Fish Ageing Services

AOTTP OTOLITH PREPARATION, AGE ESTIMATION AND OTC ANALYSIS

FISH AGEING SERVICES PTY LTD

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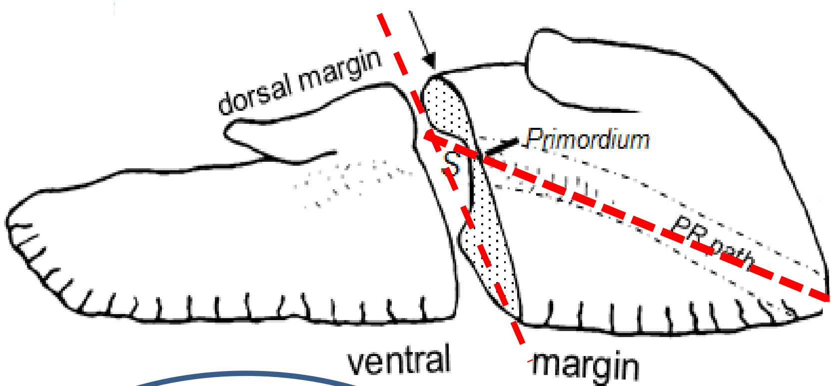
1. PREPARATION AND AGEING OF AOTTP SAMPLES

2. OTC DETECTION AND ANALYSIS

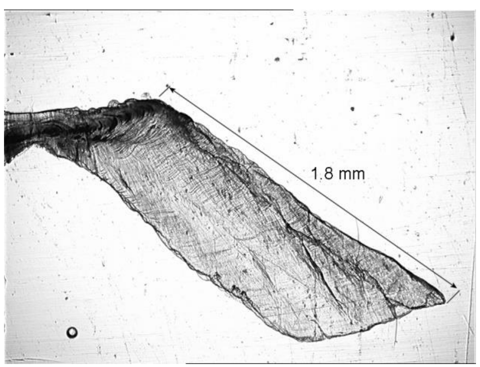
1 – Samples and ageing methods

Species	N	Min length at release (cm FL)	Max length at Release (cm FL)	Min length at Recapture (cm FL)	Max length at Recapture(cm FL)	Time at Liberty range (cm FL)
Yellowfin Tuna	40	38	135.4	40	159	19 -650
Bigeye Tuna	38	36	108	49.5	122	46 -839
Skipjack	5	-	-	-	-	
Little Tuny	1	-	-	-	-	

- Sectioning planes

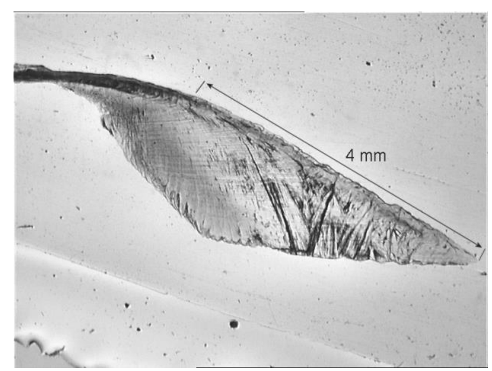


Transverse section



Daily age
Annual age

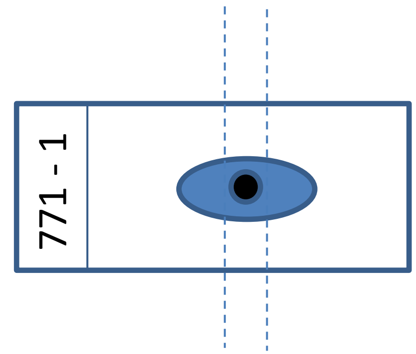
Frontal (longitudinal) section

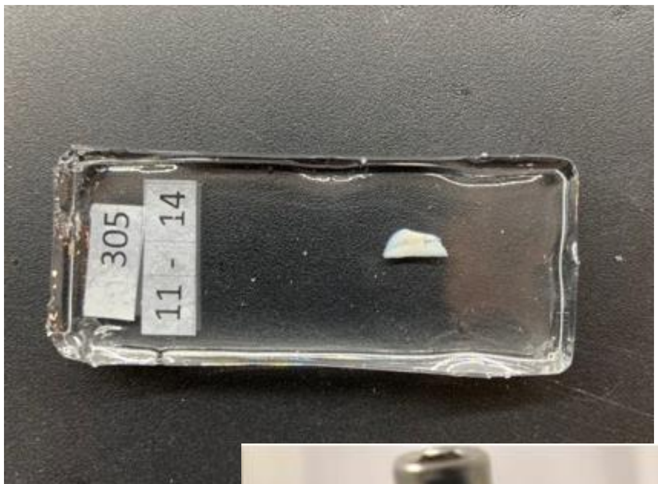


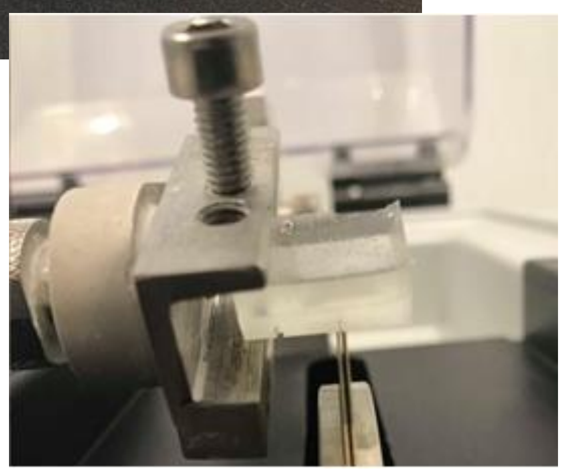
Daily age

- For AOTTP samples transverse sections were used in the age estimation and the OTC detection
  - To be consistent with previous daily ageing studies such as
    - Shuford *et al* (2007) - Atlantic yellowfin
    - Stéquert *et al* (1995) - Atlantic yellowfin
    - Farley *et al* (2006) – Western and central Pacific bigeye
    - Sardenne *et al* (2015) - Indian Ocean bigeye and yellowfin
    - Lehodey and Leroy (1999) – Western pacific yellowfin
  - So that we could complete annual ageing as transverse sections have been used successfully in other tuna species
    - Lang *et al* (2017) - Atlantic yellowfin
    - Andrews *et al* (2020) – Atlantic yellowfin and bigeye
    - Adams and Kerstetter (2014) - Little tunny, skipjack and black tuna
    - Farley *et al* (2006) – Western and central Pacific bigeye
    - Gun *et al* (2008) – Southern bluefin tuna
    - Farley *et al* (2020) – Western and central Pacific yellowfin
    - Neilson and Campana (2008) – Altantic bluefin

Single section method







- Section ≈ 400µm through the nucleus

- First step – Annual preparation
  - Mounted on a slide with crystalbond 509 and ground thinner with various grades of wet dry sandpaper (600, 800,1200 grit) until the section was approximately 320-340µm.
  - Imaged for annual age and then under UV for the OTC detection

- Second step – Continue preparation to allow for daily ageing on the same section

Annual Ageing

- Methods followed those used for other Pacific Ocean tunas which we have worked previously
  - Counted completed opaque zones
  - Assigned a marginal edge category-based on the optical property of the edge - Opaque (O), Narrow Translucent (NT) or Wide translucent (WT)
  - Converted the zone count to decimal age using a birthday of 1st July and the following algorithm:

Age algorithm

From Eveson et al. (2004)

$$\mathbf{a} = \boldsymbol{(n + b) + r/365}$$

Annual ageing - Yellowfin

Sample L10YFT8 - Estimated as 8 or 9 yrs old

Annual ageing – Bigeye

- Sample BET158 – estimated as 8 yrs old

Daily ageing

- Polished on either side until the section thickness reached ≈ 80µm. Care was taken to ensure that the primordium was still contained within the section
  - Final polishing with 5 µm lapping film and then 1 µm aluminum oxide powder
- Counting of micro-increments included:
  - Total count
  - Count from OTC to the edge (detailed later)
  - Where present a count and measurement out to 365th and the 730th increment

- The techniques we used for the interpretation follow those methods we have used previously on Pacific tunas

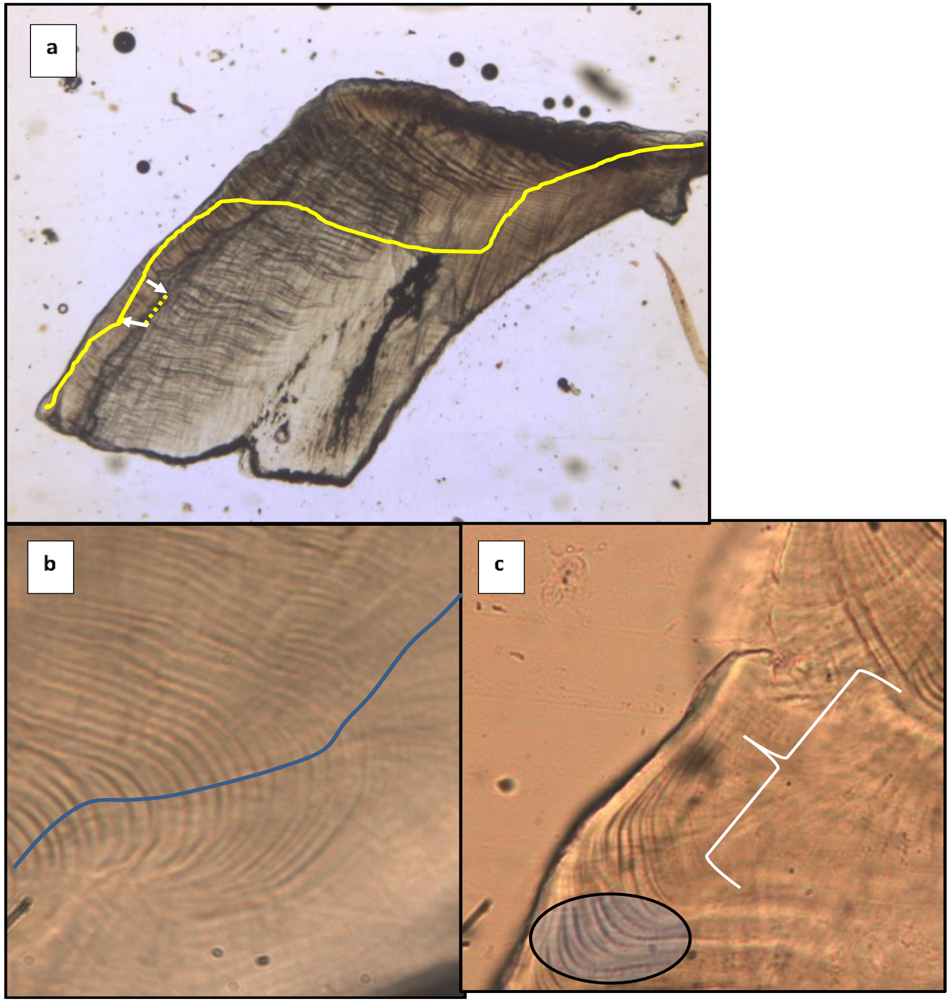


Image of a yellowfin tuna otolith prepared for daily age estimation showing (a) the preferred age reading path (yellow line) and an example were the reading path was shifted temporarily (dotted yellow line); (b) higher magnification image showing the counting path going through the maximum concavity of increments (maximal growth axis); and (c) an otolith section showing two areas where the interpretation is considered subjective. The white brace indicates an area where the microincements needed to be interpolated and the shaded circle indicates the presence of split and overlapping zones.

Not dissimilar to the method used by Sardenne *et al.* (Indian ocean samples) and Shuford *et al.* (Atlantic ocean yellowfin)

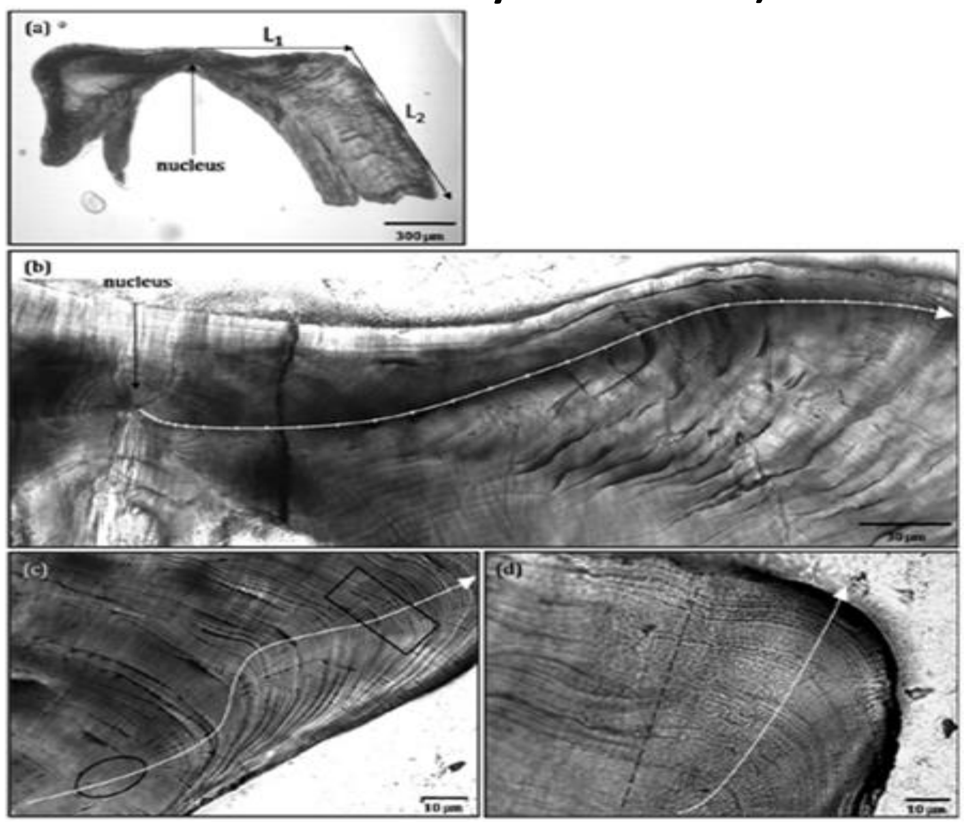
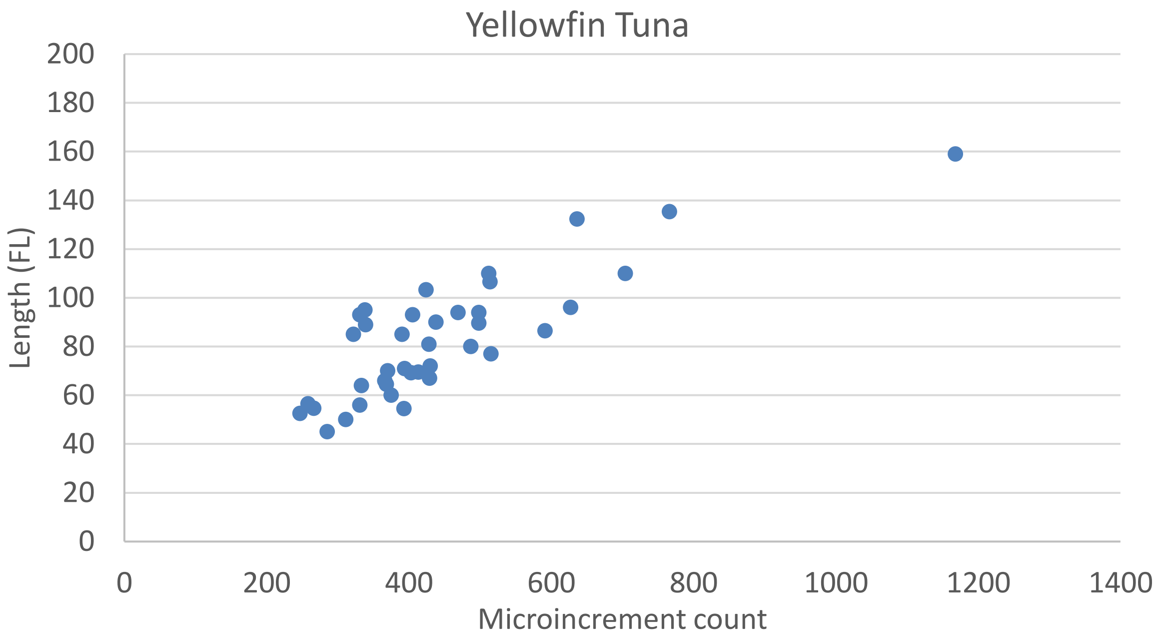
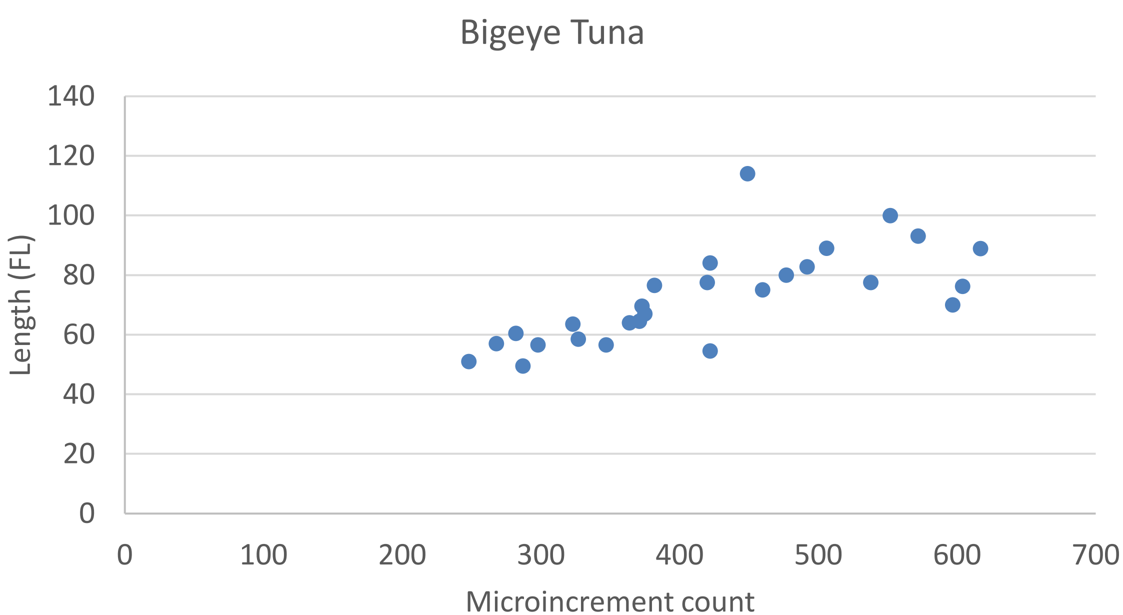
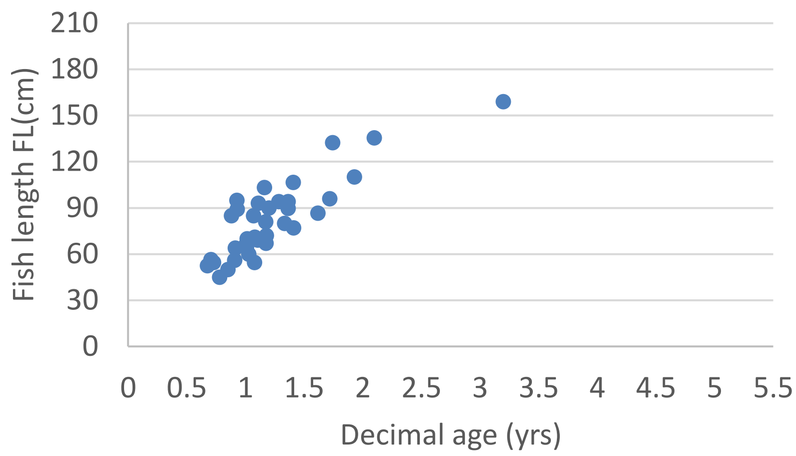


Fig. 2. Image of a yellowfin tuna otolith showing (a) the full transverse section with linear measurement segments L_1 and L_2 (used to compute L_O; black arrows); (b) an otolith section showing the counting path (maximal growth axis) with detected micro-increments (white ticks); (c) an otolith section showing two zones where subjective interpretation is required, the black oval identifying where micro-increments had to be interpolated and the black rectangle identifying where there is some overlap in the micro-increments; and (d) an otolith section (black zone represents the otolith edge) illustrating differences in image resolution (left versus right sides of the dashed line) when the microscope is focused on the reading section. White arrows indicate the reading axis (b–d).

Source: F. Sardenne et al. / Fisheries Research 163 48 (2015) 44–57


Yellowfin Tuna
Length (FL)
200
180
160
140
120
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
Microincrement count


Bigeye Tuna
Length (FL)
140
120
100
80
60
40
20
0
0
100
200
300
400
500
600
700
Microincrement count



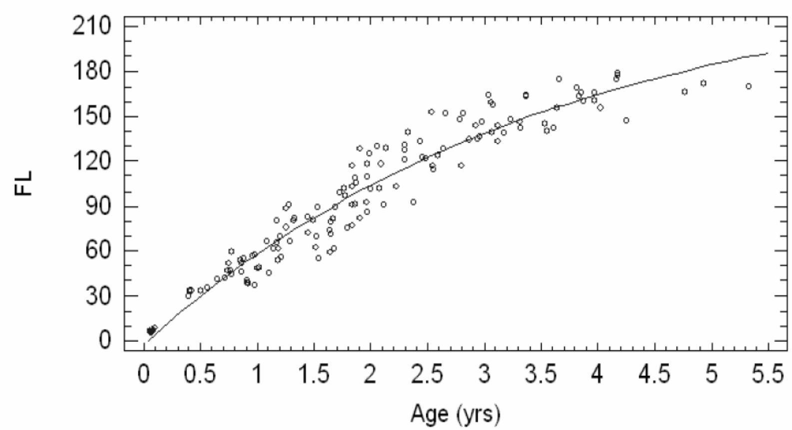
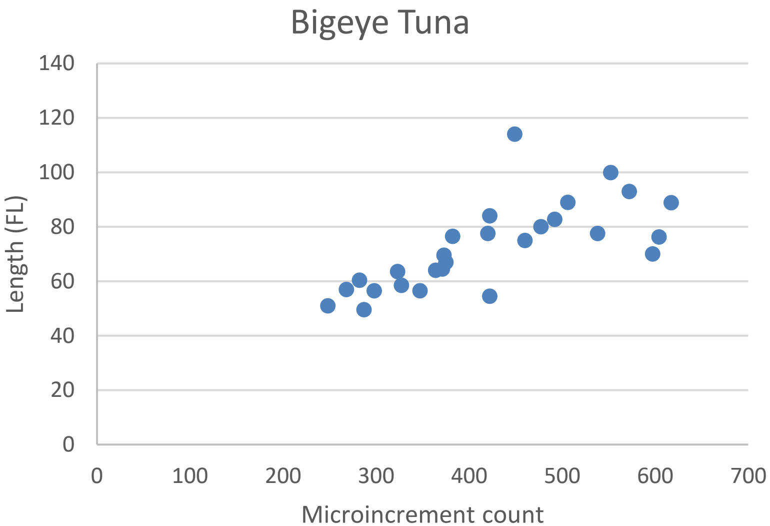
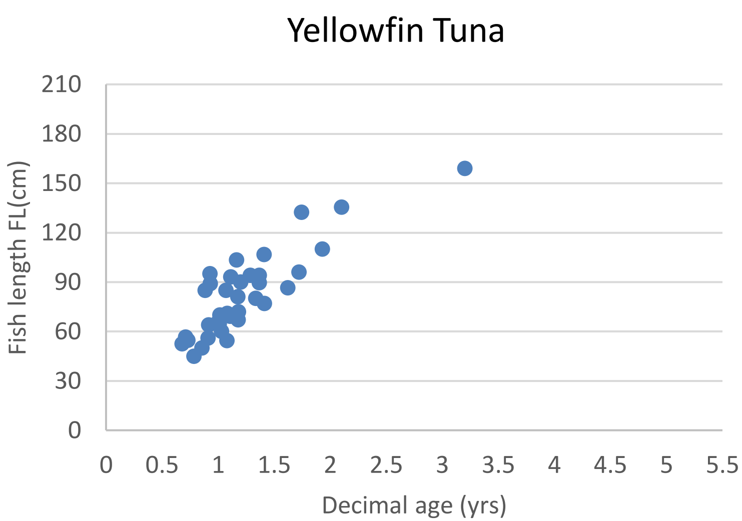


Figure 4. Von Bertalanffy growth curve for yellowfin tuna in the Atlantic Ocean, age estimated were obtained from counts of daily otolith microstructure in sagittal otoliths. The associated von Bertalanffy equation is FL_t = 245.541*(1-exp(-0.281*(t-0.0423))). FL = Fork Length at age t.

Source: Shuford et al. 2007 – Age and Growth of Yellowfin Tuna in the Atlantic Ocean. Col. Vol. Sci. Pap. ICCAT, 60(1):330-341





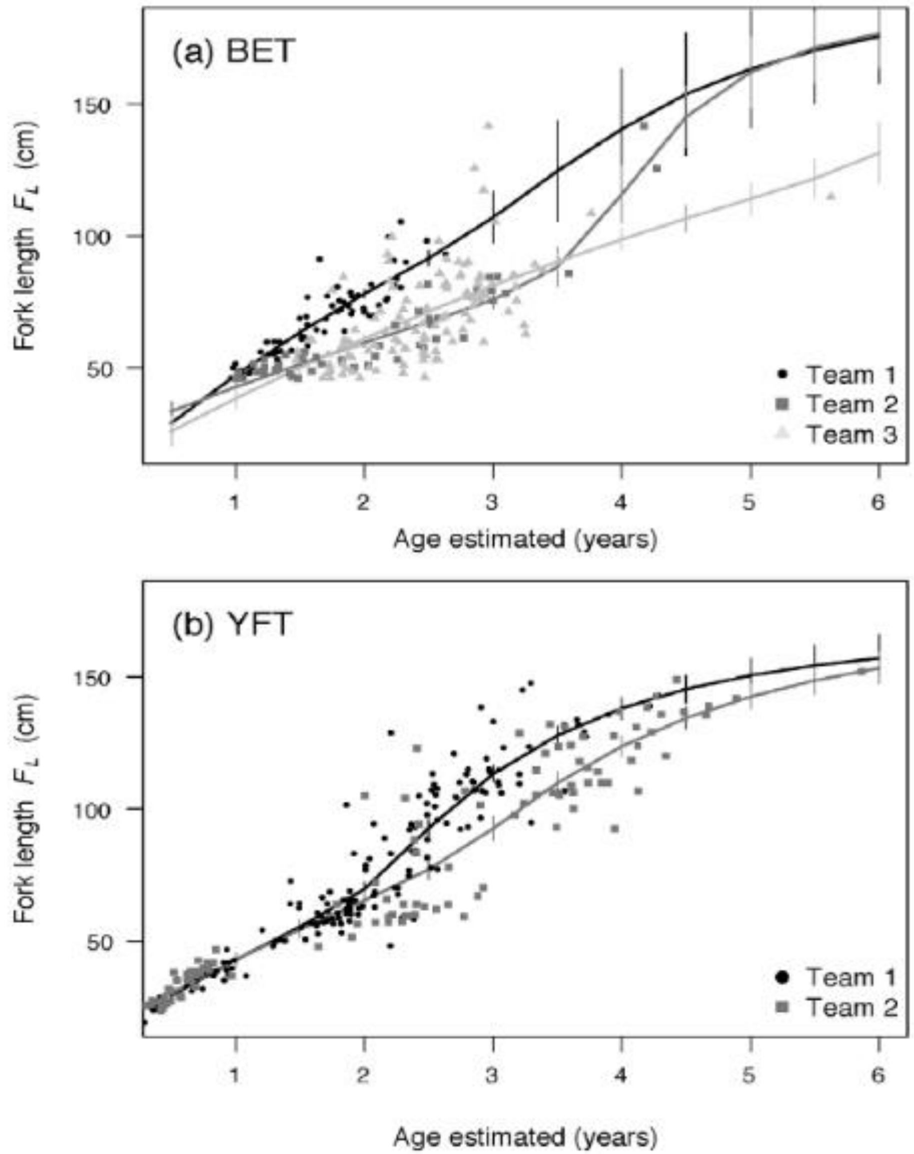
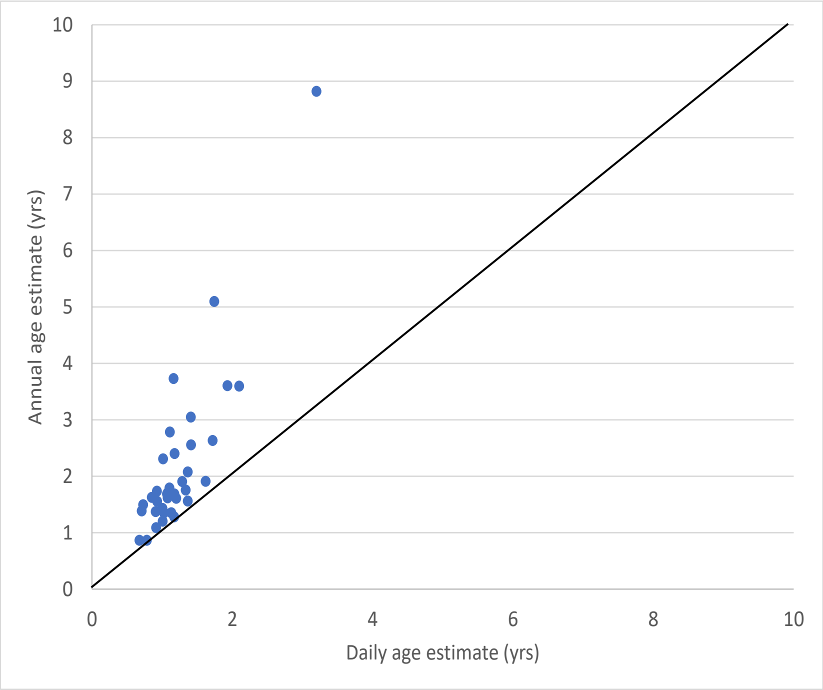


Fig. 6. Growth estimates for (a) bigeye (BET) and (b) yellowfin (YFT) based on the age estimates derived from micro-increment counts made by reading teams 1–3 for 129 BET and 215 YFT.

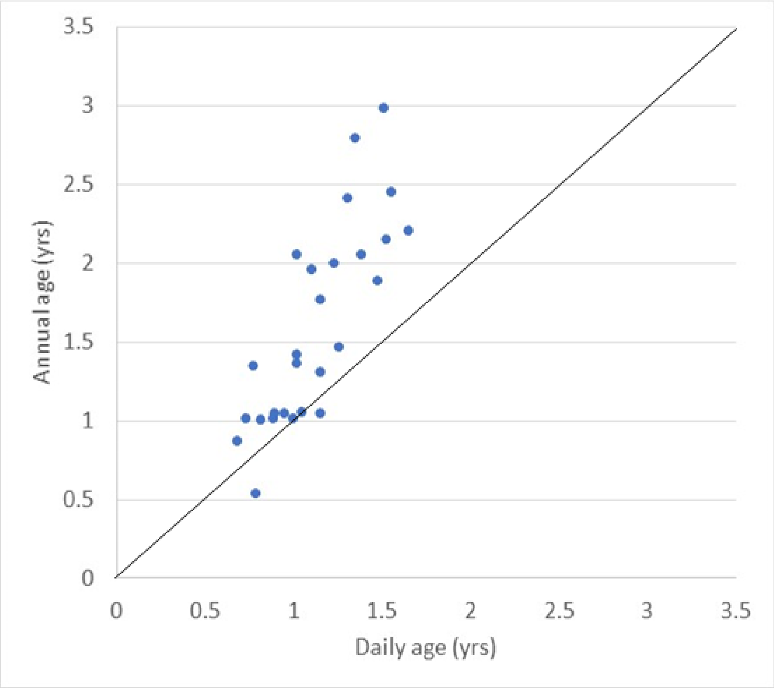
Source: F. Sardenne et al. / Fisheries Research 163 48 (2015) 44–57

Annual age compared to daily age

Yellowfin



Bigeye

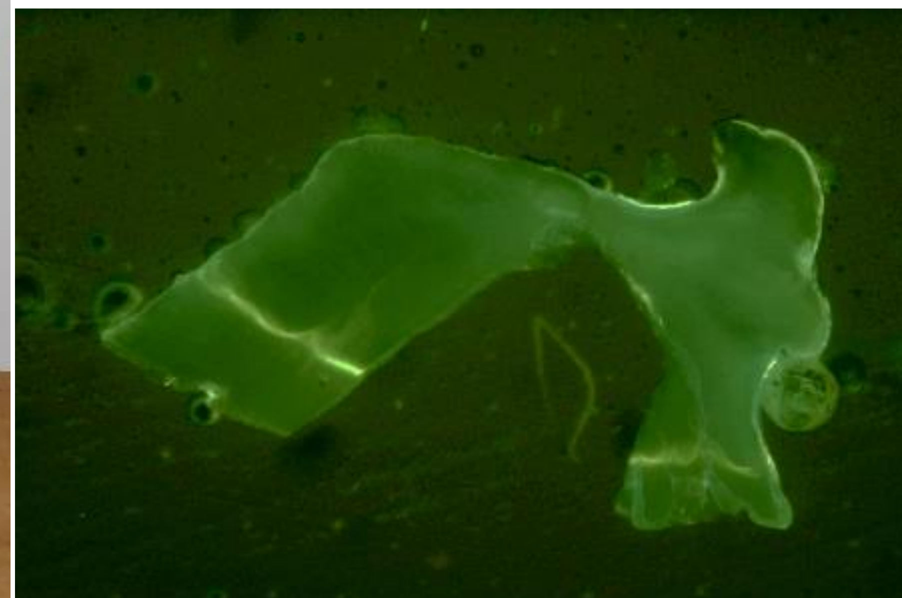


2 - OTC detection in transverse sections

OTC methods

- The annual preparation was illuminated with transmitted light and an image was captured at 25x magnification
- The light source was changed to UV/darkfield to determine the presence or absence of the OTC and an image was captured at the same magnification
- This allowed us to then determine the relative position of the OTC mark within the transmitted image so that we could record the number of opaque zones present between the OTC mark and the otolith margin
- Once the section had been prepared for daily ageing, the number of microincrements between the OTC mark and edge were also counted and recorded

OTC marks were examined for fluorescence under a Leitz Laborlux compound microscope fitted with a 100W UV light source, and a D filter block (excitation filter 450–520 nm) to suit the fluorescent properties of OTC



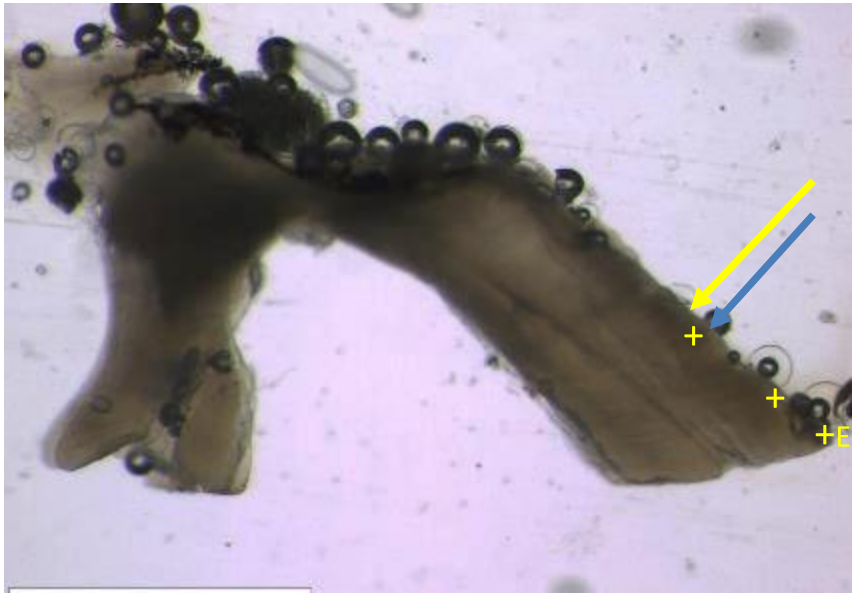
Leitz Diaplan Fluorescent Microscope with Leitz HBO 100 watt mercury fluorescent lamphouse illuminator

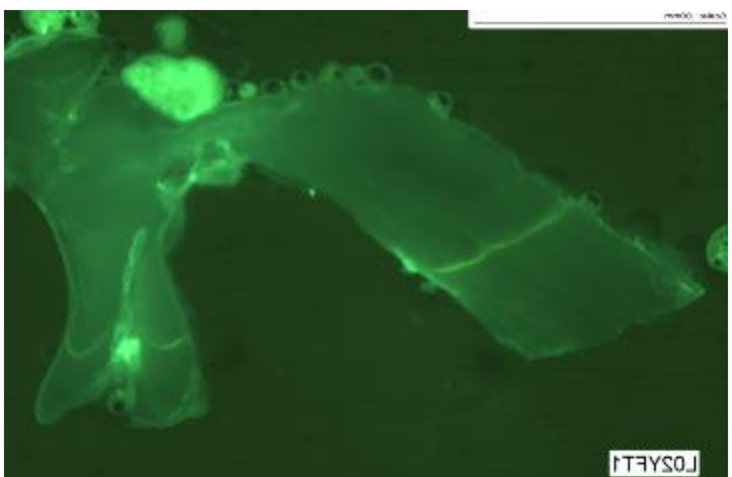
Estimating time at liberty from annual ageing

- Comparing just the number of annual increments present after the OTC mark against time at liberty can be misleading
  - The count of annual increments does not provide a fractional age
  - The timing of the opaque and translucent zone formation is currently unknown

- Attempted to provide an estimate of expected time at liberty which uses:
  - the assumption that opaque and translucent zones are formed on an annual basis
  - The position of the OTC mark relevant to the closest opaque zone
  - The number of completed opaques between the OTC mark and the edge
  - The relative distance between the last opaque zone and the otolith edge
  - Expressed as a decimal age

Yellowfin tuna example





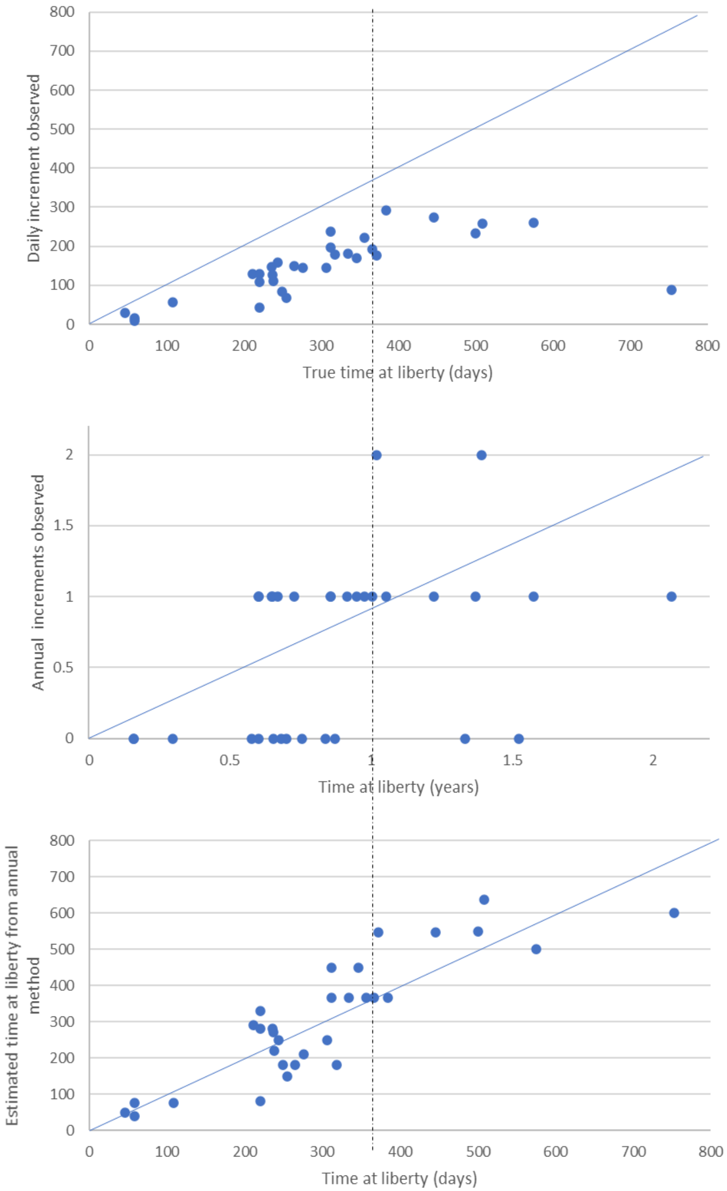
L02YFT1 - (305_009_001)

- Time at Liberty 575 days (31/10/16 – 29/5/2018)
- Estimated time at liberty from annual ageing = 500 days
- Total microincrement count = 591
- Zone count between OTC and edge = 261 (estimated daily age at tagging = 330).
- Distance (inside measurement) to 365 = 1.018mm.
- 51 FL (cm) at release and 86.5 FL (cm) at recapture

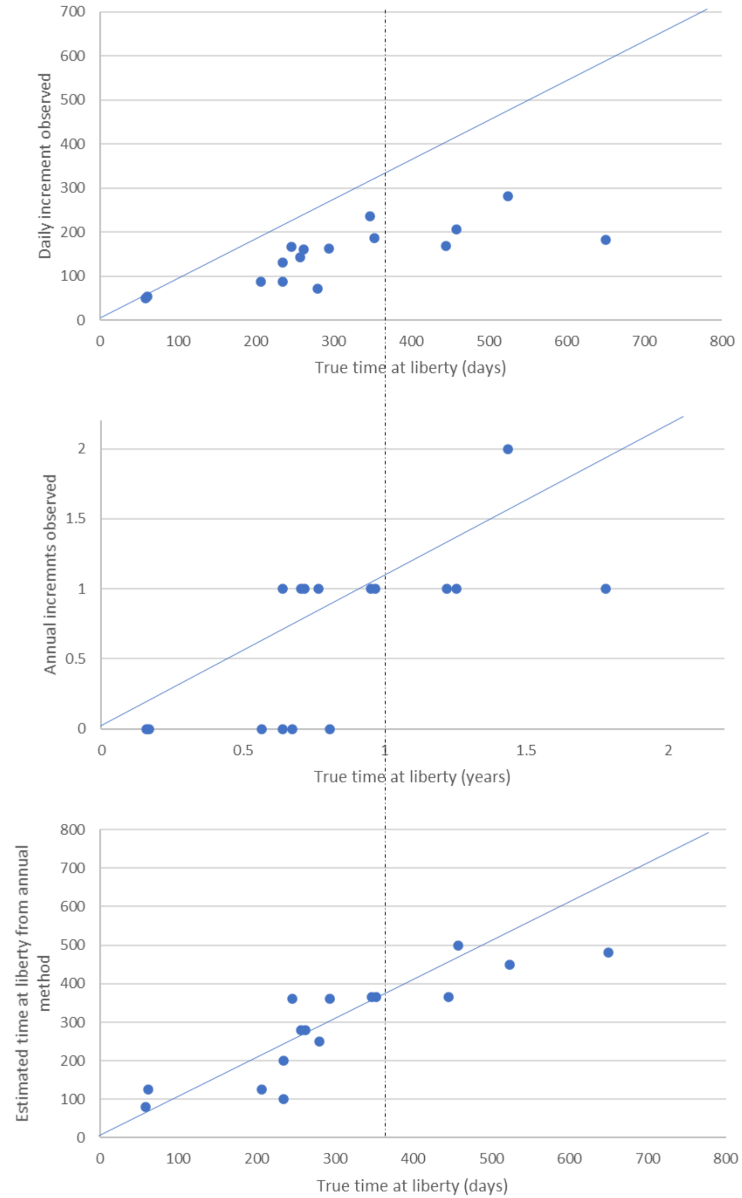
Yellow arrow – OTC mark

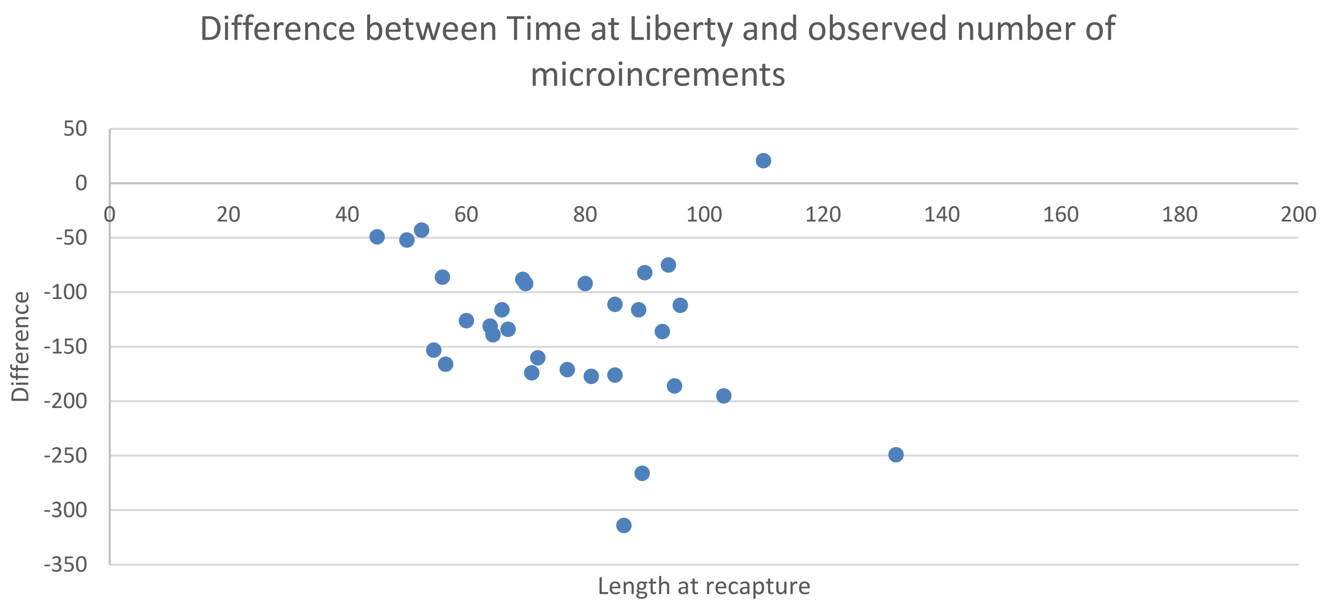
Blue arrow – First Opaque increment

Yellowfin Tuna

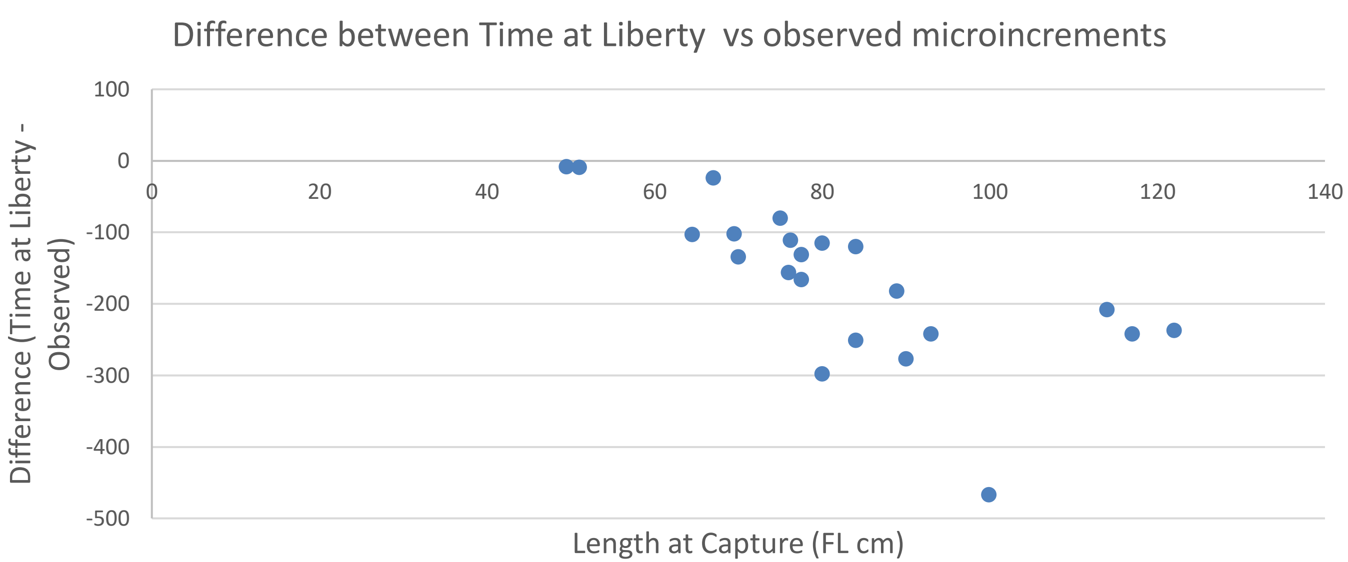


Bigeye Tuna





Yellowfin



Bigeye

Conclusion

- Counting microincrements in samples >50cm may lead to an underestimation of age.
  - Daily ageing for samples <50cm looks to still be a valid method and is still the best method for determining juvenile growth.
  - Also extremely useful for verifying the age at which the first opaque zone is deposited (perhaps also 2nd).
- Tropical tuna otoliths do contain annual growth increments which have been validated in the this study in Yellowfin up to 159cm and in Bigeye up to 122cm

Future work

- Hoping to publish this work
  - Add more samples to the analysis which should extend the size range and times at liberty
  - Compare transverse counts with frontal counts for both species on a small number of samples
- As time goes by, we need to recognise that there will be ongoing recaptures from the AOTTP and valuable samples/otoliths may still need to be analysed.

Relevant References

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