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#### Preliminary Aging of Striped Marlin in the Southwest Pacific using Otoliths

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# Preliminary ageing of striped marlin in the southwest Pacific using otoliths

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### **Executive summary**

Accurate estimates of age and growth are required for age structured stock assessments and to develop management advice. For most billfish species, sectioned anal fin spines are used to estimate the age and related population parameters, such as growth. However, spines are subject to vascularisation and studies have shown that they may underestimate true age in older individuals. A recent study of swordfish in the southwest Pacific showed that otoliths do contain opaque and translucent zones, and that the pattern of zone formation suggests that they may be formed on an annual basis. This study showed that age estimates from swordfish otoliths is possible and that the technique may be useful for other billfish species.

The aim of this work was to evaluate the use of otoliths to estimate the age of striped marlin (*Kajikia audax*) in the southwest Pacific, using a collection of otoliths from a previous study. The previous study did examine otoliths; however, the analysis was for only very small/young samples and used counts of microincrements to provide growth estimates for the assumed YOY, while annual age of larger fish was estimated age using fin spines.

In this study age from counts of opaque zones in transversely sectioned otoliths was estimated for 17 fish ranging in length from 222 to 269 cm lower jaw fork length (LJFL). The preliminary results indicate that the otoliths of striped marlin do show opaque and translucent zones which are similar to those of swordfish otoliths and suggest that striped marlin may live longer than previously estimated based on dorsal fin spines. Direct validation of the accuracy of the ageing method used (for spines or otoliths) is required to confirm the age estimates. The otolith-based age estimates were provided to SPC for use in the 2019 stock assessment for striped marlin in the southwest Pacific.

# 1 Introduction

Accurate estimates of age and growth are required for age structured stock assessments and to develop management advice. Although fish size is an indicator of age, variability in length-at-age within a species makes it difficult to determine the age of individual fish from length alone. Estimates of age can, however, be obtained from validated counts of annuli in hard-parts, such as otoliths, spines and vertebrae.

Sectioned fin spines are almost universally used to estimate the (annual) age of billfish worldwide as they are easy to collect as otoliths are considered too small and fragile to analyse. In the case of spines, however, it is often difficult to differentiate annual zones from split zones and to account for zones obscured by vascularisation and resorption (Kopf et al. 2010). When this occurs, incorrect interpretation can lead to ageing errors and systematic bias. In contrast, otoliths grow throughout the life of a fish and are not vascularised and, therefore subject to resorption, and are generally considered to be a more accurate structure to age fish (Mugiya &Uchimura 1989; Campana et al. 1995).

In the southwest Pacific, the age of striped marlin has been estimated using counts of assumed annuli in sectioned fin spines (Kopf et al. 2011). A maximum age of 7 years for males and 8.5 years

for females was obtained in the study and indirect validation methods were used to indirectly verify the annual age estimates obtained. Growth parameters were estimated by fitting von Bertalanffy growth models to observed and back-calculated length-at-age data (Kopf et al. 2011). The 2012 stock assessment of striped marlin the southwest Pacific used fixed growth parameters from the "back-calculated 1" growth model (for both sexes combined) from Kopf et al. (2011).

A recent study estimated the age of swordfish (*Xiphias gladius*) in the southwest Pacific using spines and otoliths from the same fish (Farley et al. 2016). A direct comparison of age from the two structures showed no bias in young age classes, however, a clear bias was evident in the older age classes (>7 years for females and >4 years for males), with age from spine counts lower on average than counts from otoliths. Although direct validation of the ageing methods is still required, the study provided strong arguments supporting the use of otolith-based age estimates rather than ray-based estimates, and recommended that otolith-based age estimation is investigated for other billfish stocks (Farley et al. 2016).

Otoliths from striped marlin were collected and analysed by Kopf et al. (2011) to estimate length at (assumed) daily age of fish <214 cm LJFL and to confirm the locations of the first annulus in spines. However, no attempt was made to use otoliths to estimate annual age. This paper provides the results of using the otoliths from large fish from the Kopf et al. (2011) to assess their suitability to provide estimates of annual age of striped marlin.

### 2 Methods

Whole sagittal otoliths (n=19) were obtained from Kopf et al (2011) for striped marlin caught in the southwest Pacific (Australia and New Zealand). Fish ranged in size from 222 to 269 cm LJFL. Otoliths were prepared following the procedure described by Farley et al. (2016) for swordfish. In short, individual transverse sections were prepared and ground down in four step process. Otoliths were initially fixed on the edge of a slide using thermoplastic mounting media (crystalbond 509) with the anterior side of the otolith balanced over the edge. The primordium was positioned on the slide approximately 100  $\mu$ m in from the edge. The otolith was then ground down to the edge using 800 and 1200 grit wet and dry paper. The slide was heated and the otolith was removed before being "glued" (ground side down) to a new slide. The otolith section was ground horizontally with varying grades of wet and dry sandpaper until the section was 250-300  $\mu$ m thick and finally polished with 5  $\mu$ m lapping film and 3  $\mu$ m polishing media (aluminium oxide). The process can also be found in Robbins & Choat (2002). The sectioning plane used is shown in Figure 1.

All otolith preparations were examined with transmitted light and each otolith was viewed once before any attempt was made at age estimation. Many of the otoliths have some areas that appear to lack structure (white arrow in Figure 1). The effect is the result of there being less otolith material to work with (yellow arrow in Figure 2). If further investigation into the potential for sagittal otoliths is deemed warranted, then alternate sectioning planes should be investigated to determine the best sectioning plane for this species. Even though striped marlin otoliths were very small and difficult to prepared, several sections showed clear opaque/translucent zones not dissimilar to those observed in swordfish otoliths (Appendix 1). An image analysis system was used to make counts of sectioned otoliths and to capture images each section. This system counts and measures the distance of each manually marked increment from the primordium and retains an annotated image from each sample aged. The start of each opaque zone was marked and distance from the primordium to each mark and from the final mark to the otolith edge was collected. Opaque zones at the terminal edge of the otolith were counted only if some translucent material was evident after the opaque zone, signifying the completion of the opaque zone. The otolith edge was classified as either narrow translucent, wide translucent or new opaque.

Age estimates from spines were obtained from Kopf et al (2011) for direct comparison with the current otolith-based age estimates. The spine ages were based on counts of annuli (observed and estimated as missing due to vascularisation) plus 0.5 years, if the distance from the last zone counted was more than half the width of the previous increment.

### 3 Preliminary results

Age was estimated (counts of opaque zones) for 17 of the 19 otoliths supplied. The other two otolith could not be read (e.g., top otolith in Figure 2). As only one reading was made of each otolith by one reader, the precision of age estimates could not be estimated from this preliminary study.

Counts of presumed annuli from otoliths ranged from 2 to 11 years, while ages from spines for the same individuals ranged from 3 to 7. Figure 3 shows a comparison of ages from spines and otoliths. A bias was apparent where otolith-based counts were lower on average than counts for spine for the youngest age classes (Figure 3A). However, otolith-based estimates were higher on average than spine-based age estimates for fish > 4 years. One fish had an otolith age of 11 and a spine age of only 3; it was unknown if the wrong sample was analysed (see STM15 in appendix A). Examples of paired structures (otoliths and spines) are shown in Appendix A. Areas of vascularisation are evident in all spine sections, which result in inner growth zones being obscured. Even when methods were applied to estimate lost growth zones in fin spines (see Kopf et al. 2011), the otolith-based annuli count were consistently higher and diverged most in older age classes.

### 4 Summary

Studies indicates that age estimates from otoliths are likely to be more reliable for billfish because fin rays are subject to bone remodelling and resorption. Preliminary analysis of otoliths in the current study indicate that annual like zones can be observed in transverse sections and that resultant count of opaque zones suggest that striped marlin in the southwest Pacific may live longer than Kopf et al. (2011) estimated from dorsal fin spines. The maximum age of striped marlin estimates in previous studies is ~11 years based on analysis of spines (Melo-Barrera et al., 2003) but may have been higher if otoliths were analysed for the same fish. Direct validation of the accuracy of spine and otolith ageing method has not been undertaken in any study, and is

required to validate the annual periodicity of the opaque zones counted in this study. Validation of longevity from small amounts of material in billfish otoliths is now technically feasible using bomb radiocarbon dating (Andrews et al. 2017). The otolith-based age estimates here were provided to SPC for inclusion in the 2019 stock assessment (see Ducharme-Barth et al 2019) as an alternate to the growth and longevity parameters estimated in Kopf et al (2011).

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## 6 Figures

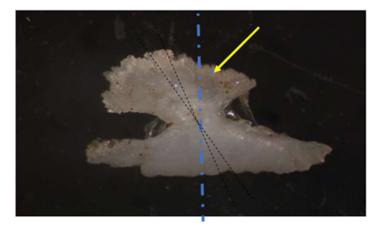


Figure 1. Striped marlin otolith with transverse section plane (blue line) and suggested alternate section planes (black line) that could be investigated. Yellow arrow indicates the area on the otoltih that seemed to lack structure in some otoliths.

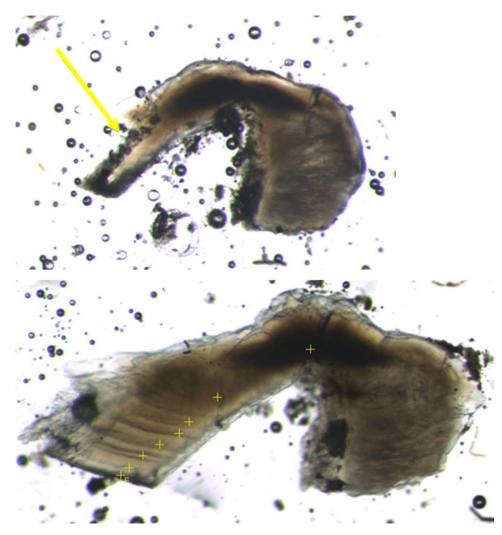


Figure 2. An example of a transverse section of an (top) unreadable otolith indicating an area that lacked structure (yellow arrow) (top) and an otolith with reasonably clear zones (bottom).

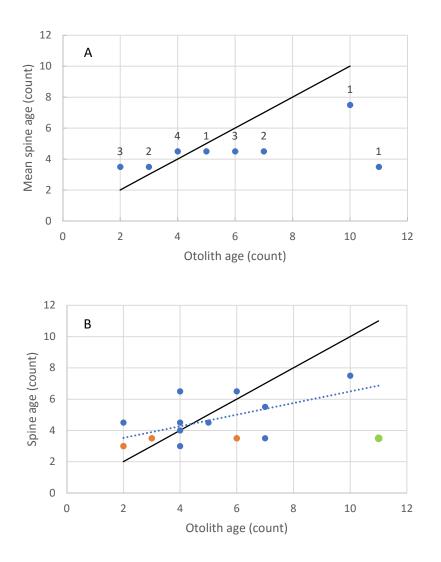


Figure 3. A: Age bias plot for comparison of counts in otoliths and spines sampled from the same fish. B: Comparison of individual counts from spines and otoliths sampled. The orange points designate two fish. Green point indicates one fish with uncertain otolith/spine age comparison (see text). A linear regression line (blue) and 1:1 line (black) is shown (excluding the 'uncertain' fish in red). N=17.

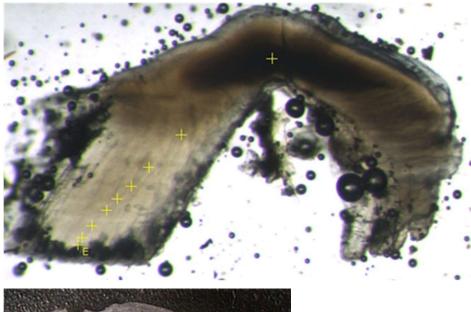
# Appendix A Images of sectioned otoliths and spines of striped marlin with increments counted marked by FAS (+)

Examples of transverse sections of otoliths (yellow +'s mark the opaque zones counted) and sectioned spines of striped marlin examined in the study. Vas = area of vascularisation in the spine section. Fish length, sex and count of increments in otoliths and spines given. Otolith images are all taken at the same scale. Spines images are also all taken at the same scale, but at a different scale to the otoliths.



Swordfish sample ID 324\_002\_055 of a similar age to STM06.

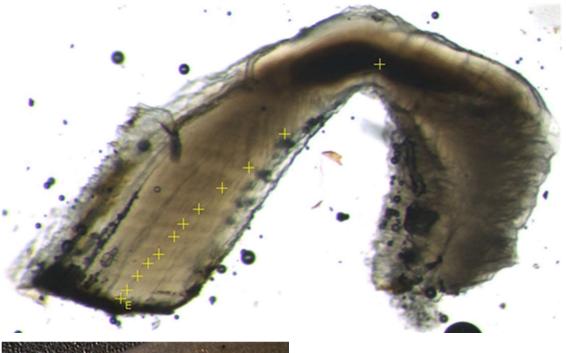
STM06: Length = 255.5 cm, LJFL, Sex = Female Otolith = 6 Spine = 3 (age 3.5)





STM07:

Length = 232.2 cm LJFL, Sex = Male Otolith = 7 Spine = 3 (age 3.5)



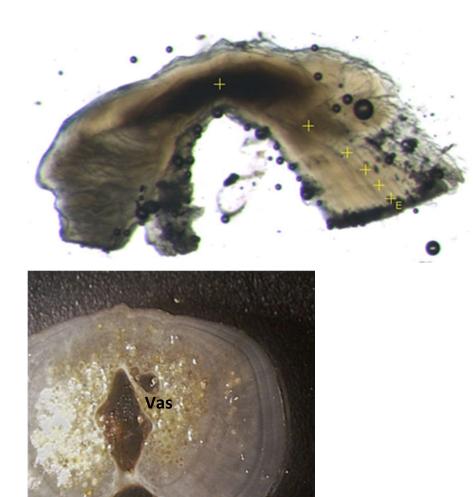


STM09:

Length = 263 cm LJFL, Sex = Female Otolith = 10 Spine = 7 (age 7.5)

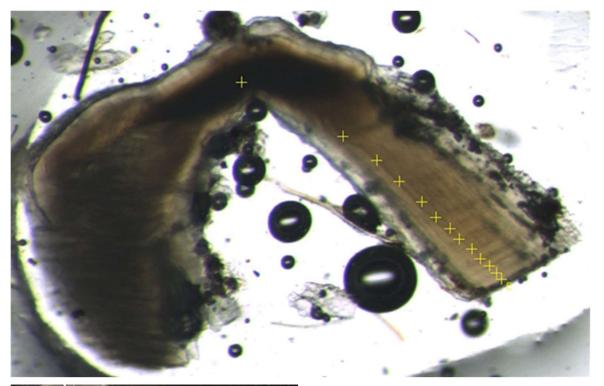


STM10: Length = 242 cm LJFL, Sex = Female Otolith = 4 Spine = 4 (age 4.5)



#### STM13:

Length = 251.8 cm LJFL, Sex = Female Otolith = 4, Spine = 6 (age 6.5)





### STM15:

Length = 244 cm LJFL, Sex = Male Otolith = 11 Spine = 3 (age 3.5)

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