REPORT OF THE INTERNATIONAL WORKSHOP ON THE AGEING OF SKIPJACK TUNA FROM INDIAN OCEAN

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EXECUTIVE SUMMARY

A recent study found differences in age estimates of skipjack (Katsuwonus pelamis) (SKJ) based on otoliths and dorsal fin spines obtained from the same fish caught in the Indian Ocean. This difference highlighted the need to develop standardized and validated aging criteria for both hard structures. In response, an international workshop was conducted in February 2023, with the objective of discussing and reviewing preparation protocols, reading criteria, validation methods to ensure consistent and comparable age data across laboratories. This document summarizes the key points of discussion and the progress achieved during the workshop on the following topics: 1) what constitutes annual growth zones in both the spine and otolith sections, 2) where are the inconsistencies between otoliths and fin spines readings, 3) assessment and adoption of edge type criteria for fin spine and otolith readings, and 4) age interpretation differences between readers. Protocols for fin spine and otolith preparation were reviewed and found to be consistent with previous studies on SKJ aging, with a few exceptions. The group agreed to revise the fin spine and annuli diameter measurements approach following an agreed age interpretation method. Results from a previous OTC mark and recapture study in the Indian Ocean suggested that daily increment counts in SKJ otoliths were unreliable. As the marked OTC otoliths have been prepared for daily age reading, they are not useful in directly validating the annual increments. However, the group suggested that the measurement of the amount of otolith growth after the OTC mark may be useful in verifying the annual increment widths and these samples have been selected for further investigation. A novel age algorithm was discussed to determine the fractional age using counts of opaque zones and otolith measurements. This algorithm uses the relationship between daily age and otolith size to estimate the age of the fish when the first opaque zone is deposited, which needs further investigation. The group recognized the difficulty of assigning the edge type, particularly in otolith sections. A more simplified set of edge type criteria was suggested. In addition, as a general criterion, only fully formed translucent and opaque areas on fin spines and otoliths, respectively, would be counted as rings. Participants identified crucial follow-up actions (tasks) that must be addressed as a stepwise process before any age estimates obtained from hard structures can be included in SKJ age-structured stock assessment models. The group also concluded that further age validation studies (e.g., using post bomb C^{14}radiocarbon chronologies) would be highly beneficial for this species.

Keywords

Skipjack tuna, fin spine, otolith, ageing, OTC daily age validation

1. Rational for workshop

Estimating the age of skipjack tuna (*Katsuwonus pelamis*) (SKJ) from counts of micro-increments has been challenging and remains uncertain due to considerable variation in age estimates among otolith readers and a lack of validation that micro-increments are deposited daily basis (Eveson et al. 2012, 2015). Investigating alternate methods of ageing, such as counts of assumed annual growth zones in otoliths (as opposed to daily zones) or other hard structures (such as dorsal fin spines), has been scarcely explored for this species in the Indian Ocean. In 2021, for the first time, as part of the GERUNDIO project[1], researchers explored the feasibility of using the annual ageing method on otoliths and fin spines collected from the same fish in the Indian Ocean to determine the most appropriate structure to estimate the age of SKJ (Luque et al. 2021). Preliminary comparative analysis showed a high discrepancy in age estimates (i.e., zone counts) among otoliths and fin spines from the same fish. This highlighted the need to examine ageing methods among structures and to develop appropriate ageing criteria, age validation and verification methods to accurately estimate the age of SKJ in the Indian Ocean. Given this, and generalized uncertainty in SKJ ageing using annual aging methods regardless of stocks and oceans, it was important to hold a workshop of ageing experts to determine if an agreed and validated ageing method can be developed to improve the comparability of ageing data sets, to standardize fin spine and otolith preparation protocol and ageing criteria, and to ensure consistency across labs when ageing this species. The workshop allowed participants to share knowledge, methodologies and assess the different aspects of ageing determinations that contribute to discrepancies in annual ageing among hard structures. A three-day workshop was scheduled on Feb 24, 27, and 28, 2023 in AZTI, Pasaia, Spain. After opening remarks and introductions the workshop began with presentations reviewing of SKJ biology including current knowledge/research on life history (age, growth, maturity, spawning, diet, and trophic behaviour) as well as abiotic factors (e.g., SST, currents, circulation, and monsoons in the Indian Ocean) that play a role in its individual/population growth dynamics. The workshop allowed participants to share knowledge, methodologies and assess the different aspects of ageing determinations that contributed to the discrepancies in estimates of age obtained from both hard structures.

[1] Collection and analysis of biological samples of tropical tunas, swordfish, and blue shark to improve age, growth and reproduction data for the Indian Ocean Tuna Commission (IOTC), FAO Contract No. 2020/SEY/FIDTD/IOTC - CPA 345335.

2. Presentations and Discussions

Day 1

Life history and stock structure of skipjack tuna in the Indian Ocean (I. Artetxe- Arrate, AZTI).

Here, we reviewed the available literature regarding the stock structure and life history strategics of skipjack tuna in the Indian Ocean. Regarding habitat preferences, skipjack tuna inhabit the epipelagic zone, preferentially occupying the surface mixed layer above the thermocline of tropical and subtropical water around the world, although larvae require warmer waters (>24ºC) and are confined to narrower habitat ranges. In warm equatorial waters, skipjack spawn year-round while further away from the equator, spawning season is limited to the warmer months. As with other tuna species, skipjack tuna are highly opportunistic so that their diet is mainly determined by body size, period and location, and are "energy speculators" meaning that high rates of energy are invested in order to obtain even higher rates of energy. Skipjack tuna is considered the fastest growing species of all tuna. In the Indian Ocean, there has been 19 different regional growth curves described, but they reported a wide range of growth rate coefficients (k), with the latest studies (Murua et al. 2017) suggested a two-stanza growth model; a rapid growth in the first stage, followed by a slower growth in the second stage. In terms of stock structure, studies performed to date show inconclusive results. Tagging results describe fast and large-scale skipjack tuna movements in the Indian Ocean, but also regional differences. Otolith chemistry studies did not sed any light into the stock structure of skipjack tuna in the Indian Ocean, and studies carried out with genetic markers show co-existence of genetically differentiated groups of skipjack tuna but no clear spatial pattern.

Reproductive strategies of skipjack tuna in the Indian Ocean (M. Grande, AZTI)

The reproductive strategy of skipjack in terms of oocyte growth, recruitment and reproductive traits were presented. The estimated length at which 50% of the female population reached maturity is 39.9 cm (equivalent to ~1.5 years). The shape of the maturation curve indicates that when fish overcome threshold levels for growth or energy stores, individuals are submitted to a strong selection pressure to initiate maturation. Around 70% of the mature population is in spawning capable phase year around, with period of more intense sexual activity mainly during the monsoon events (i.e., the north-east and south-east monsoons). However, high variability of the condition indices suggests a continuous recruitment to the spawning population. The reproductive capacity of skipjack is the highest during north-east monsoon and is positively related to hepatosomatic index. As such, skipjack might increase the energy invested to reproduction when food quality and/or availability is enhanced. Lipids are not stored in somatic tissues prior to the spawning season in order to be used as fuel provisioning of reproductive expenditures. Skipjack might finance oocyte recruitment mainly by using energy gained concurrently by feeding and, therefore, seems to be oriented towards an income breeding strategy. Responding to the energy allocation strategy (income breeding) adopted by the species, peaks in the reproductive activity of the population are related to seasonal increase in food quality and abundance.

Overview of SST, currents, circulation & monsoons in the Indian Ocean (J. Farley, CSIRO).

This presentation mainly focused on ocean currents and the influence of the monsoon in the Indian Ocean (IO) surface circulation. Maps and monthly time series of sea surface temperature (SST), surface salinity, average dissolved oxygen was presented from Davies et al., 2020; PSTBS-IO project Final Report[2]) for 2017-2019. It was noted that during the winter monsoon, a prominent feature south of the equator is the development of SE Counter Current flowing eastward. In contrast, during the summer monsoon, the South Equatorial Current and East African Coast Current supply the northward- flowing Somali Current. The Intertropical Convergence Zone changes its position with the seasons – it is south in December to March and north in June to September. Of interest was the SST plots that indicated a bimodal distribution with temperatures peaking approximately in April/May and again in October /November in the western and central IO. In the northwest IO, SST peaks occurred in May/June and October.

Age and growth of skipjack tuna from the Atlantic Ocean: comparison of age from otolith, fin spine and vertebrae (R. Agnissan, CRO).

This presentation provided preliminary results of a PhD about age and growth of skipjack tuna from the east Atlantic Ocean by comparing estimates of age obtained from otolith, fin spine and vertebrae collected from the same fish. This study reviewed the preparation methodology for the three hard structures and the age criteria used. Fork length ranges from 33 and 70 cm. Preliminary results of marginal increment analysis in fin spines showed that translucent bands are forming once a year between June and September, validating the seasonal periodicity of annuli formation. The estimates of age obtained varied from 0 to 7 years from fin spine, from 1 to 6 years from vertebra, and from 0 to 4 years for otoliths. In addition, the comparisons of estimates of age from otolith vs. fin spines and otolith vs. vertebrae showed an underestimation of age by the otolith for most age classes. The comparison between estimates of age obtained from otolith counts vs vertebra shows that the age is underestimated by the otolith for individuals older than 2 years. Regarding the comparison of estimates between otoliths and spines, the age bias graph also showed an underestimation of age at 2 years old by otolith. While the comparison between the spine and the vertebra shows that the age is overestimated by the vertebra for individuals aged younger than 4 years and underestimated for individuals aged older than 4 years.

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[2] Population Structure of IOTC species and sharks of interest in the Indian Ocean: Estimation with Next Generation Sequencing Technologies and Otolith Micro-chemistry. FAO contract.

Skipjack growth from tag-recapture data in the Indian Ocean (J. Farley, CSIRO).

During this presentation, the research conducted by Eveson et al. (2015) on estimating the growth of skipjack in the Indian Ocean (IO) using tag-recapture data was introduced. Three common sources of growth information were highlighted: changes in length and time at liberty data from tag-recapture experiments, direct age and length data from hard-parts such as otoliths, and length-frequency data from commercial catches. However, in Eveson et al. (2015), only the data obtained from the Regional Tuna Tagging Project of the Indian Ocean (RTTP-IO) was analyzed. The presentation included information on the size of the fish at release and recapture, the time at liberty, daily growth rates, and length-at-age. The estimated growth curve was then compared to other growth curves from the IO based on length frequency and tag-recapture analyses. This highlighted the substantial variability in growth among the studies.

Decimal/fractional age estimation: A novel age algorithm (J. Farley, CSIRO).

In this presentation, a novel age algorithm was introduced to determine the fractional age of tuna by using counts of opaque zones and otolith measurements. The algorithm follows a three-step process using (1) the relationship between daily age and otolith size to estimate the age of the fish when the first annual opaque zone is deposited; (2) the number of complete annual increments; and (3) the marginal increment size, to estimate the total age of the fish. 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. Further information was be obtained from Farley et al. (2020) (See WCPFC-SC16-2020/SA-WP-02 https://meetings.wcpfc.int/node/11692.

Overview GERUNDIO project: Summary of samples analyzed, locations, years, length frequency (I. Artetxe, AZTI).

This presentation provided a brief update of the biological sampling achieved during the GERUNDIO project[1] that aimed to develop and implement a sampling scheme to support the collection of biological samples and conduct analysis on these samples to facilitate the estimation of age, growth and reproduction of tropical tunas, swordfish, and blue sharks. Samples of previous projects were collected (505 otoliths and 184 spines) and a new sampling was also conducted (805 otoliths and 592 spines). However, samples are not evenly distributed across the whole Indian Ocean, with the eastern region still being underrepresented. Fish size of available samples ranged from 30 to 79 cm, and all months are well represented except May and December.

Fin Spines: preparation, reading method, nucleus vascularization, edge assignment (P. L. Luque, AZTI).

Here, a brief background was provided on the status of knowledge about the fin spine as a bone-like structure and their use for annual ageing in tuna species. The presentation reviewed the fin spine preparation methodology (i.e., sample extraction, cleaning, sectioning, digitalization, and ageing criteria) used in previous work on SKJ fin spines, following the protocol developed for Atlantic bluefin tuna (*T. thynnus*) (Luque et al. (2014)) and how it compared with similar studies on SKJ age in other stocks (e.g., SW Atlantic). In contrast to what has been commonly reported in other tuna and tuna-like species (Murua et al. 2017 for review), the fin spine nucleus vascularization that causes the loss of the earliest growth marks is moderate or partial in the skipjack tuna and does not seem to be a limitation in terms of ageing, and hence no age correction would be needed. This was similar with other SKJ ageing studies in the SW Atlantic (e.g., Garbin and Castello, 2014, Soares et al. 2019, Cunha-Neto et al. 2022) where the fin spines have proven useful for direct age determination of large individuals. The presentation highlighted that age validation using fin spines are limited to only a few tuna species such as Atlantic and Mediterranean albacore (Ortiz de Zarate et al. 1996; Megalofonou, 2000) and further research must be undertaken in this area.

Other methods such as edge type and marginal increment analysis (MIA) will help in determining (indirectly validate) the seasonal periodicity of annuli formation as long as the samples available are representative and covers all year around. In addition, noted that fin spine-based reading protocols need to be consistent with other ageing studies of SKJ in other stocks to establish inter-laboratory and inter-reader calibrations and ensure that standards are maintained and to assess whether further standardization is required.

Day 2

Otolith: Daily/Annual ageing method. OTC Age validation *(K. Krusic-Golub,* FAS*).*

This presentation provided an update to the presentation previously presented during the last online SKJ workshop meeting (5th July 2022). That presentation provided a background into what has previously been reported in the literature regarding otolith-based skipjack age and growth and the work conducted so far on SKJ by Fish Ageing Services (FAS). Previous studies based on otoliths have mainly focused on counts of daily increments either on the surface of the otolith or within thin transverse sections, however the resultant growth estimations have varied between studies. Whether these differences are methodological or geographical is unknown, however, a validation study conducted in the Indian Ocean reported that the increments observed in transverse sections of skipjack otoliths that had been marked with OTC and later recaptured were not deposited daily for the range of sampled fish that were available to the study (Sardenne et al. 2015). More recently, the annual ageing methods of otoliths from a range of other tuna species have been validated and given the results from those studies the annual ageing technique was applied to SKJ otoliths.

Preliminary work focused around developing the ageing method for SKJ based on transversely sectioned otoliths. Firstly, an appropriate section thickness of 280-300 µm was determined and then OTC marked otoliths (with times at liberty ≥8 months) from the Sardenne et al. 2015 study, along with several other non-OTC marked otolith sections that showed reasonable patterns of assumed alternating opaque and translucent zones were used to develop the ageing methodology. The ageing method was applied to 165 samples and the results showed that annual ageing skipjack tuna from sagittal otoliths is possible, but that the interpretation of the annuli can be difficult, and the preliminary estimates should be used with caution until further work is completed. Age estimates from those samples ranged from 0 to 3.8 years.

To increase the number of samples covering a larger sampling area and increase the size range of the sampled fish, further otoliths have recently been collected during a second round of sampling. A comparison between the two collections of otoliths was shown and while additional larger fish were able to be collected during the second round of sampling, the length frequency of the two batches were similar and the smallest fish sampled in the second round was the same as that sampled in the first round (i.e., 27 cm). Otolith weight was plotted against fish straight fork length (SFL) for all the samples and separated by the predetermined ocean blocks. Some differences were observed as follows: samples from the Central Indian Ocean (CIO) were sampled in general from smaller fish, while those from the North Indian Ocean (NIO) off Pakistan looked to have lighter otoliths for a given length than the samples from the other sampling areas and likely suggest that these samples could have different growth compared to the other areas. Lastly, an image of an otolith section from one of the larger fish sampled from the Northwest Indian Ocean (NWIO) was shown and the group agreed that it could be interpreted as at least 6 or 7 years of age (Figure 1).

Skipjack tuna group ageing exercise

Prior to the workshop, participants conducted an image-sharing exercise for reading otoliths and fin spines collected from the same fish caught in the Indian Ocean (mostly from western IO). The exchange collection includes a total of 50 images, i.e., 25 fin spine and 25 otolith sections from the nearly 500 fin spines and 200 otoliths already prepared and read by experts from AZTI and the FAS, respectively. For both structures, images were captured using transmitted light and were selected to ensure that the whole length range for SKJ in the Indian Ocean were covered.

The first reading (prior to the workshop) was made “blind” without prior knowledge of the individual's length, sex, month of capture, etc., It aimed to serve as data to start the group discussion and to check the reader´s initial interpretation of growth bands of otoliths and fin spines. These age data (*preliminary*) were used to test the reader's interpretation of the opaque bands (otoliths) and translucent bands (fin spines), and to identify reader bias in counting bands among hard structures. Age bias plots produced from the age estimates were produced for each reader (Figure 2) and discussed. Then, otolith *vs* fin spine images were projected, and a *reading group session* was held in attempt to resolve concerning questions such as:

- *i)* *Can we agree on locations of 'annual' growth zones?*
- ii) *What is causing the differences/ inconsistencies between otoliths and spines readings in paired samples?*
- iii) *Are the matching otoliths of "easy to read" spine also clear with good zone pattern?*
- iv) *Can we identify exemplar clear fin spines and otoliths to help develop reading protocols and reference collection?*
- v) *Can we develop reading protocols for both structures?*

(NOTE: during the *reading group session*, the group was informed of the fish length):

Regarding <u>annulus identification</u>, results of the preliminary reading exercise suggested that there is a general overestimation of counts using fin spines, particularly for age 1, 2, and 3, except for two reader (JF, AG) that showed a consistent agreement of reading among structures regardless of age. The group agreed that these differences between otoliths and spines are very likely due to the presence of a first translucent band that can be observed in the spines but not the otoliths, including in small specimens (28-30cm SFL) and located within the vascularized area that was considered as "annulus zero". The group discussed whether it might truly represent an annulus zero or in contrast it might represent the first annulus and must be counted. In this regard, the group suggested further daily ageing of otoliths from small fish was needed to then compare the size of fin spines at various (daily) ages (see task 3 in the Follow-up actions section) by comparing the fin spine diameter of fish with the matching otolith.

<u>Spine reading discussion</u>.

The group commented that in certain fin spines there is variance in width of the translucent zones (TZ) that were observed in different forms: fine or thick single zones, and double or triple translucent rings that were separated by narrow opaque rings (Figure 3). While in others noted, the absence of a systematic (periodic) pattern in the deposition of the translucent bands. This might be likely linked to the known opportunistic strategy associated with the growth or, discontinuity to the reproduction of the species (Grande et al. 2012, Artetxe-Arrate et al. 2021). In addition, the group also noticed difference in growth patterns among structures and pointed out that the matching otoliths for fin spines with clear increment pattern do not always show a similar clear pattern in the otolith, and vice versa.

The group largely discussed whether we might consider the formation of one or two rings (translucent bands) per year. Based on other SKJ age studies using fin spines, for example, Vilela and Castelo (1991) reported the formation of two rings per year for the SKJ from southwestern Atlantic. While in Andrade et al. (2004) study, the marginal increment analysis revealed that the band formation pattern is different with one ring per year for

SKJ from the same region. This difference may occur because they analyzed pooled data from different years. Luque pointed that based on her experience on reading (preliminary) obtaining by counting translucent band in fin spines (n=627) from SKJ caught in the Indian Ocean it might be the case and this could explain why fin spine counts were higher on average compared to otolith counts (see Figure 11 in IOTC-2021-SC24-INF02). The group set out to determine the periodicity of growth increment formation on fin spines using available samples that spanned the entire year.

The group also noticed that the vascularization of the nucleus might not be a limitation in terms of ageing in this species in contrast as it has been reported in other tuna and tuna like species (Panfili et al. 2002, Drew et al. 2006, Kopf et al. 2010). Even in those spines where the nucleus appeared vacularized, translucent bands were visible and could be counted/measured. However, the group agreed that we need to quantify them before ruling them out for any age correction. Besides, the group also noticed that for certain fin spine-sections, the images were not taken on the “widest” section face of the section affecting the identification of translucent bands, particularly at the edge of the fin spine. Therefore, before measuring any bands in these samples the fin spine images need to be flipped and re-imaged.

<u>Annual otolith age reading discussion.</u>

Several OTC otoliths from the tagging study have been prepared and examined for annual ageing reading to help validate the annual otolith age reading protocol. Unfortunately for the samples available, the size range of the tagged fish at release was limited to between 48-55 cm SFL and the maximum time at liberty was only 1.57 years. While these samples were informative and generally supported the assumed increment pattern observed in the otoliths, the direct the validation of the age reading method was not considered conclusive. Even though the time at liberty was not extensive and generally limited to less than 1 year for most samples recaptured, the distance between the OTC mark and the otolith edge can be used to determine the mean otolith growth of that individual since it was tagged and the 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 could be informative. At the time of the workshop, only five specimens were available to be included in the analysis (Figure 3). A further 7 samples were prepared and imaged for OTC examination prior to the workshop, however the measurement data had yet to be collected. It was suggested that some of the otoliths analyzed in the daily increment validation study (Sardenne et al. 2015) may offer a wider size range at tagging and larger times at liberty than those selected for the annual examination and could possibly be re-examined. While these sections will not be useful for directly validating the annual increments post OTC mark, the group agreed that it would be useful to include the measurement data from these samples in the analysis.

<u>Reference collection</u>. The group identified from the exchange images set, clear fin spine images that have matching otoliths from the same fish and annotated images will be used as a preliminary reference set.

Follow-up actions

The group concluded that before developing reading protocols for each calcified structure, certain tasks need to be achieved in the coming months. In this context, the group identified the following forthcoming follow-up actions as:

1. Daily analysis→Further daily ageing with otolith measurements must be done based on selected otolith samples (n=30) from fish size range~28-30 cm SFL. This will include starting first with smallest fish to see whether any of the otoliths contain the first annual opaque zone and if not, it might be considered whether to include new extra otoliths from larger specimens and provide daily counts from the primordium to the start of the first opaque zone. We propose to use the age data to calculate daily age to otolith size relationship to be used in step 1 of the novel otolith age algorithm.

2. Measure additional OTC marked otoliths selected for analysis for annual age validation. Measurement from the otolith core to OTC mark and from the OTC mark to otolith edge along the annual ageing transect. By plotting the relationship between otolith growth during time at liberty and fish size at release and recapture from all the OTC marked otoliths measured we may be able to use that relationship to estimate the approximate age of the fish (in days) after the first opaque zone if the relationship is strong. Together this will help estimate a probably maximum age of the fish. For example, if the otolith growth rate is relatively consistent over time (i.e., as the fish grows/ages), then it may be possible to approximate the age of the fish after the first opaque zone based on the size of the otolith and mean otolith growth rate. Otherwise, it will be needed to discuss the best approach to use the OTC measurement data. e.g., to group the data by recapture length class and determine the mean otolith growth by release length class.

3. Measure the fin spine diameter of fish with matching otolith (SFL<30 cm) with daily age, to determine the size of fin spines at various (daily) ages and to help confirm the position of the first translucent zone.

4. Determine the best approach to measure fin spine diameter and translucent bands width to be consistent and in general agreement with other SKJ age studies. Then, new criteria will be applied on the whole fin spine sections database (n= 627).

5. Determine the maximum width of the vascularized area where an annual structure it is not expected to be counted. This is based on daily age to otolith size relationship in otoliths (See point 3 above). Use this as a proxy when following the proposed fin spine reading criteria.

6. 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.

Recommendations and conclusions from SKJ ageing workshop.

Estimating age and growth of SKJ in the Indian Ocean is an important scientific milestone that should be pursued to reduce the uncertainties in the stock assessment model. The selection of an incorrect growth model for the stock assessment can result in inaccurate biomass and fishing mortality estimates, as well as incorrect estimates of associated reference points and stock status (Kolody et al. 2016). Hence, the recommendations identified by the group as priorities are:

- The implementation of coordinated international research sampling programs (as the one developed for the GERUNDIO project in the Indian Ocean) is highly needed to obtain new biological samples that are representative of the species life history attributes (e.g., spatial-temporal distribution, size range, sex, seasons). Therefore, further sampling in areas such as Northeast and Southeast of the Indian Ocean (NEIO, SEIO), and the Arabian Sea (NIO) with limited or no samples is highly recommended, and it must be prioritized.

- Further sampling includes as many fish sizes as possible to obtain a sufficiently representative number of fish at both ends of the size distribution, for fish belonging to both sexes, throughout the year is highly recommended. This will be essential to better understand whether the spatial /seasonal variation in growth should be accounted for in future SKJ tuna Indian Ocean stock assessments.

- A more comprehensive study on age and growth in SKJ in the Indian Ocean would need to consider using update spine/otolith-age derived data and be revised from time to time to enhance and provide best available scientific evidence as possible. Additionally, it is also recommended the application of the novel ageing algorithm developed in other tuna species (Farley et al. 2020) in SKJ too.

- Validation of age estimates from fin spines and otoliths may be limited. Hence, the use of either structure should be carefully validated to ensure they can be used for accurate age reading. To this, the periodicity of band formation (i.e., indirect validation) could be analyzed by using Marginal Increment and Edge type analysis. Other age validation (direct) approaches such as bomb radiocarbon C14 method is highly recommended to explore and considered.

- Reference Collection: Exchange of fin spine and otoliths among annual ageing experts is needed to ensure consistency in datasets used in the assessment models.

- OTC validation work needs to be completed: The analysis of more OTC marked otoliths would help to determine whether the number of increments after the OTC mark is consistent with the time at liberty.

- Other: The presence of double or multiple growth bands in fin spines and otolith are not fully understood but they have been associated with periodic events indicative of slow growth (e.g., changes in the environment, such as cycles in temperature and food availability and linked to migrations, trophic and spawning events (e.g., Compeán-Jimenez and Bard 1983, Megalofonou, 2000, Sun et al. 2001, Morales and Panfili, 2002). By developing trace element maps on sister otoliths of very clear sectioned otoliths of a range of ages might help to identify any peaks that correspond to opaque/translucent zones.

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Figures

Figure 1 The image of an otolith section collected from one of the larger fish sampled from the Northwest Indian Ocean (NWIO). The age is assumed to be 6 or 7 years based on the number of opaque zones (white dots) assumed to be deposited annually. The question mark indicates the position of one potentially missed annual increment.

Figure 2 Age bias plots comparing zone counts from otoliths and fin spine from the same individuals (n=25) for each reader. Bars show 95% confidence intervals.

Figure 3 Images of transverse sections of three *K. pelamis* fin spine sections under transmitted light from different specimens indicating (red dots) the intermediate zones counted at the transition between opaque (dark) and translucent (light) zones.

Figure 4 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 years.

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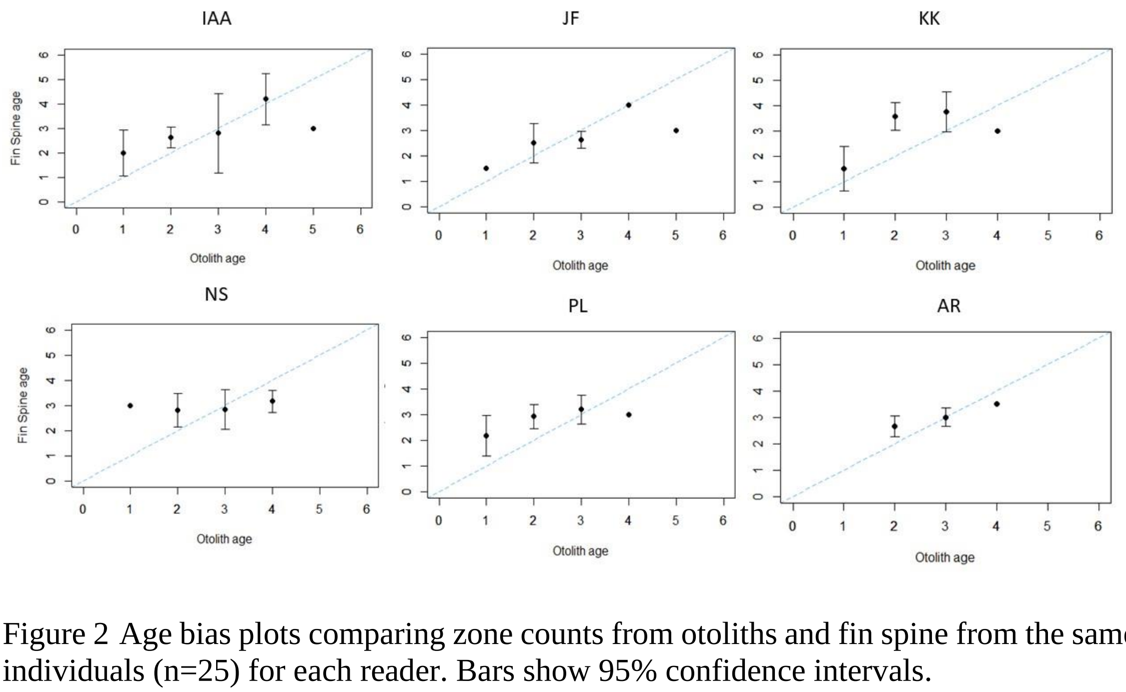

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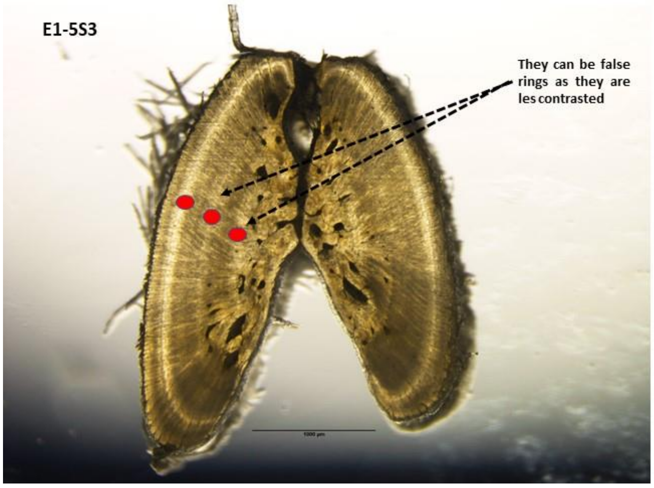

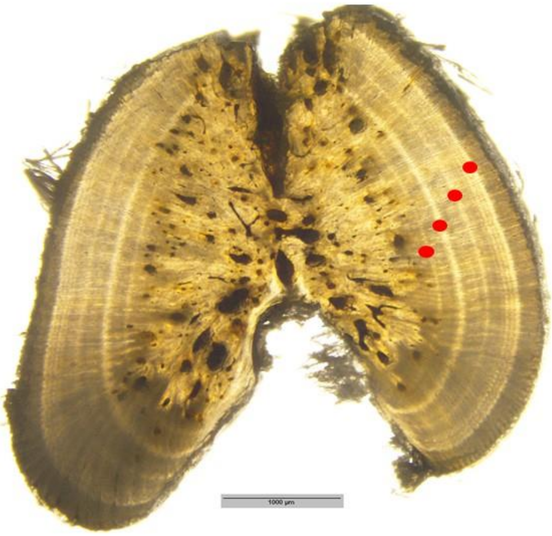

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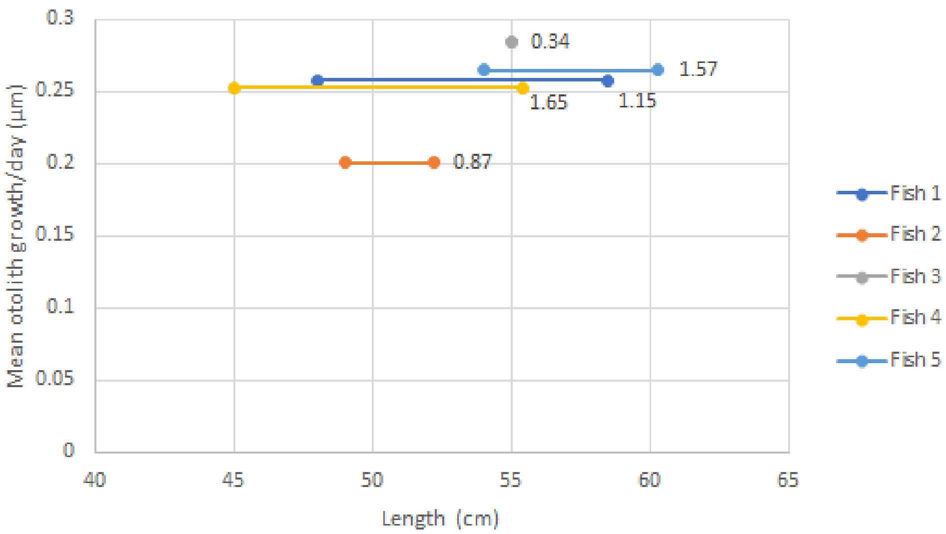

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