A comparison of direct age estimates from otolith and fin spine sections of skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean

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EXECUTIVE SUMMARY

This paper describes work to investigate the potential of two fish hard structures, otoliths and first dorsal fin spines, to estimate the annual age of skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean, as part of the "GERUNDIO" project[1]. The 2020 stock assessment model for skipjack tuna in the Indian Ocean used a Richards curve approximation of the growth model in Eveson et al. (2012), with an asymptotic length (L_∞) of 70 cm straight fork length (SFL), growth rate coefficient (k) of 0.34, and an inflexion parameter fixed at 2.96 (Fu 2020). To date, the inclusion of otolith age data into the skipjack growth model has been challenging due to considerable variation in daily age estimates among otolith readers and lack of validation that micro-increments are deposited on a daily basis (Eveson et al. 2012, 2015). Moreover, there was a recommendation by Sardenne et al. (2015) to explore alternate ageing methods such as annual ageing of otoliths (as opposed to daily ageing) or strucures (such as dorsal fin spines), which have rarely been studied for this species in the Indian Ocean (but see Romanov et al. 1995). The aim of the current study was to investigate and compare (assumed) annual age estimates from both otolith and fin spine of skipjack tuna.

A total of 943 otoliths and 613 fin spines from skipjack tuna collected in the current and previous projects in the Indian Ocean were available for analysis. The fish ranged in size from 25 to 77 cm straight fork length (SFL). Of the samples available, 159 otoliths and 120 spines were analysed, of which 86 were paired samples from fish ranging in size from 28 to 73 cm SFL. Comparison of annual ages (i.e., counts of growth zones) based on both structures resulted in very low agreement (only 8.14% of individuals were aged the same with both structures). Based on otolith opaque zone counts, analysed skipjack tuna were estimated to be between 0 and 3 years, while based on fin spine translucent zone counts fish were estimated to be between 0 and 6 years. Decimal age estimates were calculated for 159 fish based on the counts of opaque zones and otolith measurements; ages ranged between 0.14 and 3.59 years. Otolith-based age estimates showed greater variability in length-at-age than the fin spine-based estimates. Overall, the otolith age estimates suggest a very fast initial growth, with a transition to slower growth at around age two years, whereas the fin spine ageing method suggests skipjack tuna grow is linear. Preliminary age validation work using otoliths and data from the IOTTP provides some evidence that the otolith ageing method used in this study is accurate for fish tagged at 48 to 53 cm SFL and recaptured up to 1.65 year later.

Based on these results, we recommend that direct comparisons of otoliths and fin spines from the same fish are performed to help determine where the differences in counts occurred. We highly recommend that this work is undertaken when images of the sectioned structures, with the zones marked, are available. This is also necessary in order to determine the position of the first ring of the fin spines. This study highlights the current need to develop appropriate age validation and verification methods before conducting a large-scale age and growth study for skipjack tuna in the Indian Ocean.

[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.

1. INTRODUCTION

Skipjack tuna (*Katsuwonus pelamis*) is a cosmopolitan species inhabiting tropical and subtropical waters of the Indian, Pacific and Atlantic Oceans (Collette & Nauen 1983). It is one of the most commercially valuable fish; it accounts for more than the half of global tuna production, and is the third most harvested marine species worldwide (FAO 2020, McKinney et al. 2020). Five stock of skipjack tuna are recognized for stock assessment and management; eastern Pacific Ocean, and western and central Pacific Ocean, eastern and western Atlantic Ocean, and a single stock in the Indian Ocean (ISSF 2021). There is some indication, however, of a more complex stock structure of skipjack tuna within the Indian Ocean than is currently assumed for assessment purposes, but the lack of understanding of its population dynamics and connectivity at oceanic scale hinders finer stock structure (Rodríguez-Ezpeleta et al. 2020, Artetxe-Arrate et al. 2021).

The most recent stock assessment of skipjack tuna in the Indian Ocean shows that skipjack tuna stock of the Indian Ocean is not overfished and is not subject to overfishing (IOTC 2020). These results are derived from a Stock Synthesis (SS) model, which is a highly parameterized statistical age-structured population modeling framework (Methot & Wetzel 2013). Specifically, growth models are used in stock assessments to convert length measurements of the catch to age measurements, and to model biomass and fishing processes over time (Murua et al. 2017). Thus, an incorrect growth model can bias the estimated age structure of a population, which in turn, can reduce the reliability and confidence in stock assessment outcomes and undermine the scientific advice for sustainable management of fish stocks and fisheries.

Skipjack tuna is considered the fastest growing species of all tunas (Murua et al. 2017). Previous studies of skipjack tuna growth in the Indian Ocean based on tag-recapture data found skipjack to have an initial phase of rapid growth, followed by a phase of slower growth (Eveson et al. 2012, 2015). The 2020 stock assessment model for skipjack tuna in the Indian Ocean used a Richards curve approximation of the growth model in Eveson et al. (2012), with an asymptotic length (L_∞) of 70 cm straight fork length (SFL), growth rate coefficient (k) of 0.34, and an inflexion parameter fixed at 2.96 (Fu 2020). To date, the inclusion of otolith age data into the skipjack growth model has been challenging due to considerable variation in daily age estimates among otolith readers and lack of validation that micro-increments are deposited on a daily basis (Eveson et al. 2012, 2015). Moreover, Sardenne et al. (2015) found that skipjack tuna otoliths were not suitable for daily age estimations of fish during the adult phase, due to the poor agreement found between micro-increment counts and days-at-liberty from tag-recapture experiments. Therefore, Sardenne et al. (2015) recommended that alternative methods be explored for skipjack tuna growth studies in the Indian Ocean, such as annual age estimation (as opposed to daily), and alternative structures, such as dorsal fin spines. While otoliths are composed mainly by calcium carbonate in the form of aragonite, fin spines are hydroxyapatite, and form part of the bony skeleton of a fish (Clarke et al. 2007). Both structures grow by accretion onto the marginal surface and have been used to estimate age and growth in bony fishes.

In this context, in 2020 the European Union and the Indian Ocean Tuna Comission (IOTC) supported the “GERUNDIO” project for the “*collection and analysis of biological samples of tropical tunas, swordfish, and blue sharks to improve age, growth and reproduction data for the IOTC*”. The project developed a sampling scheme to collect new biological samples from across the Indian Ocean and carried out the analysis of new samples and existing materials from previous research initiatives. This paper provides preliminary results of both otolith and dorsal fin based age estimates for skipjack tuna in the Indian Ocean, undertaken within this project.

2. METHODS

2.1 Sample collection

A total of 943 sagittal otoliths and 613 fin spines from skipjack tuna of the Indian Ocean were available for analysis (as of November 15th 2021) (Figure 1). Of these samples, a total of 496 sagittal otolith and 424 dorsal fin spine samples were collected in 2021 within the GERUNDIO project, of which 415 were paired samples (Table 1). Fish ranged from 36 to 77 cm straight fork length (SFL) (Figure 2a). Of all the GERUNDIO skipjack tuna samples, 80 otoliths and 92 spines were selected for age analyses, of which 67 were paired samples. Additional 79 otolith and 28 spine samples (of which 19 were paired samples) were provided from the “Population Structure of Tuna, Billfish and Sharks in the Indian Ocean” (PSTBS-IO) and “Estimation of Maternal effects upOn the susTainability of large pelagIc pOpulatioNs”(EMOTION) projects (Bodin et al. 2018, Davies et al. 2020). These fish ranged from 27 to 73 cm SFL (Figure 2b) and were captured between 2017 and 2019 for PSTBS-IO samples and in 2013 for EMOTION samples. In addition, 8 otoliths available from the “Indian Ocean Tuna Tagging Program” (IOTTP, Murua et al. 2015) were included for ageing analyses. These otoliths were marked in 2006 with oxytetracycline (OTC) and recaptured later between 2007 and 2008 and ranged from 52 to 61 cm SFL (Figure 2b). At this stage of the project, the final number of analysed samples was of 159 otoliths and 120 spines, of which 86 were paired samples (Table 1), ranging from 28 to 73 cm SFL.

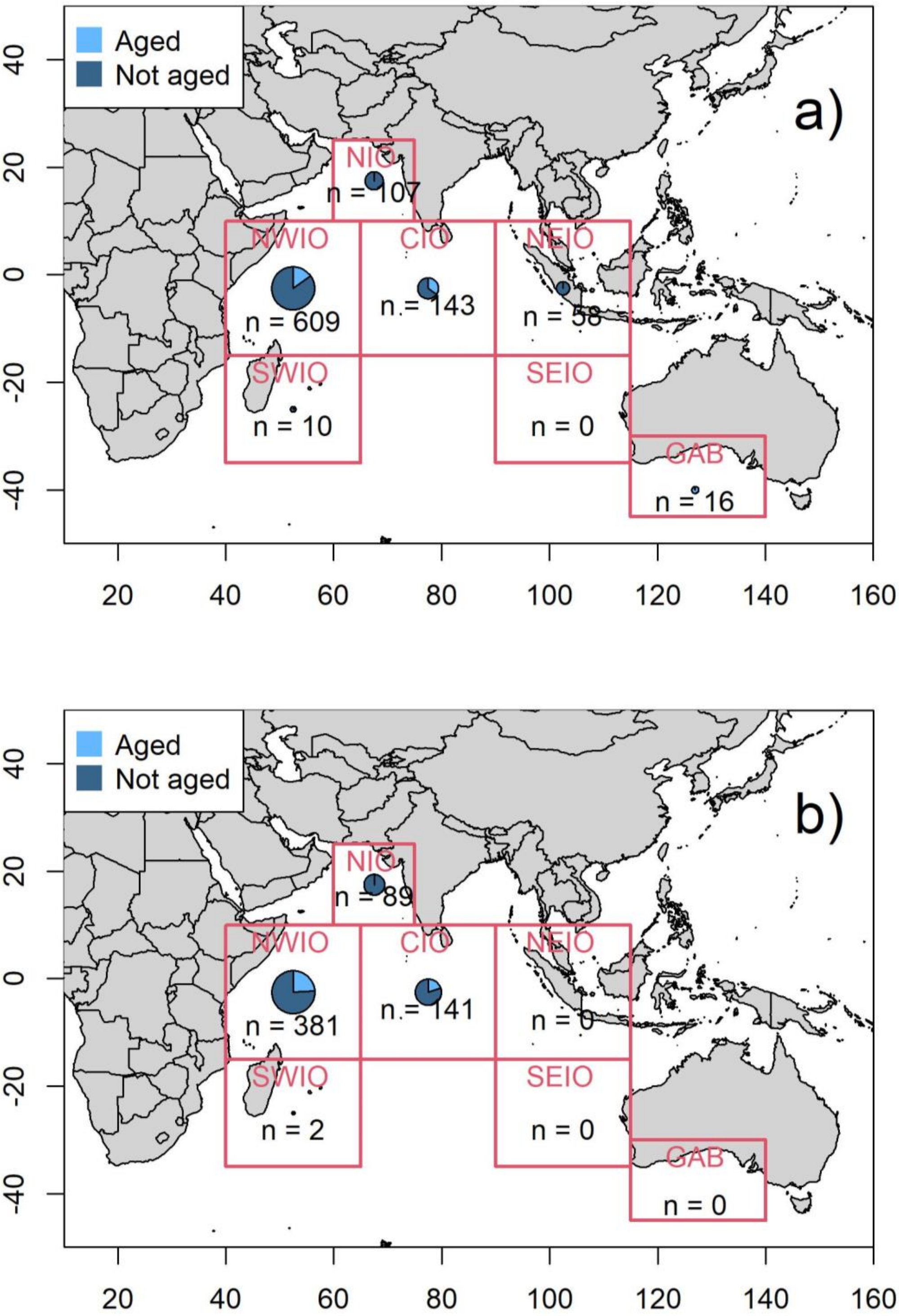

Figure 1. Map showing sampling locations and number otolith (a) and fin spine (b) samples available for skipjack tuna (Katsuwonus pelamis) in the Indian Ocean. Circle size is proportional to sample size, and colours indicate the proportion of otoliths and spines that have been aged in this study analysis (light blue) and the proportion remaining in the collection for future analysis (dark blue). The total number of samples collected is in brackets. Longitude is shown in degrees east. Sampling regions across the Indian Ocean were defined as, Southwest Indian Ocean (SWIO), Northwest Indian Ocean (NWIO), North Indian Ocean (NIO), Central Indian Ocean (NIO), Northeast Indian Ocean (NEIO), Southeast Indian Ocean (SEIO) and Great Australian Bight (GAB).

Table 1. Number of skipjack tuna (*Katsuwonus pelamis*) otolith and skipjack samples collected, selected, and analysed in this study.

Project	N Otoliths	N Fin spines	N Otoliths + Fin spines
GERUNDIO sampled	496	424	415
GERUNDIO analysed	80	92	67
Other projects analysed	79	28	19
Total analysed	**159**	**120**	**86**

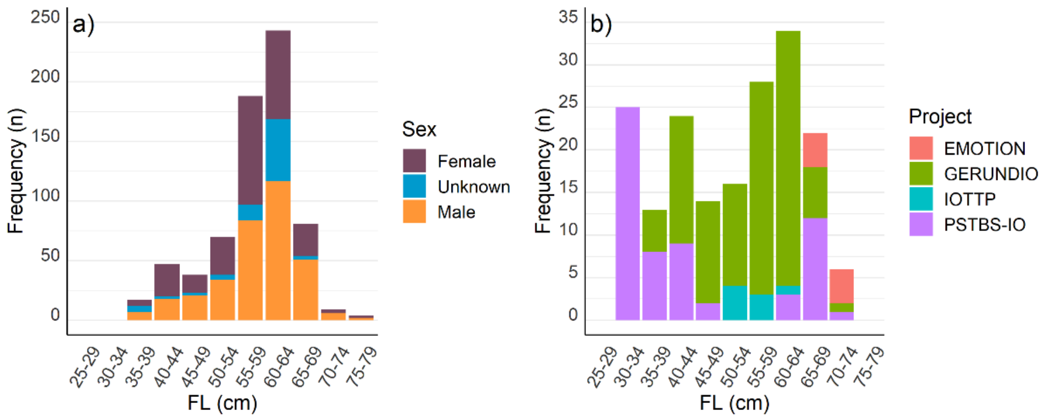

Figure 2. Length class frequency (SFL) of skipjack tuna (*Katsuwonus pelamis*) sampled within the GERUNDIO project (a), and those included in final analyses from various projects (b).

2.2 Fin spine preparation and reading

A total of 120 dorsal fin spines of skipjack tuna ranging in size from 32-72 cm SFL were prepared following the procedure described in Luque et al. (2014) for Atlantic bluefin tuna (*Thunnus thynnus*). An illustration of the workflow for fin spine preparation is displayed in Figure 3. After extraction, the first dorsal fin spine ray was carefully cleaned of the remaining skin tissue and washed with Milli-Q water and air dried at room temperature for 24h. Prior to sectioning, the maximum width of the condyle (Dmax) was measured (±0.1 mm) with a digital caliper (Figure 4a) and marks were made at the point 1.5 times the condyle base width (1.5Dmax) (Figure 4b) where sections were removed. This sectioning axis has proved to enhance the contrast of annuli and may reduce the effect of nucleus vascularization (Luque et al. 2014). Two consecutive transversal sections of 0.7 mm thickness were obtained with an Isomet low-speed saw provided with a diamond blade 0.2 x 0.5 x 12.7 mm (Series 15HC; Ref. 11-4244) and working at 250-300 rpm (Buehler, Lake Bluff, Illinois, USA).

Fin spine sections were examined under transmitted light and a single unmarked digital image (.tiff) was captured for each section with a binocular lens magnifier connected to a digital camera (for additional details see Luque et al. 2014). Age interpretation was performed using an image analysis system (Nis-elements D 3.0, Nikon software; www.nikon.com) that counts and measures the total diameter of the fin spine section and the diameter of each visible translucent zones at its external border all around the section perimeter. An annulus was defined as a bipartite structure consisting generally of a wide opaque zone followed by a thin translucent zone (which were sometimes present in groups of two) and were interpreted as representing periodic events indicative of growth and presumably formed on a yearly basis (Figure 3).

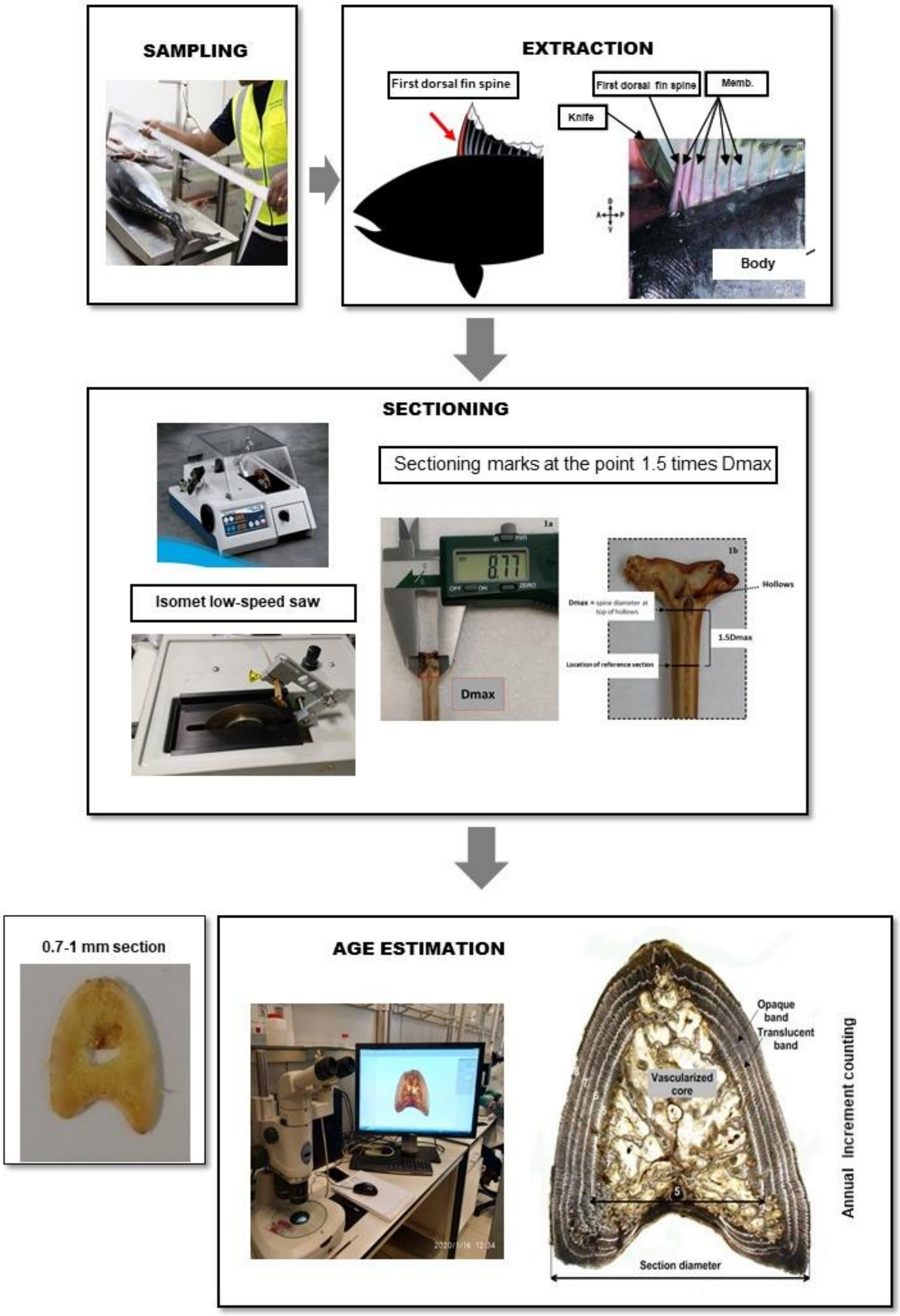

Figure 3. Illustration of fin spine section preparation for ageing analyses.

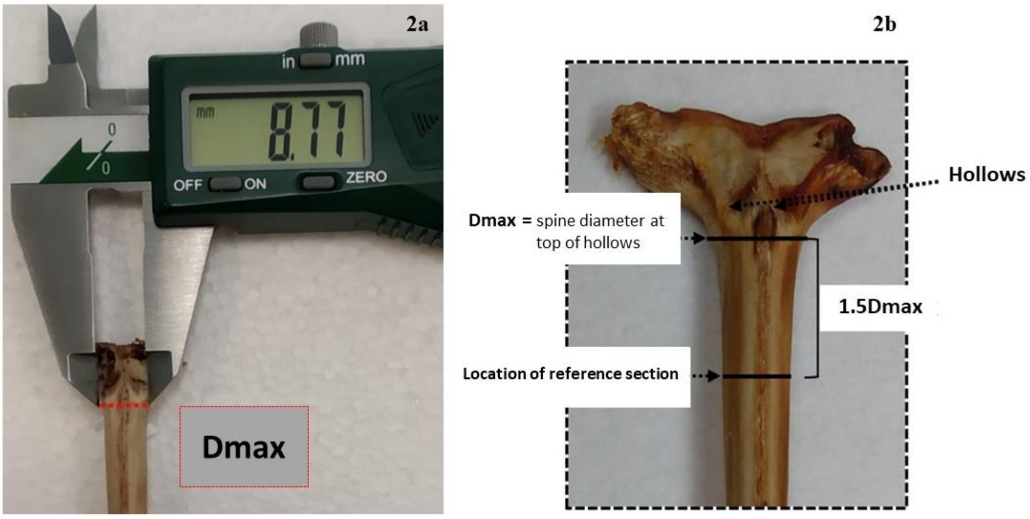

Figure 4. Description of the sectioning axis location: a) an anterior view of the condyle base and the location of Dmax, which is manually measured with the calliper along the imaginary line below the hollows, b) an anterior view of the condyle base and the location of the 1.5 cutting axis at 1.5Dmax from the same imaginary line.

The total number of translucent zones and their diameter (measured when they were fully formed and continuous around the section perimeter) was determined by a single reader twice with no knowledge of the individual's size. The fin spine edge was classified as narrow or wide opaque, and when the new translucent zone appeared at the fin spine edge, its measurement was not counted unless there was a narrow opaque zone afterward.

A replacement method modified from Hill et al. (1989) was used to estimate the number of translucent zones lost due a vascularized core (which increases with size) based on the average position of translucent zones in fin spines from young fish without vascularized cores. Once the number of translucent zones lost was estimated, final assumed ages were calculated by adding the number lost to the number of visible translucent zones between the vascularized area and the spine edge (Luque et al. 2014).

2.3 Otolith preparation and reading

Annual ageing

The 159 sagittal otoliths (fish size 28 to 73 cm FL) selected for annual ageing were embedded individually in blocks of Polyplex Clear Ortho Casting Resin. Each otolith was sectioned on the transverse plane. A single section approximately 320 µm thick was cut through the primordium with a Pace Technology slow speed saw precision saw (Pico-155P©). The saw uses two 300 µm thick diamond impregnated blade separated by a 380 µm spacer. Sections from each sample were cleaned, dried and were mounted in lots of threes on glass microscope slides (25 x 75 mm) under glass coverslips using resin.

The sections were illuminated with transmitted light at 40 x magnification (Leica MZ125). All sections were viewed once before an attempt to estimate age and a single unmarked tiff image was captured for each section. An image analysis system was then used to read the sectioned otoliths. The system counts and measures the distance of each manually marked opaque zone from the primordium and collects an annotated image from each sample read. The increment measurements were taken at the start of each opaque zone. The otolith edge was classified as new opaque, narrow translucent or wide translucent and each reading was assigned a readability score of 1-5 (poor-good).

Daily ageing

Otoliths were selected from four small (30 to 40 cm SFL) and two large (65 and 69 cm SFL) skipjack tuna for preparation for daily age reading. Single transverse sections were prepared for each otolith through the primordium following the methodology of Williams et al. (2013). The number of visible microincrements (assumed daily growth zones) were counted, and measurement were taken, from the primordium to the 25^{th}, 50^{th}, 75^{th} and 100^{th} increment (i.e. days) under high magnification on a compound microscope. The aim of obtaining measurements along the sectioned otolith from the primordium to specific age estimates (i.e., 25, 50, 75 and 100 days) was to determine the relationship between age and otolith size for use in the decimal age algorithm (see below). For the four smallest fish, total counts of micro-increments were made. Although counts of micro-increments in adult skipjack is known to underestimate age, there is evidence to suggest that counts of micro-increments in young fish may be accurate (Tanabe et al. 2003).

Biological (decimal) age calculation

Decimal age was calculated for all 159 fish based on the method developed for bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*T. albacares*) in the western Pacific Ocean (Farley et al. 2020). First, the age of each fish when the first opaque zone was beginning to be deposited was calculated. This was done using the relationship between daily age and otolith size. Daily age estimates were obtained as described above and otolith size was the measurement from the primordium to the proximal edge of the first opaque zone. The daily age-otolith size relationship was estimated using the exponential curve shown in Figure 9 (see Results).

Second, the number of complete annual increments in the otolith was calculated. A complete annual increment is one opaque zone plus one translucent zone, which is assumed to represent one year of growth, and is calculated as the total count of opaque zones minus 1.

Third, the time elapsed after the last counted opaque zone was deposited and when the fish was caught was estimated. This was calculated using the width of the marginal increment in the otolith prepared as a proportion of the mean width of the complete annulus for that age group. The mean increment width was calculated using the otolith measurements taken routinely for each otolith included in the annual ageing. The distance between the terminal edge of each opaque zone was calculated, and the mean width estimated for each age group.

The total age of each fish was estimated by adding together the age components estimated in each of these three steps. Note that for otoliths with no opaque zones, age was estimated using only the otolith measurement (i.e., only step 1).

2.4 Comparisons of zone counts among structures

Age comparison among structures was based on counts of translucent zones (in fin spines) and opaque zones (in otoliths), and not biological (decimal) ages. Comparisons were made for 86 individual skipjack tuna containing information on the two types of hard structures. Consistency between the fin spine and otolith based counts was evaluated by percent of agreement and also by both Average Percent Error (APE) and Average Coefficient of Variation (ACV) estimates (Beamish & Fournier 1981, Chang 1982). These measures are believed to be more accurate than just the percent of agreement (Campana 2001), where APE is defined as:

$$APE(j) = 100\% \, x \, \frac{1}{R}\sum_{i=1}^{R}\frac{|Xij - Xj|}{Xj}$$

where X_{ij} is the *i*th age determination of the *j*th fish, X_j is the mean age estimate of the *j*th fish, and R is the number of times each fish is aged. This can be averaged across fish to get a mean APE. When the standard deviation is substituted for the absolute deviation from the mean age, an estimate of ACV is produced:

$$ACV(j) = 100\% \frac{\sqrt{\sum_{i=1}^{R}\frac{(Xij - Xj)^2}{R-1}}}{Xj}$$

where ACV(j) is the age precision estimate for the *j*th fish. As for APE, this can be averaged across fish to produce a mean ACV.

Age agreement between otolith and spine counts was also evaluated by individual t-test and an age bias plot.

2.5 Age validation with otoliths

Age validation for skipjack tuna was undertaken using otoliths from fish that had been tagged and recaptured as part of the IOTTP project (Murua et al. 2015). A subset of the otoliths was selected for annual ageing, which consisted of 8 OTC marked samples ranging from 0.34 to 1.65 years at liberty and 52.2 to 60.3 cm SFL length at recapture. Transverse sections were prepared using the same method as described in section 2.3. The only modification was that during the processing of the OTC samples, the blocks and slides were not subject to heat and were stored in a dark box while the resin cured as both heat and direct light can reduce the intensity of the OTC mark prior to examination.

Otoliths marked with OTC were examined for the presence and position of the OTC mark using a Leitz Diaplan compound microscope fitted with a 100-W incident ultraviolet light source, and a Leitz D filter block (excitation filter 450–520 nm) to suit the fluorescent properties of OTC. One image was captured of the otolith under fluorescent illumination and another under transmitted light for direct comparison.

Since the time at liberty was known for each fish (i.e., a truly a blind read was not possible at this time) and the maximum time at liberty was just over 1 year, we used an objective method to verify the annual age estimation method used in this study. For each sample the distance from the primordium to the OTC mark and the distance from the OTC mark to the edge was measured. The distance between the OTC mark for each sample and the outer edge of the otolith was plotted against time at liberty. Measurements were taken along a similar transect used for measuring annuli widths in the annual ageing process described in section 2.3. These measurements were also used as "yardsticks" that were overlaid on otolith sections from non-marked otoliths from fish with a similar length at capture, to determine if the amount of otolith growth for a known amount of time (based on OTC mark and tagging data) was visually comparable to that estimated by annuli increment counts.

3. RESULTS & DISCUSSION

3.1 Fin spines ageing

Out of the 120 fin spines analysed for age estimation, 75 (62.5%) were assessed to have between one to four missing translucent zones due to vascularization of the core. Fin spine diameter increased linearly with increasing fish size, although the goodness of fit (R^2) was higher in the case of males (Figure 5). This strong relationship shows that fin spines continue to grow during the life cycle of skipjack tuna collected in the Indian Ocean and hence support the use of this structure for ageing studies in this species.

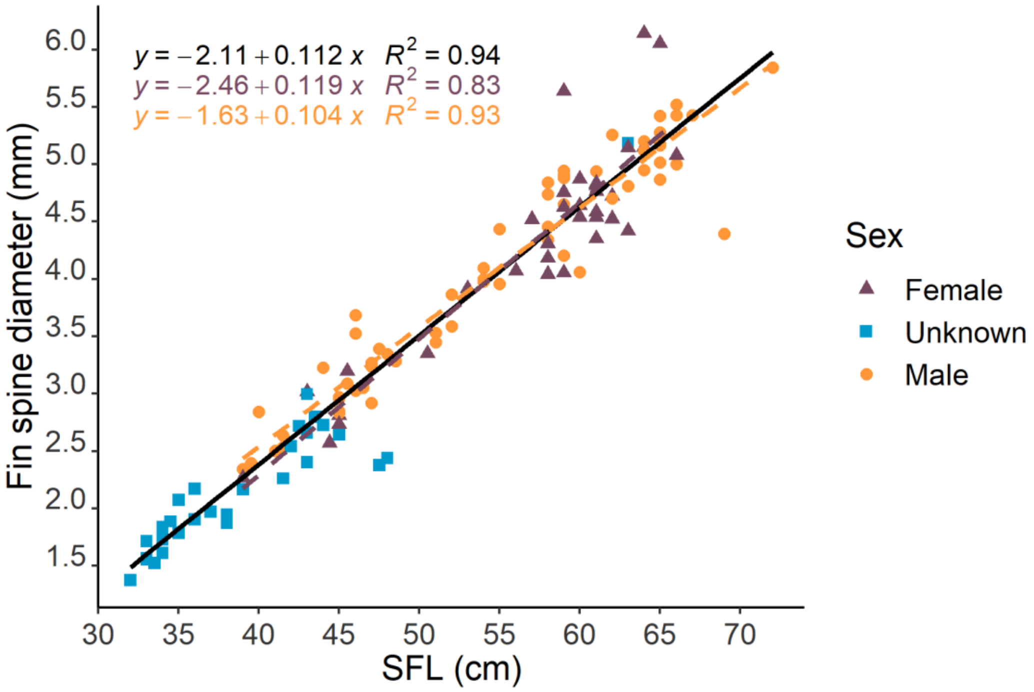

Figure 5. Relationship between body size (SFL) and fin spine diameter for female (purple triangles, n=33), and male (orange circles, n=58) and unknown sex (blue squares, n=29) skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean. Linear regression model trend and equation are shown for all individuals together (solid black, n=120), and females (dashed purple, n=33) and males (dashed orange, n=58) separately.

Age estimates based on counts of visible translucent zones in fin spines ranged from 0 to 6 years. Age estimates for fish within each 5-cm length class spanned 2 to 3 age classes, except for fish from 70-74 cm SFL size class, which were all classified as age 6 (Table 2). These results are similar to those obtained by Romanov et al. (1995), which reported estimated ages between 1 to 6 for skipjack tuna individuals of the Indian Ocean ranging 32 to 72 cm FL.

Table 2. Proportion of translucent zone counts by size class obtained in fin spines of skipjack tuna (*Katsuwonus pelamis*) from the Indian Ocean (n=120).

Size class (SFL cm)	Fin spine translucent zone counts						
	0	1	2	3	4	5	6
30-34	80.0	20.0	0.0	0.0	0.0	0.0	0.0
35-39	0.0	88.9	11.1	0.0	0.0	0.0	0.0
40-44	0.0	8.3	50.0	41.7	0.0	0.0	0.0
45-49	0.0	0.0	7.1	64.3	28.6	0.0	0.0
50-54	0.0	0.0	0.0	12.5	62.5	25.0	0.0
55-59	0.0	0.0	0.0	0.0	0.0	38.5	61.5
60-64	0.0	0.0	0.0	0.0	0.0	33.3	66.7
65-69	0.0	0.0	0.0	0.0	0.0	15.4	84.6
70-74	0.0	0.0	0.0	0.0	0.0	0.0	100.0

In the case of sexed individuals (n=91), estimated ages ranged from 1 to 6 years. There was not a clear pattern of size at age differences by sex for fin spine age estimation, but females estimated to be age 2 and 4 by fin spine translucent zone counts attained greater sizes than males whereas age 6 estimated males were larger than females (Figure 6).

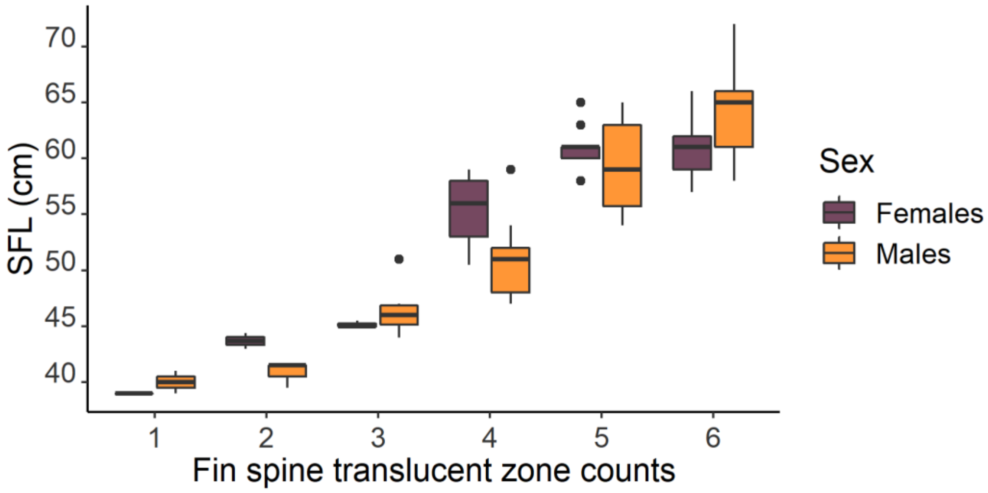

Figure 6. Fin spine translucent zone counts by fish size (SFL) in female (purple, n=33) and male (orange, n=58) skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean.

The mean radius of each translucent zone was relatively constant for fish belonging to different age classes (Table 3). Mean radius of annual translucent zone increased with estimated age, ranging from 1.61 to 4.53 for ages 1 to 6, respectively (Table 3).

Table 3. Mean radius of the distal edge of each translucent zone in fin spines of skipjack tuna (*Katsuwonus pelamis*) from the Indian Ocean (n=120). Mean and standard deviation (SD) for all age classes at each translucent zone are also shown.

Age (yr)	Sample size	Mean spine radius (mm) to each translucent zone					
		1	2	3	4	5	6
0	9						
1	11	1.70					
2	10	1.55	2.22				
3	22	1.55	2.05	2.62			
4	15	1.69	1.96	2.64	3.31		
5	22	1.55	2.19	2.65	3.40	4.19	
6	33		2.00	2.69	3.45	3.99	4.53
Mean		*1.61*	*2.09*	*2.65*	*3.39*	*4.09*	*4.53*
SD		*0.07*	*0.10*	*0.03*	*0.06*	*0.1*	-

3.2 Otolith ageing

Annual ageing

Age estimates (counts of opaque zones) were achieved for all 159 otoliths prepared for annual ageing. The average confidence score of the remaining otoliths was 3.08. Otolith weight was obtained for 153 individuals. Otolith weight increased linearly with increasing fish size when all individuals were considered (R^2=0.84), indicating that otoliths continue to grow throughout the life of the fish, although there was a large amount of variability between individuals (Figure 7). Goodness of fit decreased when sexes were analysed separately, being lower in the case of females (R^2=0.52 in females vs R^2=0.70 in males), but this was probably due to low sample size of females (n=22).

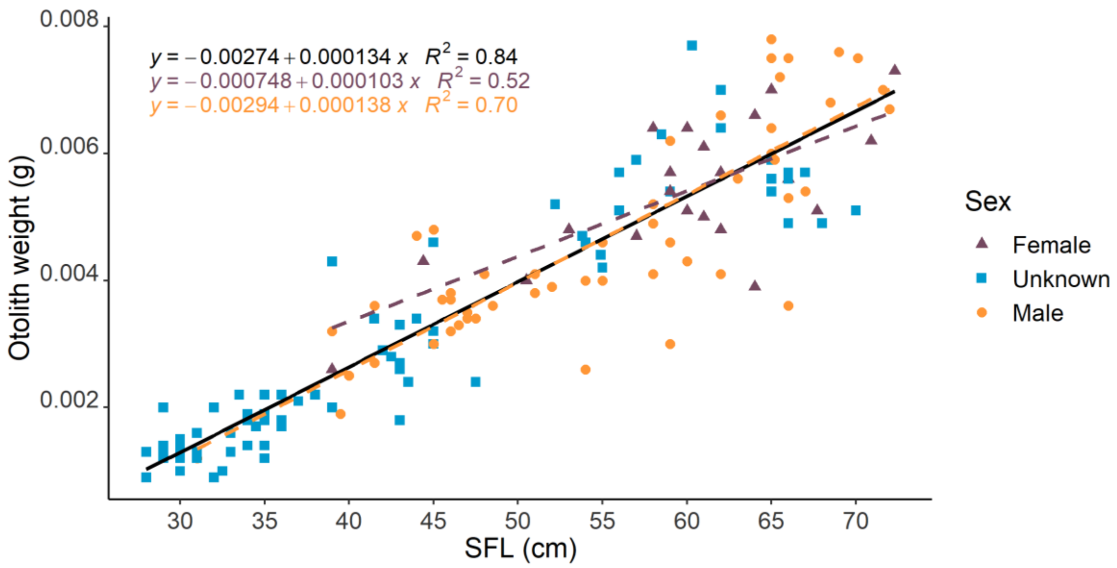

Figure 7. Relationship between body size (SFL) and otolith weight: for female (purple triangles, n=22), male (orange circles, n=55) and unknown sex (blue squares, n=76) skipjack tuna (*Katsuwonus pelamis*) in the

Indian Ocean. Linear regression model trend and equation are shown for all individuals together (solid black, n=153), and females (dashed purple, n=22) and males (dashed orange, n=55) separately.

Age estimates based on counts of opaque zones in otoliths ranged from 0 to 3 years (Table 4). All individuals ≤ 34 cm SFL and 89.5% of fish belonging to the 35-39 cm SFL class were aged as 0. The majority of 35-39 cm SFL size class were also age 0 (76.5%). Then, almost all individuals from 40 to 54 cm SFL were classified as age 1. Greater variability was observed for fish with size ranges greater than 60 cm FL, which spanned 2 to 3 year classes.

Table 4. Proportion of age estimates by size class obtained from opaque zone counts in otoliths of skipjack tuna (*Katsuwonus pelamis*) from the Indian Ocean (n=159).

Size class (SFL cm)	**Otolith opaque zone counts**			
	0	**1**	**2**	**3**
25-29	100.00	0.0	0.0	0.0
30-34	89.5	10.5	0.0	0.0
35-39	76.5	23.5	0.0	0.0
40-44	7.1	92.9	0.0	0.0
45-49	0.0	100.0	0.0	0.0
50-54	0.0	90.9	9.1	0.0
55-59	0.0	55.0	45.0	0.0
60-64	0.0	13.3	73.4	13.3
65-69	0.0	6.5	67.7	25.8
70-74	0.0	0.0	57.1	42.9

Although the length-at-age otolith data by sex is insufficient to be conclusive, preliminary data suggest that there is no difference in growth between sexes (Figure 8).

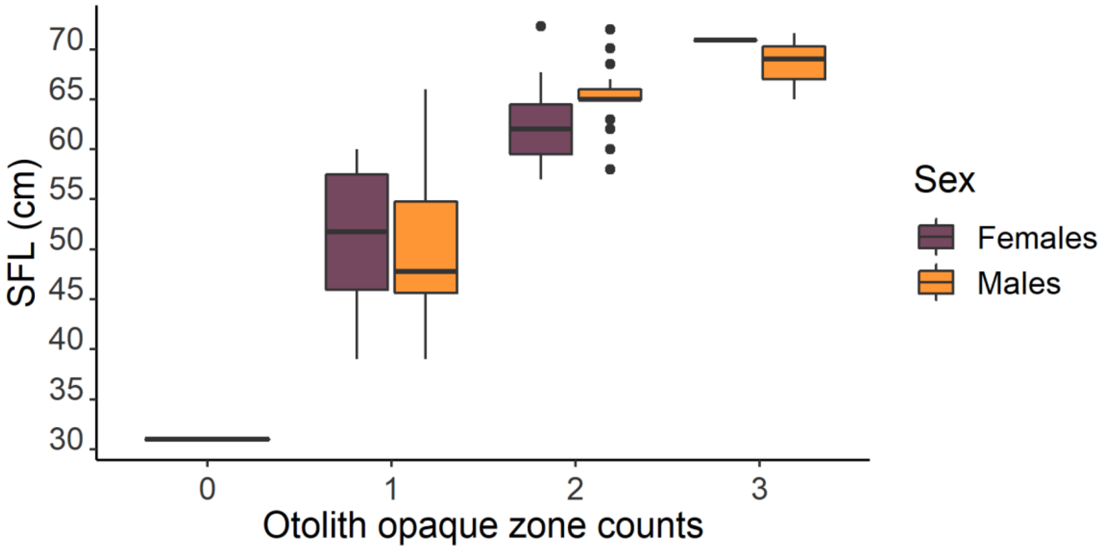

Figure 8. Otolith opaque zone counts by fish size (SFL) in female (purple, n=22) and male (orange, n=55) skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean.

The mean length to each opaque zone was consistent for fish belonging to different age classes and increased with estimated age (Table 5). The mean length to the first opaque zone was 0.36 ± 0.01, 0.50 ±0.02 to the second opaque zone and 0.60 ±0.00 to the third opaque zone.

Table 5. Mean size of the otolith from the primordium to each opaque zone in skipjack tuna (*Katsuwonus pelamis*) from the Indian Ocean (n=159). Mean and standard deviation (SD) for all age classes at each opaque zone are also shown.

Age class (yr)	Sample size	Mean otolith length (mm) to each opaque zone		
		1	2	3
0	38			
1	62	0.35		
2	46	0.36	0.48	
3	13	0.38	0.51	0.60
Mean		*0.36*	*0.50*	*0.60*
SD		*0.01*	*0.02*	*0.00*

Daily ageing

Figure 9 shows the preliminary relationship between daily age and otolith size for skipjack based on the analysis of six otoliths ranging 30 to 69 cm SFL. By measuring each otolith at multiple locations, corresponding to different age estimate, a larger number of points could be used to estimate the relationship. The four small fish with total age estimates and ranging from 30 to 40 cm SFL were between 98 and 122 days old.

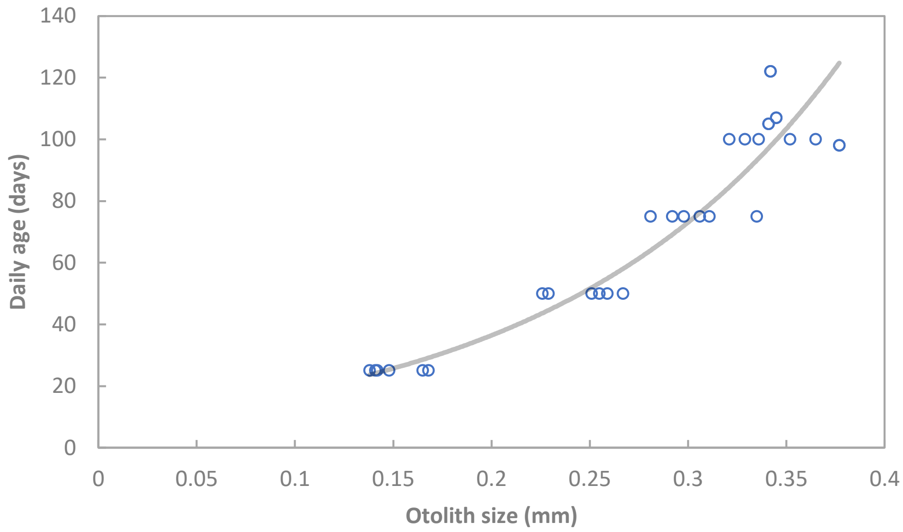

Figure 9. Relationship between daily age and otolith size with fitted exponential curve for skipjack tuna. Otolith size is the distance from the primordium to the edge in transverse sectioned otoliths. R^2=0.9437.

Decimal age

Decimal age was calculated for all 159 otoliths read for annual ageing. High individual variability in length at otolith decimal age was observed (Figure 10). This might be due to spatial and/or temporal variability in growth among individuals. There was insufficient information to estimate region-specific, sex-specific, or year-specific decimal age or to investigate the variability in growth, but this issue should be further investigated to understand the source of variability among fish when further data is available. Observed data suggest that growth is very fast initially before slowing.

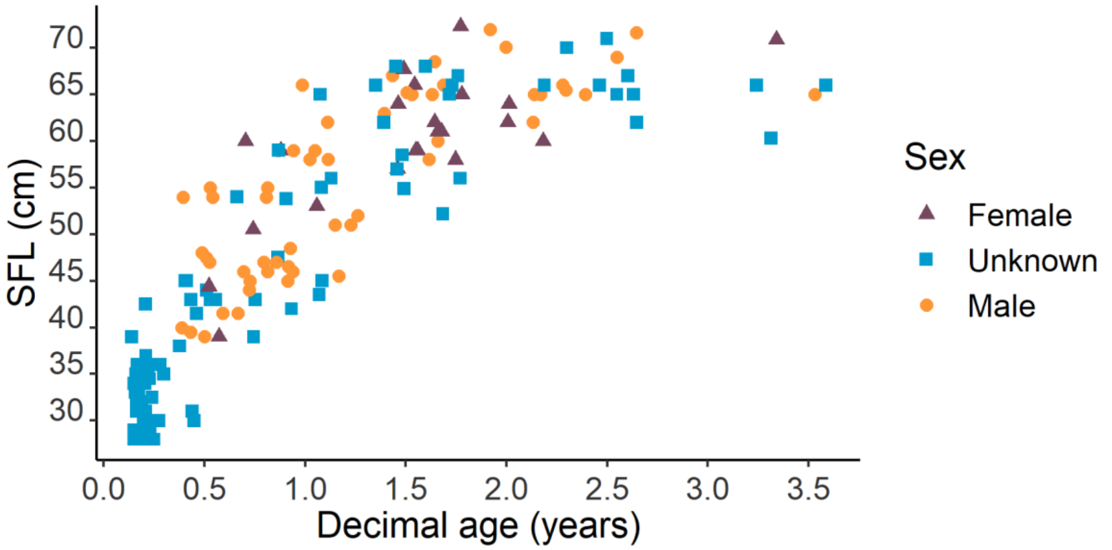

Figure 10. Relationship between body size (SFL) and decimal ages calculate for female (purple triangles, n=22), male (orange circles, n=55) and unknown sex (blue squares, n=82) skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean.

3.3 Comparison of zone counts among structures

The precision of assumed age estimates varied between otolith and fin spines zone counts (Table 6). Only 8.14% of individuals were estimated to be the same age, and ACV and APE were 73.87% and 52.24% respectively.

Table 6. Precision for age estimates (zone counts) between otoliths and spines of the same individuals (n=86) of skipjack tuna (*Katsuwonus pelamis*). ACV: Average Coefficient of Variation, APE: Average Precision Error.

Comparison	% Agreement	ACV	APE
Otolith age vs Spine age	8.14	73.87	52.24

Significant differences in zone counts between otoliths and spines were evident for all age classes (Table 7), fin spine counts being higher on average compared to otolith counts (Figure 11). In particular, 14.9% of individuals were estimated one year older based on spine counts, 17.2% two year older, 20.7% three year older, 31.0% four year older and 6.9% five year older.

Table 7. Summary table of paired t-test results for age (zone counts) estimates obtained from otoliths and fin spines from the same individuals (n=86) of skipjack tuna (*Katsuwonus pelamis*). The results are shown otolith-age. Significant differences among ages are indicated by *.

Otolith age (years)	N	Min spine age	Max spine age	Mean spine age	P

0	11	0	3	1.0	0.005*
1	47	1	6	3.55	<0.001*
2	26	5	6	5.77	<0.001*
3	2	6	6	6	NA

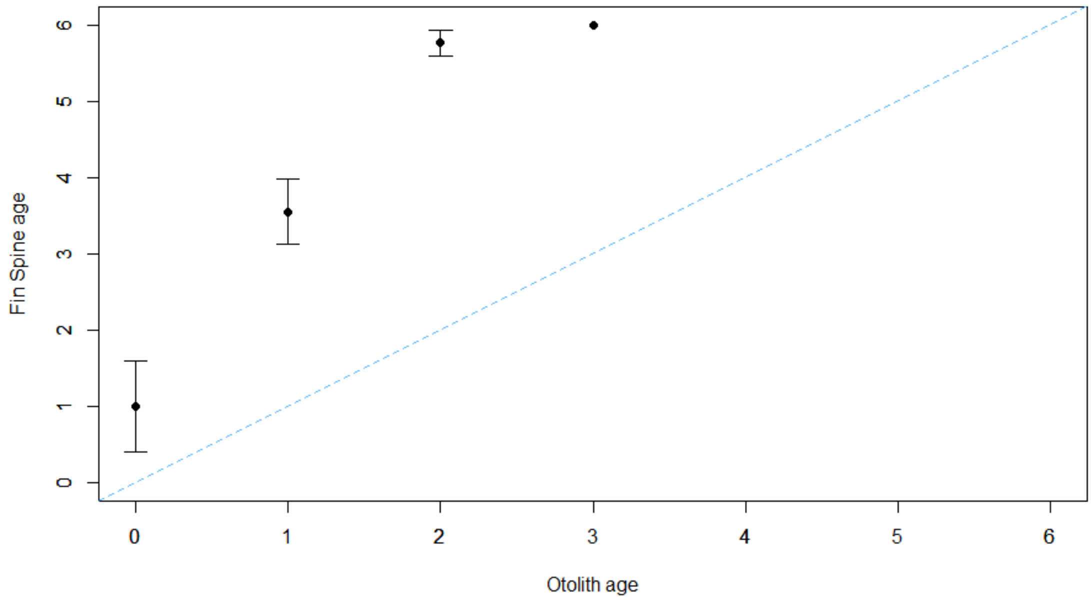

Figure 11. Age bias plot comparing zone counts from otoliths and fin spine from the same individuals (n=86) of skipjack tuna (*Katsuwonus pelamis*). Bars show 95% confidence intervals.

Otolith age estimates suggest greater variability in length at age than the fin spine estimates (Figure 12). The fin spine ageing method suggests skipjack tuna grow is linear, whereas the otolith age estimates suggest a very fast initial growth, with a transition to slower growth around two age years.

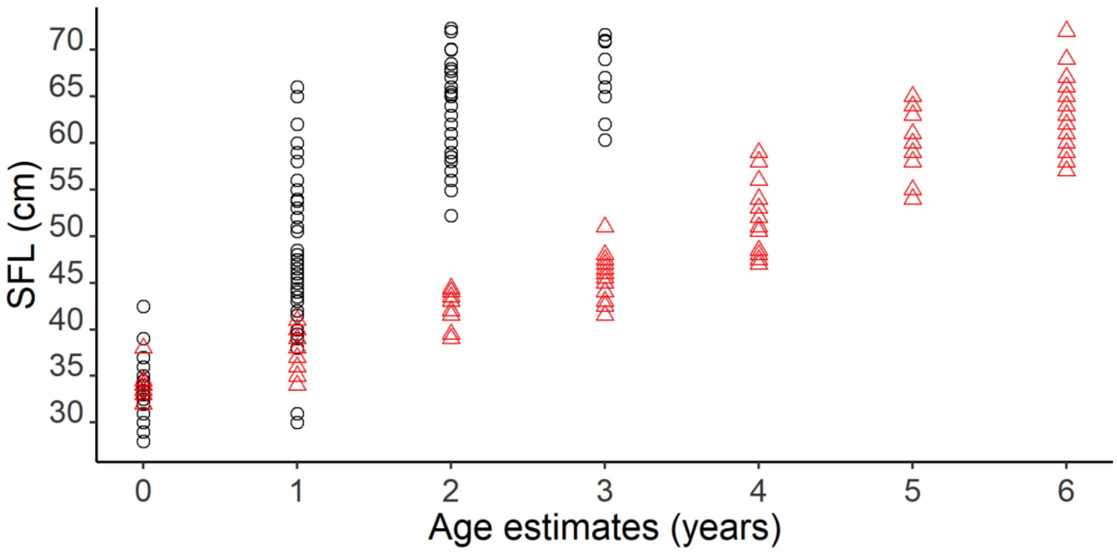

Figure 12. Relationship between fish body length (SFL) and assumed age of skipjack tuna (*Katsuwonus pelamis*) from the Indian Ocean based on age estimates obtained from fin spines translucent zone counts (n=120, red triangles) versus otolith opaque zone counts (n=159, black circles).

The disagreement in zone counts between structures can be due to several factors. For instance, it is possible that more than one increment is deposited annually in the case of fin spines, or contrary, that increments are not deposited annually on otoliths after a certain age (Green et al. 2009). As far as we are aware, there are no studies to date that compare age estimates from otoliths and fin spines in skipjack tuna. However, in other billfish and temperate tunas (e.g. bluefin tuna: Rodríguez-Marín et al. 2007, albacore and swordfish: Farley et al. 2013, 2016), higher age estimates have been obtained from otoliths compared to fin spines from the same fish, contrary to what was observed in the present study. Due to the fact that skipjack tuna predominantly inhabit equatorial environments, it is possible that growth cycles are not related to environmental conditions, as the seasonal contrast in equatorial waters might be less pronounced than in tropical waters. Sardenne et al. (2015) found high variability in the deposition rate of micro-increment counts of adult skipjack tuna OTC marked otoliths and times-at-liberty and hypothesized that the reproductive income breeder strategy (Grande et al. 2012)(i.e., reinvest the majority of acquired energy to reproduction, while breeding all year round) of skipjack tuna may be affecting the variability observed in otolith deposition rates among fish belonging the same age-classes. Likewise, other authors have also suggested that skipjack tuna maturation process and reproductive events may influence their otolith growth (Wild et al. 1995, Leroy 2000), although the evidence to support this hypothesis is limited. Alternatively, studies on billfish and other tuna species using fin spines have reported the existence of two or three translucent zones per year, which were referred to as “doublets” or “triplets” (Megalofonou 2005, Farley et al. 2016).

3.4 Age validation with otoliths

Oxytetracycline marks were observed in 7 of the 8 otolith samples available from the IOTTP tag-recapture program. An example of one of the samples is shown below in Figure 13. In two samples the otoliths were broken into several parts and measurements to and after the OTC mark was not possible. For the remaining 5 samples, the measured distance and the time at liberty showed a positive linear relationship with a high goodness of fit (Figure 14). Since all samples were tagged at similar lengths (48 to 53 cm SFL), the results are useful in the context of providing information on the expected amount of otolith growth for fish in that size range. Using this data, the expected amount of otolith growth for one year is approximately 95 µm. This compares reasonably well to the average distance measured between the 2nd and 3rd opaque zones of the annually aged otolith samples (0.097 mm) and provides some weight of evidence for the otolith-based age estimates, albeit only in fish greater than 48 cm SFL and potentially 2 years of age. As the tagging data, time at liberty and the length range of the fish tagged was limited, it would still be recommended that age validation be extended to a wider range of length fish, particularly for fish smaller than 48 cm SFL.

Figure 1(TAG_ID/FISHID OT12894– Skipjack tuna (*Katsuwonus pelamis*) OTC marked and tagged at 45 cm (SFL), recaptured at 55.4 cm, 602 days later (1.65 yrs). White arrows indicate the OTC mark. The white and black line in the bottom image is the “yardstick” line used comparison with non-OTC marked otoliths.

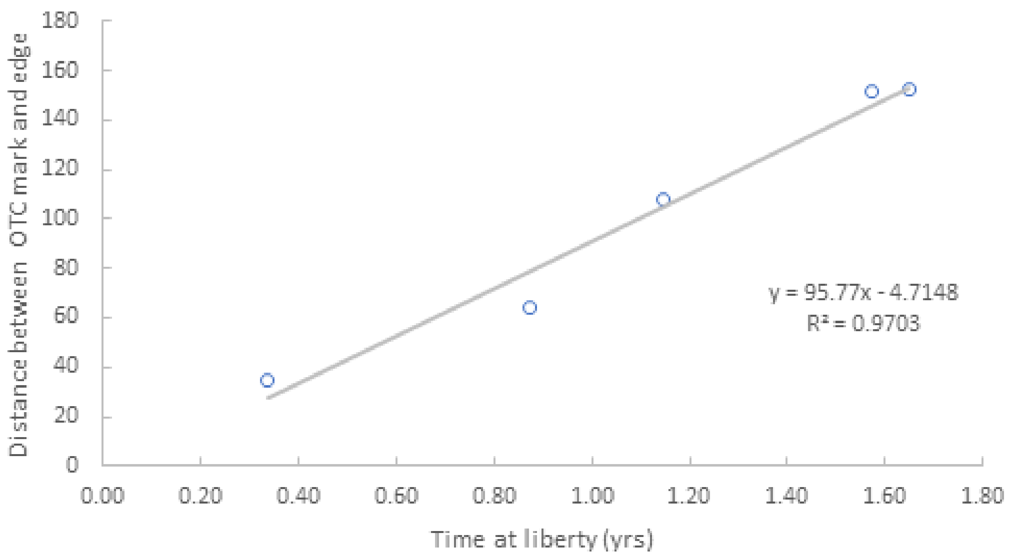

Figure 14. Relationship between time at liberty and the distance between the OTC mark and the otolith edge for 5 OTC tagged and recaptured skipjack tuna (*Katsuwonus pelamis*) of the Indian Ocean.

4. FINAL REMARKS

This study explored for the first time two hard structures, sectioned otoliths and first dorsal fin spines, collected from the same individual skipjack tuna from the Indian Ocean in an attempt to estimate annual ages. The preliminary results show that different age estimates (i.e., zone counts) can be obtained from otoliths and fin spines from the same fish, highlighting the need to develop appropriate age validation and verification methods to determine the most appropriate structure and ageing criteria to accurately estimate the age of skipjack tuna. Unfortunately, due to the short lifespan of this species, it is unlikely that bomb radiocarbon decline could be a suitable technique for age validation, as it has been successfully proven for other tropical tuna species (Andrews et al. 2020). Time did not permit a direct comparison of images between otoliths and fin spines from the same fish to help determine where the differences in counts occurred. We highly recommend that this work is undertaken when images of the sectioned structures, with zones marked, are available. This is also necessary in order to determine the position of the first ring of the fin spines, and to better estimate the number of translucent zones lost due to vascularized core. Given the low number of samples analysed and the variability in sampling location and year, it was also not possible to undertake marginal increment analysis (MIA) to examine the periodicity of zones formation (i.e., indirect validation). We recommend that MIA is investigated for both structures in the future when higher sample sizes are analysed. It is also important that both otoliths and fin spines are read again, preferably by different readers, as the subjective interpretation of annual increments could have played a role in the observed differences between otolith and spine counts. Likewise, 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. Additional research is also required to determine the accuracy of the daily age estimates obtained for very young skipjack (up to age ~100 days). The accuracy of these daily age estimates is critical for determining the decimal age of fish with an annual otolith read. Studies undertaken in other oceans may be useful for this daily age validation/verification work.

This work must be undertaken prior to conducting a large-scale age and growth study for skipjack tuna in the Indian Ocean. Once age estimates are validated, the preparation of reference collections could be useful for future studies. Estimating age and growth of skipjack tuna 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).

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