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**Progress on yellowfin tuna age and growth in the WCPO
WCPFC Project 82**

WCPFC-SC14-2018/SA-WP-13

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1 Executive summary

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. In December 2017, the Western and Central Pacific Fisheries Commission (WCPFC) endorsed a new project “Yellowfin tuna age and growth” (Project 82). The aims of the project are to develop ageing protocols 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 paper summarises the preliminary work undertaken in the project.

Over 4,000 sets of yellowfin tuna otoliths have been collected and archived into the WCPFC specimen tissue bank since 2009 and are available for analysis in the project. Almost all were collected from fish caught within the WCPO stock assessment region. Additional otoliths have been collected by Japan and Taiwan in the WCPO and are also available to the project.

Forty yellowfin tuna were selected for preliminary analysis to determine if otoliths and/or dorsal fin spines were suitable for age estimation. Fish ranged in size from 30 to 172 cm fork length (FL). All otoliths were prepared and read by Fish Ageing Services (FAS). One otolith from each fish was prepared for annual ageing and the sister otoliths from 10 of the 40 fish was prepared for daily ageing. FAS also prepared 40 fin spines from the same fish for comparative ageing. The spines were examined by CSIRO.

The results indicate that otoliths are a suitable structure for estimating annual age of yellowfin tuna with preliminary counts of opaque zones ranging from 0 to 13. The fin spines examined showed resorption and vascularisation leading to a “loss” of early increments, and were not suitable for annual age estimation beyond three years. However, they were useful to corroborate the location of the first three increments in sectioned otoliths. The daily ageing work found clear ‘daily’ zones could be detected along the ventral arm of otoliths out to the edge, even in very large fish. However, the areas with clear zone patterns were interspersed with areas that were difficult to interpret and did not show the “classic” daily zone structure, which is likely to lead to an underestimation of age. Therefore, we are not confident in age estimates beyond the first transition point (150-180 increments).

A priority for the remainder of the project is to undertake an inter-lab daily ageing workshop to compare otolith preparation and reading methods between the eastern and western Pacific. This was also a recommendation of the SPC pre-assessment workshop in April 2018. A key activity will also be the analysis of two strontium marked otoliths and two otoliths from fish tagged (but not marked) for direct age validation. We will then finalise the selection, preparation and reading of otoliths (and some spines) for the project and undertake edge type and/or marginal increment analysis as indirect validation of age. It is anticipated that length at age data and preliminary growth parameters will be delivered to the SPC pre-assessment workshop in 2019.

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 (Tremblay-Boyer et al. 2017). This recommendation arose given how influential new growth estimates for bigeye tuna (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 of 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). This is the first comprehensive age and growth study for yellowfin tuna in the WCPO using otoliths. This paper summarises the preliminary work undertaken in the project.

3 Background - otolith ageing in the WCPO

A variety of techniques have been applied to yellowfin tuna to estimate age and growth, including analysis of length frequency data, tagging data and hardparts (see review by Murua et al. 2017). Three studies have analysed the microstructure of otoliths in the WCPO to estimate daily age and have also undertaken direct age validation (Lehodey and Leroy 1999, Yamanaka 1990, Uchiyama and Struhsaker 1981) (Table 1).

Uchiyama and Struhsaker (1981) ‘tentatively’ validated a daily deposition rate for increments in whole otoliths of two small (52 cm FL) captive held yellowfin tuna in Hawaii (Table 1). In that study, the amount of daily ration appeared to influence the formation of microincrements. When the fish were fed once per day but not to satiation, daily increments did not form. However, when the fish were fed to satiation each day, daily increments did form. A further 14 fish were then aged, but from a wider range of sizes (7-93 cm FL) than those two validated samples (Table 1; Fig. 1).

Yamanaka (1990) also directly validated a daily deposition rate for otolith microincrements for 12 small (25-40 cm FL) yellowfin tuna held captive in Hawaii using whole otoliths (Table 1). The fish were fed twice a day and were in captivity for 2-39 days. An additional 139 otoliths were read from fish 16-79 cm FL.

Lehodey and Leroy (1999) is the only study that examined microincrements in sectioned otoliths. They attempted to validate daily age through a tagging program and an oxytetracycline (OTC) marking experiment. Three marked otoliths were ‘recaptured’ from fish 39-90 cm FL that had been at liberty for 21-175 days (Tables 1 and 2). A daily deposition rate of increments was validated in the three otoliths when viewed under a scanning electron microscope (SEM). A SEM allows the examination of otoliths at much higher magnifications than light microscopy to identify the otolith microstructure, although the microstructure in the yellowfin tuna otoliths examined show that

increments split and merge across the otolith (see Fig. 1 from Lehodey and Leroy, 1999). Lehodey and Leroy (1999) also validated the daily deposition rate of increments in one otolith from the smallest yellowfin tuna (39 cm FL) at liberty for 21 days using light microscopy (Table 2). However, counts of increments underestimated days at liberty by ~10% in the other two otoliths examined using light microscopy (Table 2). A further 180 fish were aged using light microscopy, but from a wider range of sizes than validated using light microscopy (20-145 cm FL) (Table 1; Fig. 1). Their growth curve is, therefore, likely to be biased (age underestimated).

There are differences in the growth curves estimates from the three studies above (Fig. 2). It is unclear whether these differences are the result of spatial/temporal variation in growth or differences in the preparation and reading methods used by the laboratories. In the Indian Ocean, Sardenne et al. (2012) found that daily age estimates varied among reading teams for yellowfin and bigeye tunas, highlighting the potential for incorrect interpretation of otolith microstructure. In the eastern Pacific Ocean, Wild and Foreman (1980) validated daily age estimates of yellowfin tuna through an OTC mark-recapture experiment for fish 40-110 cm FL and at liberty for 3-389 days. Dr. Wild was also the otolith reader from Lehodey and Leroy (1999) that used light microscopy to examine the three OTC-marked otoliths. Dr Wild noted 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 differences in otolith ‘readability’ may help to explain the reason direct age validation was possible for larger fish in the EPO but not in the WPO using light microscopy.

Table 1. Summary of studies that have used otolith microstructures to estimate the age and growth of tropical tunas in the western and central Pacific Ocean. SEM = scanning electron microscope; OTC= oxytetracycline.

Reference	Type	Size range	N	Reading method	Validation method	Days at liberty or captivity	Age validation successful
Uchiyama and Struhsaker 1981	Age validation	52	2	Whole otolith	Captive experiments	24-30	Yes
	Ageing	7-93	14	Whole otolith			
Yamanaka 1990	Age validation	25-40	12	Whole otolith light microscope	Captive experiments	2-39	Yes
	Ageing	15-28	68	Whole otolith light microscope			
	Ageing	16-79	139	Frontal section light microscope			
Lehodey and Leroy 1999	Age validation ¹	39-90	3	Transverse section SEM	OTC mark-recapture	21-175	Yes
	Age validation ¹	39	1	Transverse section light microscope	OTC mark-recapture	21	Yes
	Age validation ¹	43, 90	2	Transverse section light microscope	OTC mark-recapture	49, 175	No
	Ageing	20-145	180	Transverse section light microscope			

¹ See Table 2 for results of age validation.

Table 2. Comparison between numbers of microincrements counted by two independent readers and the numbers of days at liberty for sagittal otoliths of OTC-marked yellowfin tuna in the western Pacific. Readers were (1) Dr Wild using light microscope and acetate replica of the otolith surface; and (2) Dr Stequert using SEM and transverse sections. Table adapted from Lehodey and Leroy (1999).

Sample	FL (cm) release	FL (cm) recapture	Days at liberty	Count (1) (mean number) Light microscopy	% difference	Count (2) SEM	% difference
T00105	42	43	49	44.4	-9.4	50	2.0
T00138	35	39	21	21.3	1.4	21	0.0
T00159	62	90.5	175	157	-10.3	175	0.0

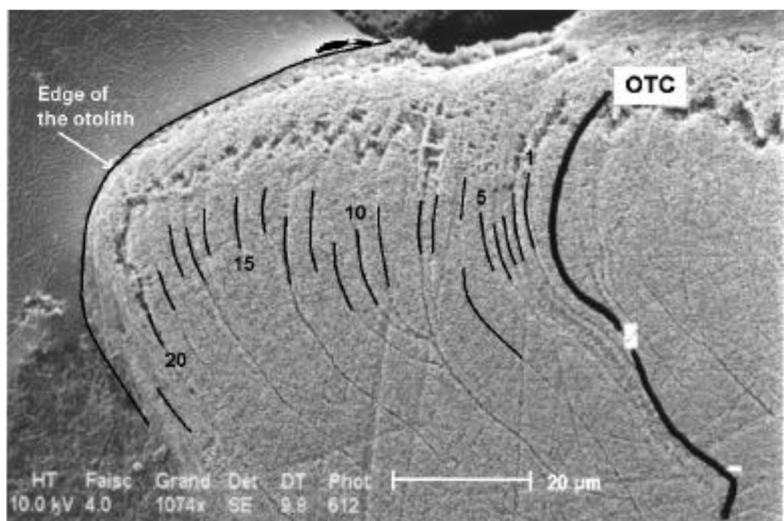


Figure 1. SEM view of the transverse cross-section of an OTC-marked otolith of yellowfin tuna. The fish was recaptured after 21 days at sea and 21 increments were counted since the OTC mark (from Stequert, LASAA, Brest, France). Figure from Lehodey and Leroy (1999).

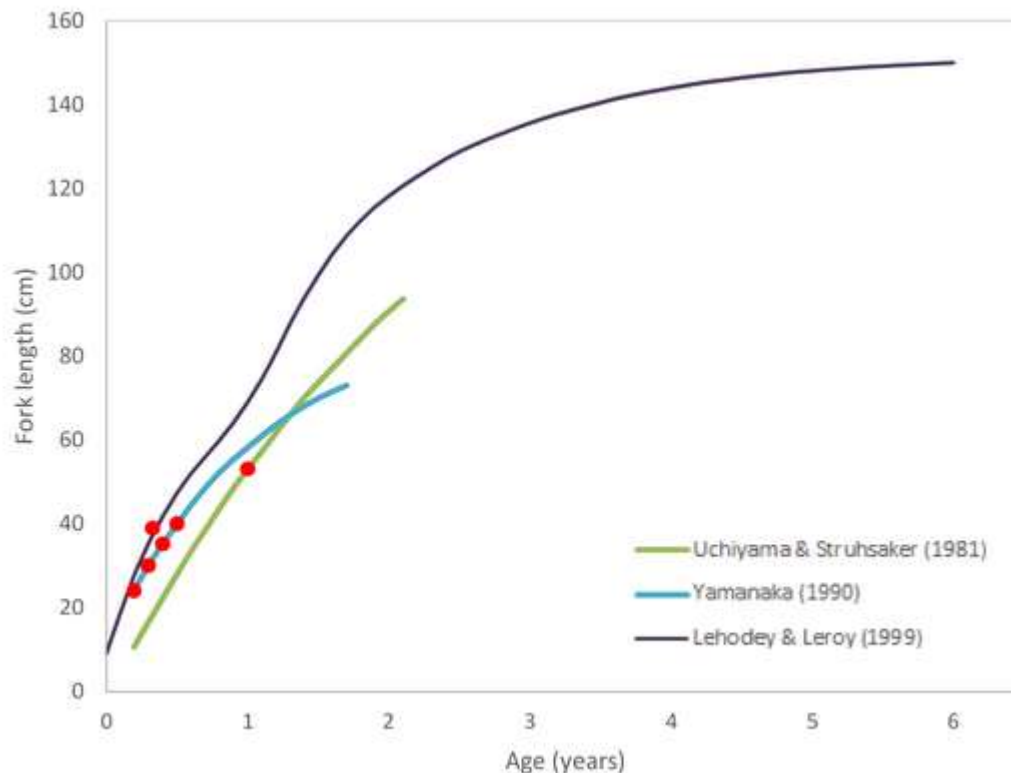


Figure 2. Growth curves for yellowfin tuna in the WCPO from otoliths (daily ageing methods). The red dots indicate the size range for which age validation was successful in each study.

4 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 will:

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

5 Preliminary analysis

5.1 Hardparts available for analysis

Over 4,000 sets of yellowfin tuna otoliths have been collected and archived into the WCPFC specimen tissue bank since 2009, and nearly 3000 since 2014. Almost all were sampled from fish caught in the WCPO stock assessment region (Fig. 3). The majority are from fish between 30 and 150 cm FL (Fig. 4). Additional otoliths have also been collected by Japan and Taiwan in the WCPO and are available for the project. A large proportion of the sampled fish also have dorsal fin spines available in the tissue bank. These samples should be adequate to complete a comprehensive and robust study of yellowfin tuna age and growth.

Forty fish were selected from the tissue bank for preliminary analysis to determine if otoliths and/or dorsal fin spines were suitable for estimating age in yellowfin tuna (Fig. 3). Otoliths and spines were obtained from each fish and the fish ranged in size from 30 to 172 cm FL.

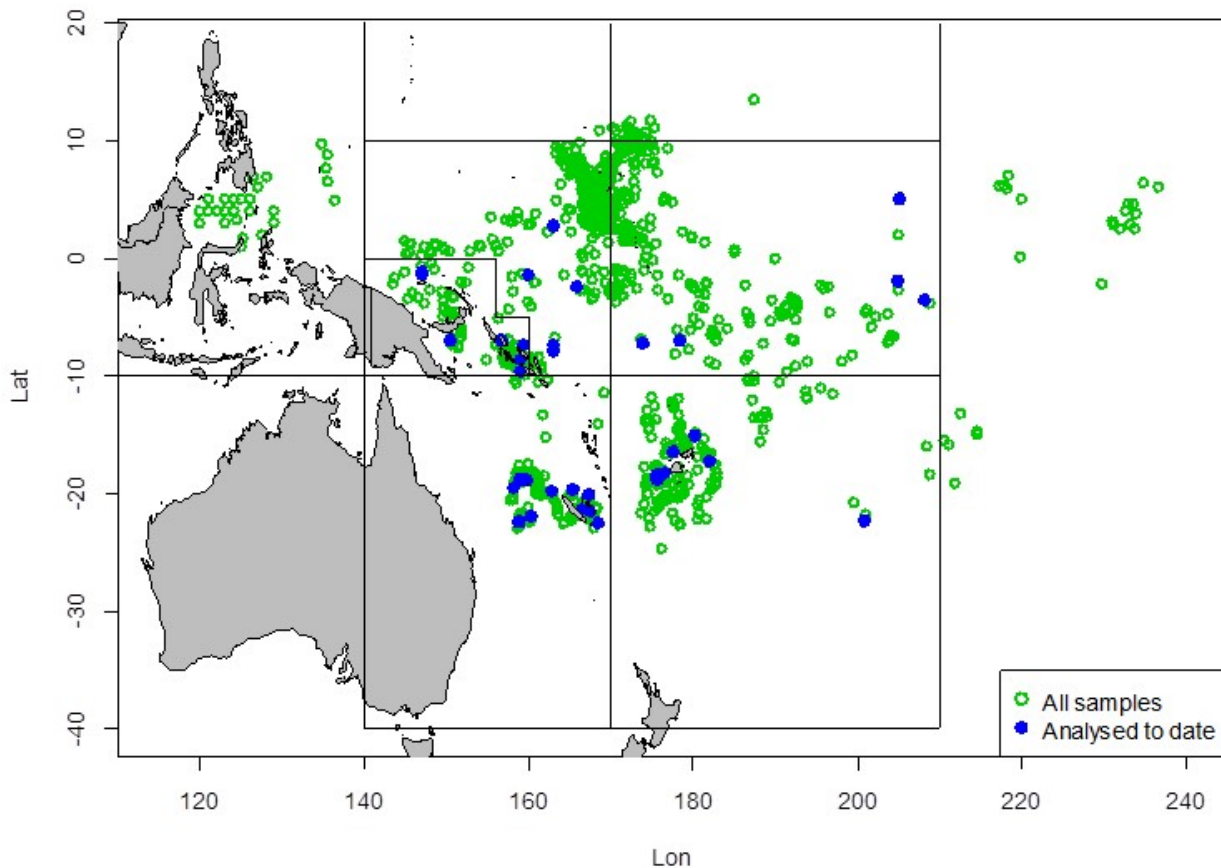


Figure 3. Map of the sampling locations for otoliths in the WCPFC tissue bank available for analysis. The blue dots indicated the sampling locations for 40 fish that otoliths and spines were selected for preliminary analysis. Longitude shown in degrees east. Note that otoliths collected east of the yellowfin tuna stock assessment region (210° longitude) will not be selected for analysis.

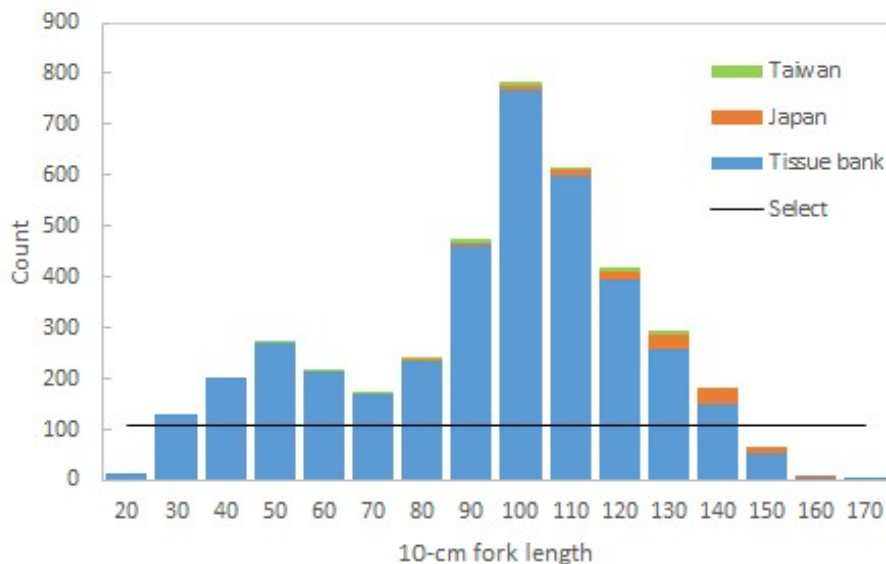


Figure 4. Length frequency of yellowfin tuna with otoliths available for analysis from the WCPFC tissue bank (since 2009) and from Japan and Taiwan. Only otoliths from fish caught within the yellowfin tuna stock assessment regions are included. The lower boundary length value of the bin is shown. The black line represents the number of otoliths per 10-cm length bin that would be analysed to obtain ~1500 age estimates for the project. Data shown as at April 2018.

5.2 Annual age estimation from otoliths

One otolith from each pair was selected for annual age estimation and weighed (if whole). The otoliths were sent to Fish Ageing Services (FAS) for sectioning and reading using protocols developed for other tuna species (e.g., bigeye) and for yellowfin tuna (Williams et al., 2013). Transverse sections were prepared from each otolith following the methods outlined in Anon. (2002) and Farley et al. (2017). Otoliths were embedded in clear casting polyester resin and four or five serial transverse sections approximately 280-300µm thick 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 had been cut. The otolith sections were read at 25x magnification illuminated with transmitted light.

The otolith structure was relatively translucent for most of the otolith samples. Even though a standard thickness was used for the sections, this species may benefit from thicker sections. Translucent and opaque zones were observed on both the ventral and dorsal arm on the transverse section; however, the zones on the ventral (long) arm appear clearer and are also more widely spaced. As with other species of tuna, the first one to three annuli were difficult to interpret as there was little difference in the optical properties between the translucent and opaque zones.

A customised image analysis system was used to mark the opaque zones on the image and also measure the distances between the end of each opaque zone and the first inflection point on the ventral arm (Fig. 5). The position of the first and second opaque zones (~0.85 mm and ~1.25 mm from the first inflection) were relatively consistent between samples. The second inflection along

the ventral arm seemed to coincide with the position of the 5th or 6th opaque zone. Figure 5 shows an example of a sectioned yellowfin tuna otolith aged 12.

Counts of opaque zones ranging from 0 to 12 were obtained for 37 of the 40 otoliths examined. Length at age estimates are shown in Figure 6. Note that the “ages” are counts of opaque zones and not biological (decimal) ages that take account of birth and catch date (see section 3.1.3 of Farley et al. 2018 for further details).

Although very preliminary, a von Bertalanffy (VB) growth model was fitted to the age and length data following the methods described in Farley et al. (2017) (Project 35). The VB model has the form:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

where L_t is the fork length at age t , L_∞ is the mean asymptotic length, k is a relative growth rate parameter (year^{-1}), and t_0 is the age at which fish have a theoretical length of zero. We used maximum likelihood estimation assuming a Gaussian error structure with mean 0 and variance σ^2 .

The result of fitting the VB growth curve to the length at age data is shown in Figure 6. The VB parameters were $L_\infty = 173.65$, $k = 0.221$, $t_0 = -1.027$.

Hampton (2000) estimated VB growth parameters for yellowfin tuna in the western tropical Pacific from tagging data ($L_\infty = 166.4$ cm; $k = 0.25$). The method was based on the change in fish length between the time of tagging and recapture. Although t_0 is not estimated in the analysis, if it is set so that the VB curve has a length at age 1 year equal to length at age 1 from daily ageing by Lehodey and Leroy (1999) (i.e., 68.9 cm FL), then the VB curve estimated from tagging data is consistent with the curve estimated using our preliminary otolith annual age data (Fig. 7). Note that length at age 1 from Lehodey and Leroy (1999) and the current study are very similar (Fig. 7). However, further validation of the otolith age estimation method for yellowfin tuna is still required.

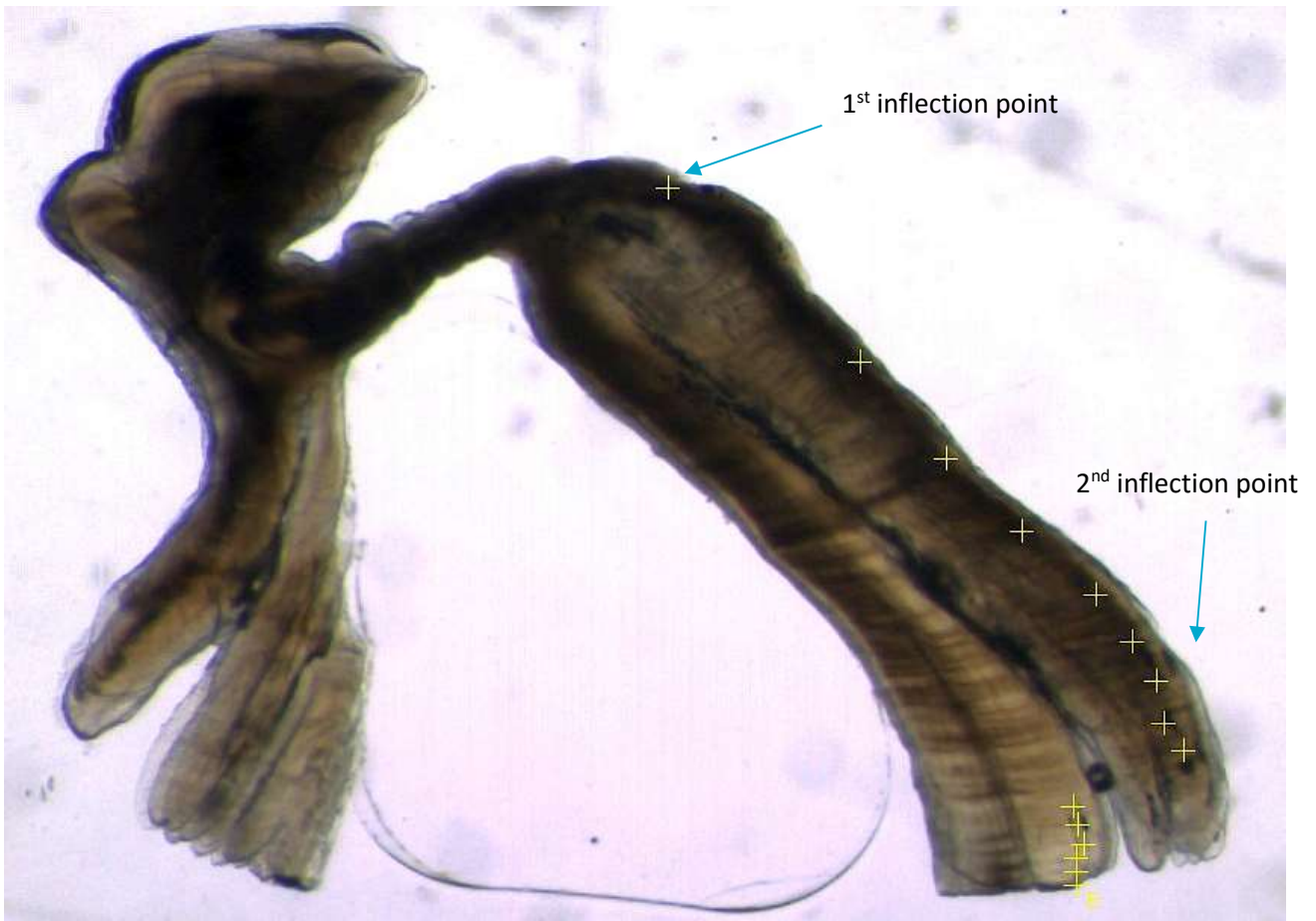


Figure 5. Transverse section of a yellowfin tuna otolith viewed under transmitted light. The yellow +'s mark the 12 opaque zones counted and the otolith edge. See more examples in Appendix A.

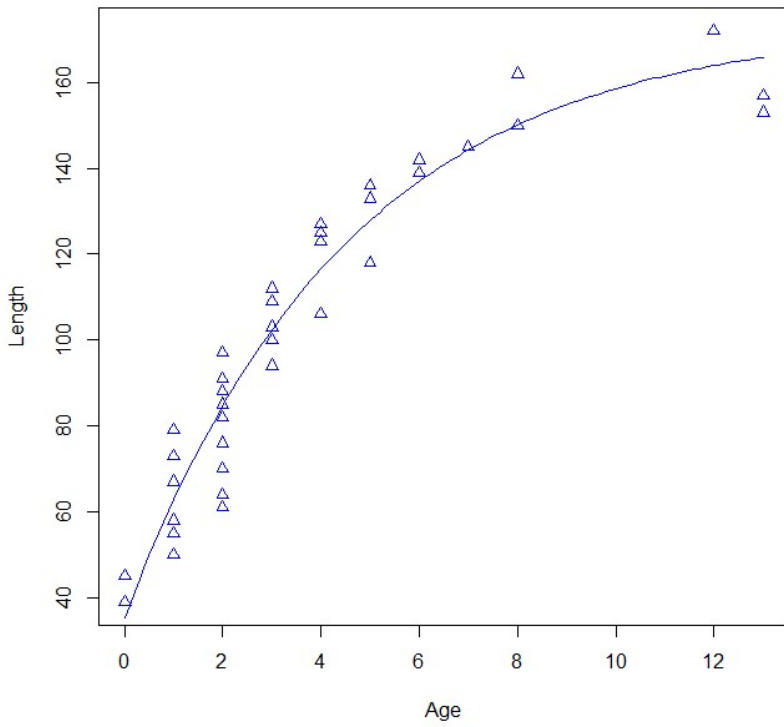


Figure 6. VB growth model fit to the preliminary length at age data. VB parameters $L_{\infty} = 173.65$, $k = 0.221$, $t_0 = -1.027$

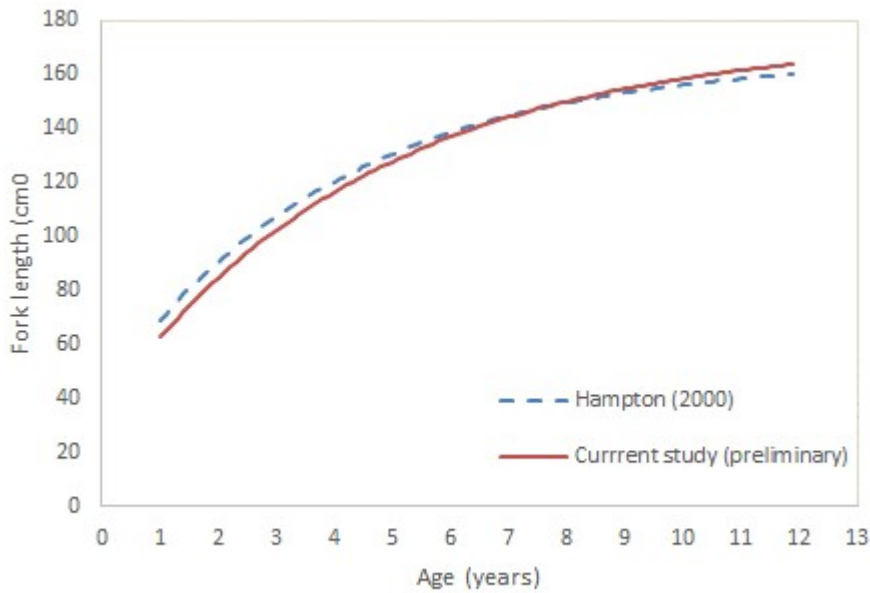


Figure 7. Comparison of VB growth curve from the current study and from Hampton et al. (2000) for yellowfin tuna in the WCPO.

5.3 Fin spine comparison

Fin spines were examined as they have been useful for ageing small fish in other species (e.g., swordfish in the southwest Pacific; Farley et al. 2016, WCPFC-SC12-2016/ SAWP-11) and may be useful to corroborate the otolith ages. The 40 spines selected for ageing were sent to FAS for sectioning. The spines were sectioned based on the protocols outlined in Rodríguez-Marín et al. (2007). The first cut of the spine was then made near the hollows (Fig. 8) and several serial sections 500 μm thick were made, cleaned and placed on microscope slides in order that they were cut. A 'reference' section was identified as the section cut at distance along the spine at half the diameter above the 'hollows' (Fig. 8) (Rodríguez-Marín et al. 2007). The sections were embedded in clear casting polyester resin and mounted on glass slides with resin.

The sectioned spines were examined at CSIRO under both transmitted and reflected light. The clarity of the sectioned spines varied among individuals and many contained split growth zones, which were difficult to interpret (Fig. 9). Unlike otoliths, spines are a vascularised structure (i.e., connected to the circulatory and nervous systems), and are subject to resorption and vascularisation as the fish grows, leading to a "loss" of early increments (Fig. 9). All spines examined had some level of vascularisation and resorption present near the core. The spines from large fish, in particular, had increments "missing" due to vascularisation (see Appendix A for examples). Some spines, however, had clear growth increments that were not obscured by vascularisation. These were generally from small/young fish (ages 1 to 3 years) and were useful to corroborate the locations of the first one to three annual increments in sectioned otoliths (see examples in Appendix A).

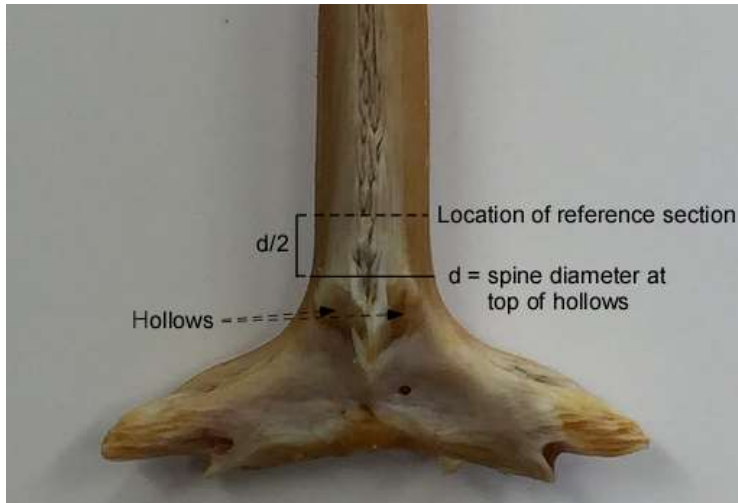


Figure 8. Image of a yellowfin tuna fin spine showing the position of the reference section for ageing.

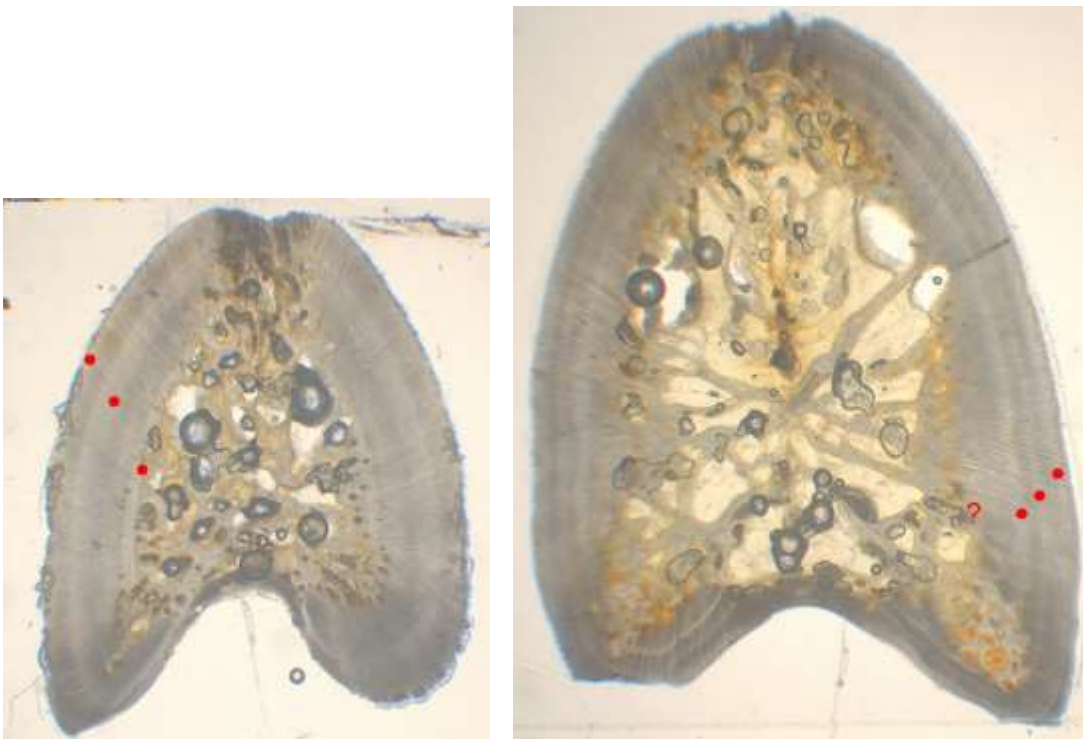


Figure 9. Sectioned fin spines from a (left) 109 cm FL and a (right) 139 cm FL yellowfin tuna viewed under transmitted light. The red dots indicate annual zones. Vascularization has led to a ‘loss’ of growth zones in the right-hand spine. See Appendix A for more examples.

5.4 Daily age estimation from otoliths

The ‘sister’ otoliths of 10 yellowfin tuna that had been aged by counting annual increments were selected for microincrement analysis (daily ageing). Otoliths were analysed to determine the position of the 365th increment and if possible the 730th increment and to provide measurements to these points (taken from the first inflection on the long arm). A secondary aim was to determine when the age estimated from the daily counts diverged from the age estimated from the annual counts.

All otoliths were sent to FAS for sectioning and reading. Transverse sections were prepared so that a direct comparison of otolith measurements could be made with the otoliths sectioned for annual ageing. The otoliths were prepared following the methods outlined in Williams et al. (2013). The otoliths were ground down manually until they were 35µm thick. Even at 35µm the opaque daily zones were quite difficult to read. Therefore, we recommend that to age young fish in the future (i.e., <150 days), the preferred method would be to section to 50-80µm. The microincrements were counted along the ventral (long) arm.

As with other species of tuna we have examined, the area of the otolith close to the primordium displays relatively clear and consistent microincrement structure, followed by an area where the structure becomes difficult to interpret and does not show the “classic” daily zone structure. For the yellowfin tuna otoliths we examined, this interruption occurred at around 150-180 increments. Even in the sample from the largest otolith, clear ‘daily’ zones could be detected along the ventral arm of the otolith out to the edge but the pattern was interrupted with areas that were difficult to interpret. If the microincrements formed in these hard to interpret areas are not formed on a daily cycle, as we suspect, then they will lead to an underestimation of age. Naturally

the more of these areas that exist in the otolith, then the greater the magnitude of the bias. Therefore, we are not confident in age estimates beyond the first transition point (150-180 increments).

Despite this, total counts of microincrements were attempted for all otoliths and ranged from 177 for a 30 cm FL male to 538 for a 130 cm FL female (Fig. 10). Figure 12 shows the microincrements visible in the yellowfin tuna aged 538 days. Unfortunately, the age of this fish was not obtained from annual ageing of the otolith but, based on our growth curve (Fig. 6), this sample was likely to be around age 5, providing further evidence that the daily age of large yellowfin tuna is like to be underestimated. In all fish where daily and annual age estimates from sister otoliths were available, counts underestimate daily age when compared to counts of annual zones (Fig. 12).

The distance from the first inflection to the 365th increment was measured in four of the 10 otoliths prepared for daily ageing. The measurements ranged from 1.34 mm to 1.62 mm (mean 1.42 mm). Annual opaque zones were counted and measured on the sister otolith of three of these fish, and in all cases the distance from the first inflection to the first and second opaque zones occurred before the 365th increment. This suggests that either the microincrements counted that were not daily increments, or the first annual opaque zone in otoliths is a 'false' zone. Daily and annual age validation work is required to resolve this. An inter-lab daily ageing workshop is recommended to compare otolith preparation and reading methods in the Pacific.

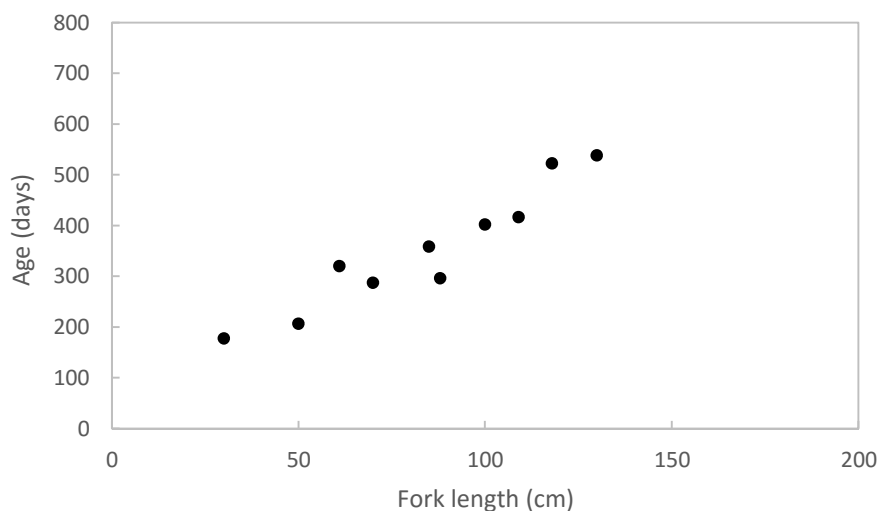


Figure 10. Relationship between fork length and daily counts from transverse sectioned otoliths for yellowfin tuna.

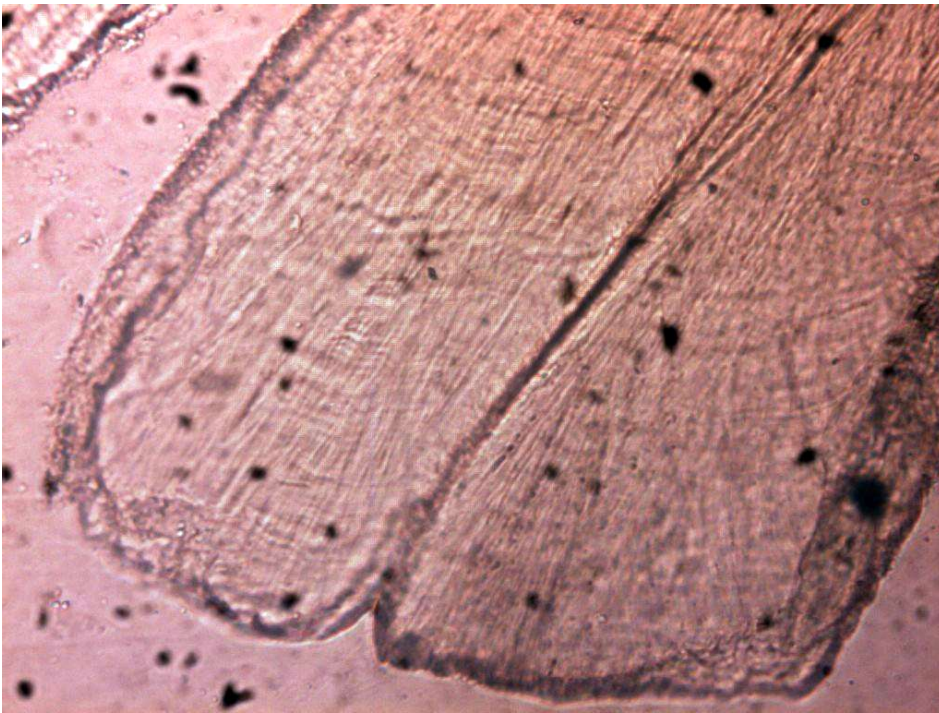


Fig. 11. Microstructure visible at the edge of an otolith from a large (130 cm FL) yellowfin tuna estimate to be 538 days old (1.47 years) from daily ageing.

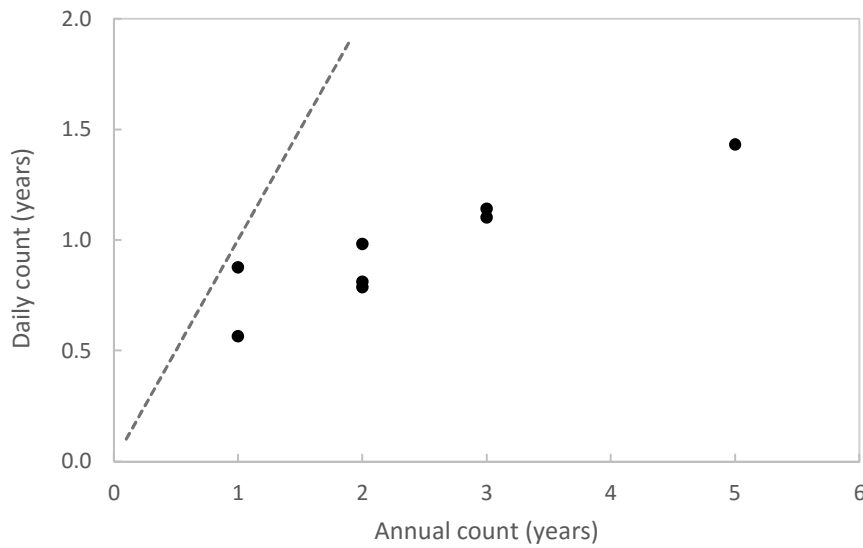


Figure 12. Comparison of daily and annual counts from transverse sectioned otoliths sampled from the same fish (n=8). The 1:1 line is shown (dotted).

6 Future work

Only preliminary results of the analysis of yellowfin tuna hardparts are presented here. The priorities for the remainder of the project are to:

- Undertake a small inter-laboratory daily age workshop to compare otolith preparation and reading methods between the eastern and western Pacific. This was also a recommendation of the SPC pre-assessment workshop in April 2018.
- A key activity of the inter-laboratory workshop would be to analyse two sets of strontium chloride (SrCl_2) marked otoliths and two sets of otoliths from tagged (but not marked) fish for direct age validation. These otoliths may be useful for both daily and annual age validation purposes. The marked otoliths were from yellowfin tuna released during a tagging programme in the Coral Sea (off northeast Australia) in the 1990s.
- Complete the daily ageing work (n=150) after the above workshop. We may also need to consider longitudinal sections of otoliths, although a direct comparison of the locations of the 365th and 730th increments are more difficult as the sectioning planes for annual and daily ageing would be different. This work will help confirm the location of the first and second annual opaque zones in otoliths.
- Complete the selection, preparation and reading of the otoliths for annual ageing (n=1500). Figure 4 indicates the number of otoliths that will be selected by 10-cm length class to obtain 1500 otoliths for analysis, although selection of otoliths would be based on 1-cm length classes. We suggest that all otoliths that had been collected from small and large fish are selected, as well as a fixed number of otoliths from each of the remaining 1 cm length classes. These will be selected randomly from each yellowfin tuna stock assessment region in proportion to the abundance/catch of yellowfin in that region. Note that if additional otoliths from yellowfin tuna >150 cm FL are provided by other project partners within the next 5 to 6 months, they will be incorporated into the analyses.
- Undertake edge type and/or marginal increment analysis as a method indirect validation of age, and daily age estimation will be used to corroborate length at age at one year.
- Weigh all otoliths (if whole) and use generalized additive models (GAMs) to investigate spatial variation in otolith growth across the Pacific, based on the approach presented for bigeye tuna by Farley et al (2018).
- Undertaken additional analysis of fin spines from small fish, where we are confident there has been no loss of increments, to help confirm the location of the first one to two annual opaque zones in otoliths.

It is anticipated that length at age data and preliminary growth parameters will be delivered to the SPC pre-assessment workshop in 2019.

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8 Appendix A: Example images of otoliths and fin spines prepared for annual ageing

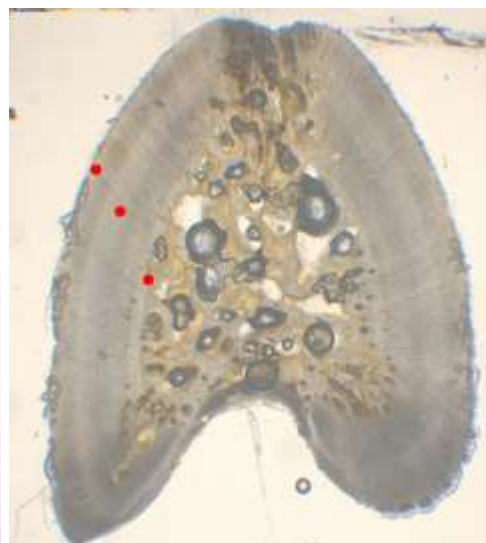
Red dots = zones counted. Red circle = incomplete zone (not counted). The first inflection point and the terminal edge are marked with a + on each otolith. All are shown under transmitted light.



YFT_38, 45 cm FL, count = 0 (nearly 1). Otolith – distance from inflection to the otolith edge is 0.70 mm. Spine diameter at the widest point is 2.6 mm.



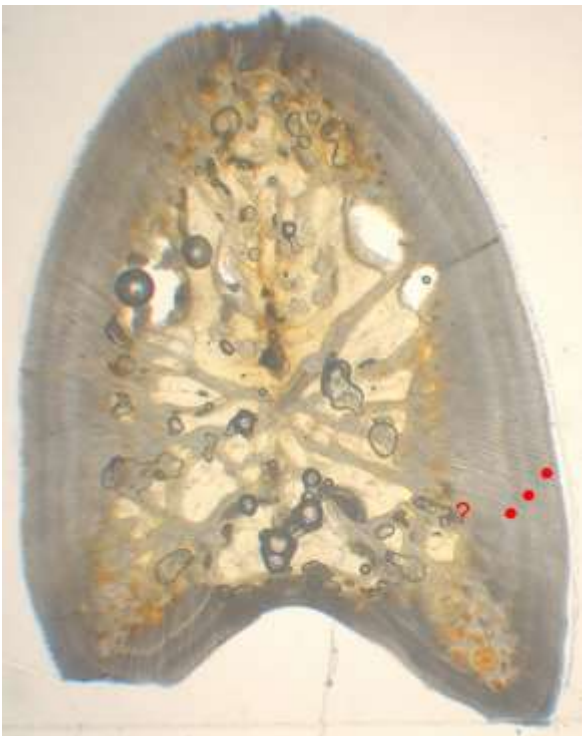
YFT_25, 76 cm FL. Otolith count = 1 opaque or 2 narrow translucent. The spine confirms 1 wide as the 2nd zone is not fully formed. Otolith - distance to the 1st zone is 0.85. Spine - diameter of the 1st zone is 3.6 and the spine diameter is 4.4 mm.



YFT_1, 109 cm FL, count = 3. Otolith - distance to the 1st and 2nd zones are 0.85 and 1.25 mm respectively. Spine - diameter of the 1st and 2nd zones are 3.8 mm and 5.4 mm. No zones appear to be 'missing' due to vascularisation as the 1st zone has a diameter similar to the 1st zone in YFT_25 above. Daily age estimated for this yellowfin was only 416 days, and is likely to be an underestimate of true age.



YFT_34, 139 cm FL, count = 6. Distance to the 1st and 2nd zones are 0.76 and 1.12 mm respectively.



YFT_34, 139 cm FL, count is unknown as several increments are 'missing' due to vascularisation. The 1st three visible zones are marked. The diameter of the 1st marked zone is 6.8 mm.



YFT_4. 172 cm FL. Count = 12. The distance to the 1st and 2nd zones are 0.95 and 1.37 mm respectively.



YFT_4. 172 cm FL. Count is unknown. Five increments are marked but several increments in the core of the spine are 'missing' due to vascularisation. Diameter of the 1st marked zone is 6.7 mm

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