**INTER-AMERICAN TROPICAL TUNA COMMISSION**

**WORKSHOP ON METHODOLOGIES FOR ESTIMATING AGE OF BIGEYE AND YELLOWFIN TUNAS FROM OTOLITHS**

**La Jolla, California (USA)**
**25-26 June 2019**

# REPORT OF THE WORKSHOP

**CONTENTS**



## 1. BACKGROUND

Because of valid concerns raised regarding differences in the estimated length-at age data, growth models, and $L_\infty$ estimates used in recent Inter-American Tropical Tuna Commission (IATTC) and Western and Central Pacific Fisheries Commission (WCPFC) stock assessments for bigeye tuna (BET) from the eastern Pacific Ocean (EPO) and the western and central Pacific Ocean (WCPO), along with the differences in those stock status determinations, the IATTC held a workshop in January 2019 to evaluate the basis for the age estimation methods and growth models used in current stock assessments of bigeye tuna by the IATTC and WCPFC. Since the stock assessments of yellowfin tuna (YFT) from the eastern Pacific Ocean (EPO) and western and central Pacific Ocean (WCPO) are also greatly influenced by length-at age data, growth models, and $L_\infty$ estimates, that species was also included in the workshop.

A technical workshop on methodologies for estimating age of BET and YFT from otoliths which had been scheduled to take place by a small group of experts immediately preceding the main workshop was postponed due to the US Federal Government partial shutdown which prevented access to the IATTC laboratory. The technical workshop was rescheduled and eventually held at the IATTC laboratory, La Jolla, California, USA, on 25-26 June 2019, chaired by Kurt Schaefer, head of the IATTC Life History and Behavior Group. Participation was limited to a small group of scientists from IATTC and those whom are involved in BET and YFT age and growth studies in the WCPO in association with the WCPFC (see list of participants, Appendix 2). Presentations were given by participants to address specific issues associated with ageing BET and YFT, as background for discussions (see abstracts in Appendix 3).

The workshop addressed the following topics:

1. Otolith preparations for estimating age from daily and annual increments in otoliths from BET and YFT;
2. Validation of daily and annual increment deposition rates for BET and YFT;
3. Methodologies being employed for counting daily and annual increments in the otoliths of BET and YFT from the EPO and WCPO

This report summarizes the key points noted by the chair and other participants during presentations and discussions, along with the recommendations of the workshop.

## 2. KEY POINTS UNDER EACH TOPIC

### 2.1. Otolith preparations for estimating age from daily and annual increments in otoliths from BET and YFT

The methodology being employed at IATTC and Fish Ageing Services (FAS) for preparation of otoliths for estimating age from daily increments for BET and YFT is time consuming and requires considerable experience, particularly the polishing and etching so as to obtain suitable sections mounted on microscope slides for counting daily increments. However, FAS does not currently etch otolith sections for daily ageing.

The methodology being employed at FAS for preparation of otoliths for estimating age from annual increments for BET and YFT allows for many samples to be prepared in a timely and efficient manner. This is due to the multiple otolith sectioning technique (cutting 5 otoliths at a time) and also the method used to mount the sections on slides negates the need for any polishing of the otoliths.

### 2.2. Validation of daily increment deposition rates for BET and YFT in the EPO

Several oxytetracycline-hydrochloride (OTC) marked otoliths from BET and YFT, derived from OTC injection mark-recapture experiments conducted in the EPO, were viewed by participants through a compound microscope to demonstrate the criteria for what constitutes a daily increment. It was discussed how daily increment depostion rates have been validated and published for 70 BET from 38 cm to 135 cm FL at liberty for 15 to 551 days, and for 127 YFT from 40 cm to 148 cm FL at liberty for 3 to 515 days from OTC injection mark-recapture experiments in the EPO. However, there is less confidence in age estimates for large BET and YFT due to the lower numbers of OTC marked otoliths analyzed for larger individuals.

### 2.3. Validation of daily increment deposition rates for BET and YFT in the WCPO

Digital images of strontium chloride (SrCl) marked otoliths from 2 BET (94 and 109 cm FL at recapture) and 2 YFT (77 and unknown cm FL at recapture) derived from SrCl injection mark-recapture experiments conducted in the Coral Sea were presented and discussed regarding daily increment deposition rates for those species in the WCPO. Frontal sections of otolith pairs were prepared, one of which was evaluated under scanning electron microscope to identify the SrCl mark and obtain a measurement from the SrCl mark to the post-rostral (PR) tip. The other otolith was read (daily increments counted) from the estimated location of the SrCl mark to the PR tip prior to the workshop using light microscopy. The counts of daily increments in frontal sections of each of those 4 otoliths were substantially less than the number of days the fish were at liberty, indicating that daily increment counts may not be a reliable method for age determination for BET and YFT of the lengths examined. The results for BET are similar to earlier results obtained for 7 SrCl marked otoliths from the Coral Sea by CSIRO and SPC, where counts of daily increments in transverse sections also substantially underestimated days at liberty.

However, considering only 9 SrCl marked otolith samples are available for BET and 2 for YFT from mark-recapture experiments in the Coral Sea, no valid conclusions can be reached about the daily increment deposition rates in otoliths of BET and YFT in the wider WCPO until substantially more otolith samples are obtained from throughout a suitable length range of fish, preferably 30 to 150 cm, derived from SrCl or OTC injection mark-recapture experiments.

It was suggested that there is no reason to suspect that the physiology and rate of daily increment deposition would differ for BET and YFT between the EPO and the WCPO. However, it was previously reported that for a few YFT otoliths examined from the WCPO it appeared more difficult to discern daily increments compared to those from the EPO. This finding was confirmed in the workshop as increments in the BET and YFT otoliths from the WCPO prepared for the workshop were more difficult to discern than increments in BET and YFT otoliths from the EPO. Whether this is related to differences in the structural growth of YFT otoliths due to oceanographic differences between the EPO and WCPO is unknown.

### 2.4. Validation of annual increment deposition rates for BET and YFT in the WCPO

Digital images of SrCl marked otoliths, from BET mark-recapture experiments conducted in the Coral Sea, were viewed by participants to evaluate and discuss how annual increment deposition rates have been validated and published for 9 BET from 85 cm to 157 cm FL at recapture and at liberty for 260 days to 6.6 years. As for YFT, there currently is very little direct validation of the annual increment deposition rate from marked otoliths derived from SrCl or OTC injection mark-recapture experiments. There are only two YFT SrCl marked otoliths obtained from mark-recapture experiments in the Coral Sea which were at liberty for just 61 and 261 days. The time at liberty of the former fish was insufficient for validation purposes, but the annual increment deposition rate was validated in the later fish.

### 2.5. Methodologies being employed for counting daily and annual increments in the otoliths of BET and YFT from the EPO and WCPO

Daily increment counts along frontal sections of BET and YFT otoliths, for fish up to about 4 to 5 years of age, from the EPO are fairly straightforward. The counts are repeatable, but only after training with an expert to learn what constitutes a daily increment, and then how to follow an optimal counting path particularly when encountering some of the common problematic areas of the otolith. Daily increment counts provide much lower error rates in absolute ages than do annual increment counts for EPO caught BET and YFT up to at least 135 cm FL and 148 cm FL, respectively.

Annual increment counts in transverse sections of BET and YFT otoliths from the WCPO are subjective, even for experienced readers, because there are many opaque zones which may not be annual bands. For the first two years of otolith growth the annual increments are often indistinct. For this reason, in BET otoliths from the WCPO the approximate location of the first and the second annuli has been verified based on distances measured from the primordium of reference otoliths to the location of the 365$^{th}$ and 730$^{th}$ daily increment counts. It was discussed how similar verification for YFT is needed and that utilizing pairs of otoliths from some EPO caught YFT which have daily increment counts along frontal sections of about 365 and 730 days, and preparing transverse sections of the paired otoliths to enable measurements to the outer edge of the otolith would provide some verification of the expected location of the first and second annuli.

IATTC scientists suggested that without direct validation of the annual increment deposition rate throughout the length range for which absolute ages are to be estimated, it would not be appropriate to proceed in estimating ages of YFT from annual increment counts as proposed for the WCPO. This was a viewpoint that was not shared by FAS or CSIRO scientists. However, it was noted that the current YFT ageing program in the WCPO includes work to verify the first two annuli for WCPO caught YFT using daily increment counts and indirect age validation methods such as marginal increment analysis. Additional YFT and BET otolith mark-recapture tagging experiments were also discussed for providing direct validation of increment deposition rates, as well as the potential application of bomb radiocarbon (carbon-14) dating with otoliths for corroboration of longevity of YFT and BET.

## 3. RECOMMENDATIONS

1. Validation of daily and annual increment deposition rates for otoliths of BET and YFT should be expanded spatially across the Pacific, and throughout the length range for which estimates of absolute age are to be determined, by incorporating OTC or SrCl injection mark-recapture experiments within tuna tagging programs.
2. Methodologies employed and their validation for estimating absolute ages of BET and YFT from otoliths through daily or annual increment counts should be well described and documented within peer-reviewed scientific publications.

**Appendix 1. Agenda**

**INTER-AMERICAN TROPICAL TUNA COMMISSION**

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# AGENDA

**Tuesday 25 June**

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

**Wednesday 26 June**

| | |
|---|---|
| 0900 – 1000 | *Jessica Farley*: Microscope time –Strontium marked otoliths for validation of annual increment deposition rates and defining annual band pairs in bigeye and yellowfin tunas |
| 1000 – 1130 | *Kyne Krusic-Golub*: Microscope time – Estimating age of bigeye tuna from annual band pair counts |
| 1130 – 1200 | Discussion |
| 1200 – 1300 | Lunch |
| 1300 – 1430 | *Daniel Fuller*: Estimating age from daily increment counts in yellowfin tuna |
| 1430 – 1500 | Coffee Break |
| 1500 – 1630 | *Kyne Krusic-Golub*: Microscope time – Estimating age of yellowfin tuna from annual band pair counts. |
| 1630 – 1700 | Discussion and recommendations |

**Appendix 2. Participants**

| Name | | Affiliation | | Email |
|---|---|---|---|---|
| Farley | Jessica | CSIRO | Australia | Jessica.Farley@csiro.au |
| Fuller | Daniel | IATTC | USA | dfuller@iattc.org |
| Krusic-Golub | Kyne | CSIRO | Australia | kyne.krusicgolub@fishageingservices.com |
| Satoh | Keisuke | FSFRL | Japan | kstu21@fra.affrc.go.jp |
| Schaefer | Kurt | IATTC | USA | kschaefer@iattc.org |

**Appendix 3. Abstracts**

## Otolith preparation for estimating daily age in frontal sections of bigeye and yellowfin tuna

**Daniel W. Fuller and Kurt M. Schaefer**

Quality preparation of otolith sections is critical for discerning daily increments along the primordium to post-rostral counting path. Steps to prepare a sectioned otolith will be discussed outlining the complete process from casting to polishing and acid etching.

The process of preparing a clean, bubble free, epoxy casting of a sagittal otolith will be described, including materials required. A well-prepared casting of the otolith is critical, as if bubbles are present the otolith can break or crumble during sectioning. Once cured, the casting is viewed under a stereo microscope where the location of the priomordium and post-rostral tip are marked on the surface of the casting, using a fine point sharpie. Marking these locations allows for precise alignment on the Pace Technologies, Inc. Pico 155 slow speed diamond wafering saw. Otolith castings are sectioned at ~1mm thickness, at approximately 225-275 RPM to not generate excessive frictional heat as the blades rotate through the epoxy. Excessive heat can lead to a breakdown of the epoxy, warping of the section, or may cause damage to the otolith itself.

The ~1mm section containing the primordium and post-rostral tip is affixed to a microscope slide heated to ~250˚F on a hot plate using Crystalbond® 509 thermoplastic mounting adhesive. Once cool, slides are viewed under a compound microscope at 100x magnification to evaluate the depth of the primordium and post-rostral tip. Initial polishing starts with 600 grit wet/dry 3M lapping film. The section is polished in a mostly circular motion for about 10-15 seconds and then viewed again under the compound microscope. This process is repeated until the primordium and post-rostral tip are visible, but still below the polished surface. As material is removed from the surface of the section, the coarseness of the abrasive is reduced to 1200, 1800, and finally 5000 grit to remove as many scratches as possible. If necessary, the section is placed back onto the hotplate, heating the Crystalbond® 509 to its melting point and the otolith section flipped. Polishing is repeated until the section is 50 – 150 microns in thickness with both the primordium and post-rostral tip visible. Final polishing is completed with 0.3-micron diamond polishing solution on a microfiber polishing pad removing nearly all scratches.

The polished section is etched for 90 seconds using 0.5M ethylenediaminetetraacetic acid. This process removes proteins from the calcium matrix of the otolith and improves contrast and readability.

## OTC marked otoliths to describe daily increments in bigeye tuna otolith frontal sections and discuss increment deposition rates

**Daniel W. Fuller and Kurt M. Schaefer**

Five Oxy-tetracycline (OTC) marked otoliths from bigeye tuna mark-recapture experiments conducted during 2000 – 2002 will be used describe what constitutes a daily increment. These bigeye tuna otoliths originated from fish ranging in length from 58 – 108 cm and 32 – 80 days at liberty. Each otolith was sectioned in the primordium to post-rostral axis, mounted to a microscope slide and polished using methods previously described. Sections will be viewed with an Olympus Vanox light microscope under high magnification (400 and 600x) using both transmitted ultra violet and visible light.

Each participant will be instructed on methods to both find the UV mark and how to determine which increment the OTC mark is associated with. The OTC mark will be located by fluorescence induced by ultraviolet light from a 200-watt mercury burner and then the specific increment will be illuminated by controlling the brightness of the transmitted visible light so that both the fluorescent mark and increments are visible. Following instruction each participant will have the opportunity to make blind counts from the OTC mark to the post-rostral tip for each otolith.

**Estimating age of bigeye tuna from daily increments on otolith frontal sections**

**Daniel W. Fuller and Kurt M. Schaefer**

Five otoliths from bigeye tuna were selected from sampling conducted in the eastern Pacific Ocean (EPO) during January 2001 to September 2004. This subset of bigeye tuna otoliths originated from fish ranging in length from 64 – 133 cm. Each otolith was sectioned in the primordium to post-rostral axis, mounted to a microscope slide and polished using methods previously described. Sections will be viewed with a Nikon OPTIPHOT compound light microscope under high magnification (600 and 1000x) using transmitted visible light. Images of otoliths will be taken using a Canon 7D digital SLR camera, mounted to the microscope using a c-mount adapter.

Each otolith will be viewed in sections, as due to the high magnification employed, it is not possible to view the entire counting transect in one image. Beginning at the primordium and counting increments towards the post-rostral tip, each section of the otolith will be imaged and discussed. Generally, the first 9 to 12 daily increments are well defined and easy to discern. However, as the count proceeds away from the primordium, after about increment 12, they become progressively wider and diffuse which can make for difficult interpretation of daily increments. At times there will be a necessity to chase increments around the otolith surface, looking for visual ques that will indicate where the next increment may be. This can be either along the distal surface or along the sulcus ridge.

Progressing along the counting transect, at about the 90$^{th}$ increment the wide and diffuse section transitions to become more regular and defined. However, at times there are sections where increments are occluded and not clearly visible which requires some interpretation. In these cases, using other areas along the otolith surface to help inform the reader where increments may be, as well as using the pattern or periodicity both prior to, and after these occluded sections, will be used to inform the reader on the location of increments. Interpretation of these challenging sections will be discussed with participants and the decision processes for increment counting will be fully described.

**Otolith preparation and age estimation procedures used by Fish Ageing Services for the annual ageing of tunas**

**Kyne Krusic-Golub**

Fish Ageing Services (FAS) have developed a method for the preparation and sectioning of up to 5 otoliths at one time. This method increases the efficiency over traditional single section methods and allows for the preparation of large numbers of samples in a cost effective and timely manner (>200 per week). This preparation method has been used for the various tuna species routinely aged at FAS; southern bluefin tuna, Western and Central bigeye tuna and albacore. A description of the preparation method, including relevant images from each step in the process was presented to the group and is summarized below.

Prior to any preparation either the left or the right sagittal otolith from each sample is weighed to the nearest 0.0001g. Otolith weight is a useful diagnostic tool in assessing potential errors in age estimates and for examining patterns of otolith growth. Otolith weight also has a strong relationship with fish size and age and large variation about the relationship may indicate a lack of precision in the estimates. Also, an outlying data point in the otolith weight/age relationship may indicate incorrect assignment of age and/or length and otolith weight measurements.

Once weighed, otoliths selected for preparation are embedded in rows of five within blocks of Polyplex Clear Ortho Casting Resin, ensuring that the primordium of each otolith is in line. Each row of otoliths is sectioned on the transverse axis. Up to five serial sections, approximately 300µm thick, are cut through the otolith centers with a modified high-speed gem-cutting saw with a 250µm thick diamond impregnated blade. Sections from each sample are cleaned, dried and mounted in resin on clear glass microscope slides (50 x 76 mm) which are then covered by two glass coverslips (22 x 60 mm).

Sectioned otoliths are examined using transmitted light. Each section of the otolith is inspected, and the section with the clearest zone pattern is chosen for ageing. This is usually, but not necessarily the section closest to the primordium (the biological center of the otolith). The age of each sample is determined by counting the number of completed annual opaque zones along a count path from the primordium to the otolith edge along the ventral arm. To reduce potential for biasing age estimates, all counts ware made without knowledge of fish size, otolith weight, sex and location.

In addition to the zone count, a readability score which rates the otoliths on a sale of 1 to 5 (1 poor – 5 excellent) and the marginal edge type is recorded. The marginal edge type classification (WT, NT or O) is subjectively assigned by the reader and is based on the optical properties of the edge using the following categories:

- **WT** - Wide Translucent (translucent material past last opaque zone is generally greater than 1/3 of previously completed annuli)
- **NT** - Narrow Translucent (translucent material past last opaque zone is generally less than 1/3 of previously completed annuli)
- **O** - Opaque edge (opaque visible on edge)

The edge is an indicator of the likelihood of zone formation in the next period of deposition. An edge type WT indicates that an opaque zone is likely to form soon, edge type NT indicates that the opaque zone has recently finished forming and an O edge type indicates that the last opaque zones is still forming on the edge. The edge type information is required to allow zone counts to be converted to age estimates.

The developed protocols for annual ageing of tuna require that the same reader reads the samples twice and then provides a third and "final" zone count. As a further test for accuracy/precision a subset of 10% is required to be read by an additional reader (usually Jessica Farley).

## Inter-lab ageing work to jointly examine bigeye and yellowfin tuna otoliths from the western Pacific Ocean

**Jessica Farley**

A description of recent work by CSIRO, FAS and IATTC to examine and validate daily and annual increment formation in otoliths of bigeye and yellowfin tuna in the western Pacific was presented. One component of this work was to prepare and read otoliths marked strontium chloride as part of a previous mark-recapture experiment in the Coral Sea. Paired (left and right) otoliths were analyzed from four fish (two bigeye and two yellowfin) tagged and injected with strontium chloride (SrCl) in the early 1990s, and recaptured 61 to 427 days later. Given the sources of the release and recapture information, we are confident that the data are accurate.

Frontal sections were prepared from each otolith, which included the primordium and the post rostral tip. One otolith was prepared by CSIRO (Australia) and the 'sister' otolith was prepared by IATTC (USA). CSIRO used a scanning electron microscope (SEM) to detect and locate the SrCl mark in the otolith section, and to obtain high resolution images. For all fish, the SrCl mark was clearly visible. IATTC used the SEM images to locate the expected position of the SrCl mark on the sister otolith section, and counted the number of assumed daily increments from the mark to the otolith tip. Two counts were obtained and the mean was calculated. A comparison was made between the mean daily count and the known days at liberty.

In summary, 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%). Yellowfin #2 was 97 cm at release and an unknown length at recapture, and was at liberty for 261 days. The mean increment count was 114.5 days (-54.1%). 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%). Finally, 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%). These results provide 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. The results are consistent with previous daily ageing results for bigeye tuna (using transverse sections) from the Coral Sea.

In addition to the daily age work, CSIRO also prepared transverse sections from each yellowfin otolith 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 because only the area after the SrCl mark is required for validation purposes. An image of each otolith section was obtained prior to the SEM imaging to locate the SrCl mark. Yellowfin #1 was only at liberty for 61 days, so the subsequent growth was not enough to validate annual ageing. However, yellowfin #2 was at liberty for 261 days and showed one opaque zone and one translucent zone after the SrCl mark. The results provide evidence that counts of annual growth increments may be a reliable source of age information for yellowfin in the western Pacific Ocean, although further mark-recapture age validation studies are needed.

Additional work was not undertaken to validate estimates of annual age of bigeye in the western Pacific using mark-recapture otoliths as a previous study had shown that the first nine increments are deposited annually. The study analyzed 11 otoliths from bigeye 72-125 cm FL at release and 85-157 cm at recapture. Time at liberty ranged from 260 to 2420 days (0.7 to 6.6 years). A brief description of this earlier work was presented and images of the marked otoliths were examined and discussed.

The final component of the inter-laboratory ageing work presented was transverse sectioned otoliths from three bigeye and three yellowfin prepared and read by FAS (annual age estimated). Frontal sections of the 'sister' otoliths from the fish had been prepared and read by IATTC (daily age estimated). A comparison of age estimates showed that for small fish (<~110 cm), annual and daily ages were generally similar. However, for larger fish, annual age was higher than daily age, and the otolith sections were substantially larger than expected from the estimate of daily age. This provides further evidence that counts of daily increments in bigeye and yellowfin otoliths in the western Pacific may not provide reliable age estimates.

**Age reading and interpretation of bigeye tuna from the Western and Central Pacific Ocean**

**Kyne Krusic-Golub**

This portion of the workshop presented information on the annual age estimation process and the interpretation of bigeye otolith sections developed for the WPO samples. A small number of otolith sections from WPO and EPO caught fish were examined and discussed. Additionally, images of several different samples were directly compared to show some of the variability in size and shape that is observed for samples collected from fish with similar lengths, or to conversely show that samples obtained from different length fish can have similar weight and size.

The annual interpretation of bigeye tuna is considered difficult. This is mainly due to the inner structure of the otolith relating to the assumed first 1 – 3 years of growth. Unfortunately, in this region of the otolith there can be little difference in the resolution between the assumed annual opaque and translucent zones. Reasons for this may be a function of their physiology (pelagic, fast growing), environmental conditions (follow temperature gradients) or a combination of a number of different influences. Even though there has been significant research into the mechanisms behind why otoliths lay down growth zones, the specifics are still poorly understood. Certainly, it is plausible that otoliths from tropical tunas are more difficult to interpret than other species (including other tunas) because they may not be subject to the large temperature gradients that other species are subject to. This lack of temperature fluctuation between summer and winter period could certainly influence the clarity (or lack thereof) of the zones deposited within their otoliths.

Generally, the interpretation of the mid to outer part of the bigeye otolith section is considered easier. The growth zones (both opaque and translucent) within this area show a far more consistent pattern in both optical property and pattern. The pattern of zone formation observed in the outer areas of bigeye otolith sections is also consistent with those observed in other tuna otoliths, specifically those from southern bluefin where direct validation is far more extensive and has directly validated the deposition of the annual zones out to 13 years.

It was recognized early on in the annual ageing method development for the WPO bigeye, that interpreting the inner structure correctly would likely be the main challenge. Various methods were used to verify and validate the position and timing of annual zone formation which included micro-increment counts (daily ageing) in otoliths of smaller fish to verify the $1^{st}$, $2^{nd}$ and if possible, the $3^{rd}$ annuli, analyzing otoliths from 11 mark-recaptured samples and results from marginal increment and edge type analysis. While the latter two techniques are not direct validation techniques, they do provide further support that the zones that are being interpreted as annual are formed on an annual basis.

Even though various methods of validation have been utilized in developing the ageing method, a large amount of the ageing protocol was developed from viewing many sections (>500) before any attempt to initiate age estimation. This allowed examples of clear samples to be documented and also allowed for the identification of any consistent morphological characteristics that may help with the ageing process. i.e. the first major change in the angle on the distal surface of the section often corresponds with the position of the first annual opaque zone.

**Estimating age of yellowfin tuna from daily increments on otolith frontal sections**

**Daniel W. Fuller and Kurt M. Schaefer**

Wild (1986) and Wild et al. (1995) demonstrated, from mark-recapture studies using oxy-tetracycline, that yellowfin tuna from 40 to 148 cm deposit daily increments in sagittal otoliths in the primordium post-rostral axis. This fact is the basis for the current frontal sectioning methodologies employed to age yellowfin tuna in the eastern Pacific Ocean (EPO).

Six otoliths from yellowfin tuna were selected from sampling conducted in the EPO during January 2009 to December 2016. These yellowfin tuna otoliths originated from fish ranging in length from 49 – 80 cm. Each otolith was sectioned in the primordium to post-rostral axis, mounted to a microscope slide and polished using methods previously described. Sections will be viewed with a Nikon OPTIPHOT compound light microscope under high magnification (600 and 1000x) using transmitted visible light. Images of otoliths will be taken using a Canon 7D digital SLR camera, mounted to the microscope using a c-mount adapter.

Each otolith will be viewed in sections, as due to the high magnification employed, it is not possible to view the entire counting transect in one image. Beginning at the primordium and counting increments towards the post-rostral tip, each section of the otolith will be imaged and discussed. Generally, the first 9 to 12 daily increments near the primordium are well defined and easy to discern. However, as the count proceeds away from the primordium, after about increment 12, increments become progressively wider and diffuse which can make for difficult interpretation of daily increments. At times there is a necessity to chase increments around the otolith surface, looking for visual ques that will indicate where increments should be. This can be either along the distal surface or along the sulcus ridge.

Progressing along the counting transect, at about the 90th increment the wide and diffuse section transitions to become more regular and defined. However, at times there are sections where increments are occluded and not clearly visible which also requires some interpretation. In these cases, using other areas along the otolith surface to help inform the reader where increments may be, as well as using the pattern or periodicity both prior to, and after these occluded sections, will be used to inform readers on the location of increments. Interpretation of these challenging sections will be discussed with workshop participants and the decision processes for increment counting will be fully described.

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**Age reading and interpretation of yellowfin tuna from the Western and Central Pacific Ocean**

**Kyne Krusic-Golub**

This portion of the workshop presented information on the annual age estimation process and the interpretation of yellowfin tuna otolith sections used in the ageing of a small sample of 40 otoliths from the WPO and another 68 samples from the EPO. The latter batch were the sister otoliths to a batch of otoliths already aged using the daily ageing method. All samples prepared for annual age reading had been prepared using the multiple thin section method outlined earlier in the otolith preparation for annual ageing summary. Several images were shown and the corresponding ages from the daily ageing and the annual ageing methods were discussed. This summary provides details on the annual ageing method for yellowfin tuna, challenges faced in the interpretation of otolith sections and the potential ways that the method can be verified and improved.

Similar to other tuna species, the annual interpretation of yellowfin tuna otoliths is considered difficult. The areas considered most challenging to interpret relate to the inner part of the otolith that is assumed to relate to the first 1 – 3 years of growth (Area A) and also the area of the otolith around the second inflection (Area B).

Within area A, the main difficulty is that in many samples there is little difference in the resolution between the assumed annual opaque and translucent zones. This is particularly evident within the first assumed 2 annuli and to a lesser extent the third. This is not to say that there weren't examples where the zone structure was evident and there were discernible differences between the assumed annual opaque and translucent zones. Several images were examined where annual like opaque and translucent zones were relatively easy to interpret and several examples were shown where they were relatively difficult to interpret. It should be noted that the word "relatively" is very important in this discussion, because experience in the annual ageing of fish, including other tunas, does have an important influence in the classification of readability and also on what otolith structure should and should not be considered as annual.

What we also do know about fish otoliths is that in almost all species the width of the annual increments decreases as the fish becomes older. The width of the first increment is usually the widest followed by the second and so on. This pattern of zone formation has been shown to hold true in other tuna species. Once there is a reasonable process for accurately locating the position of the first opaque zone, then the location of the proceeding zones becomes easier. In the current yellowfin ageing method, the location of the first zone is based on visual cues (presence of an annual opaque zone and also a change in the growth axis on the distal surface) but also relies on knowledge gained from other work completed; yellowfin tuna in the Atlantic and the development of the ageing method for bigeye tuna in the WPO. It was discussed that additional work on verifying the position of the first zone in yellowfin tuna should be a priority. It was suggested that one way to do this relatively easily would be to obtain the sister otoliths from several EPO samples that have already been estimated close to 365 days and prepare the otoliths for annual age reading.

The second area (Area B) is difficult to interpret because of the presence of numerous relatively fine opaque and translucent zones around the 2nd inflection. Based on other tuna ageing methods it's likely that each annual increment within this part of the otolith section consists of several smaller opaque and translucent zones. Within the discipline of fish ageing these are commonly referred to as "split zones". The challenge in correctly interpreting areas that contain split zones is not unique to just tuna and often in ageing studies, the presence of split zones can lead to an over-estimation bias. It was suggested that this could be the case in the current yellowfin ageing method, and that perhaps refinement in this area of the otolith interpretation may lead to zone counts slightly less than initially recorded (in samples with zone counts > 3).

The zones in the outer area of the yellowfin tuna otolith sections (post 2$^{nd}$ inflection) show striking similarity to those from other tuna species. The zone pattern is generally more consistent in both optical property and pattern. This zone pattern has been validated as annual in southern bluefin and bigeye tuna, where direct validation is far more extensive and has validated the deposition of the annual zones out to 13 and 9 years, respectively. There seems to be little reason to believe that the zones in the outer parts of the yellowfin are also not formed on an annual cycle.

To close the session a small number of otolith sections from WPO and EPO caught fish were examined. Additionally, images of several different samples were directly compared to show some of the variability in size and shape that is observed for samples collected from fish with similar lengths (even at smaller length classes) or to conversely show that samples obtained from different length fish can have similar weight and size.