yellowfin by FAS are shown as increment counts, not decimal ages. The annual age estimate for B14504 had a readability of only 1 (low).

Species	Fish number	Fork length (cm)	IATTC age using daily counts (y)	FAS age using daily counts (y)	FAS age using annual counts (y)
BET	BET3933	39	0.62	0.39	NA
BET	B12427	107	2.24	1.52	2.57
BET	A1422	119	2.55	1.68	3.64
BET	B9376	150	3.18	NA	8.12
YFT	B14504	112	1.95	NA	3
YFT	B5080	142	2.18	NA	6
YFT	B15371	150	2.87	NA	8

BET FAS_ID 302_014_0942 SampleID B12427
Fork length 107 cm
Otolith weight 0.0461 g
IATTC daily age: 2.24
FAS annual age: 2.57

BET FAS_ID 302_014_0907 SampleID A1422
Fork length 119 cm
Otolith weight 0.0565 g
IATTC daily age: 2.55
FAS annual age: 3.64



BET FAS_ID 302_014_0528 SampleID B9376
Fork length 150 cm
Otolith weight 0.0916 g
IATTC daily age: 3.18
FAS annual age: 8.12)

Figure 1. Transverse section of three bigeye otoliths from the WPO viewed under transmitted light. The yellow +'s mark the opaque zones counted in the otoliths. Fish length, otolith weight and estimated age from annual counts by FAS and daily counts on the sister otoliths by IATTC are given for each otolith. Images are taken at the same scale.

YFT FAS_ID 305_005_014 SampleID B14504
Fork length 112 cm
Otolith weight 0.0397 g
IATTC daily age: 1.95
FAS annual age: Maybe 3 (low confidence)

YFT FAS_ID 305_005_028 SampleID B5080
Fork length 142 cm
Otolith weight 0.0804 g
IATTC daily age: 2.17
FAS annual age: 6 WT (or 7)

YFT FAS_ID 305_005_021 SampleID B15371
Fork length 150 cm
Otolith weight 0.0990 g
IATTC daily age: 2.87
FAS annual age: 8 WT

Figure 2. Transverse section of three yellowfin otoliths from the WPO viewed under transmitted light. The yellow +'s mark the opaque zones counted in the otoliths. Fish length, otolith weight and estimated age from annual counts by FAS and daily counts on the sister otoliths by IATTC are given for each otolith. Images are taken at the same scale.

4.3 Annual ageing of EPO yellowfin by FAS

Annual ageing of EPO yellowfin tuna by FAS was undertaken to compare to estimates of length at age derived from daily increment counts on sister otoliths by IATTC. Similar work has already been completed for bigeye in the EPO (Anon 2019).

A total of 67 sagittal otoliths were selected from samples in which the other otolith had been aged by IATTC using counts of daily increments. The otoliths were selected from fish ranging in size from 80-157 cm FL and all were female. Of the 67 samples sent by IATTC, only 66 were received as one otolith was lost in transit. All samples were caught between Jan 2009 and Nov 2012 from an area between 6-16°N and 92-140°W. Information on fish sizes and IATTC daily ages was not provided at this time. Therefore, subsequent preparation and analysis was conducted “blind”.

The otolith samples were prepared and aged following the methods used for the trial batch of WPO yellowfin (Farley et al. 2018b). Transverse sections were prepared from each otolith following the methods outlined in Farley et al. (2017). Otoliths were embedded in clear casting polyester resin and four or five serial transverse sections approximately 280-300µm in thickness were cut from each otolith (around the primordium). The otolith sections were set on glass microscope slides (50x75mm) in further resin and covered with 2 coverslips (25x50mm). This method negates the need for any polishing after the sections have been cut. The otolith sections were read at 25x magnification illuminated with transmitted light.

As the annual ageing method is still in development for yellowfin, there is no defined protocol or reference set to use. The interpretation used in the ageing of the first trial batch of 40 WPO yellowfin was based on the readers’ experience with reading otoliths of other tunas, including the validated methods developed to age WPO bigeye (i.e., Farley et al. 2017, 2018a).

To ensure that the interpretation used in ageing the EPO samples was consistent with how the trial set of WPO yellowfin were interpreted, the images from that set were viewed several times before any attempt was made to age the EPO samples. Opaque zones were counted on the ventral arm and the distances between each opaque zone was measured starting from the first inflection point. The otolith margin was classified either as Narrow Translucent (NT), Wide Translucent (WT) or Opaque (O) to allow IATTC to convert zone counts to decimal age estimates based on an algorithm developed for bigeye (Farley et al. 2017; 2018a). Using algorithms developed for other species and regions is not ideal, however, in the absence of any other data it was considered better than comparing daily counts to unadjusted age estimates. The comparison should be redone once the annual ageing protocol and age conversion algorithm for yellowfin has been properly developed.

The results of the comparisons of daily and annual age estimates for yellowfin from the EPO (and bigeye) were presented at the IATTC Workshop to Evaluate Bigeye and Yellowfin tuna ageing methodologies and growth models in the Pacific Ocean, January 2019 (Anon 2019). For both species for fish >~120-130 cm FL, higher age estimates were generally (but not always) obtained from the annual ageing method compared to the daily ageing method. Interestingly, yellowfin of similar sizes could be quite different ages based on the annual ageing method. For example 120-130 cm fish were as young as 1.8 years (otolith weight 0.0354 g) or as old as 5.2 years (otolith weight 0.0686 g). The daily ageing method estimated the age of these fish as 2.7 and 2.5 years, respectively.

Figure 3 shows examples of otoliths prepared for annual ageing of yellowfin from the EPO; the annual increments counted by FAS are marked, and IATTC and FAS ages are indicated. As noted above, FAS estimates of annual ages were higher than IATTC estimates of daily ages for fish larger than 120-130 cm (2-3 years). Further work is needs to resolve the factors underlying these differences.

YFT FAS_ID 305_007_009 SampleID 895
Fork length 106 cm
Otolith weight 0.0404 g
IATTC daily age: 2.06
FAS annual age: 2 NT

YFT FAS_ID 305_007_031 SampleID 1234
Fork length 136 cm
Otolith weight 0.0553 g
IATTC daily age: 3.03
FAS annual age: 3 WT

YFT FAS_ID 305_007_034 SampleID 1270
Fork length 150 cm
Otolith weight 0.0912 g
IATTC daily age: 3.67
FAS annual age: 6 NT (or 5O)

Figure 3. Transverse section of three yellow otoliths from the EPO viewed under transmitted light. The yellow +'s mark the opaque zones counted in the otoliths. Fish length, otolith weight and age estimated from annual counts by FAS and daily counts on the sister otoliths by IATTC are given for each otolith. Images are taken at the same scale.

4.4 Prepare and analyse 50 small bigeye otoliths.

Otoliths for this component have not been collected yet (area 7) but it is anticipated that they will be available and will be analysed by the completion of the project.

4.5 Ageing methods workshops

Two workshop were held at IATTC that relate to ageing methodologies for bigeye and yellowfin tuna in the Pacific. These workshops are discussed below.

1) IATTC Workshop to Evaluate Bigeye and Yellowfin tuna ageing methodologies and growth models in the Pacific Ocean, La Jolla, 23-25 January 2019 (Anon, 2019).

One of the aims of this workshop was to evaluate differences in methods for age estimation (daily and annual) for bigeye and yellowfin in the eastern and western Pacific. It was anticipated that the results of the technical workshop (see #2 below) would be presented and discussed, but that was not possible because the technical meeting was postponed due to the US Federal Government shutdown. However, as noted in section 4.3, comparisons of daily and annual ages of yellowfin and bigeye were presented at this broader workshop.

They key recommendations of the workshop regarding ageing methodologies were to (Anon 2019):

a. Hold a technical workshop to compare methodologies, and exchange additional otoliths from the EPO and WCPO, as soon as possible.
b. Include the following elements in the work plan:
    - Improve and document the protocols for daily and annual ageing.
    - Conduct spatial analyses based on otolith weight, using all available otoliths
    - Extend the validation of daily and annual otolith counts across the Pacific by incorporating some oxytetracycline (OTC) marking in tagging programs.
    - Extend the spatial/temporal/size/sex distribution of EPO daily increment otolith data.

2) Inter-lab ageing workshop, IATTC La Jolla, 25-26 June 2019.

The (postponed) two-day inter-laboratory technical workshop was held in June at IATTC, La Jolla. The agenda of the workshop and participants are given in Appendix C. A report of this workshop is being prepared by the chair (Kurt Schaefer) and it will be available on the IATTC website when finalised.

The workshop was not long enough to fully investigate and discuss all aspects of our respective ageing methods. These discussions and collaboration are ongoing. However, important similarities/differences in ageing methods were found through practical sessions (microscope work) and discussions.

One such outcome was that we determined that the otolith preparation method for estimating daily age is relatively consistent between FAS and IATTC laboratories, and hence was considered unlikely to be the cause of the different age estimates from daily counts (see section 4.2). It was determined that higher daily age estimates obtained by IATTC are likely the result of the different interpretations method used. For example, in an area of the otolith where daily increments are missing or overlapping, the number increments is interpolated based on the density of increment before and after the area in question. A similar approach was taken by Sardenne et al (2015) for yellowfin and bigeye in the Indian Ocean. They describe the method and note that interpretation of increment patterns is subjective and that in areas with illegible sub-sections, the counts were interpolated from surrounding sub-sections. This is based on the increment density in the proceeding/subsequent legible section and the width of the illegible section. It was suggested that the use of acid etching should help the daily ageing process for WPO otoliths and FAS agreed to use this method in subsequent daily ageing work.

The workshop also recognised that the micro-structure otoliths from the WPO is more difficult to interpret compared to EPO otoliths. There were more areas of overlapping or irregular increments, making interpretation difficult. Some of the counting paths were difficult due to the presence of nodules, and increments were not always visible. Dr. Alex Wild also found yellowfin otoliths from the WPO difficult to interpret. He concluded “that yellowfin otoliths from the western Pacific were much more difficult to interpret and showed greater variability in increment spacing than those from the eastern Pacific” (Lehodey and Leroy, 1999). These difficulties may explain, to some extent, why the daily ageing method was not validated for bigeye and yellowfin in the WPO for the otoliths examined.

IATTC also kindly agreed to provide otolith samples from the EPO, which may help with the first three zone verification process for yellowfin (see WCPFC SC15-SA-IP-03).

The underlying cause of the differences in age estimates from counting daily and annual increments from the same fish were not resolved, and may only be resolved through further direct age validation studies of daily and annual deposition rates for both species across the Pacific.

5 Summary

Bigeye and yellowfin tuna otoliths from both the WPO and EPO were analysed in this project, and the results discussed at a dedicated technical workshop in June 2019 with the aim of improving consistency in ageing methods between IATTC and FAS/CSIRO methods. Important similarities/differences were found in ageing methods through practical sessions (microscope work) and discussions. Preparation of otoliths for daily ageing is similar between labs, however, the interpretation of the otolith in “problematic” areas differs. It was also clear that the micro-structure of otoliths from the WPO is more difficult to interpret for counts of daily rings compared to EPO otoliths. The basis for differences in age estimates from counting daily (IATTC) and annual (FAS) increments in sister otoliths from the same fish were not resolved. Increasing the number and size/age representation of chemically marked otoliths through large-scale direct age validation studies as part of the regular mark-recapture experiments would provide a the basis for resolving the underlying mechanisms and providing consistency of methods and length at age estimates.

Although very few otoliths were available for analysis for this study, some validation work was undertaken in the project using SrC_2l/OTC marked otoliths available from previous mark-recapture studies. The results provide preliminary evidence that daily growth increments are not a reliable source of age information for yellowfin >74 cm and bigeye > 82 cm in the western Pacific Ocean. In the case of annual counts, the available evidence suggests that counts of annual increments in yellowfin otoliths may be a reliable source of age information for yellowfin in the western Pacific Ocean. This is the first direct validation of annual ageing methods for yellowfin in the Pacific. Recent bomb radiochemical work in the Atlantic has provided validation of annual ageing methods for bigeye and yellowfin in the Gulf of Mexico (Andrews et al. 2019) and may be a useful approach for validating annual ageing of bigeye and yellowfin in the Pacific. Further direct age validation studies for bigeye and yellowfin daily and annual ageing methods, spanning the entire size range and expected range of longevity, are urgently needed in the Pacific.

6 References

Andrews AH, Pacicco A, Allman R, Falterman BJ, Lang ET, Golet W. (2019). Validated longevity of yellowfin (*Thunnus albacares*) and bigeye (*T. obesus*) tuna of the northwestern Atlantic Ocean. (abstract). Proceedings of the 70th Annual Tuna Conference, May 20-23, Lake Arrowhead, California.

Anon (2019). IATTC Workshop to Evaluate Bigeye and Yellowfin tuna ageing methodologies and growth models in the Pacific Ocean. 25-26 June, La Jolla California.

Clear N, Davis T, Carter T (2000) Developing techniques to estimate the age of bigeye and broadbill swordfish off eastern Australia: a pilot study. Final report for Fisheries Research Development Corporation project 98/113, Canberra, Australia.

Farley, J., Clear, N., Leroy, B., Davis, T., and McPherson, G. 2003. Age and growth of bigeye tuna (*Thunnus obesus*) from the eastern and western AFZ. Final report No. 2000/100 for the Fisheries Research and Development Corporation, Australia

Farley J, Eveson P, Krusic-Golub K, Clear N, Sanchez C, Roupsard F, Satoh K, Smith N, Hampton J. (2018a). Update of bigeye age and growth in the WCPO. WCPFC Project 81. WCPFC-SC14-2018/SA-WP-01, Busan, Republic of Korea, 8-16 August.

Farley J, Eveson P, Krusic-Golub K, Sanchez C, Roupsard F, McKechnie S, Nicol S, Leroy B, Smith N, Chang S-K (2017). Project 35: Age, growth and maturity of bigeye tuna in the western and central Pacific Ocean. WCPFC-SC13-2017/ SA-WP-01, Rarotonga, Cook Islands, 9–17 August 2017.

Farley J, Krusic-Golub K, Clear N, Eveson P, Smith N. (2018b). Progress on yellowfin tuna age and growth in the WCPO. WCPFC Project 82. WCPFC-SC14-2018/SA-WP-13, Busan, Republic of Korea, 8-16 August.

Lehodey P, Leroy B (1999) Age and growth of yellowfin tuna (*Thunnus albacares*) from the western and central Pacific Ocean as indicated by daily growth increments and tagging data. Working paper YFT-2, 12th meeting of the Standing Committee on Tuna and Billfish, Tahiti.

Sardenne F, Dortel E, Le Croizier G, Million J, Labonne M, Leroy B, Bodin N, Chassot E (2015) Determining the age of tropical tunas in the Indian Ocean from otolith microstructures. Fish Res 163:44–57.

Schaefer KM, Fuller D (2006). Estimates of age and growth of bigeye tuna (*Thunnus obesus*) in the eastern Pacific Ocean, based on otolith increments and tagging data. Inter-Am Trop Tuna Comm Bull 23:1-31

Appendix A: Preparation of otoliths for SEM

A new method for sectioning otoliths was developed for this study to enable age estimates to be made along two axes of the same otolith. Sectioning of tuna otoliths is normally along one of two axes:

1. a frontal or longitudinal section is produced by cutting a section that includes the primordium and postrostrum (Figure A1), or:
2. a transverse section is produced by cutting a vertical section that includes the primordium and the ventral margin (Figure A2).

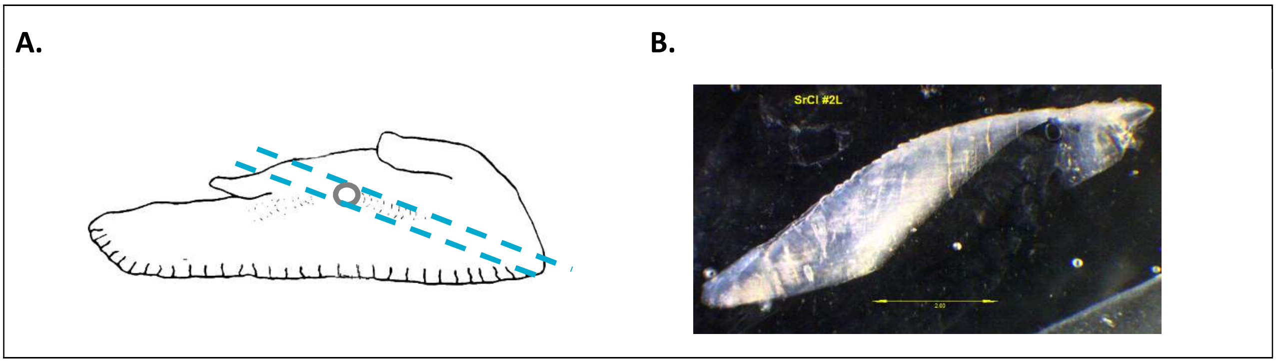


Figure A1. (A) Diagram of a frontal or longitudinal section that contains the primordium and post rostrum. (B) Light microscope view of the resulting frontal section, using reflected light.

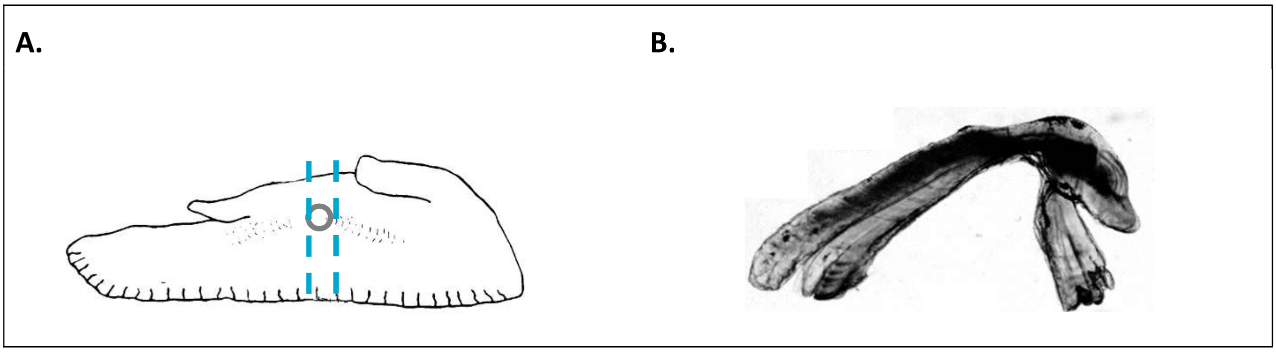


Figure A2. (A) Diagram of a transverse section that contains the primordium and ventral margin. (B) Light microscope view of the resulting transverse section, using transmitted light.

Before sectioning and analysis in the SEM, otoliths were embedded in epoxy resin — EpoFix resin and hardener— and left to harden for a minimum of 24 hours. The resulting resin blocks containing the specimens were sectioned on an Accutom rotary saw with Buhler diamond-edged blades to produce sections that were approximately 0.8 mm thick. During cutting, Milli-Q water was used as a coolant and run across the blade and specimen (Figure A3).

The resulting sections were ground down on one side by hand to expose the growth axis using two progressively-finer grades of silicon carbide wet-and-dry paper (1000 and 2400 grit) that were lubricated with Milli-Q water. The sections were then turned over and adhered permanently to glass slides using resin. The grinding was repeated on the other side of the section using the two grades of wet-and-dry paper; then polishing was done with 5 µm aluminium oxide lapping film until the primordium was at the surface. To achieve this, we used a compound microscope fitted with both transmitted and incident lighting, which allowed focussing both on the surface and within the section and hence we could determine how far the primordium was below the surface of the section. During the polishing stage of preparation, the section was checked regularly under the microscope until the correct depth was reached. After each stage of grinding and polishing the mounts were cleaned ultrasonically for 3 minutes: one minute in each of 3 beakers of Milli-Q water.

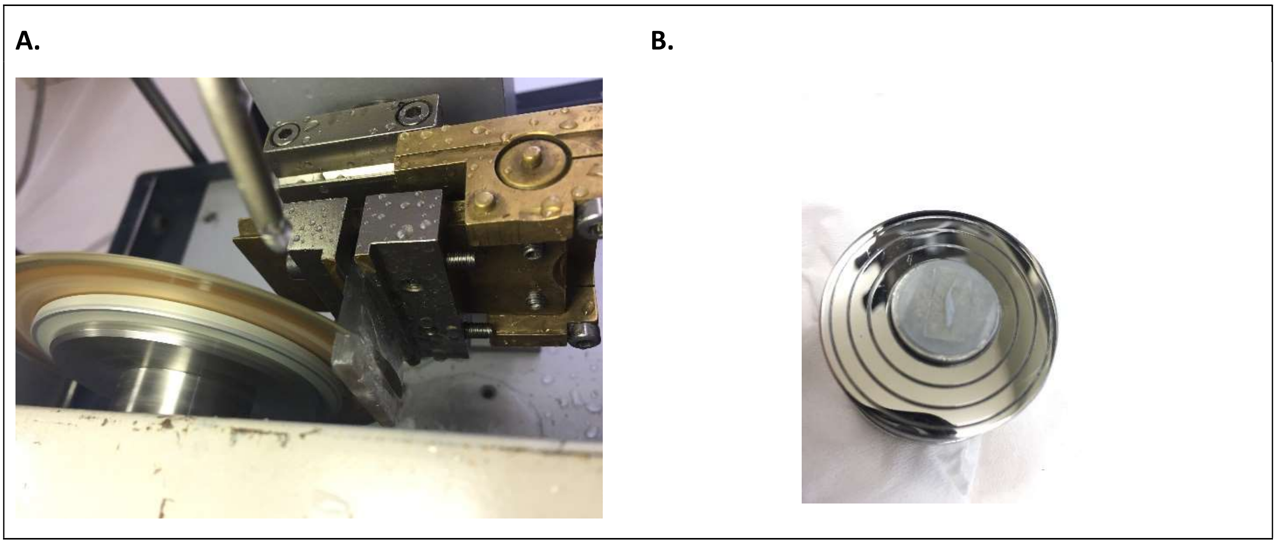


Figure A3. (A) The resin blocks containing otoliths were mounted in the saw jig at the correct angle to produce frontal or transverse sections. (B) The resulting sections were held in a metal round for grinding and polishing down to the primordium.

To compare daily and annual age estimates from the same otoliths, a frontal section was cut from the primordium using the normal sectioning method. The remaining block was removed from the saw jig, rotated and then a cut was taken in the transverse plane (Figure A4). This transverse section did not include the primordium but did contain the area of interest: the strontium mark and the growth beyond the mark out to the otolith edge, where the most recently-deposited otolith material is located.

CSIRO prepared all otoliths that underwent SEM analysis and the sister otoliths were prepared for ageing by IATTC (see Tables A1 and A2). Both labs prepared one sister from the OTC-marked otoliths (see section 4 above).

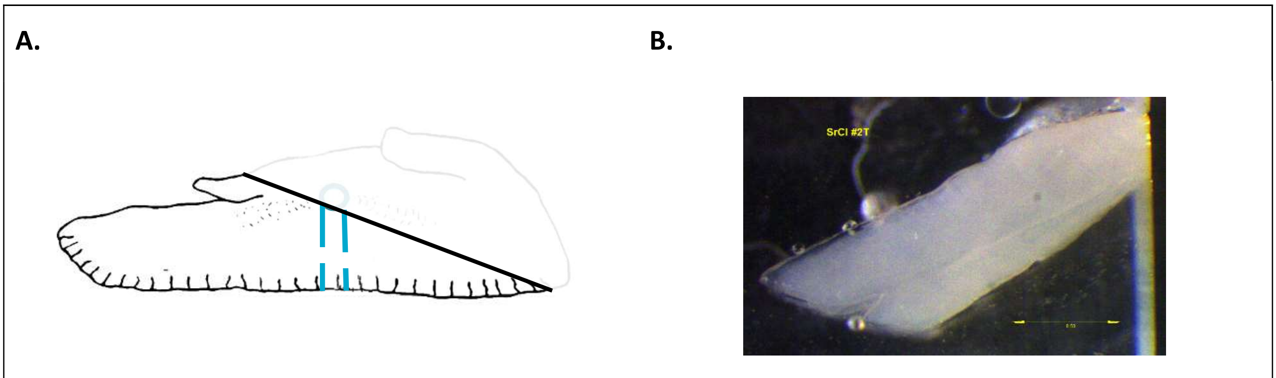


Figure A4. (A) A diagram showing the position and angle of the second, transverse section that was cut to produce (B), the resulting section that did not contain the primordium.

Table A1. Yellowfin otoliths prepared for aging. Indicated for each sister otolith are: the lab at which the otolith was prepared, the marking agent and the axes along which the otolith was sectioned.

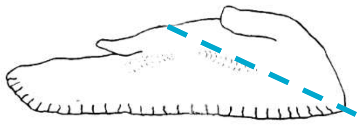
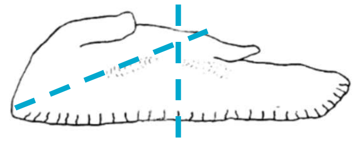
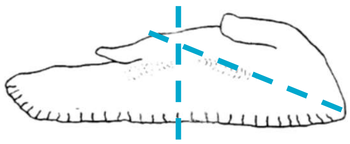
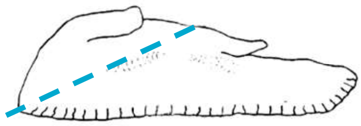
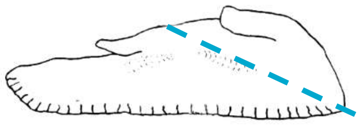
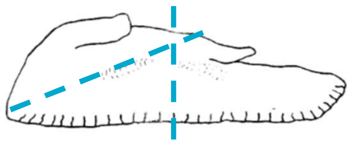
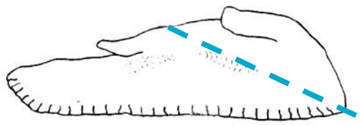
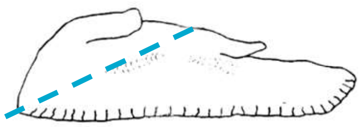
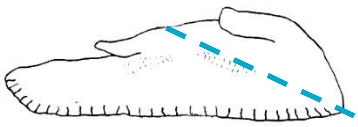
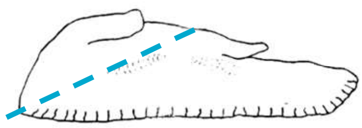
OTC	**YFT 83883 left IATTC**  frontal/longitudinal section	**YFT 83883 right CSIRO**  frontal/longitudinal and transverse sections
SrCl	**otolith number 1 left CSIRO**  frontal/longitudinal and transverse sections	**otolith number 1 right IATTC**  frontal/longitudinal section
SrCl	**otolith number 2 left IATTC**  frontal/longitudinal section	**otolith number 1 right CSIRO**  frontal/longitudinal and transverse sections

Table A2. Bigeye otoliths prepared for aging. Indicated for each sister otolith are: the lab at which the otolith was prepared, the marking agent and the axes along which the otolith was sectioned.

SrCl	**otolith number 3 left IATTC**  frontal/longitudinal section	**otolith number 3 right CSIRO**  frontal/longitudinal section
SrCl	**otolith number 4 left IATTC**  frontal/longitudinal section	**otolith number 4 right CSIRO**  frontal/longitudinal section

Prior to analysis in the SEM, the otolith sections were coated with 20nm of carbon using a Ladd 40000 vacuum evaporator. Analysis took place at the University of Tasmania, using a Hitachi SU-70 field emission SEM, coupled with a backscattered electron detector, a Hitachi photo-diode solid state BSE detector (Hitachi calls it PDBSE), with an accelerating voltage of 20kV, and a beam current of 3nA. The back-scatter detector coupled to the SEM visualized the strontium-rich bands in the otoliths, which appeared as bright bands across the otoliths, due to differences in atomic weight between the strontium and calcium (Figure A5). Confirmation that the bright band was, in fact, strontium-rich, came from energy-dispersive x-ray spectroscopy (EDS), which was used to scan across the area of the strontium mark. The EDS spectra were acquired using an Oxford AZtec 3.3 microanalysis system with an Oxford XMax 80 silicon drift detector (SDD). The EDS spectra indicated strontium levels are enhanced compared to background levels immediately before the bright band and calcium levels are correspondingly reduced in the area of the bright band (Figure A6).

The distance between the strontium mark and the edge of the otolith was measured in the SEM and then used to identify the position of the Sr mark under light microscope for that otolith and its sister otolith. The observed number of increments (daily and annual) after the position of the strontium mark was compared with the expected number, calculated from the known time-at-liberty after tagging (see section 4).

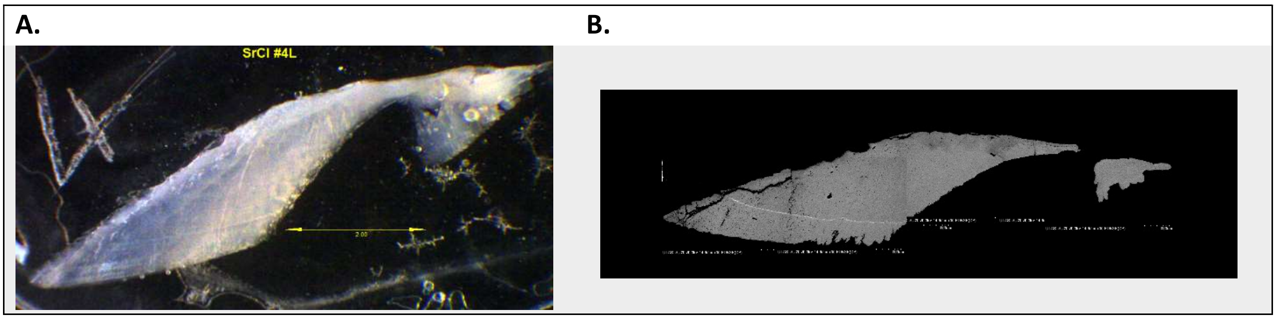


Figure A5. (A) Otolith section under light microscope and (B) SEM micrograph of the same section.

A.

B.

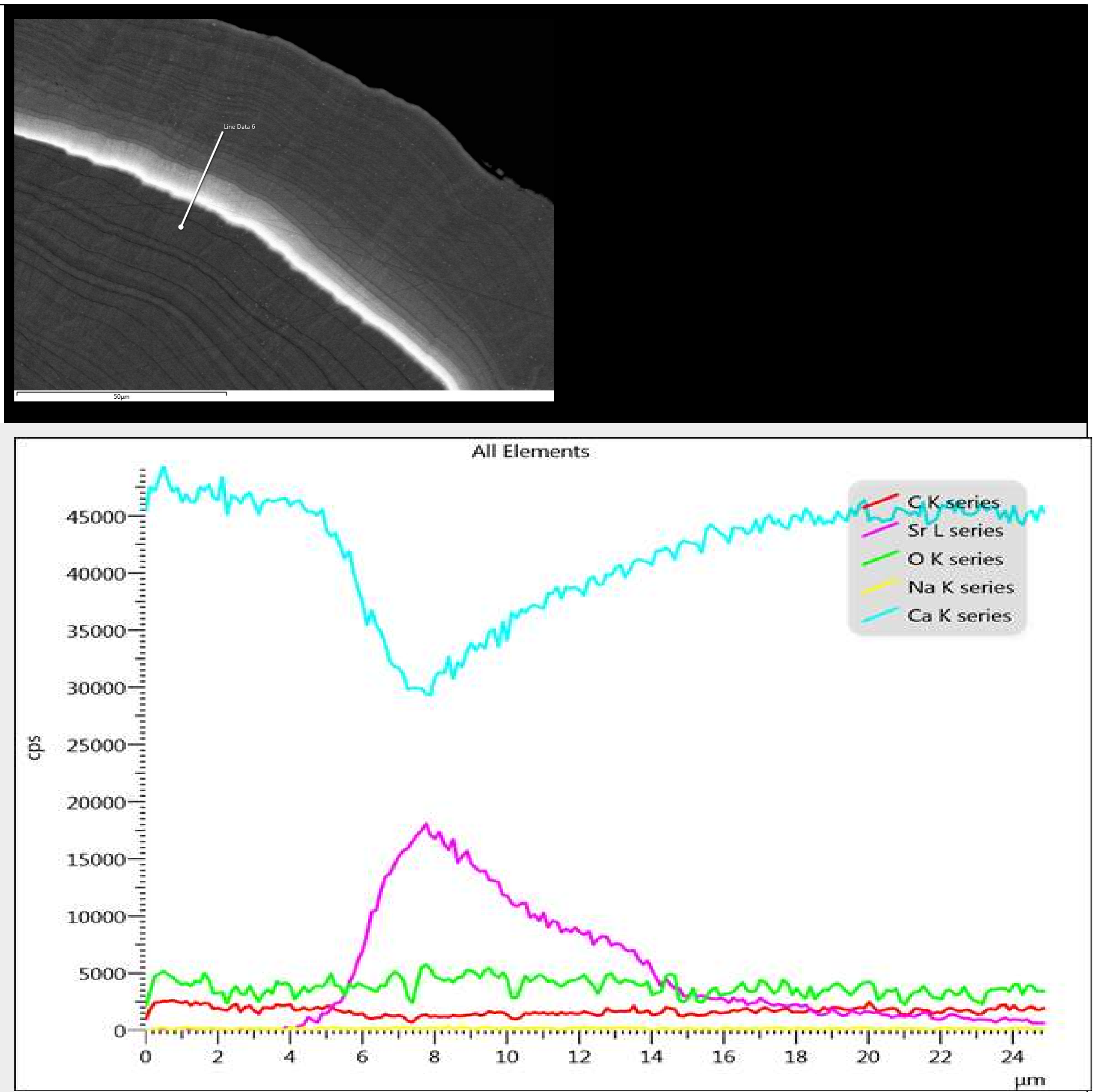


Figure A6. (A) EDS line scan across the bright band in the SEM and (B) elemental levels from the EDS scan across the bright band confirmed the presence of elevated levels of Sr.

Appendix B: Images of marked yellowfin and bigeye otoliths under SEM and light microscope.

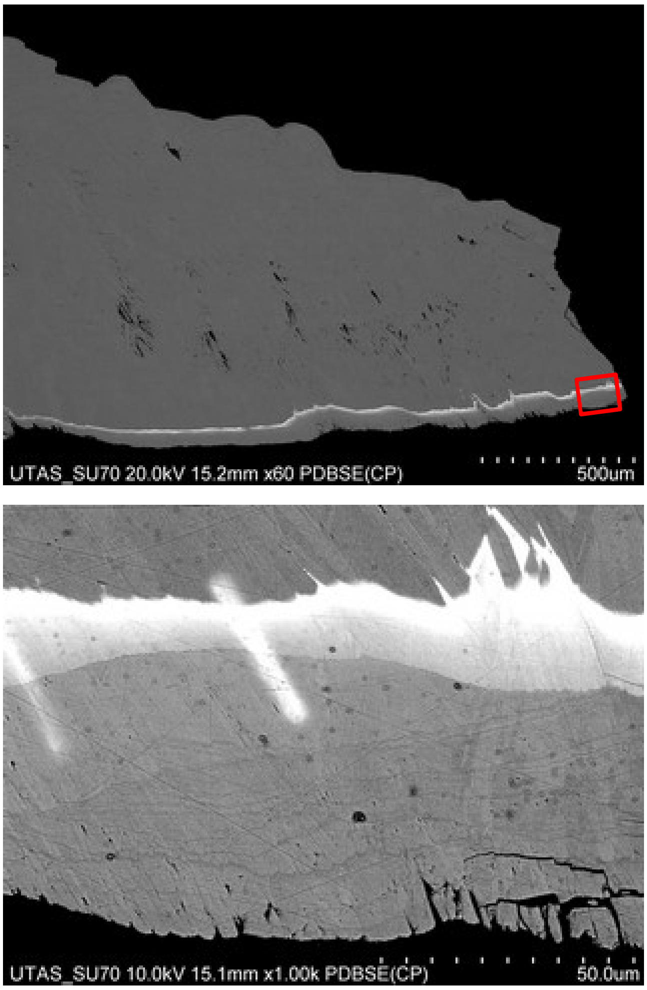
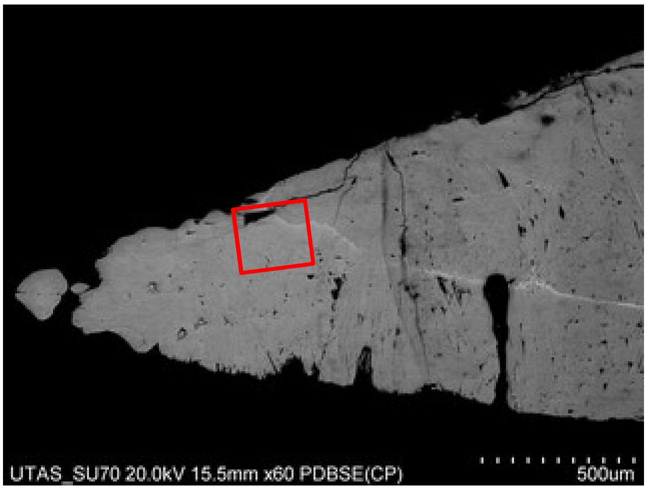


Figure B1. Top: frontal section (post rostral tip) of YFT #1 otolith marked with SrCl (white line) for daily age validation. The fish was at liberty after tagging/marking for 61 days and the mean count of daily increments was 35.5 after the mark. Bottom: Enlarged image of otolith edge (red box) accentuated under the SEM to try to detect the growth increments.



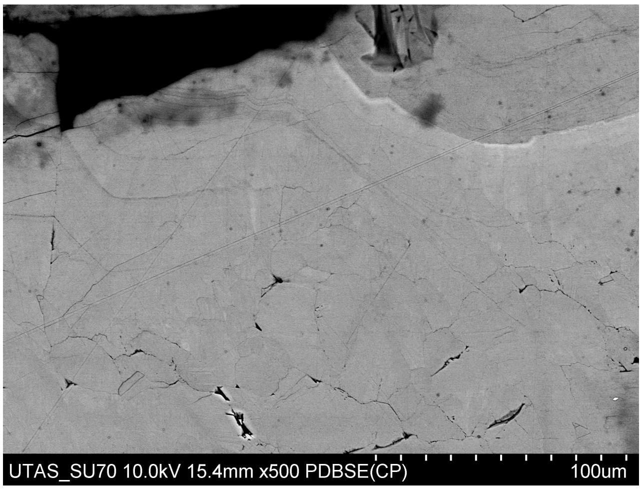


Figure B2. Top: frontal section (post rostral tip) of YFT #2 otolith marked with SrCl (white line) for daily age validation. The fish was at liberty after tagging/marking for 261 days and the mean count of daily increments was 114.5 after the mark. Bottom: enlarged image of part of the otolith (red box) accentuated under the SEM to try to detect the growth increments.

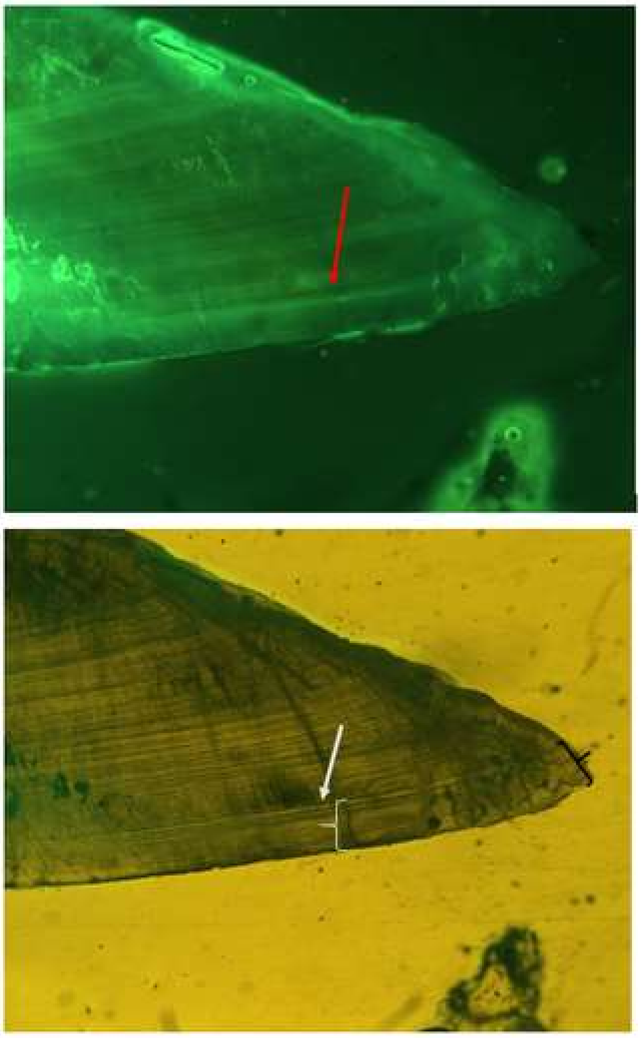


Figure B3. Frontal section (post rostral tip) of YFT #3 otolith marked with OTC for daily age validation. The location of the mark is visible under UV (top image) and is indicated in the image under light microscopy (bottom image). The arrow marks the OTC mark. The fish was at liberty after tagging/marking for 375 days and the mean count of daily increments was 147 after the mark.

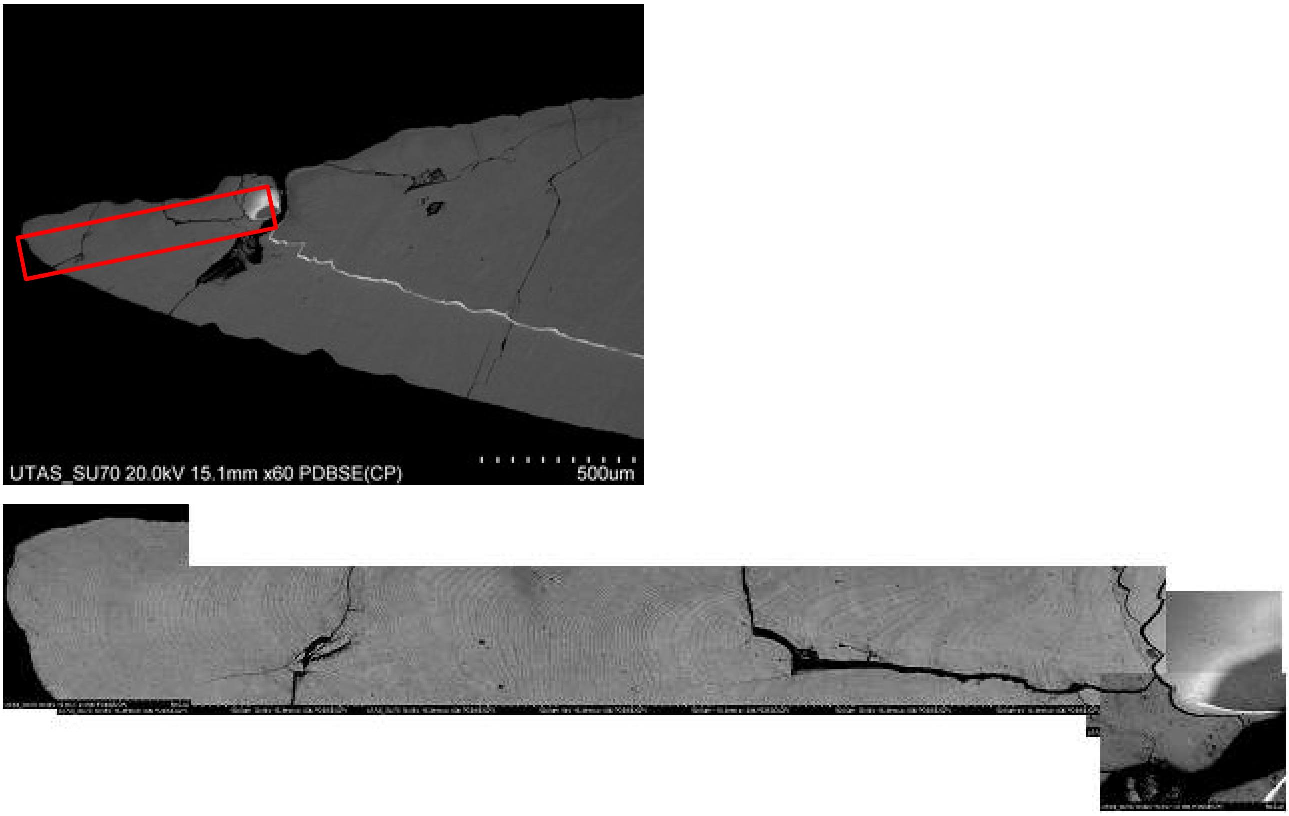


Figure B4. Top: frontal section (post rostral tip) of BET #1 otolith marked with SrCl (white line) for daily age validation. The fish was at liberty after tagging/marking for 245 days and the mean count of daily increments was 185 after the mark. Bottom: enlarged image of part of the otolith (red box) accentuated under the SEM to try to detect the growth increments.

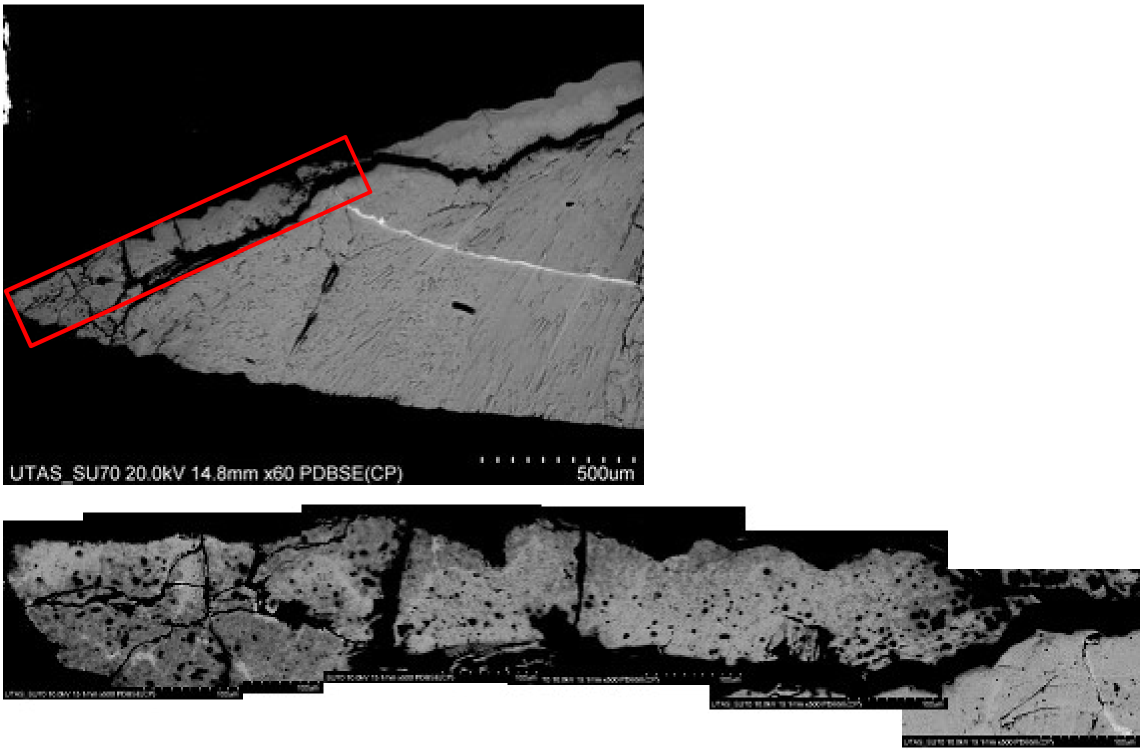


Figure B5. Top: frontal section (post rostral tip) of BET #2 otolith marked with SrCl (white line) for daily age validation. The fish was at liberty after tagging/marking for 427 days and the mean count of daily increments was 287.5 after the mark. Bottom: enlarged image of part of the otolith (red box) accentuated under the SEM to try to detect the growth increments.

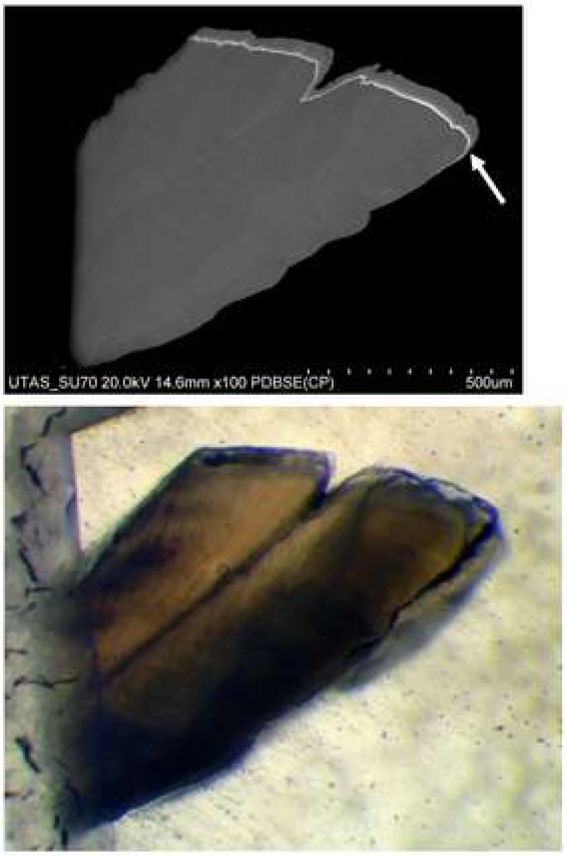


Figure B6. Transverse section of YFT #1 otolith marked with SrCl for annual age validation. The location of the mark is visible under SEM (top image). The arrow marks the SrCl mark. The fish was at liberty after tagging/marking for only 61 days so the subsequent growth was not enough to validate annual ageing.

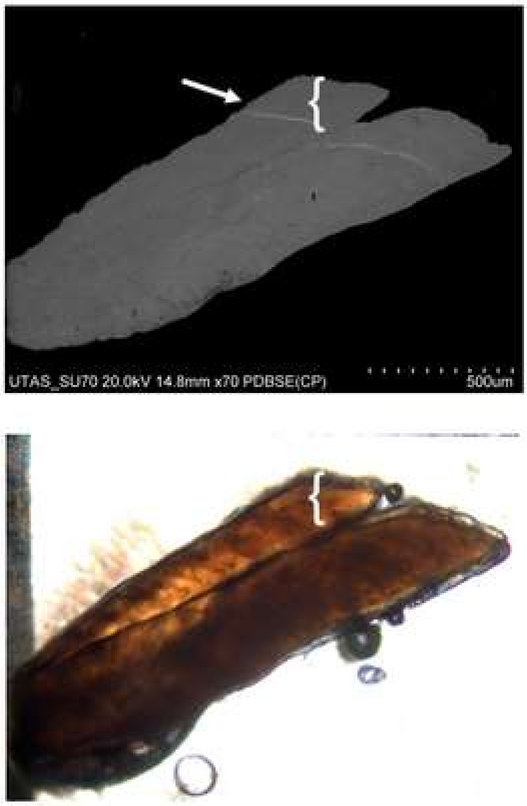


Figure B7. Transverse section of YFT #2 otolith injected with SrCl for annual age validation. Top image: the location of the SrCl mark is visible under SEM (arrow). Bottom image: The location of the SrCl mark is indicated (bracket) under light microscopy. The fish was at liberty for 261 days after tagging/marking and one opaque zone and the start of a translucent zone are visible after the SrCl mark.

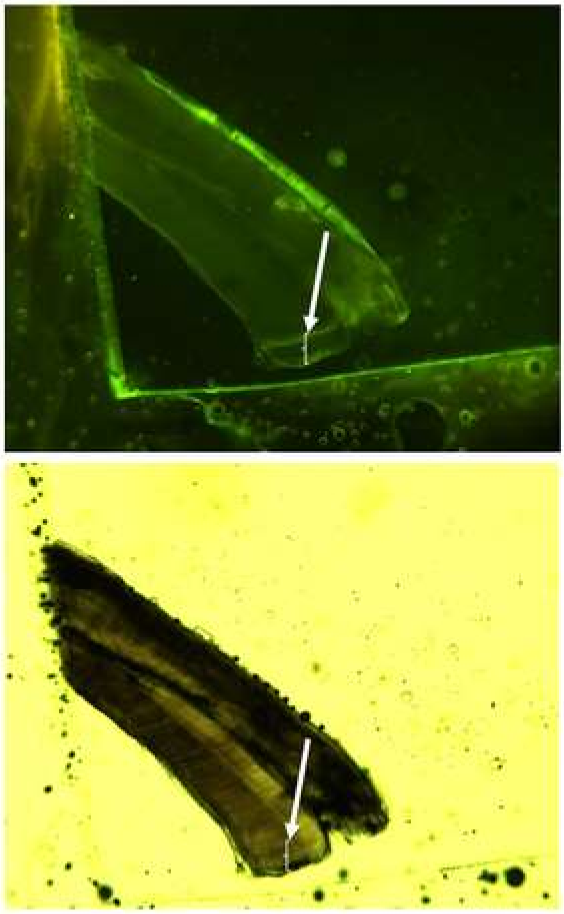


Figure B8. Transverse section of YFT #3 otolith injected with OTC for annual age validation. Top image: the location of the OTC mark is visible under UV light (arrow). Bottom image: the location of the OTC mark is shown (arrow) under light microscopy. The fish was at liberty for 375 days after tagging/marking and one opaque zone and one translucent zone is visible after the mark. The OTC mark is 111 µm in from edge.

Appendix C: IATTC technical workshop agenda

Methodologies for estimating age of bigeye and yellowfin tunas from otoliths

June 25-26, 2019, LA JOLLA, CALIFORNIA, USA

Proposed Agenda

Tuesday, June 25, 2019

0900 – 0930	**Introduction**
0930 – 1030	Fuller: Otolith preparation (embedding, sectioning, polishing) for estimating daily age in frontal sections of bigeye and yellowfin tuna
1030 – 1200	Fuller: Microscope time – View OTC marked otoliths to describe what constitutes an increment in bigeye tuna otolith frontal sections and discuss increment deposition rates.
1200 – 1300	**Lunch**
1300 – 1500	Fuller: Microscope time – Estimating age from daily increments on an otolith frontal section.
1500 – 1530	**Coffee Break**
1530 – 1630	Krusic-Golub: Otolith preparation for estimating age from annual increments.
1630 – 1700	**Discussion**

Wednesday, June 26, 2019

0900 – 1000	Farley: Microscope time –OTC marked otoliths to validate annual increment deposition rates and define what constitutes an annual band pairs in yellowfin and bigeye tuna. (We know you don't have many, if any OTC marked otoliths, however, if you have some, we plan to have our UV burner functional)
1000 – 1130	Krusic-Golub: Microscope time – Estimating age of bigeye tuna from annual band pair counts.
1130 – 1200	**Discussion**
1200 – 1300	**Lunch**
1300 – 1430	Fuller: Estimating age from daily increment counts in yellowfin tuna
1430 – 1500	**Coffee Break**
1500 – 1630	Krusic-Golub: Microscope time – Estimating age of yellowfin tuna from annual band pair counts.
1630 – 1700	**Discussion**

Participants: Jessica Farley (CSIRO), Daniel Fuller IATTC), Kyne Krusic-Golub (FAS), Keisuke Satoh (FSFRL) and Kurt Schaefer (IATTC).

CONTACT US

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

AT CSIRO, WE DO THE EXTRAORDINARY EVERY DAY

We innovate for tomorrow and help improve today – for our customers, all Australians and the world.

Our innovations contribute billions of dollars to the Australian economy every year. As the largest patent holder in the nation, our vast wealth of intellectual property has led to more than 150 spin-off companies.

With more than 5,000 experts and a burning desire to get things done, we are Australia's catalyst for innovation.

CSIRO. WE IMAGINE. WE COLLABORATE. WE INNOVATE.

FOR FURTHER INFORMATION

Oceans and Atmosphere
Jessica Farley
t +61 0 0362 325189
e Jessica.farley@csiro.au
w www.csiro.au