Preliminary age and growth of blue shark (*Prionace glauca*) in the southwest Indian Ocean

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Executive summary

This paper describes preliminary work to assess the age and growth of blue shark in the southwest Indian Ocean as part of the 'GERUNDIO' project[1]. A total of 262 vertebrae samples were available for analysis and 98 were selected for ageing as part of this initial phase of the project. The vertebrae were collected from sharks ranging in size from 96 to 276 cm straight fork length and were caught in the southwest Indian Ocean (close to the coast of South Africa). The maximum age (paired band count) was 17 years for males and 12 years for females. The youngest fish was aged two years.

Direct validation of the accuracy of the ageing methods used was not possible in the current project. However, our preliminary length at age data is consistent with the results of Andrade et al. (2019) for blue shark in the southern Indian Ocean. Limited age validation has been done using bomb radiocarbon dating (^{14}C) methods and we think further consideration should be given to this method to continue efforts to validate the annual periodicity of the bands being counted. Without direct age validation, it is impossible to determine if the age estimates are accurate. We also recommend that additional vertebrae are collected from blue shark in the western and other areas of the Indian Ocean to provide further age information. Given the difficulty of reading blue shark vertebrae, an exchange of vertebrae

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sections (or images) among reading laboratories and an ageing workshop may help to standardise the approaches used for counting growth increments.

Introduction

Blue shark (*Prionace glauca)* is a pelagic species with a widespread distribution in temperate and tropical waters. It is one of the most heavily fished shark species and catches from pelagic longline fisheries have been estimated to be ~10 million individuals annually worldwide (Clark et al. 2009), with ~28 thousand tons taken annually in the Indian Ocean in the most recent period. Genetic studies have previously indicated there is one global population of blue shark with little differentiation within and between oceans (Bailleul et al. 2018). A lack of detection of population structure, however, may be due to insufficient samples or resolution by the method used. A recent study using Single Nucleotide Polymorphisms (SNPs) indicated two genetic clusters for blue shark: one in the north Atlantic Ocean including the Mediterranean Sea and the other in the Indo-Pacific region (Nikolic et al. 2020). The study indicated that the southeast Atlantic may be a mixing area for blue sharks from the two regions.

For stock assessment purposes, blue shark is considered a single stock in the Indian Ocean. The most recent stock assessments indicated that the total biomass is declining but that the stock was not overfished and not subject to overfishing (IOTC 2017). It was recognised, however, that current catches are likely to result in the stock becoming overfished and subject to overfishing in the near future (IOTC 2017). Due to the large catches and declining numbers, blue shark is assessed as Near Threatened by the IUCN (Rigby et al. 2019).

Accurate life-history parameters such as age and growth are required for robust stock assessments and management advice. The age and growth of blue shark has been studied widely in the Pacific but there are only three studies in the Indian Ocean. All studies estimate age using counts of assumed band pairs (annuli) in vertebrae. In the Indian Ocean, two studies obtained a maximum age of 16 years for blue shark in the southwest region (Jolly et al. 2013; Rabehagasoa et al. 2014). The third most recent study (Andrade et al. 2017, 2019) collected vertebrae from 679 blue shark across the southern Indian Ocean between ~40-90^{o}E. A maximum age of 25 years (301 cm fork length) was obtained and although this estimate is not validated, it is the oldest estimate for a blue shark worldwide. The sex-specific growth curves from Andrade et al. (2017, 2019) were included in the most recent blue shark stock assessment in the Indian Ocean (Rice, 2017).

In 2020, the European Union and the 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 Indian Ocean Tuna Commission (IOTC)*". This paper provides preliminary results of age and growth work for blue shark undertaken in this project.

Methods

Sample collection

A total of 202 samples were collected in 2021 from the southeast coast of South Africa (Figure 1). The vertebrae were removed from an area over the gills or from the inter-dorsal space (see Appendix A). An additional 60 vertebrae were provided from the "*Population Structure of Tuna, Billfish and Sharks in the Indian Ocean*" project (PSTBS-IO; Davies et al. 2020). The PSTBS-IO samples were collected from the southern coast of South Africa (Figure 1) in 2018. The vertebrae were removed from the area under the dorsal fin. Natanson et al (2018) found that although age estimates can vary along the vertebrae column, this was less evident in blue shark. All vertebrae samples were collected by observers on board commercial longline vessels.

In the current project, straight fork length (SFL) was measured for all sharks and the interdorsal space (IDS) for 189 fish. The IDS-SFL relationship was estimated using linear and nonlinear (power function) regression models. The power function was of the form:

$SFL = a \times IDS^b$

In the PSTBS-IO project, the interdorsal space (IDS) was measured for all sharks. IDS was converted to SFL using the power function conversion factor (see Results). The sex of each fish was determined in 2021 samples. All vertebrae samples were sent to Fish Ageing Services (FAS) in Australia for preparation and reading.

FAS received two batches of vertebrae; the first batch was 60 samples from the PSTBS-IO project and the second batch was 202 samples from the current project. Samples were sent as four separate vertebrae from each animal. All samples received were meticulously cleaned of muscle tissue. The first batch was not frozen and was discoloured while the second batch was received frozen and in good condition.

In this initial phase of the project, all the first batch and a subset of the second batch was prepared and read (aged), for a total of 98 samples. The samples chosen from the second batch were representative of the length frequency of the samples provided to FAS. All of the samples provided to FAS will be prepared and read in the next phase of the project.

Each vertebra was cleaned, bleached, and embedded in resin before being sectioned (see full detailed in Appendix B). Prepared sections were rinsed and dried using tissue, then mounted on a 75x25mm microscope slide using polyester casting resin and allowed to dry overnight. A cover slip was applied to each slide the next day using polyester casting resin. Completed dried slides were stored in a slide box.

To age the vertebrae, slides were placed on the stage of a Leica M80 dissecting microscope fitted with a 0.5x plan objective. Slides were illuminated using reflected light against a black background using a Leica CLS 150XE light source with fibre optic arms at a magnification of 0.5x. Initially each slide was imaged using the ICMeasure program from The Image Source. Images of each vertebra with a scale bar were taken as a tagged image format file (TIFF) at a resolution of 1024x768 pixels (Figure 2).

Age estimation

To estimate the age from the vertebrae collection, each slide was placed on the stage of the dissecting microscope using the same illumination and magnification that was used to image the sections with the scale bar. The software used for ageing is in-house software developed by FAS. Distance measurements, along with sample meta data, notes and readability score were recorded automatically in MS Access.

FAS uses a readability score of 1 to 5, where 1 is unambiguous exemplar of the given age class, 2 represents a sample with clearly defined zones, 3 may be open to a different interpretation, 4 is very difficult (plus or minus 10%), and 5 means the sample is too difficult to read or is missing.

To age the sample, a point was marked at the beginning of the corpus calcareum (Figure 2). The birth band was then marked, and each subsequent zone marked with a screen marker. The edge of the vertebrae was marked and any notes regarding the sample were entered along with a readability score. The birth band was identified as the first band in the vertebrae. The position of the birth band is also indicated by a small 'bump' on the centrum. A combination of these two morphological features is used to identify the birth band. Each of the zones appear as darkened bands in the corpus calcareum, often these can also be traced through the intermedialia (the cartilaginous tissue at the vertebral centra). Samples were examined on screen, down the oculars of the microscope. The remaining block from which the section was taken was often also examined to aid in the identification of the birth band and subsequent zones. Images of each section, along with screen markers for the identified zones were automatically saved during the reading process.

All samples were read by the same reader a second time to determine ageing error. Samples were examined on screen and using the microscope, but no second image was taken of the 2nd examination of the sample. An average percent error (APE) was calculated and age difference between the first and second readings were examined (Beamish and Fournier 1981). If the successive readings agreed, this estimate was used as the final age. If the readings differed, a further reading was made with knowledge of the previous readings to decide on a final count. Note that, in this study, an age estimate is a count of assumed annuli. No attempt was made to estimate a decimal (fractional) age, except that 0.5 years was added to all counts for comparability with Andrade et al. (2019).

Growth analysis

A von Bertalanffy (VB) growth model was fit to the age and length data. The VB model has the form:

$$L_t = L_\infty(1 - e^{-k(t-t_0)})$$

where L_t is the fork length at age t, L_∞ is the mean asymptotic length, k is a relative growth rate parameter, and t_0 is the age at which shark have a theoretical length of zero. The model was fit using maximum likelihood estimation assuming a Gaussian error structure with mean

0 and variance σ^2. The nlminb function in the statistical software package R (version 4.0.5; R Core Team 2021) was used to minimize the negative log-likelihood.

Results

Length conversion

The nonlinear model (power function) provided a good fit to the IDS and SFL data (Figure 3; $R^2 = 0.886$). The predicted relationship was approximately linear (b parameter is close to 1.0); however, the nonlinear model provided a better fit to the data compared to the linear model (higher R^2 and an intercept closer to zero) (see Figure 3).

Samples collected

Figure 4 shows the size frequency of (a) all fish sampled and (b) fish selected for ageing for the preliminary growth analysis. Fish ranged in size from 94 to 276 cm SFL. The sex ratio of sampled fish was 0.47 females to 1 male, which is significantly different from 1:1 (Chi-square; $P<0.05$). The proportion of males increased steadily from the 190 cm length class (Figure 5). All fish >255 cm SFL were male.

Age estimation

Vertebrae sections from blue shark are extremely difficult to age (see details in Francis and Maolagáin 2016; Natanson et al. 2018). The initial interpretation of the structure was guided by Jolly et. al. (2013) and Andrade et. al. (2019). Age estimates were obtained for 98 fish and of these, 96 fish were included in the final growth analysis. Two additional age estimates were removed as they were possible outliers (i.e., the fish size was not consistent with the vertebrae size). The maximum age was 17 years for males and 12 years for females. The youngest fish was aged two years. The APE for the two readings was 5.25% with a maximum difference of two years (Table 1). Appendix C shows examples of sectioned vertebrae from similar sized fish.

Growth analysis

Figure 6 shows the observed length at age estimated for blue shark by sex with a VB growth model fit to the combined data. There was insufficient data to model growth for each sex separately. The growth curve obtained is very similar to the sex-combined VB curves from Andrade et al. (2019) for blue shark in the southern Indian Ocean.

Figure 7 compares sex-specific VB growth curves obtained in other vertebrae ageing studies for blue shark in the Indian and South Pacific Oceans. For the South Pacific, only growth curves from Manning and Francis (2005) and Joung et al. (2018) are shown because they were input to the 2021 stock assessment (sex-combined) (Neubauer et al. 2021). Note that the sex-combined growth curve by Rabehagasoa et al. (2014) was estimated using vertebrae back-calculation methods (as opposed to observed length at age estimates), which can lead to smaller length at age estimates in earlier ages (i.e., Lee's Phenomenon; Ricker 1975). The differences in growth curves may reflect spatial differences in blue shark growth rates or methodological differences in vertebrae interpretation between laboratories. Thus, an

exchange of vertebrae sections (or images) among reading laboratories may help resolve this.

Discussion

Many shark age validation studies have shown that band pair counts in vertebrae underestimate the age of older individuals (Cailliet et al. 2004). This age underestimation has been attributed to the band pairs being deposited so close together on the margin of vertebrae that they are difficult to resolve. Underestimating age and longevity is concerning as growth rates can be overestimated in slow-growing shark species.

For blue shark, estimating vertebral age seems particularly difficult and subjective due to the presence of single and multiple bands pairs, which are difficult to interpret (Francis and Maolagáin 2016; Natanson et al. 2018). Blue shark is a species where the band pairs in vertebrates have been validated as annual through only a portion of their life (Natanson et al. 2018). In the Indian Ocean, limited age validation has been done using bomb radiocarbon dating methods (Romanov et al. 2012). This radiocarbon study verified the age of two males estimated to be 18 and 22 years old and were 273 and 270 cm SFL, respectively. Other attempts to validate blue shark ageing techniques in the Pacific and Atlantic Oceans include oxytetracycline (OTC) mark-recapture, edge type analysis and marginal increment analysis (Skomal and Natanson 2003; Lesser et al. 2004; Hsu et al. 2015; Wells et al. 2017). Unfortunately, most studies were either limited or inconclusive (i.e., limited sample size, length/age range, or time at liberty).

Direct validation of the accuracy of the ageing methods used was not possible in the current project. Our preliminary length at age data is consistent with the results of Andrade et al. (2019) for blue shark in the southern Indian Ocean. However, without direct age validation it is impossible to determine if the age estimates are accurate.

Summary and recommendations

Studies have indicated that age estimates from vertebrae are likely to underestimate age of older sharks. Direct validation of the accuracy of the ageing method used, spanning the entire size range, and expected range of longevity, is required to confirm the age and growth estimates. Consideration should be given to further 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 that is well-suited to shorter lived species and may be capable of verifying blue shark age estimates.

We also recommend that additional vertebrae are collected from blue shark in the western and other areas of the Indian Ocean to provide further age information. Given the difficulty of reading blue shark vertebrae, an exchange of vertebrae sections (or images) among reading laboratories and an ageing workshop may help to standardise the approaches used for counting growth increments.

Acknowledgements

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Table and Figures

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

Difference	Frequency	% Frequency
-6	0	0
-5	0	0
-4	0	0
-3	0	0
-2	14	14.3
-1	21	21.4
0	**41**	**41.8**
1	16	16.3
2	6	6.1
3	0	0
4	0	0
5	0	0
6	0	0
	98	**100**

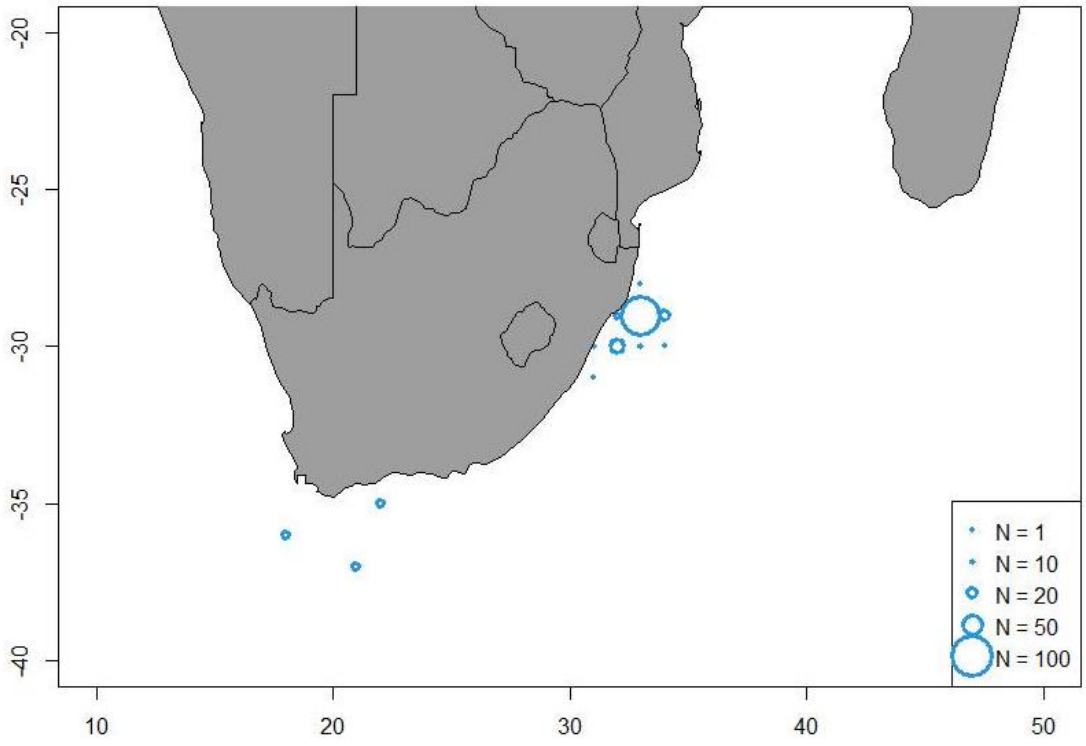


Figure 1. Map of the sampling locations for blue shark sampled. Longitude shown in degrees east.

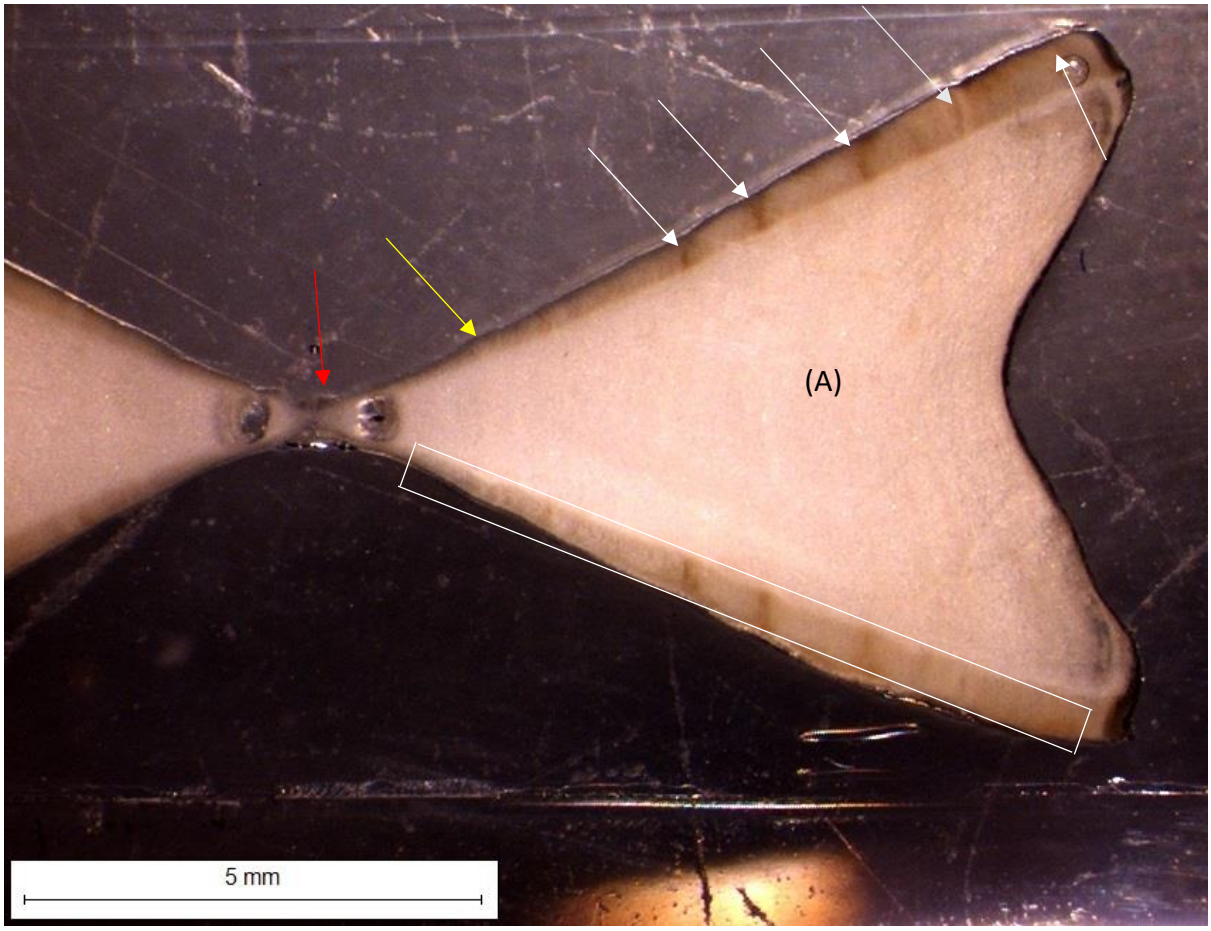


Figure 2. Vertebra from a 197cm SFL male (Original ID 3) sectioned at 500µm illuminated with reflected light. Red arrow indicates the centrum of the vertebrae, the yellow arrow indicates the position of the birth band and the white arrow represent the position of the presumed annual zones. The intermedialia (A) is marked and the corpus calcareum is marked in the white rectangle. White and yellow arrows also mark the centrum. Red arrow indicates the focus of the vertebra.

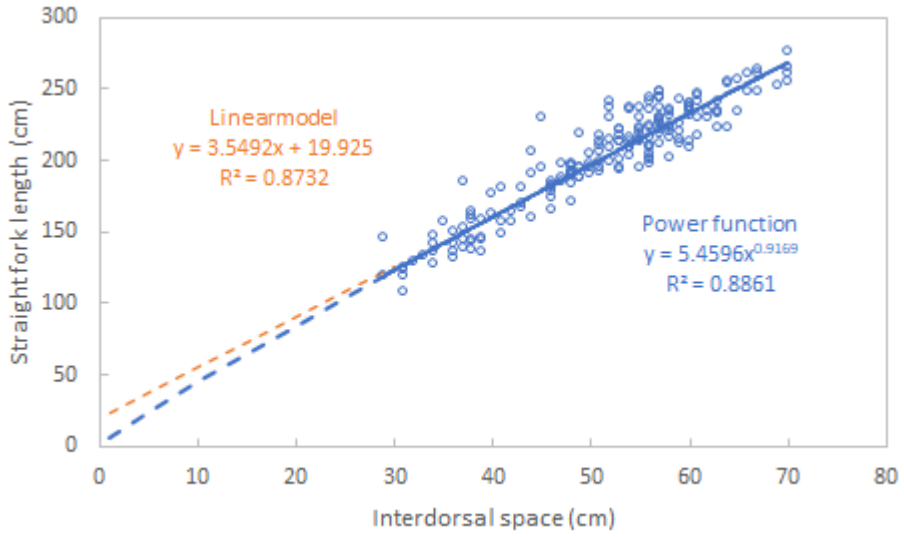


Figure 3. Relationship between interdorsal space and straight fork length for blue shark in the SW Indian Ocean. A power curve (blue line) and a linear model (orange line) fitted to the data are shown.

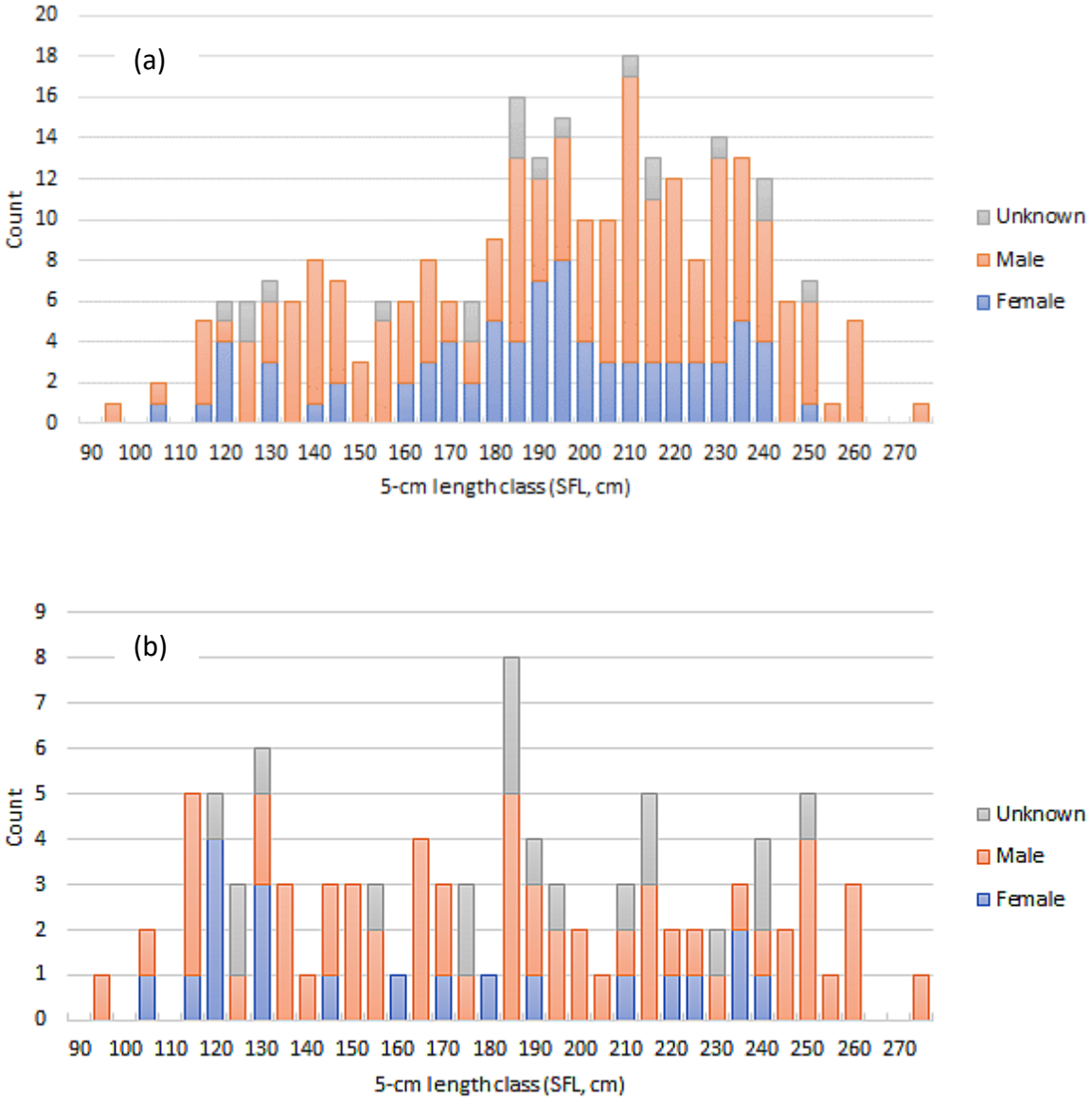


Figure 4. Length frequency (SFL) of blue shark sampled (a) and included in the preliminary growth analysis (b). When SFL was not measured, IDS was converted to SFL using the power function conversion factor shown in Figure 3. The lower boundary length value of the bin is shown.

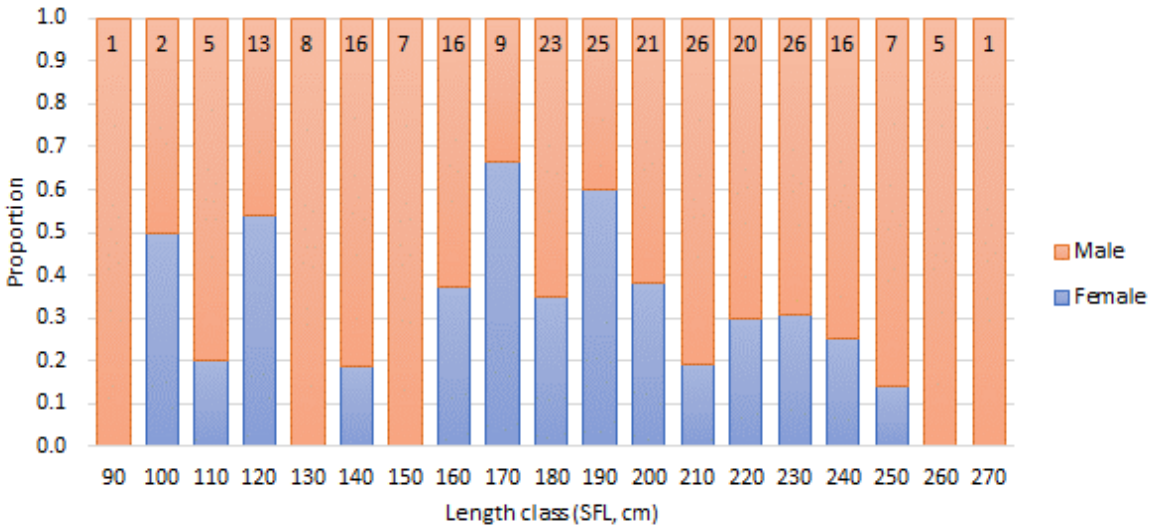


Figure 5. Proportion of blue shark sampled by sex. Sample size is indicated at the top.

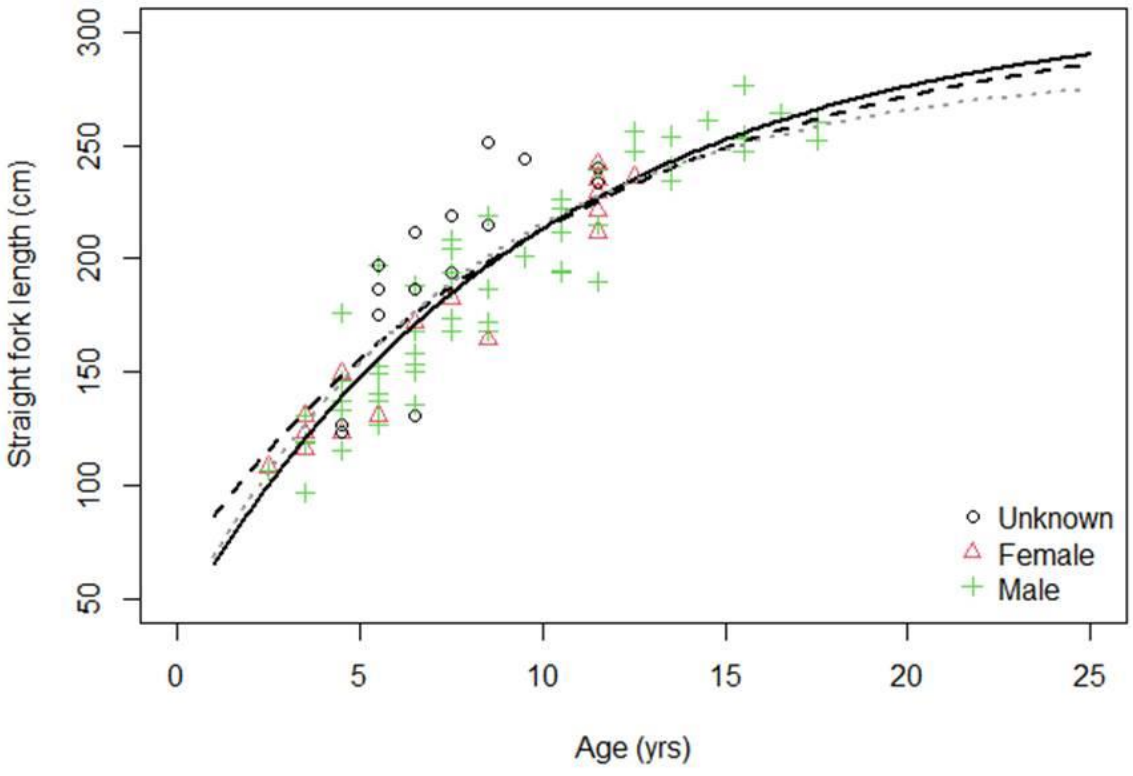


Figure 6. Von Bertalanffy growth curve fitted to length at age data for blue shark in the current study (solid line; L_∞ = 310.6 cm, k = 0.103 yr^{-1}, t_0 = -1.30 cm), and the sex-combined curves from Andrade et al. (2019) (black dashed line = three-parameter VBGF; grey dashed line = VBGF with fixed L_0).

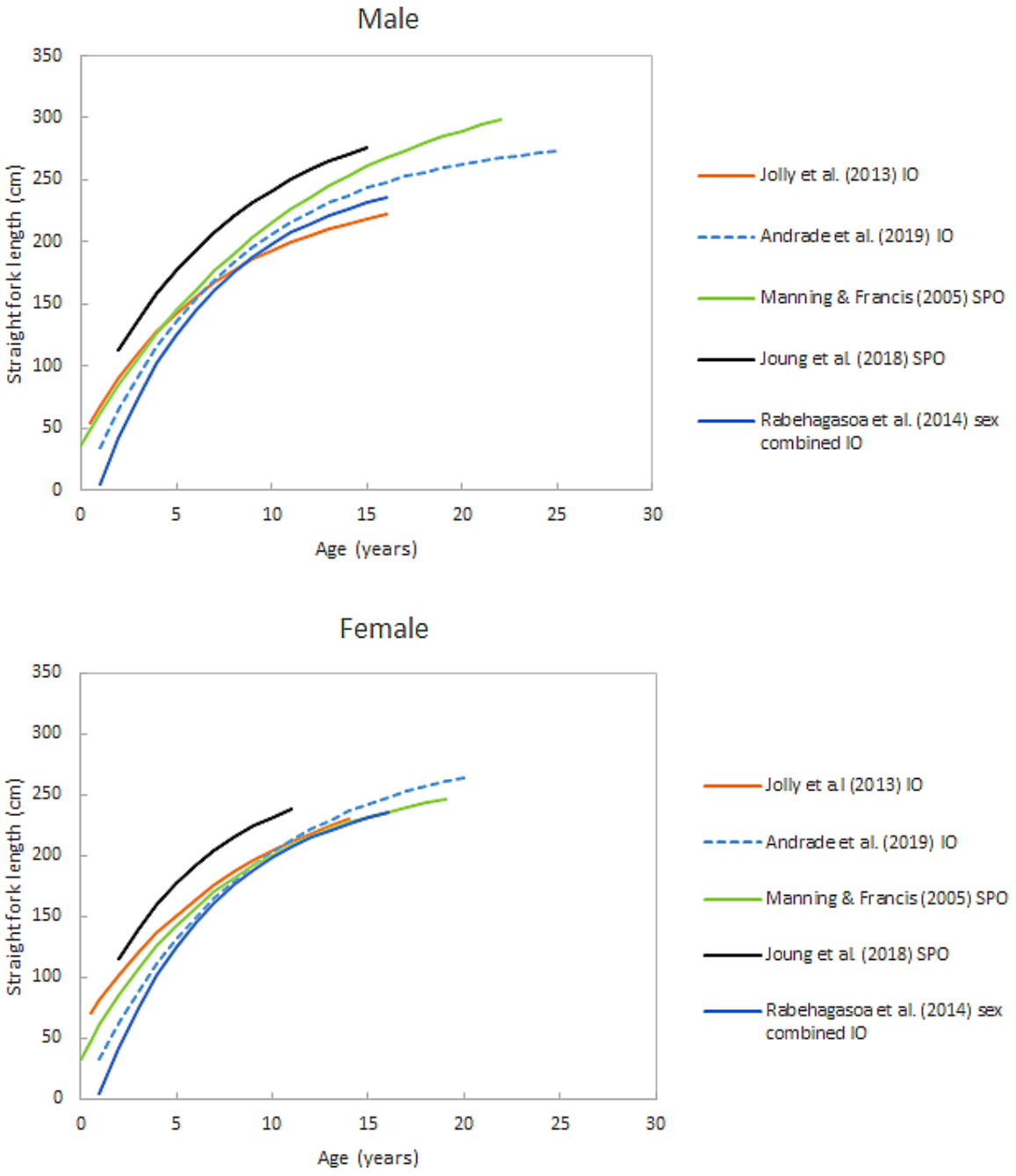
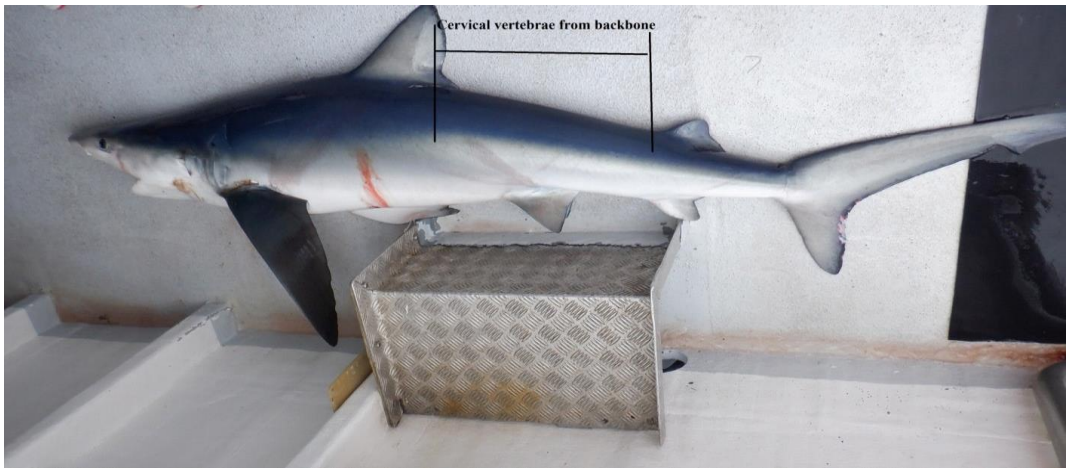


Figure 7. Estimated von Bertalanffy growth curves for male and female blue shark in the Indian and South Pacific Oceans. The sex-combined curve from Rabehagasoa et al. (2014) is included in both plots as sex-specific growth parameters were not available. For the South Pacific, only von Bertalanffy growth curves from Manning and Francis (2005) and Joung et al. (2018) are shown because they were input (sex-combined) to the 2021 stock assessment (Neubauer et al. 2021). Total length was converted to fork length, if necessary, using the conversions in 'IOTC-2020-WPEB16-DATA11-Equations' (Indian Ocean) and Francis and Duffy (2005) (South Pacific Ocean). The curves are shown for the age range of the data in each study. IO = Indian Ocean, SPO = South Pacific Ocean.

Appendix A: Sampling location of the vertebrae

Cervical vertebrae extracted from the backbone “over the gills”.



Sample of the vertebrae extracted from the backbone “posterior” area (interdorsal space).

Vertebrae sample, extracted from the backbone.

Appendix B: Method for preparing vertebrae for sectioning and ageing

To prepare the vertebrae for sectioning, vertebrae were bleached. The vertebrae were immersed in White King Concentrated Bleach. The active ingredients are sodium hypochlorite (10%) solution and sodium hydroxide (1.3%) which bleaches the vertebrae and removes tissue inaccessible using a scalpel. The immersion time varied dependant on the size of the vertebrae. The bleaching times were of shorter duration due to the excellent vertebrae preparation and removal of tissue. The vertebrae were divided into three groups dependant on size. The first group (less than 15 millimetres (mm) vertebral diameter) had a soak time of four minutes in bleach; vertebrae between 16 and 24mm diameter were soaked for eight minutes and vertebrae above 25mm diameter were soaked for 10 minutes. After the vertebrae were removed from the bleach solution, they were rinsed in fresh water to remove excess bleach then soaked in fresh water for 30 minutes. When samples were removed from the water, they were dried in an oven at 40 degrees Celsius for up to ten hours depending on the size of the vertebrae. Larger vertebrae require longer oven time. The cleaned and dried vertebrae were then placed in labelled envelopes.

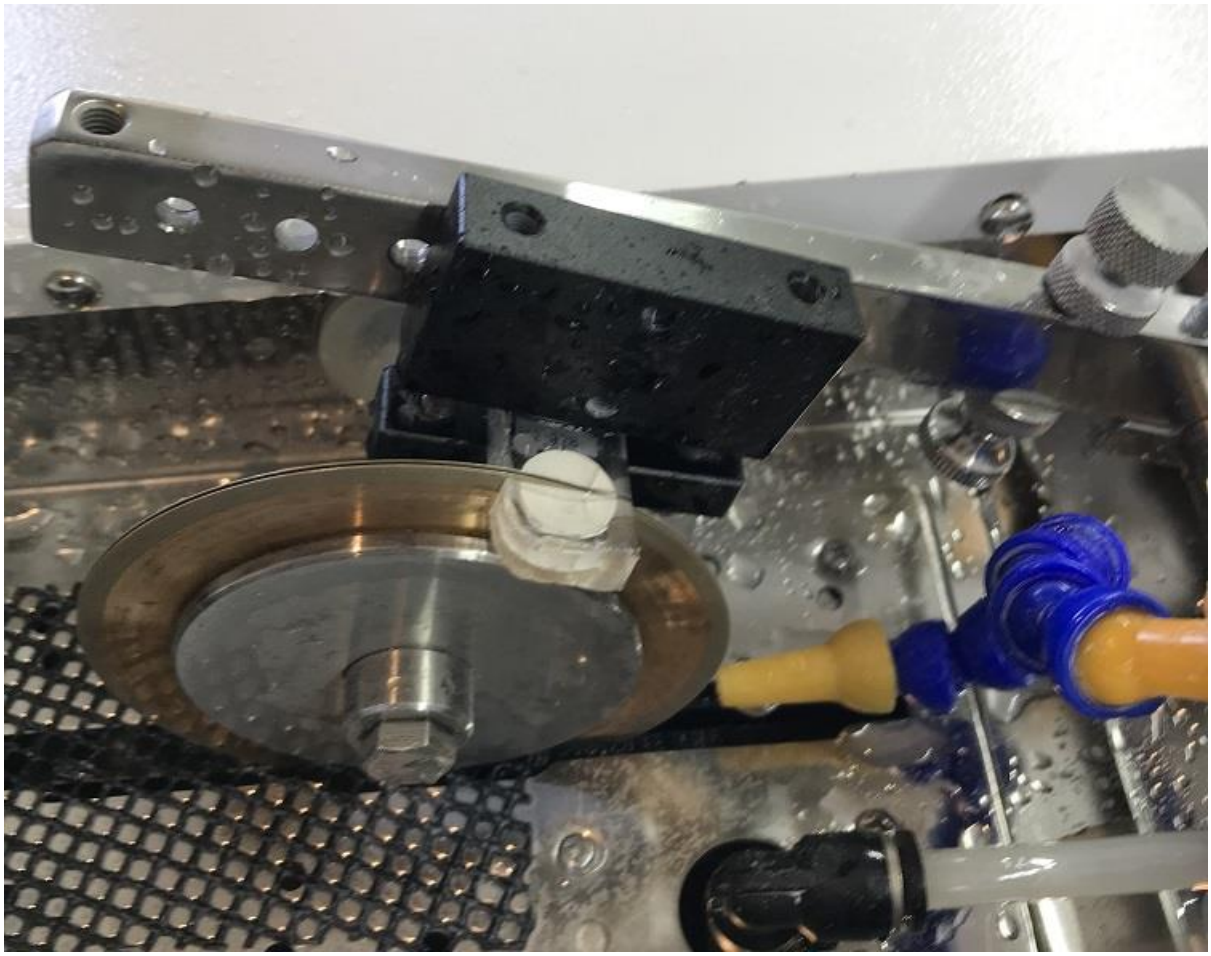
Vertebrae were embedded in the Polyplex Clear Ortho Casting Resin. A diagonal groove was also cut at one end of each vertebra to allow resin to flow into the lower articular face during embedding. A hi-speed Dremel© tool using a plastic cutting blade was used to cut the groove. Vertebrae were then embedded in two rows of four with the articular face upwards. Smaller vertebrae were embedded in FAS rubber moulds (Appendix Fig 1), while larger vertebrae were embedded in longer custom PVC channels (Appendix Fig 2). After the resin had cured, the samples were left for seven days to harden. Each block was then cut into individual elements of one vertebra.

Each block containing one vertebra was mounted in the sample jig on a Pace Technologies PICO 155P precision cutting saw. The sectioning plane for the vertebrae was transverse (proximal-distal *in-vivo*). For smaller vertebrae, 5” blades were used with a spacer between the diamond impregnated blades to cut a 500µm section through the centre of the sample (Appendix Fig 3). Larger vertebrae were cut using a single 6” blade with two passes (Appendix Fig 4). The section thickness was 500µm. All sections were cut with a blade speed of 400 rpm. The blades were lubricated with fresh water. A single section from each vertebra was cut except in the case of one sample where two were cut. This was done on one sample to ensure an ageable section was collected. All remaining pieces of the blocks were stored in labelled envelopes for further examination.

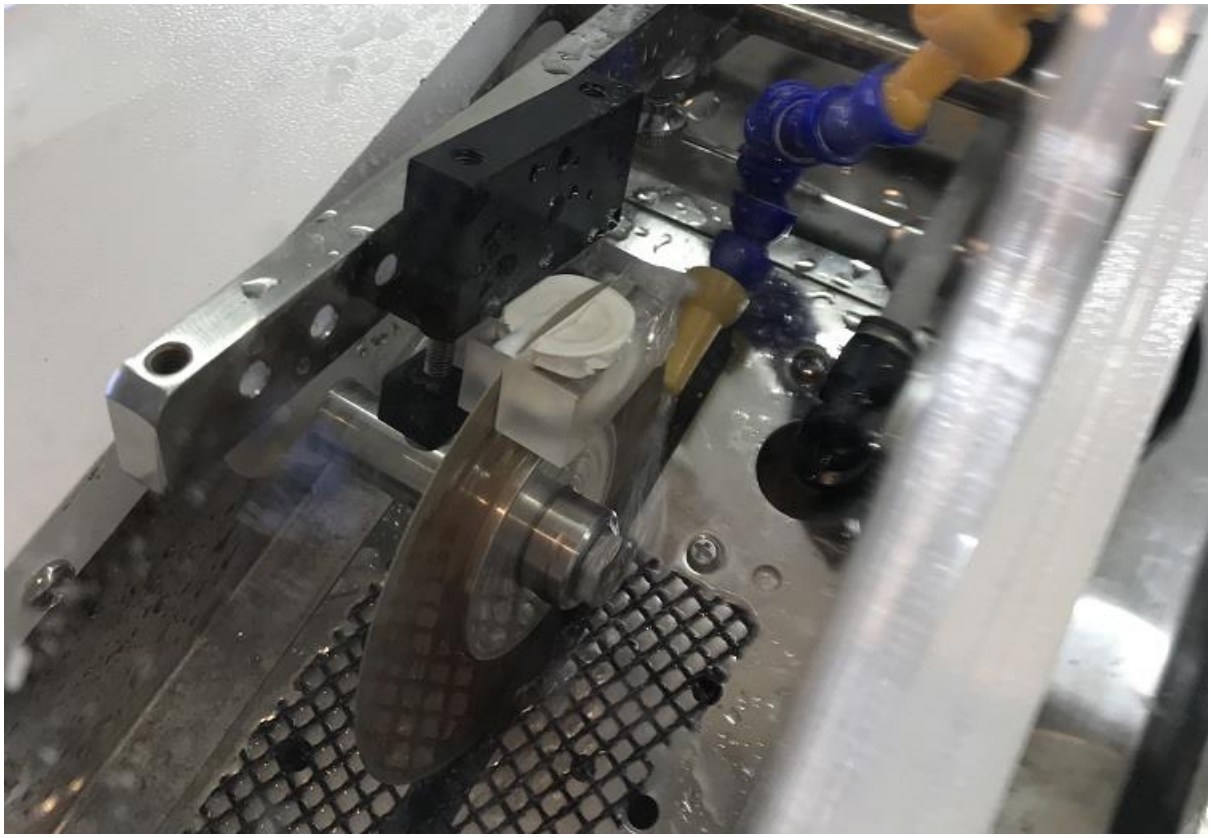
Sections were rinsed and dried using tissue, then mounted on a 75x25mm microscope slide using the polyester casting resin and allowed to dry overnight. A cover slip was applied to each slide the next day using polyester casting resin. Completed dried slides were stored in a slide box.

Appendix Figure 1. Smaller BSH vertebrae in FAS moulds being embedded in casting resin.

Appendix Figure 2. Larger BSH vertebrae in FAS channel moulds being embedded in casting resin.



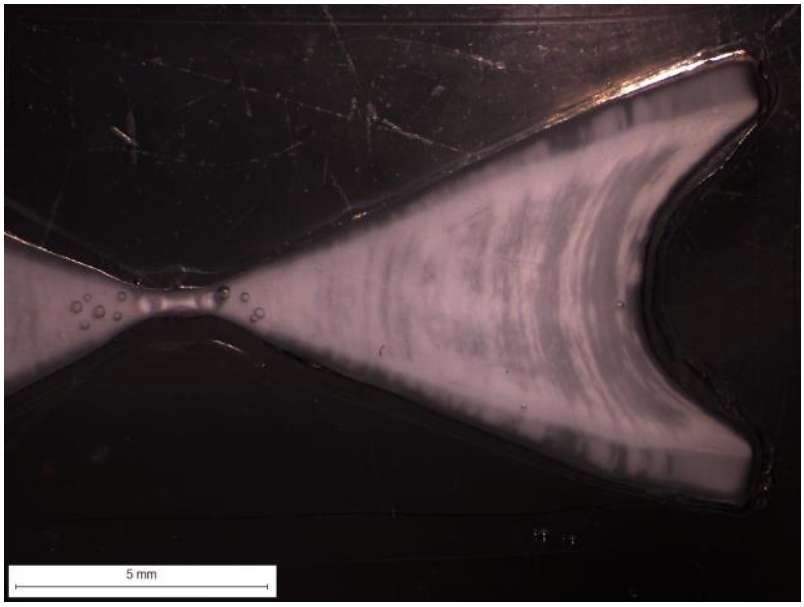
Appendix Figure 3. Sectioning smaller vertebrae using double blades on the PICO 155P.



Appendix Figure 4. Larger vertebrae being cut with single blade on PICO 155P.

Appendix C: Example images of sectioned vertebrae from large blue shark

Append Figure B1. Blue shark vertebrae sample (section #02-157) with a fork length of 236 cm. Age estimate 11 years.



Append Figure B2. Blue shark vertebrae sample (section #02-098) with a fork length of 264 cm. Age estimate 16 years.