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Progress on yellowfin tuna age and growth in the WCPO (Project 82)

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Progress on yellowfin tuna age and growth in the WCPO

WCPFC Project 82

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Contents

1 Executive summary

This paper describes work undertaken to develop ageing protocols for yellowfin tuna in the western Pacific Ocean (WPO). Preliminary work was undertaken in 2018 and the results were presented at SPC's 2018 Pre-Assessment Workshop (PAW) and at SC14. The results indicated that otoliths were a suitable structure for estimating annual age of yellowfin tuna and preliminary counts of (assumed annual) opaque zones ranged from 0 to 13 for fish ranging in length from 30-172 cm fork length.

Review of this work at the PAW and SC14 identified differences in ageing methodologies between laboratories in the Pacific and it was recommended that an inter-laboratory ageing workshop be undertaken to compare the respective ageing techniques. The proposed workshop could not be held until June 2019 due to the US Federal Government shutdown in late 2018 - early 2019 (Farley et al. 2019), which has delay caused some flow-on delays to the current project.

All otoliths have been selected for ageing (n=1569) and registered into the Fish Ageing Services sample monitoring system. Otoliths, if whole and undamaged, were weighed and a digital image taken for potential use in future stock discrimination using otolith shape. Serial sections of otoliths have been prepared for some of the largest fish but it was decided not to prepare additional otoliths from the remaining fish until after the inter-laboratory ageing workshop with IATTC was complete as this was likely to influence the choice of the most appropriate ageing method.

The results of the age validation work suggest that daily ageing methods under-estimate age for yellowfin greater than 74 cm FL from the WCPO (Farley et al. 2019). Notwithstanding this result, frontal sections of otoliths will be prepared and read for daily increments for fish <74 cm for age and growth information. In addition, some fish 74-130 cm FL will also be prepared and read for daily counts for further comparison with other studies and to provide some information on the location of the first few increments. Before the final annual age readings for this study are completed, additional collaborative work is planned with IATTC aimed at more accurately determining the location of the first few annual increments in transverse sectioned otoliths.

A draft ageing protocol document will be developed that provide a summary of the results of the daily ageing work, including the results from the first zone verification work, useful information developed from collaborative work with IATTC and other labs, and various images and documented information that might be useful in the age reading process. Consideration should be given to bomb radiocarbon (^{14}C) dating in addition to SrCl/OTC mark-recapture programs to validate the annual periodicity of the bands being count using these protocols. An updated work plan to complete the project is provided.

2 Introduction

The 2017 stock assessment for yellowfin tuna in the western and central Pacific Ocean (WCPO) recommended that new estimates of age and growth be developed for yellowfin tuna. At SC13, the Western and Central Pacific Fisheries Commission (WCPFC) endorsed a new project "Yellowfin tuna age and growth" (Project 82). This recommendation arose given how influential the new

growth estimates for bigeye tuna based on counts of annual increments in otoliths (Farley et al. 2017) were on the assessment in 2017, noting the similarities in the fisheries for the two species. In addition, the current assessment model for yellowfin tuna predicts a decline in the selectivity for large fish for longline fisheries, a counter-intuitive result that can occur if the growth is incorrectly specified within the assessment model.

In December 2017, the Western and Central Pacific Fisheries Commission (WCPFC) endorsed the project "Yellowfin tuna age and growth" (Project 82). The aims of the project are to develop protocols annual ageing for yellowfin tuna, create a reference otolith collection, and prepare and read 1500 otoliths for annual age estimation and 150 otoliths for daily age estimation. This is the first comprehensive age and growth study for yellowfin tuna in the WCPO using otoliths.

Preliminary work was undertaken in 2018 and the results were presented at SPC's Pre-Assessment Workshop (PAW) and at SC14. The results indicated that otoliths were a suitable structure for estimating annual age of yellowfin tuna and preliminary counts of (assumed annual) opaque zones ranged from 0 to 13 from fish ranging in length from 30-172 cm fork length.

Review of this work at the PAW and SC14 highlighted differences in ageing methodologies between laboratories in the Pacific. In the western Pacific, Fish Ageing Services (FAS) and CSIRO estimate the age of tunas using 'annual ageing' methods while in the eastern Pacific, IATTC estimate the age of tunas using 'daily ageing' methods. FAS also estimate the daily age of small fish but consider daily ageing to be difficult and that counts of presumed daily growth increments may lead to an underestimation of age for fish > 1 year old. It was recommended that an inter-laboratory ageing workshop be undertaken to compare ageing techniques between the laboratories, standardise the approaches for daily increment counts, and analyse mark-recapture otoliths for age validation.

The workshop was scheduled for January 2019 but was delayed until late June 2019 due to the US Federal Government shutdown in late 2018 and early 2019 (Farley et al. 2019). This delay has caused flow-on delays in Project 82 but the work will be completed by the end of the project. This paper described the current status of the yellowfin ageing work in the project and provides an updated work plan.

3 Objectives and scope

The objectives of the project are to provide robust age and growth estimates for yellowfin tuna in the WCPO to inform future stock assessments and related analyses.

The work plan was to:

- Conduct preliminary analysis of the suitability of yellowfin tuna otoliths for providing accurate estimates of age and growth;
- Conduct preliminary analysis of the suitability of yellowfin tuna dorsal fin spines to verify the annual increments in otoliths of small fish;
- Develop a reference collection of otoliths and protocols for reading daily and annual growth increments in yellowfin tuna otoliths;

- Prepare and read 1500 otoliths using the annual increment method;
- Prepare and reading 150 otoliths using the daily growth increment method;
- Undertake a marginal increment analysis to support estimates of decimal age and growth;
- Report estimates of age and growth for yellowfin tuna to WCPFC SC15.

4 Progress against project objectives

4.1 Preliminary analysis of otoliths and spines

- **Conduct a preliminary analysis of the suitability of yellowfin tuna otoliths for providing robust estimates of age and growth.**
- **Conduct a preliminary analysis of the suitability of yellowfin tuna dorsal fin spines to verify the annual increments in otoliths of small fish;**

Preliminary work to estimate the annual age of yellowfin tuna based on counts of opaque zones in transverse sectioned otoliths and sectioned fin spines was undertaken in 2018, and presented at SC14 (Farley et al. 2018; WCPFC-SC14-2018/SA-WP-13). Forty otoliths and 40 spines were selected for the trial work from fish ranging in size from 30 to 172 cm FL from the western Pacific. Counts of opaque zones in otoliths ranged from 0 to 13. A comparison of zone counts from otoliths and spines showed that spines are useful to verify the location of the first three increments in otoliths, but spines are not suitable for annual age estimation beyond three years of age as early zones are lost due to resorption and vascularisation.

Preliminary work was also undertaken to estimate the daily age of yellowfin based on counts of micro-increments in frontal sectioned of otoliths. Clear increments were observed in otoliths close to the primordium but at a count of ~150-180 increments this initially clear pattern was interrupted and the interpretation of the micro-increments became more difficult (Farley et al. 2018). In some areas along the count path, increments could not be detected at all. This suggests that daily ages may become less accurate for yellowfin > 6-8 months in the WCPO. Recent analysis of mark-recapture otoliths by IATTC/FAS demonstrated that age estimates based on counts of micro-increments substantially under-estimate age for fish >74 cm in the western Pacific (Farley et al. 2019).

Since the first trail batch of otoliths from the western Pacific were analysed (n = 40), samples provided by the Atlantic Ocean Tuna Tagging Program (AOTTP (n = 11) and samples provided by IATTC from the eastern Pacific Ocean (EPO) (n = 68) have been prepared and aged by counting annual increments. Prior to reading these otoliths, a study estimating the annual age of Atlantic yellowfin caught in the Gulf of Mexico was published (Lang et al., 2017). Interpretation of the otoliths in the Lang et al. (2017) study is mostly consistent with the interpretation used in our trial batch. The subsequent ageing on the Atlantic and eastern Pacific yellowfin was conducted with knowledge of the protocols used in the Lang et al. (2017) paper. However, both sets of samples were aged using the same interpretation as used for the earlier western Pacific set of otoliths.

There was potential that the interpretation of the first three zones in the EPO and Atlantic otoliths could have been inadvertently influenced by the knowledge of the Lang et al. (2017) study. To investigate this, the average distances from the primordium to the first five zones were compared to the measurements from the western Pacific trial set. For the EPO samples, the start point for measurement was the first inflection point on the otolith (as per the initial trial batch) and for the Atlantic samples, to remain consistent with Lang et al. (2017), the start point was the primordium. The measurement distances to the first five zones showed reasonable overlap between the EPO and the WPO samples. When taking into account the different start points in the zone measurements between the Atlantic and the Pacific samples, there was still reasonable overlap in zones 1 -3 but perhaps a slight bias in the 4th and 5th zone points. This suggests that there could either be a slight ageing bias (slight over ageing in the Pacific samples) or a difference in growth rates for yellowfin tuna otoliths between oceans.

The comparison suggests that the initial interpretation of (at least the first 1-5 zones) has remained relatively consistent between the readings of the two Pacific Ocean sets. The average distances from the first inflection to the first three zones are slightly higher in the EPO samples and may indicate that the EPO otoliths are growing slightly faster or that the reader slightly adjusted the interpretation between reading the two sets. This will be investigated further using multiple blind readings during the rest of the project.

The interpretation of the zone structure in the otolith area around the position of the second inflection is considered difficult. It is possible that for the preliminary readings some zones counted within this area may not be annual. Otoliths are available from OTC marked fish from the Atlantic through the AOTTP. It is hoped that some of the larger samples from tagged, OTC marked and recaptured fish will provide a more accurate basis for estimating the expected amount of otolith growth that relates to a year interval in this region of the otolith.

4.2 Reference collection and annual ageing

- **Develop a reference collection and protocols for reading daily and annual growth checks in yellowfin tuna otoliths.**
- **Prepare and read 1500 otoliths using the annual increment method.**

Otoliths will be prepared for annual age reading based on the method used by FAS for routine ageing of other tuna species. This method involves embedding a row of 5 otoliths in resin and cutting up to four serial sections, each 0.320 mm thick, through the otolith centres. The sections will then be mounted on numbered glass microscope slides and covered with glass coverslips.

Before attempting any annual ageing, all samples will be imaged and viewed. This will allow an archive of images to be developed and also provide an opportunity to identify any otoliths that might be exceptionally clear or prove informative to the reading process. Once this is complete, an ageing protocol document will be developed. It will provides a summary of the results of the daily and annual ageing work, including the results from the first zone verification work (see section 4.3 below) and useful information developed from the collaborative work with IATTC other labs. It will

also provide various images and documented information that might be useful in the age estimation process.

Otoliths will be viewed for annual ageing using stereo microscopes, illuminated with transmitted light at 25x magnification. Methods used to collect the ageing data will be consistent with previous ageing work completed on southern bluefin, bigeye and albacore tunas previously aged at FAS.

Reference collections of otoliths are a mandatory adjunct to any routine ageing programs to ensure consistency between age reading events and to ensure that bias is not introduced over time. Reference collections are usually developed after the ageing has been completed on a species for the first time and the age estimates are deemed to be within precision standards (<5% and no bias detected between the first and the second age readings). Once the annual ageing has been completed a precision set of set of 200 otoliths will be selected using age estimates that agreed between the first and second age readings. The set will include samples from the full range of ages and readability scores available.

Otolith selection

The aim of the study is to construct a WCPO-wide, sex-aggregated growth curve for yellowfin in the WCPO assessment region. Otoliths from nearly 4,300 yellowfin have been collected since 2009 and archived into the WCPFC specimen tissue bank. Almost all fish were caught within the WCPO stock assessment region (Figure 1). The majority of otoliths are from fish between 30 and 160 cm fork length (FL).

We selected otoliths for ageing using a 1-cm length-stratified approach. The number of otoliths selected from each assessment region was in proportion to the catch in the region, as far as practicable. For regions where the number of otoliths available was lower than required, additional otoliths were selected from other regions. Table 1 shows the number of fish selected for ageing by assessment region and Figure 2 shows the size frequency of fish selected for ageing. All otoliths have been sent to FAS for sectioning and reading.

Otoliths were selected for ageing using a length stratified approach to ensure age information was available across the full age range, and particularly for the oldest fish in the population. Stratified length sampling is beneficial for estimating an age-length key (conditional age-at-length, which is the form that the data would be incorporated directly into the MULTIFAN-CL assessment model), however it is well documented that it can lead to biased growth (i.e., mean length at age) estimates. Goodyear (2019) used simulations to demonstrate the extent of the biases in different situations, and found that the bias increased as the coefficient of variation in mean length at age increased and also as sampling effort was increased to compensate for low sample numbers in bins within the upper tail of the distribution of sizes in the populations. Simulations tailored to the situation for yellowfin could be carried out to evaluate the expected extent of the biases and, if they are considered too large, then measures can be taken to correct for this. For instance, Chih (2009) presents a method for weighting the otolith data based on true population proportions of fish in each length bin to get an unbiased growth curve when stratified length sampling is used. Note, however, that this assumes knowing the proportions at length in the population accurately – if length samples used to estimate these proportions are not representative of the population

then Chih (2009) shows that weighting the otolith data can still help, but does not completely correct the bias.

Table 1. Number of yellowfin with otoliths selected for ageing by assessment region.

Assessment region	% of catch	No. otolith required	No. otoliths selected
1	18.9	283	2
2	4.7	71	68
3	15.5	233	351
4	11.2	168	271
5	10.4	155	231
6	5.2	78	121
7	24.6	370	269
8	8.5	128	256
9	0.9	14	0
Total	100	1500	1569

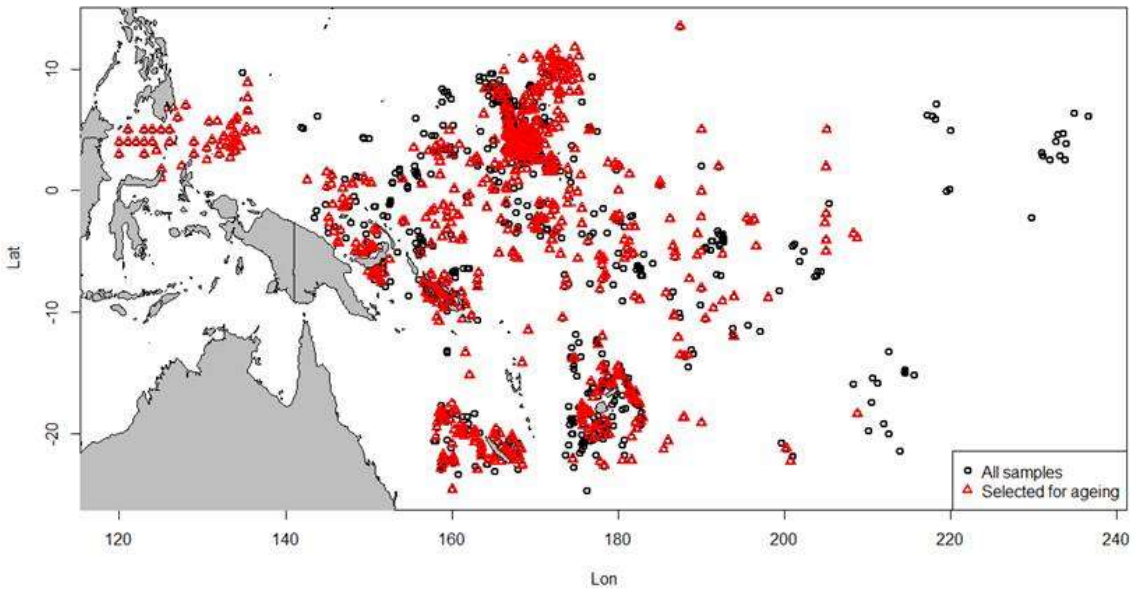

Figure 1. Map of the sampling locations for otoliths in the WCPFC tissue bank available for analysis (as at July 2019). Otoliths shown in red were selected for ageing. Longitude shown in degrees east. The area east of 210°E is outside the WCPO assessment boundary.

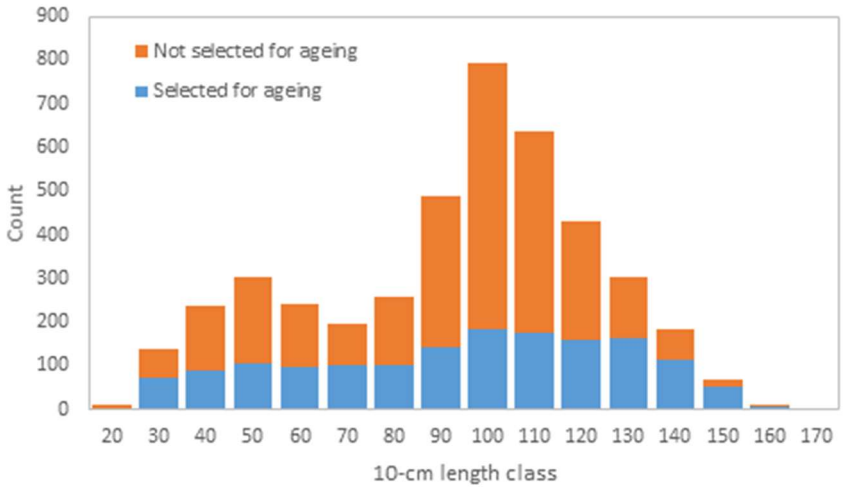

Figure 2. Length frequency of yellowfin with otoliths selected for analysis (blue) and not selected (orange) from the WCPFC tissue bank. The lower boundary length value of the bin is shown.

Otolith preparation and reading

Otoliths have been registered into the FAS sample monitoring system and weighed to the nearest 0.1 mg (if whole and undamaged). A digital image was taken of the majority of otoliths (if complete) for potential future stock discrimination work by SPC using otolith shape analysis (SPC funded). Serial transverse sections were prepared for 131 otoliths from fish >130 cm FL for annual ageing purposes. It was decided not to prepare additional otoliths from smaller fish until after the inter-laboratory ageing work program with IATTC was complete as this was expected to influence decisions on the most appropriate ageing method (annual or daily) for WCPO yellowfin. As it was not possible to complete the collaborative work and hold the inter-laboratory workshop until June, FAS has not completed the otolith sectioning work and been able to begin readings.

Before the final reading is completed, additional collaborative work is planned with IATTC to help locate the first few annual increments in transverse sectioned otoliths. As with other tunas, the first few annuli are often the most difficult to interpret as there is little difference in the optical properties between translucent and opaque zones. From the age validation work of Farley et al. (2019), it appears that daily ageing of WCPO yellowfin for samples greater than 74 cm FL substantially under-estimates age and cannot be used to help locate annual growth zones in the second and third years of life. However, assuming that otolith growth (otolith weight to fish length; Figure 3) is similar in the WCPO and EPO, and that otoliths from the EPO appear easier to read than WCPO otoliths (Farley et al., 2019), it may be possible to use sister otoliths from EPO fish aged ~365 and 730 days to help locate the first two annuli in transverse sectioned otoliths for the current study. IATTC are confident that the age estimates are accurate for the size range of fish we are interested in (ages 1 and 2).

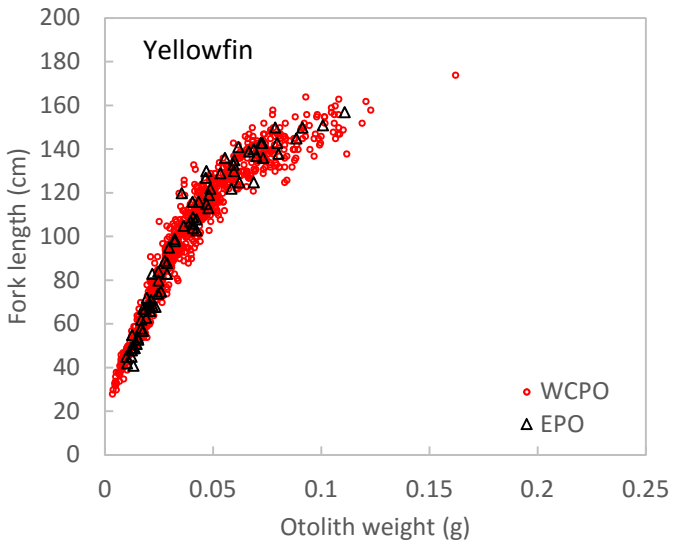

Figure 3. Otolith weight to fork length for yellowfin for fish caught in the WCPO and EPO. EPO data from Schaefer and Fuller, unpublished data.

4.3 Daily ageing

Prepare and reading 150 otoliths using the daily growth increment method

As noted above, the results of the inter-laboratory ageing work by IATTC, FAS and CSIRO to examine and validate daily ageing methods for yellowfin in the western Pacific indicated that daily increments counts substantially under-estimate age for yellowfin > 1 year (see Farley et al. 2019). Daily increment counts from mark-recapture otoliths underestimated days at liberty by ~40-55% for YFT 74 cm and 97 cm FL at release. Therefore, we are only confident in daily age estimates for yellowfin < 1 year using frontal sections. The analysis of the AOTTP OTC otoliths is due to be completed late September 2019. This will also provide a further indication the accuracy of daily ageing (albeit for samples in the Atlantic).

While this result will mean a reduction the amount of daily aging from that originally proposed, daily ageing should still provide valuable information for fish <74 cm. Transverse daily ageing should provide for verification of the first annual zone. The ageing of frontal sections on samples at least up to 74 cm FL will likely provide better age and growth estimates than the annual ageing can for the small fish. From those samples less than 50 cm, back calculated spawning dates can be estimated and compared to the (limited) reproduction information available.

A revised plan for the daily ageing is proposed below:

- Prepare and age 50 frontal sections from samples < length at age 1 for age and growth information.
- Prepare and age at least a further 20 frontal sections from samples between 74-130 cm FL for comparison against other studies.
- Prepare and age transverse sections from the smallest available up to 130 cm with the expectation that daily zone counts can provide some form of verification on the first three increments (even though we know that daily age estimates may underestimate total age).

4.4 Marginal increment analysis

Undertake a marginal increment analysis to support the age and growth estimates;

This objective will be completed by the end of the project and reported in the final report. Note that marginal increment analysis can confirm the periodicity in increment formation over the time period examined and is not considered true validation of the age (Spurgeon et al. 2015).

4.5 Report to SC15.

Report estimates of age and growth for yellowfin tuna to WCPFC SC15.

Given the postponement of the technical meeting with IATTC, and consequent delay in analysis of the otoliths at FAS, the age and growth results are not complete. It is anticipated that the work can be completed within the project timelines and results reported in the project final report and at SPC's Pre-Assessment Workshop in 2020. However, an extension to the project will provide a better opportunity to continue collaboration with IATTC and potentially other ageing labs (see sections 5) and consideration can be given to whether bomb radiocarbon (^{14}C) dating to validate the annual periodicity zones being counted is an option for bigeye and yellowfin in the Pacific.

5 Direct validation of BET and YFT annual ages in the Atlantic

It is clear that otoliths are the accepted structure for ageing tuna. However, both daily and annual ageing methods are used for yellowfin and bigeye, and the accuracy of these structures is difficult to validate across the full age and spatial range of the species. Recently, annual ageing protocols for yellowfin and bigeye in the Gulf of Mexico were validated using bomb radiocarbon (^{14}C) dating (Andrews et al. 2019; Appendix A). The study used the decline period in the atmospheric/ocean ^{14}C levels (1980s to 2000s) to successfully validate yellowfin aged 2 to 18 years and bigeye 3 to 17 years. Previous studies have used the increasing levels of ^{14}C immediately after nuclear testing (late 1950s) to validate the age of longer loved species, including southern bluefin and Atlantic bluefin tunas (Kalish et al. 1996, Neilson and Campana 2008). This new application is suited to shorter lived species and has already been applied to Pacific bluefin tuna (Ishihara et al. 2017).

As the "bomb radiocarbon" method is considered to be one of the most rigorous techniques to validate the accuracy of otolith reading methods, consideration should be given to applying the method to bigeye and yellowfin tunas in the Pacific.

6 Work plan

Although it was anticipated that age data and preliminary growth parameters would be presented at the SPC Pre-Assessment Workshop in 2019 and the final results presented at WCPFC SC15, this

was not possible due to the postponement of the inter-laboratory ageing work and technical meeting with IATTC.

The priorities for the remainder of the project are to:

- Complete preparation of otoliths for annual ageing.
- Prepare frontal sections of otoliths from small fish up to 74 cm FL for age and growth information. Prepare and age a further frontal sections from samples between 74-130 cm FL for comparison against other studies.
- Prepare and age transverse sections from the smallest available up to 130 cm with the expectation that daily zone counts can provide some form of verification on the first three increments (even though we know that daily age estimates may underestimate total age).
- Prepare transverse sections of yellowfin 'sister' otoliths from EPO fish provided by IATTC. The otoliths will be selected by IATTC from fished aged ~1 (365 days) and ~2 (730 days) from frontal sections. Measure from the first inflection point to the otolith edge on the ventral (long) arm of the transverse section to help confirm the location of the first and second annual opaque zones in WCPO otoliths.
- Analyse additional fin spines from small fish, where we are confident there has been no loss of increments, to help confirm the location of the first and second annual opaque zones in otoliths
- Develop an ageing protocol document
- Undertake edge type and/or marginal increment analysis to examine the periodicity in annual increment formation.
- If possible, undertake inter-lab ageing workshops with other labs using annual ageing methods for bigeye and yellowfin, e.g., the Southeast Fisheries Science Centre, Panama City and Louisiana Department of Wildlife and Fisheries. These labs provided the annual age estimates used in the bomb radiocarbon age validation work of Andrews et al (2019).
- Explore the potential for bomb radiocarbon age validation.
- Develop an ageing protocol of yellowfin tuna and complete the reading of otoliths for annual ageing and estimate growth.
- Carry out simulations to evaluate the expected extent of biases in growth due to length stratified selection of otoliths for ageing and, if they are considered too large, correct for the bias.

7 References

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Appendix A: Abstract, Proceedings of the 70th Annual Tuna Conference, May 20-23, Lake Arrowhead, California.

VALIDATED LONGEVITY OF YELLOWFIN (*Thunnus albacares*) AND BIGEYE (*T. obesus*) TUNA OF THE NORTHWESTERN ATLANTIC OCEAN

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The age, growth and longevity of yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) tuna (YFT and BET) remain problematic in that attempts to validate age estimates have been limited and typically incapable of evaluating maximum age. Otolith growth zone structure can be complicated for tropical pelagic fishes because they live in a more aseasonal environment than higher latitude habitats. However, bomb radiocarbon (^{14}C) dating has evolved considerably over the last 25 years and is a well-founded approach that has been useful in accurately describing the life history characteristics of several tropical pelagic species. In this study, age reading protocols that produced maximum age estimates approaching 20 years for YFT and BET of the northwestern Atlantic Ocean were validated with bomb ^{14}C dating. A novel aspect of the method is use of the ^{14}C decline period (more recent than ~1980) — after nuclear testing and as described by regional coral records of the Gulf of Mexico — to provide valid estimates of age through ontogeny. Yellowfin tuna aged 2 to 18 years (n = 33, 1029–1810 mm FL) led to birth years that were coincident with the bomb ^{14}C decline reference, while BET aged 3 to 17 years (n = 10, 1280–1750 mm FL) were more variable but in agreement, as well. Results indicate that age reading discrepancies of previous studies may have led to truncated estimates of growth and longevity.

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