

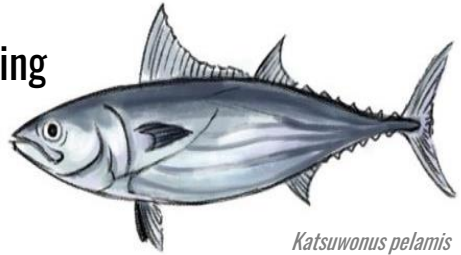


25th IOTC Working Party on Tropical Tunas Meetin (WPTT25): Oct 31st – Nov 4th, 2023



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Advances in the development age reading methods from fin spines and otoliths for Indian Ocean skipjack tuna



Katsuwonus pelamis



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1. Identification of first annulus

- Estimate daily age of fish 28-30 cm FL using otoliths.
- Measure otolith size for daily age – otolith size relationship.
- Measure fin spine diameter (of same fish) for daily age – spine diameter relationship.

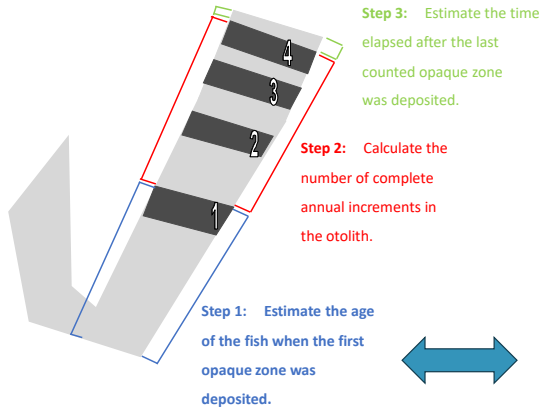
2. Validation

- Measure additional OTC marked otoliths from Sardenne et al. (2015) to examine the daily otolith growth rate by fish size & time at liberty.

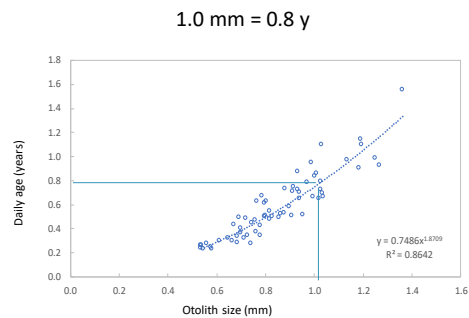
3. To help us in the otolith interpretation:

- Identify 'clear' spines with matching otoliths.

Before developing reading protocols for each calcified structure, participants identified crucial follow-up actions (tasks) that must be addressed as a stepwise process before any age estimates obtained from fin spines and otoliths can be included in Indian Ocean SKJ age-structured stock assessment models (see WD IOTC-2023-WPTT25(DP)-08, for further details).



(**This info paper focuses on progress made on step 1)



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- A novel age algorithm was discussed during the session to determine the fractional age using counts of opaque zones and otolith measurements. The algorithm is particularly valuable for species such as skipjack tuna that may spawn throughout the year, as it does not rely on a single birth date assumption for all fish.
- The *jes*timation algorithm follows a three-step process (see figure) but currently we are addressing **step 1: the relationship between daily age and otolith size to estimate the age of the fish when the first annual opaque zone is deposited.**



Previous

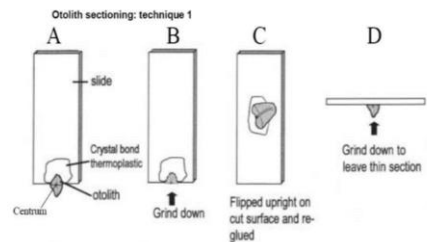
In 2021, daily age was estimated for 6 samples (31 – 65cm SFL). The distance from the primordium to the 25th, 50th, 75th, and 100th increment for each sample was also measured

Current

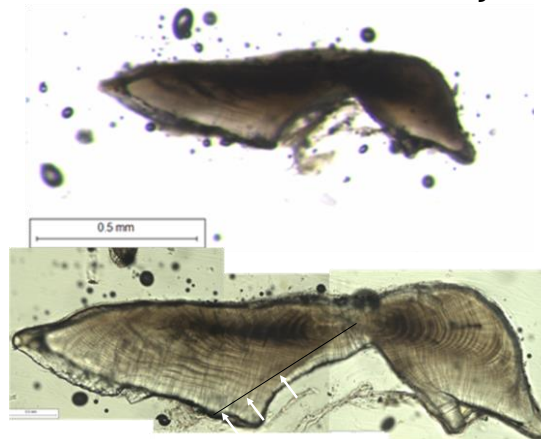
An additional 30 samples were selected for daily age reading from fish ranging between 28cm and 40cm SFL

METHODS

- Transverse sections were prepared (thickness ~ 100µm)
- Daily age was provided by counting microincrements from the primordium to the otolith edge
- Distance from primordium to edge measured



- During the grinding process once the section thickness reached 280µm we took an image so that the sample could also be used for annual ageing.
- The samples were aged twice, unless the difference was greater than 10%, then a 3rd read was conducted. The age was the average of the 2 closest readings.

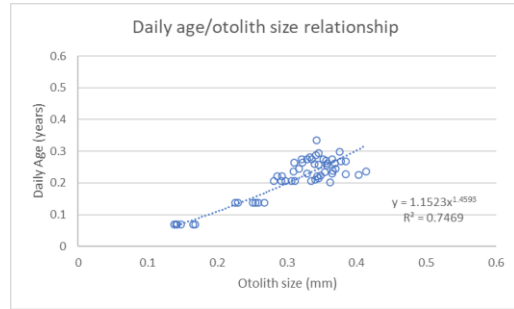
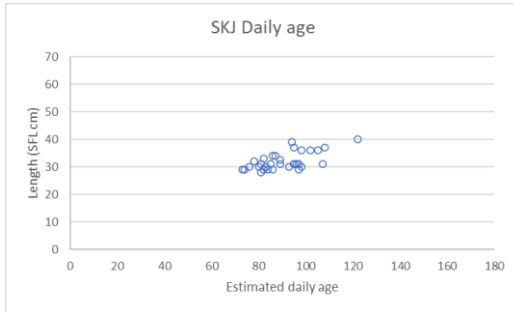


Example 315_14_142 (SKJ-AZT-NWIO-0016) - Total daily age = 76. Position of the 25th , 50th , and 75th , increment shown with white arrows

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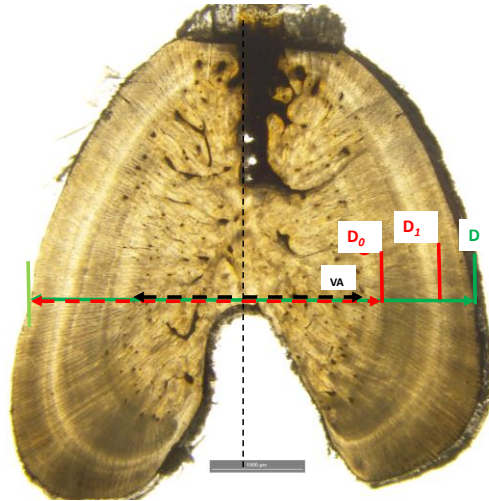
Top image is the sample at the section thickness used for annual ageing. **Bottom** image is the same sample ground thinner for daily increment counting. White arrows indicate the position of the 25th, 50th, and 75th increments. Black line indicates the transect for measuring the distance between the primordium and the edge.



Still to do – Prepare and age a few more samples between 40-45cm SFL

- Data was combined with previous data and the age at length and otolith measurement at age was calculated. Limited to what size range we have and likely limitation of the daily ageing method.
- Micro-increment counts in transverse sections >120-140 days might not be accurate.

1. IDENTIFICATION OF FIRST ANNULUS : Fin spine measurements

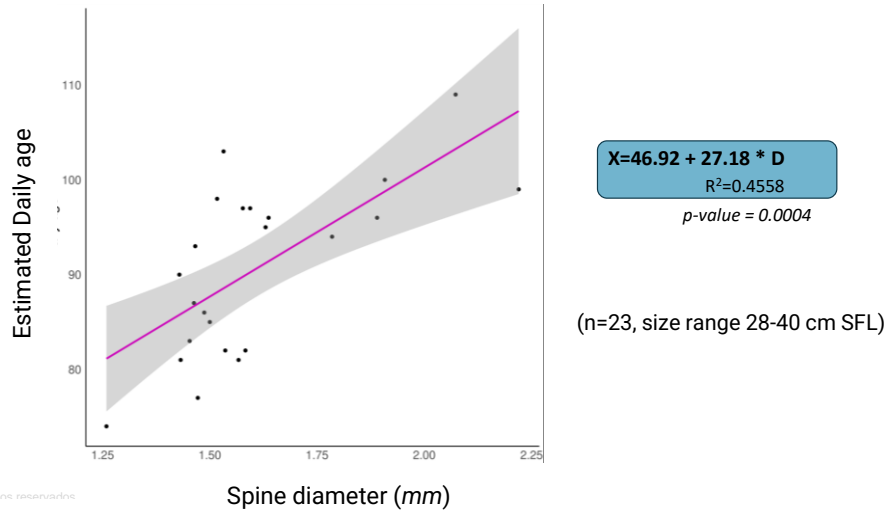


- D** Spine Diameter
- D_n** Diameter of the "n" translucent band
- VA** Vascularised Area

7 Following criteria adopted in Cunha-Neto et al., 2021, and Soares et al., 2022

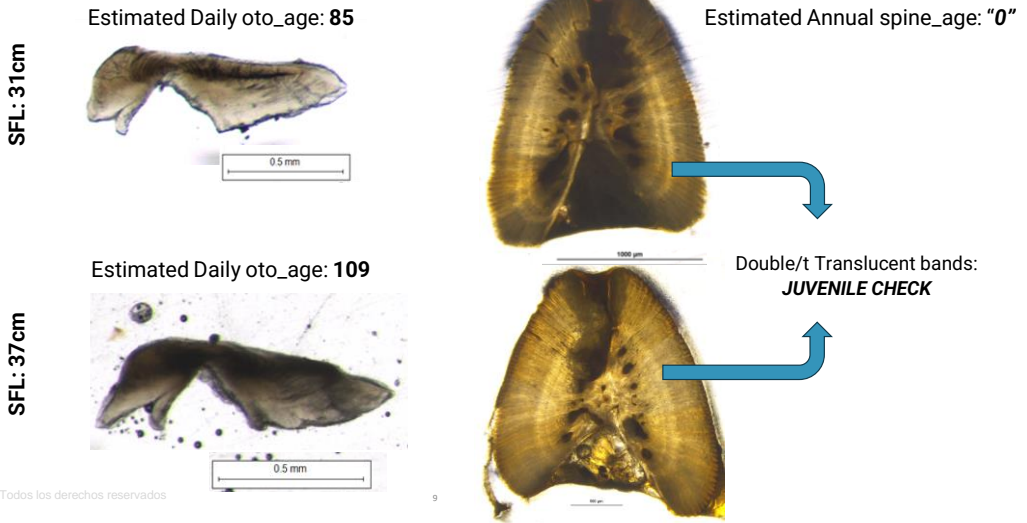
- The group agreed to determine the best approach to measure fin spine diameter and translucent bands width to be consistent with other SKJ age studies from other stocks.
- As such, measurements of fin spine cross-sections is performed following the protocols adopted by Soares et al., 2022, and Cunha-Neto et al., 2021 for ageing western Atlantic Ocean SKJ (SW Brazil),
- The figure shows a cross-section of first dorsal fin spine of *K. Pelamis* (SFL: 42 cm) showing main measurements: **D** as diameter of fin spine → the horizontal distance between the outer edges measured on the line of the caudal concavity, *i.e.*, above the posterior notch, where the least band curvature occurred to avoid errors due to the loss of material from the vascularized core area (Vilela and Castello 1991, Cunha-Neto et al., 2021, Soares et al., 2022); **D_{n=1,2,3,..}** diameter of each *annulus* → the distance taken also above the posterior notch from the outside edge of each translucent band to the opposite edge of the fin spine cross-section; **VA** (vascularized area) → the horizontal distance between the outer edges of the vascularized area measured on the same line of the caudal concavity.

1. Spine diameter-Daily ageing (Otolith) relationship



- Linear regression model (solid pink line) with smoothing based on 95% confidence intervals (grey area) for fin spine diameter (mm) and estimated daily ages from otolith measured across growth intervals in the fin spine and otolith section respectively with daily age (n=23, SFL 28-40 cm) .
- The relationship between daily otolith age and the maximum spine diameter (D) is used
 - to determine the size of fin spines at various (*daily*) ages and help to confirm whether the position of the first translucent zone.
 - to confirm the position of the first translucent zone
 - To determine the maximum width of the vascularized area where an annual structure it is not expected to be counted.

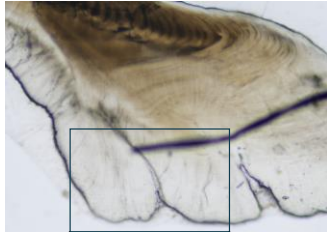
1. Otolith vs Fin spine cross-sections (collected from same individual)



Examples of otolith (left panel) and fin spine (right panel) sections from same ind.,

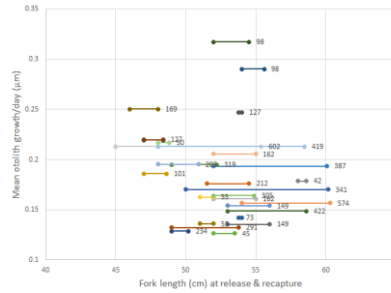
- Fin spine sections in both cases clearly shows two translucent bands (doublet pattern).
- Considering the estimated daily age, we confirm that the position of these earliest first translucent bands in matched fin spine sections do not correspond with the first year of the fish life, and cannot be counted as annual.

2. Measure otolith growth after OTC mark



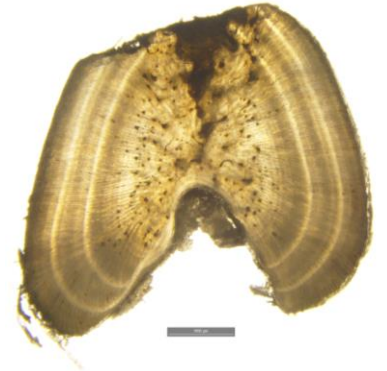
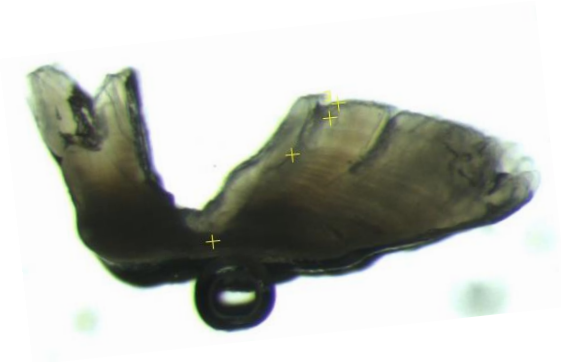
Otoliths from Sardenne et al. (2015)

- Otolith growth: 0.13 - 0.32 $\mu\text{m}/\text{day}$ (mean ~ 0.19)
- No pattern with fish length or time at liberty
- Could use otolith size to give proxy age
 - measure after max confident daily age (~ 40 cm FL)



Relationship between mean otolith growth per day after the OTC mark (i.e., otolith growth during time at liberty) and fish size at release and recapture. The label on the recapture length is the time at liberty in days.

3. REFERENCE COLLECTION: Do the "easy to read" otoliths match the fin spine also showing a clear growth zone pattern?

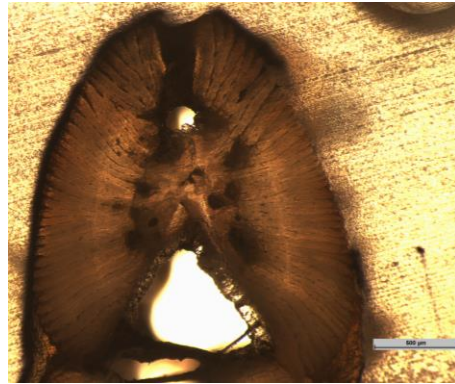
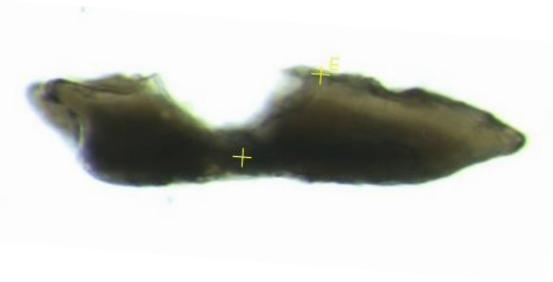


SFL: 65cm

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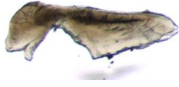
- The group identified cleared fin spines and the matched otoliths to help us in the otolith interpretation.
 - The exchange paired images set (annotated images) will be used as a preliminary reference set.
- Here it is showed two examples.

3. REFERENCE COLLECTION: Do the "easy to read" otoliths match the fin spine also showing a clear growth zone pattern?



SFL: 33 cm

How these results will integrate into the protocol

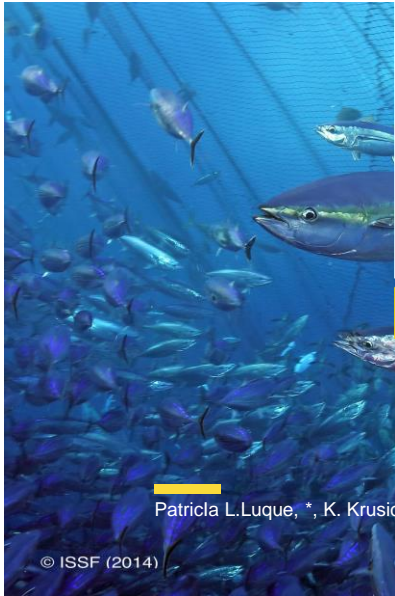


- Select some additional samples that might be 1+ that show a reasonable clear first zone and **count micro-increments to the start of the first opaque zone to determine the daily age at first increment formation.** Likely extend the daily age/otolith size/spine size relationship further.
- To include new extra otoliths from larger specimens >1+ and provide daily counts from the primordium to the start of the first opaque zone. We will use the age data to calculate daily age to otolith size relationship to be used in step 1 of the novel otolith age algorithm.

How these results will integrate into the protocol



- Annual age criteria (spine measurements) will be applied on the entire fin spine sections database (n= 627).
- To determine the maximum width of the vascularized area where an annual structure it is not expected to be counted. Use this as a proxy when following the proposed fin spine reading criteria.
- MIA and edge type analysis using the whole fin spine sample set collected all year around. Results are expected to confirm a yearly periodicity of annulus formation in fin spines.



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MANY THANKS

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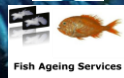


Photo: Fabien Forget