Preliminary age and growth of swordfish (*Xiphias gladius)* in the western Indian Ocean

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Executive summary

This paper describes preliminary work to estimate the age and growth of swordfish in the Indian Ocean as part of the 'GERUNDIO' project[1]. The most recent stock assessment for Indian Ocean swordfish was undertaken in 2020 using Stock Synthesis. The base case model used otolith-based growth estimates for swordfish from the southwest Pacific Ocean from Farley et al. (2016), and the sensitivity models used fin spine-based growth estimates for swordfish in the northern Indian Ocean from Wang et al. (2010). Farley et al. (2016) found that age estimates from fin spines from Pacific Ocean swordfish are likely to underestimate age of older swordfish, so the current project was undertaken to assess the suitability of otoliths to estimate age and growth for swordfish in the Indian Ocean.

A total of 317 swordfish otolith samples collected in the current and a previous project were available for analysis from the western and eastern Indian Ocean. In the western Indian Ocean, 128 otoliths were available from fish ranging in size from 59-251 cm lower jaw fork length (LJFL), although the majority were from fish <200 cm LJFL. Sex was recorded for 86 of these fish. In the eastern Indian Ocean, 189 otoliths were available from fish ranging in size from 51-309 cm LJFL, but sex was not recorded for any of these fish.

Since swordfish have sexually dimorphic growth, only samples from the western Indian Ocean were selected for analysis. Otoliths for this preliminary work were selected from fish for which the sex was reported (n=84 out of 86, since two arrived at the lab too late to be included). An

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additional 21 otoliths were selected from fish <150 cm LJFL with unknown sex, since sexual dimorphism in growth is not expected for fish under this size (see Farley et al. 2016).

A combination of daily and annual ageing was undertaken. The maximum age was 11 years for both males (183 cm LJFL) and females (251 cm LJFL). The youngest fish was aged 76 days. There was insufficient data to model growth as otoliths from large male/females were not available. However, our preliminary length-at-age data suggests that swordfish in the western Indian Ocean have similar, although perhaps slightly slower, growth to swordfish in the southwest Pacific. Fish length to otolith weight (which may be an indicator of age) data are consistent with this finding. However, the sample size is too small to be conclusive. The otolith-based length-at-age data for the western Indian Ocean does not align well with the fin spine-based growth curves from Wang et al. (2010) for swordfish from the northern Indian Ocean, particularly for small/young fish (<150 cm LJFL, ~3 years old), where we have the most data to compare.

We recommend that additional otoliths are collected and analysed from fish >200 cm LJFL, from which sex is known, in the Indian Ocean to provide further information on growth and longevity. It would be desirable to collect samples from across the range of the species including northern and southern regions to provide potential to clarify hypothesis about stock structure from the PSTBS-IO project (see Grewe et al., 2020). It may be possible to genetically determine the sex of fish with otoliths currently available, if a genetic-based sex-specific marker can be found in studies currently underway. We also recommend age validation studies to determine if the otolith age estimates obtained are accurate.

Introduction

Swordfish (*Xiphias gladius)* is a highly migratory species found between ~45°N and 45°S in all the major oceans. They spawn in tropical regions but move to higher latitudes to feed as they mature, before returning to tropical waters for winter (Collette et al. 2011). For stock assessment purposes, the population of swordfish in the Indian Ocean is considered as a single stock. There is some indication, however, of genetic heterogeneity within the Indian Ocean (Lu et al. 2006; Bradman et al. 2009; Grewe et al. 2020), with the most recent study using Single Nucleotide Polymorphisms (SNPs) indicating different northern and southern genetic groups.

The 2020 stock assessment for swordfish in the Indian Ocean indicated that the stock is not overfished and is not subject to overfishing (IOTC 2020). Biological parameters included in the assessment model, including growth and maturity, were sourced from studies in the Pacific and Indian Oceans. The base case model used growth estimates from Farley et al. (2016) for swordfish from the southwest Pacific, and the sensitivity models used growth estimates from Wang et al. (2010) for swordfish from the northern Indian Ocean. The study by Farley et al. (2016) compared age estimates from counts of assumed annuli in sectioned otoliths and anal fin spines for southwest Pacific swordfish and showed there was a divergence in counts from the two structures in age classes >7 years for females and >4 years for males; otolith counts were higher on average compared to fin spine counts. They concluded that age estimates from otoliths were likely to be more reliable than from fin spines, especially in larger/older fish. They obtained otolith age estimates from 0-17 years for males and 0-21 years for females and modelled growth using the observed length-at-age estimates. Sexual differences in growth were observed with females reaching larger length-at-age than males. The Wang et al. (2010) study estimated swordfish age and growth using counts of assumed annuli in sectioned anal fin spines collected in the northwest and northeast Indian Ocean. Age estimates ranged from 1-10 years for both males and females and growth was estimated using back-calculation methods. Spatial and sexual variation in growth was detected.

In 2020, the European Union and the Indian Ocean Tuna Commission (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*". This paper provides preliminary results of otolith-based age estimation for swordfish in the western Indian Ocean that was undertaken in this project.

Methods

Sample collection and selection for ageing

A total of 317 swordfish samples were available for otolith analysis. Of these, 34 were collected in the current project in the southwest Indian Ocean in 2021. The remaining otoliths were made available from the "*Population Structure of Tuna, Billfish and Sharks in the Indian Ocean*" (PSTBS-IO) project and were collected in northwest, southwest and southeast Indian Ocean between 2017 and 2019 (Davies et al. 2020). Figure 1 shows the sampling locations for all otoliths. Lower jaw fork length (LJFL) was measured to the nearest cm for fish in the western (southwest and northwest) Indian Ocean and orbital fork length (OFL) was measured for fish in the southeast Indian Ocean. OFL was converted to LJFL using the length conversion equations for IOTC species in 'IOTC-2018-WPB16-DATA18-Equations'. Sex data was only available for 86 fish, all of which were sampled in the western Indian Ocean.

A subset of sagittal otoliths was weighed to the nearest 0.001 g. as swordfish otoliths are particularly small, weighing only a few mg, we only weighed otoliths that were clean, dry and complete. Additional otoliths will be meticulously cleaned and dried in preparation for weighing later in the project.

Since swordfish have sexually dimorphic growth, otoliths from fish with known sex were initially selected for ageing (n=84 out of 86, since two arrived at the lab too late to be included). Additional otoliths (n=21) were selected from fish ≤150 cm LJFL with unknown sex as dimorphism in growth is not expected in fish of these sizes. All fish selected were caught in the western Indian Ocean. Figure 2 shows the size frequency of fish selected for ageing and those remaining for future analysis. All but one fish selected for ageing were <200 cm LJFL. The selected otoliths were sent to Fish Ageing Services Pty Ltd (FAS) in Australia for preparation and reading.

Otolith preparation and reading

Both daily and annual increments were examined in this study. Daily growth zones (microincrements) were used to: 1) estimate the age of very small fish (<114 cm), and 2) determine the age at first transition point observed within the otolith. In Pacific swordfish otoliths examined by Farley et al. (2016) this transition point coincided with the first assumed annually formed opaque zone that can be observed in transversely sectioned otoliths prepared for annual age reading.

The majority of samples that were available consisted either of only one otolith or the second otolith was broken and often unusable for preparation. We were, therefore, unable to use both otoliths for direct comparison of daily and annual age estimates, as was done previously for Pacific swordfish samples. Annual and daily age estimates can still be determined from the same otolith; however, once the otolith is prepared for daily ageing, the ability to interpret the assumed annual opaque and translucent zones is lost as the zones become indistinct from each other. Therefore, all samples needed to be initially prepared for annual age estimation and the annual preparations were read and/or images were captured before any further steps were completed for those samples also requiring daily ageing.

To prepare thin transverse sections of the sagittae, the otoliths were ground down in a 4-step process (Figure 3Figure 7). One otolith from each sample was positioned on the edge of a slide using thermoplastic mounting media (crystalbond 509) with the anterior side of the otolith hanging over the edge. Care was taken to ensure that the primordium was located approximately 100µm in from the glass edge. The otolith was ground down to the edge of the slide using 800 grit wet and dry abrasive sheets (Norton©). The slide was then reheated and the otolith was removed and placed (ground side down) on another slide and the crystalbond was allowed to cool. The otolith was then ground horizontally to the grinding surface using 1200 grit sheets. The thickness of the preparation was measured continuously with a digital calliper until the thickness of the prepared section was approximately 260µm. As there is a small amount of crystalbond between the otolith section and the glass slide, we estimate that the final thickness of the actual otolith section would be between 180 to 200µm. Finally, the otolith preparation was polished with 5um diamond impregnated lapping cloth. Before further preparation for daily ageing, the sections were read (aged) and an image was captured (see imaging and ageing section below).

A total of 16 samples were selected from small fish (59-114 cm LJFL) for further preparation for daily age reading. To prepare the sections for daily ageing the surface of the section was ground down using 1200 grit paper until just before the nucleus was reached. The slide was reheated and the section was flipped and the exposed side was ground further towards the primordium. During this process the otolith preparation was continuously viewed under the compound microscope (between 40x – 100 times magnification) and where necessary flipped and ground to ensure that the recommended section thickness (50-80µm) was reached and that the remaining section still contained the primordium.

Imaging and annual age

All otolith preparations were examined with transmitted light with a LeicaM125 stereo microscope set at 40x magnification. Even though swordfish otoliths are very small and difficult to prepare, several sections showed clear alternating opaque (which appear dark under transmitted light) and translucent zones. Images were captured with a 1mm scale bar using the IC Measure (The Image Source©) and were saved as tagged image format files (TIFF) at a resolution of 1024x768 pixel (Figure 4).

An image analysis system was used to read the sectioned otoliths. The system counts and measures the distance of each manually marked opaque zone (marked at the start of each opaque zone) from the primordium and collects an annotated image from each sample read. The opaque zones at the terminal edge of the otolith were only marked if they were complete and some translucent material was evident after the opaque zone. The otolith edge was classified as new opaque, narrow translucent or wide translucent based on the criteria developed for Pacific swordfish otoliths (Farley et al. 2016) and each reading was assigned a confidence score of 0-5 (poor-good). In this preliminary study, we assumed that age equalled the number of opaque zones counted; no attempt was made to convert zone counts into decimal (fractional) age.

All samples were read by the same reader a second time to determine intra-reader ageing error. Samples were examined on screen and using the microscope, but no images or measurements were taken during the second age estimation. Average percent error (Beamish and Fournier 1981) and age difference tables were used to assess the precision of readings.

Daily age estimation

All 16 samples selected for daily age reading were successfully prepared. To improve the clarity of each of the preparations, the side of the otolith that was viewed for reading was covered with non-drying immersion oil for microscopy (Cargill ©) before examination. The transverse sections were examined with transmitted light at various magnifications between 250x and 1000x. The number of opaque microincrements were counted on the dorsal lobe along the clearest path from the primordium to the otolith edge and the distance between the primordium to the dorsal tip was recorded. All samples were read twice by the same reader. If the difference in age estimates was >10%, then a third reading was completed. The average or the two closest reading was used as the agreed age.

In 8 out of the 9 largest otoliths examined for daily ageing, it was evident that there was a transition point in the otolith microstructure near the outer edge of the dorsal tip. Interpretation of the microstructure after this transition became more difficult and, while still evident from the transition to the edge, the increment structure was far less regular than prior to the transition point. For these samples, the position of the transition point on the section was noted and the distance and age from the primordia to the transition point was recorded.

Results and discussion

Age estimates were obtained from all 16 otoliths selected for daily ageing. Ages ranged from 76 to 378 days. The seven smallest samples (from fish 59-88 cm LJFL) all showed relatively clear alternating opaque and translucent microincrements, which could be counted from the primordium to the otolith edge (Figure 5). In the remaining larger samples (91-114 cm LJFL), a transition point where the daily increment became less regular and more difficult to interpret (see Figure 6) occurred at a mean count of 157 days (+/-21.6) and a mean distance of 0.51 mm (+/-0.05) from the primordium. The area post transition contained overlapping zones, merging zones and areas containing diffuse or very little structure. The mean distance from the primordium to the first annual opaque zone in (all) otoliths prepared for annual ageing was also 0.52 mm, confirming that the transition zone in the otoliths prepared for daily ageing corresponds with the completion of the first opaque zone.

Annual age estimates were obtained from all 105 otoliths selected for annual ageing (note that the 16 samples with a daily age were also given an annual age). Ages ranged from 0 to 11 years for both males and females. The average percent error between annual readings was 7.9% with a maximum difference of two years (Table 1). Considering that almost all fish were <200 cm LJFL, we would expect that the maximum longevity would be extended with analysis of more otoliths from swordfish >200 cm LJFL.

Figure 7 shows the relationship between otolith weight and estimated age for swordfish in the western Indian and southwest Pacific Oceans. The linear relationships are similar and show that otoliths continue to growth (increase in weight) throughout life. Figure 8A shows the length-at-age data obtained in this study. The daily age data aligns well with the annual age data for small/young fish. The data are insufficient to model sex-specific growth; however, Figure 8B compares the raw data with length-at-age data estimated for swordfish in the southwest Pacific by Farley et al. (2016). The data aligns relatively well, although there is some indication that swordfish in the western Indian Ocean may grow slightly slower than in the southwest Pacific. The sample size (particularly for fish >200 cm LJFL) is too small, however, to be conclusive and other factors such as size-selective fishing can result in biased data/growth curves.

Since otolith weight may be an indicator of age, we compared otolith weight to fish length for swordfish in the western Indian and southwest Pacific Oceans (Figure 9). Like the length-at-age data above, the length-at-otolith weight data is similar for the two regions. However, again there is some indication of western Indian Ocean swordfish growing slower, having slightly smaller length for their otolith weight than southwest Pacific swordfish.

Figure 10 compares the length-age data from the current study with fin spine-based growth curves from Wang et al. (2010) for swordfish from the northern Indian Ocean. The current length-at-age data does not align well with the growth curves, particularly for small/young fish (<150 cm LJFL, ~3 years old) where we have the most data to compare. Wang et al. (2010) estimated growth using back-calculated length-at-age estimates, which involves estimating fish length at previous ages (prior to capture) using increment measurement data for each spine. The method can lead to bias (Campana 1990), such as bias towards slower-growing older fish that have shorter lengths at younger age classes (i.e., Lee's Phenomenon; Ricker 1975). This may be the reason the back-calculated growth curves using spines by Wang et al. (2010) resulted in lower length-at-age compared to our observed length-at-age estimates.

Farley et al. (2016) found that age estimates from fin spines are likely to underestimate age of older swordfish sampled from the southwest Pacific Ocean. This age underestimation was attributed to: (i) the increments being deposited so close together on the margin of fin spines as a fish gets older that they become too difficult to resolve, and (ii) vascularisation and resorption of fin spines obscuring increments that are consequently not counted. Vascularisation and resorption of increments does not occur in otoliths and, compared to fin spines, the annual increments in otoliths are generally clearer at the margin. Therefore, we recommend that otoliths are used to estimate the age of swordfish in future studies.

Further work

In this preliminary work, we were unable to estimate sex-specific growth and the likely maximum age of swordfish since almost no otoliths were analysed from fish >200 cm LJFL. We recommend that additional otoliths from larger fish are collected and that sex data are collected during sampling to provide further information on growth and longevity by sex. It would be desirable to collect samples from across the range of the species, including northern and southern regions to provide potential to clarify hypothesis about stock structure from the PSTBS-IO project (see Grewe et al., 2020) (i.e., potentially spatial and/or stock differences in growth). Note that it may be possible to determine the sex of fish with otoliths currently available, if a genetic-based sex marker can be found as has been done successfully in other species (e.g., Tani et al. 2021).

In this initial study, a count of opaque zones in otoliths was used as the age estimate. It may be possible to estimate a decimal (fractional) age using a new method developed for tropical tunas by Farley et al. (2020). The method would estimate decimal age using the mean daily age to the first opaque zone (157 days), one year for each completed annuli, and the marginal increment measurements as a proportion of a completed year. The last step requires that the available data for the mean annuli width is available for each age class. As more swordfish are aged, these data should be available in the future.

Direct validation of the accuracy of the ageing method used, spanning the entire size range, and expected range of longevity was not possible in the current study and is required to confirm the age and growth estimates. Consideration could be given to bomb radiocarbon (^{14}C) dating to validate the annual periodicity of the bands being counted. The use of the decline period in the ^{14}C signal (~1980-present) is a new approach (e.g., Andrews et al. 2020) that is well-suited to shorter lived species and may be capable of verifying swordfish age estimates.

Acknowledgements

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Figures and tables

Table 1. Difference in age estimates between otolith readings by Fish Ageing Services (FAS) for swordfish.

Difference	Frequency	% Frequency
-2	2	1.9
-1	12	11.5
0	**56**	**53.8**
1	30	28.8
2	4	3.8
	104	100

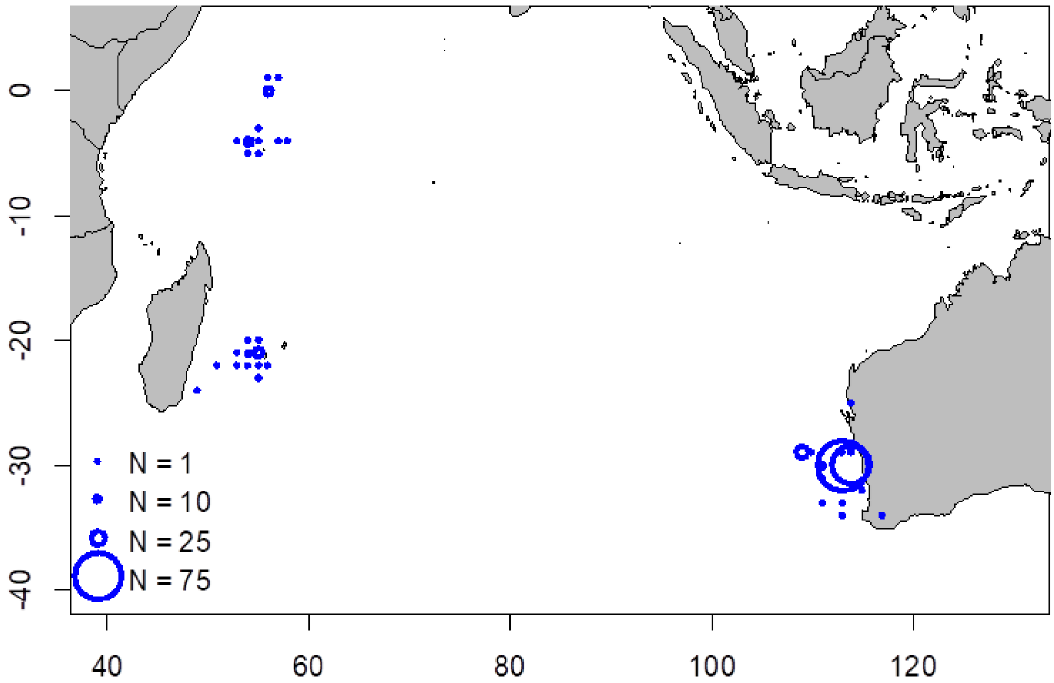

Figure 1. Map of the sampling locations for swordfish with otoliths available for analysis. Longitude shown in degrees east.

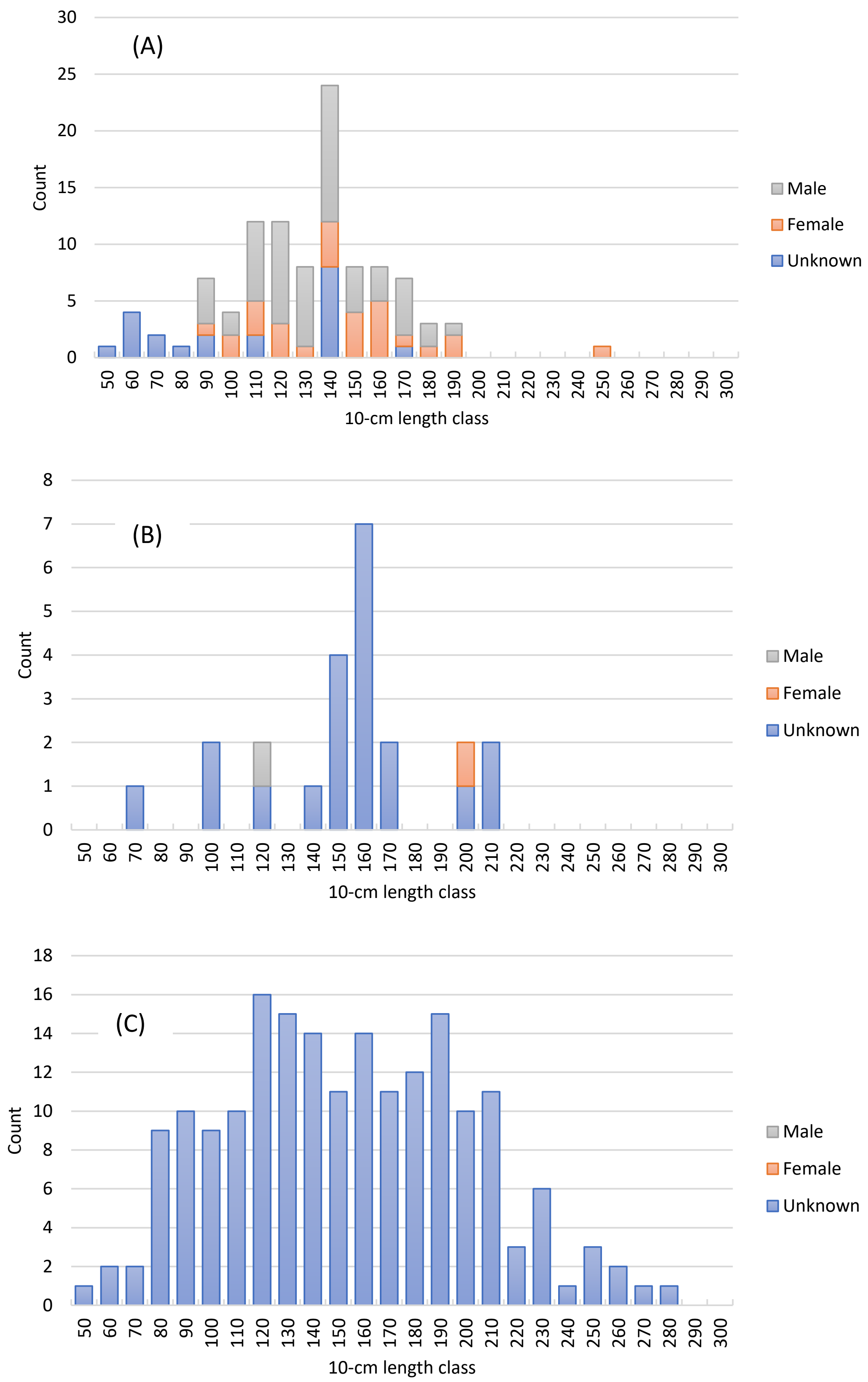

Figure 2. Length frequency (LJFL) of swordfish sampled by sex (A) included in the preliminary growth analysis for the western Indian Ocean in this study (n=105), (B) remaining in the collection from the western Indian Ocean (n=23), and (C) remaining in the collection from the eastern Indian Ocean (n=189). The lower boundary length value of the bin is shown.

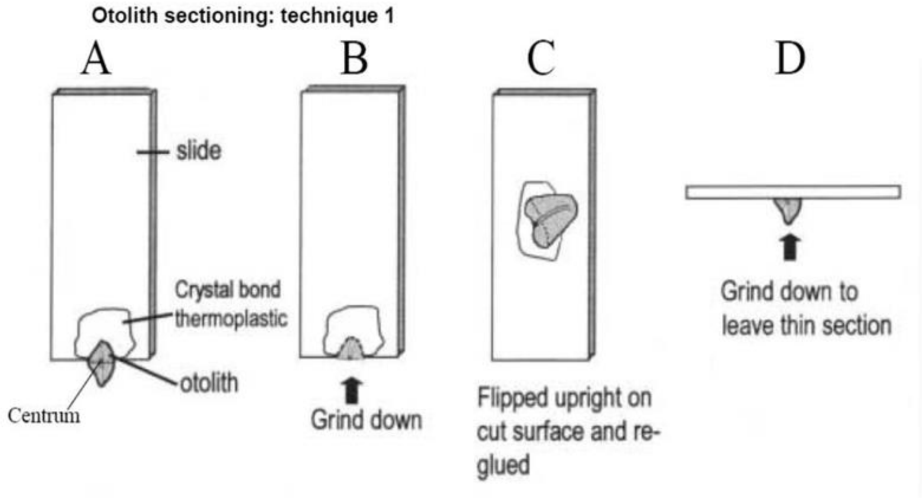

Figure 3. Illustration of the grinding process for preparation of a transverse otolith thin section (Source: Robbins and Choat, 2002)

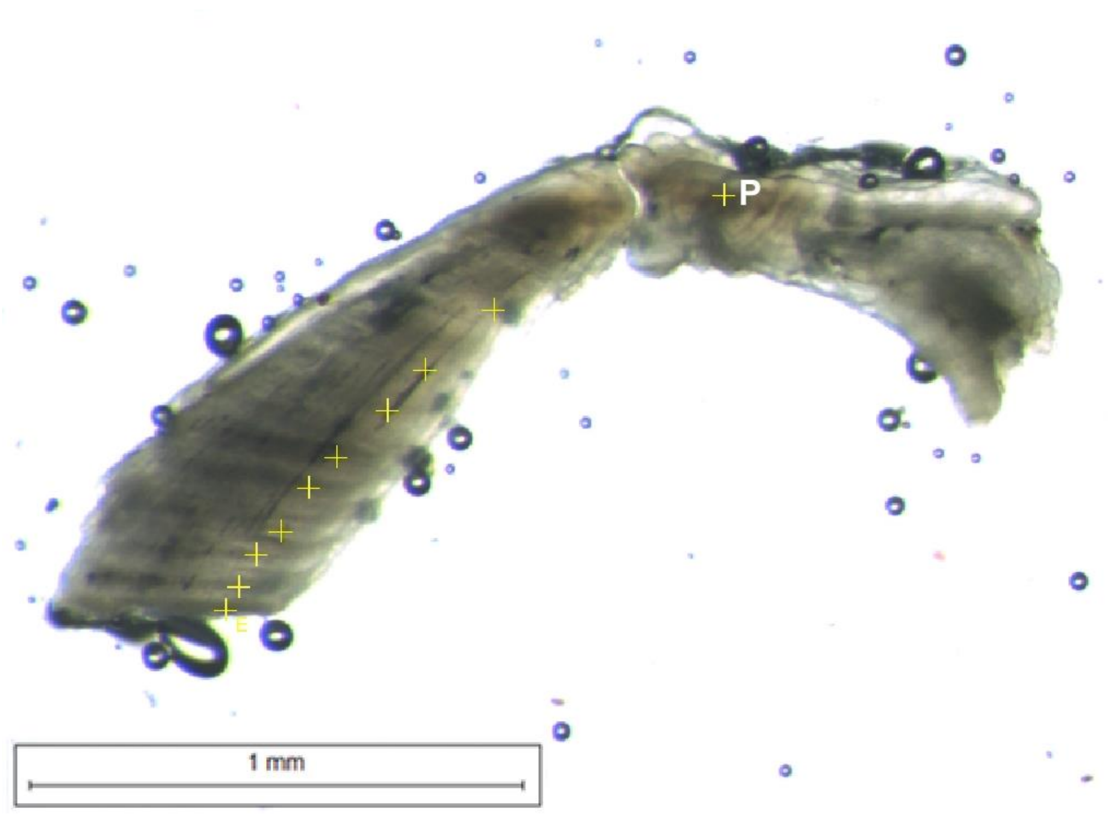

Figure 4. Transverse preparation of a swordfish otolith prepared for annual reading showing presumed annual opaque zones indicated by +, which are marked manually. The image analysis system measures the distance from the primordium (P) to each opaque zone. Sample 32349 (324_018_029), length 184 cm LJFL.

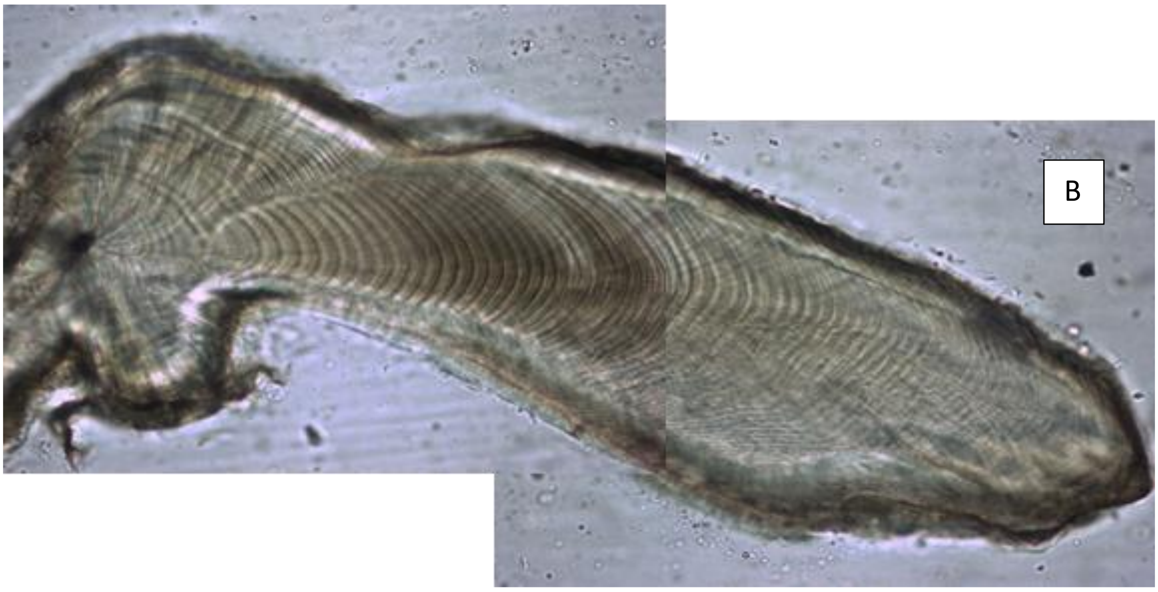

Figure 5. Image of a swordfish otolith prepared as a transverse section for daily ageing. Sample number B5-4W32 (324_017_007), 66 cm LJFL, total estimated age 95 days.

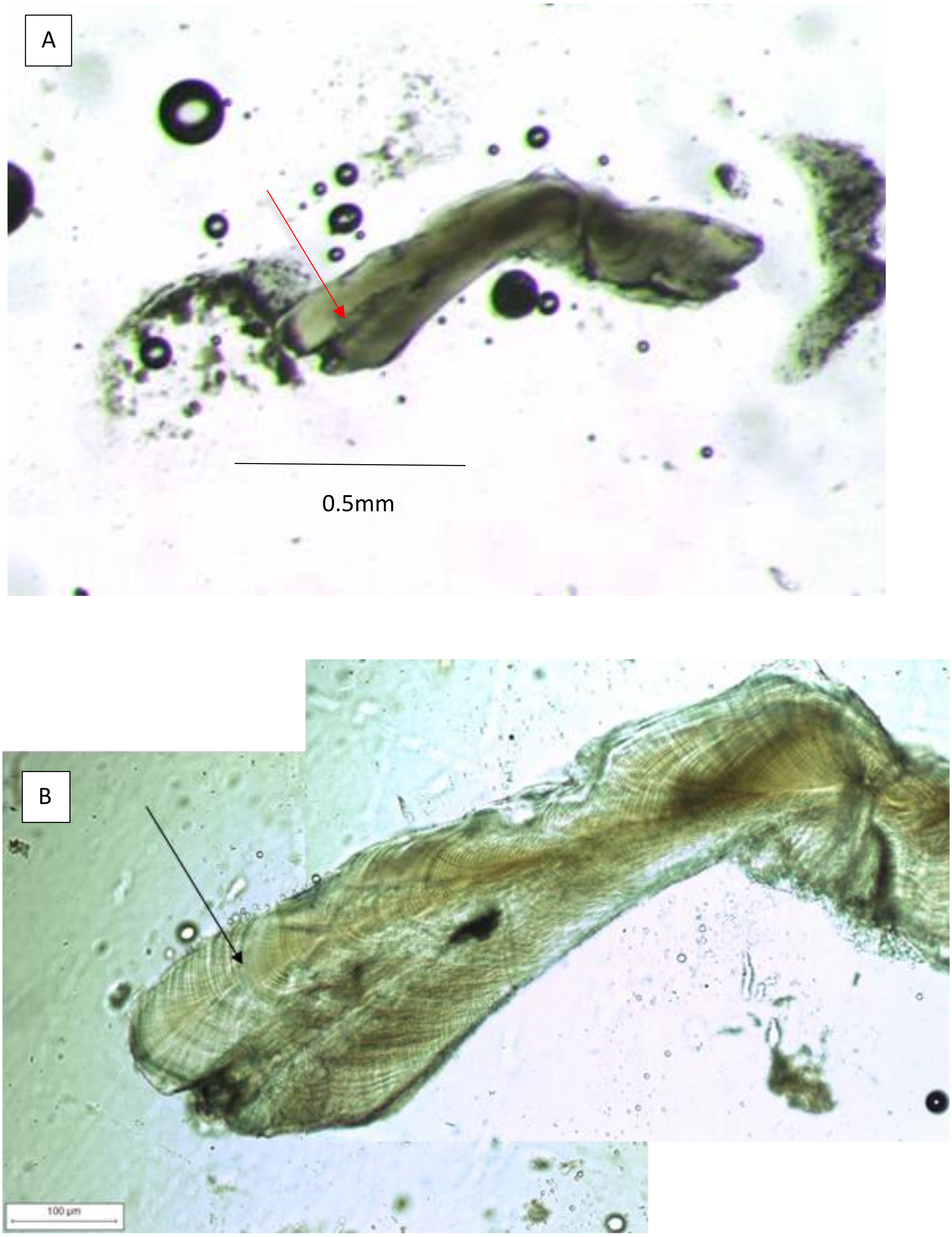

Figure 6. Two images of the same otolith prepared as a transverse section for swordfish. (A) Otolith firstly prepared for annual ageing and (B) ground thinner to reveal the daily structure. Sample number B2-2W15 (324_017_003), 95 cm LJFL, total estimated age 217 days. Transition point where the daily increment becomes less regular and more difficult to interpret is highlighted with a black arrow. This transition point occurred at a count of 160 days and corresponded with the location of the first assumed annual opaque zone observed in the annual preparation (red arrow in A).

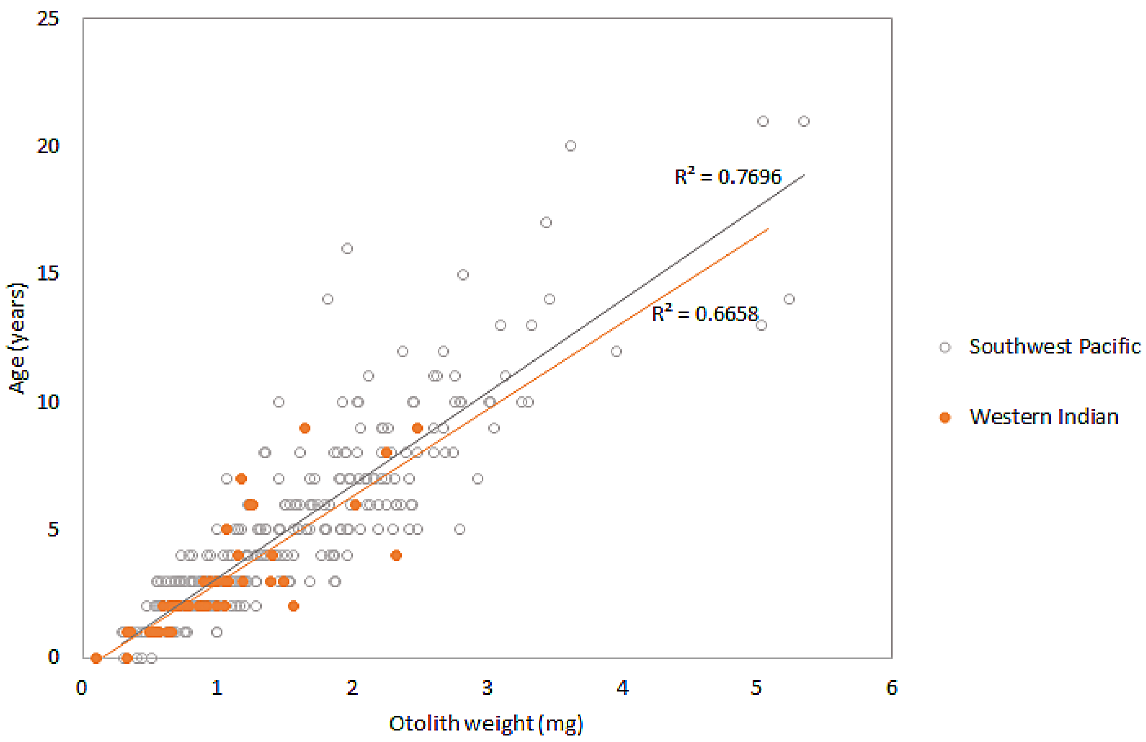

Figure 7. Relationship between otolith weight and fish age (i.e., counts of opaque zones) for swordfish sampled in the western Indian and southwest Pacific Oceans. A linear trendline is fitted to the data separately. The Pacific data is from Farley et al. (2016).

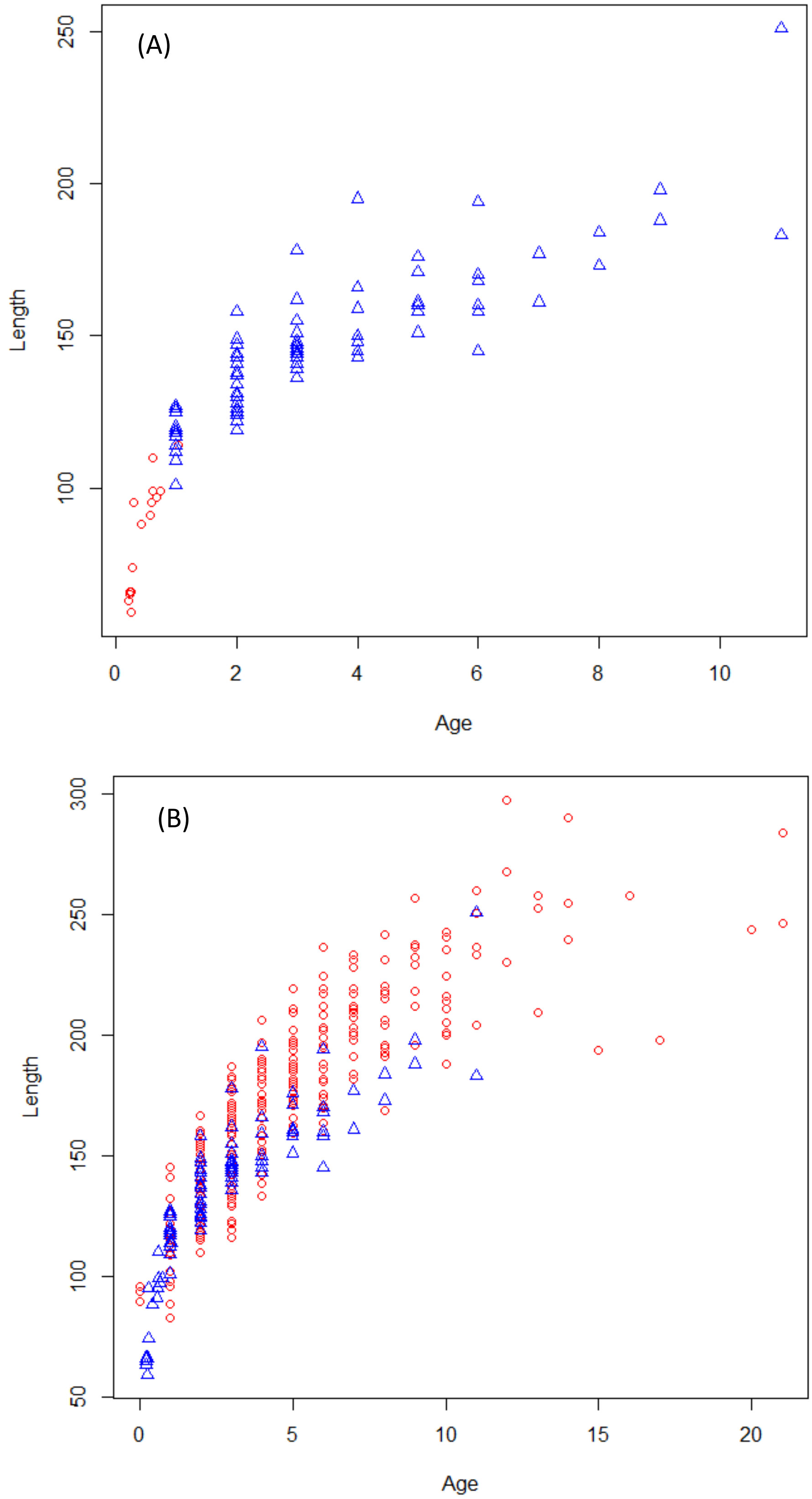

Figure 8. Length-at-age data for (A) swordfish in the western Indian Ocean (from the current study) with daily age data shown in red (circles) and annual age data shown in blue (triangles), and (B) swordfish in the western Indian Ocean (blue triangles; from current study) and southwest Pacific (red circles; from Farley et al. 2016). Length is lower jaw fork length (LJFL, cm). If both daily and annual age estimates were obtained for a given otolith/fish, only the daily age data is shown.

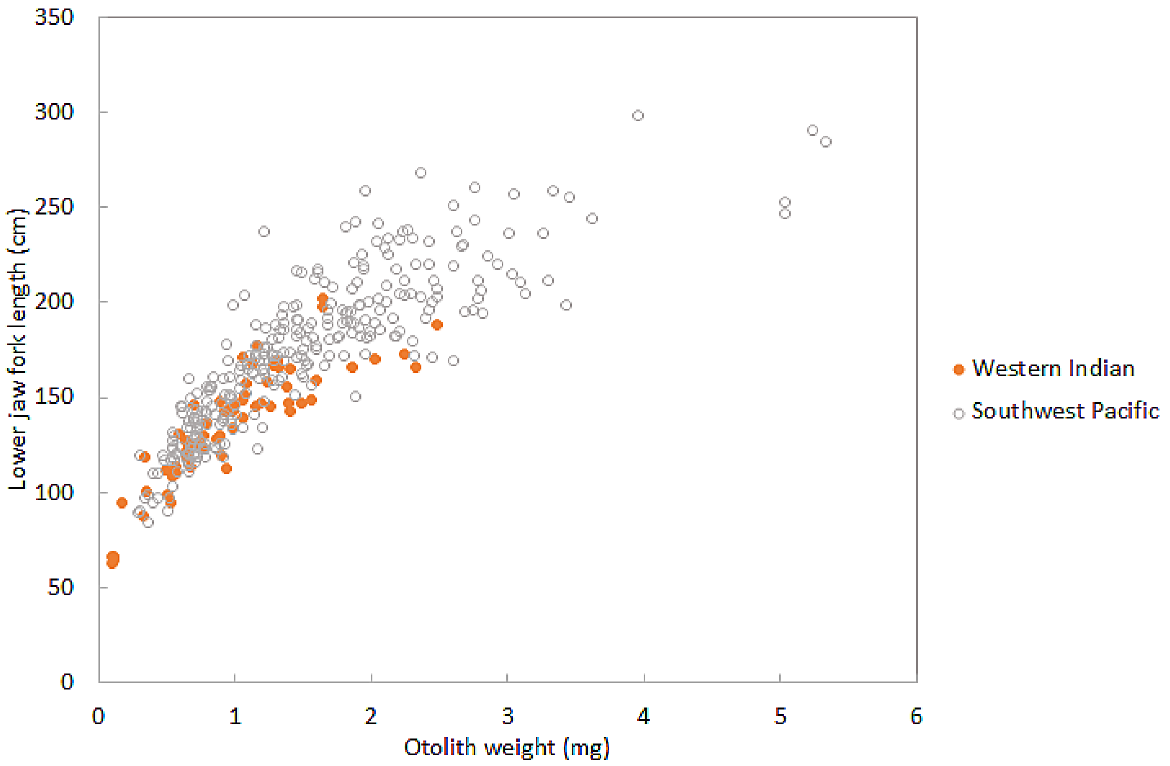

Figure 9. Relationship between otolith weight and lower jaw fork length (LJFL) for swordfish sampled in the western Indian Ocean and southwest Pacific. The Pacific data is from Farley et al. (2016) and orbital fork length (OFL) was converted to LJFL using the relationship in Davies et al. (2013) for southwest Pacific swordfish.

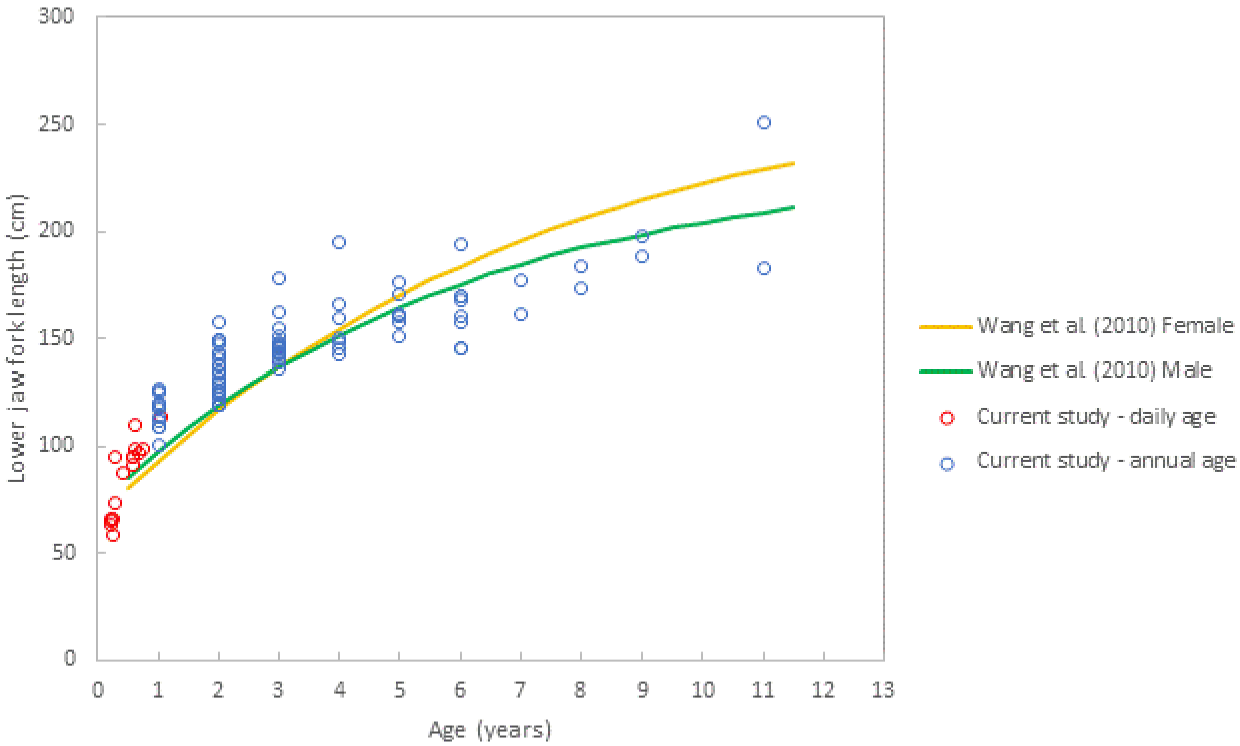

Figure 10. Length-at-age (daily and annual) data for swordfish in the current study. Also shown is the estimated von Bertalanffy growth curves from fin spines for male and female swordfish in the northern Indian Ocean (from Wang et al. 2010).