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Age and growth of the blue shark (*Prionace glauca*) in the Indian Ocean

I. Andrade[*1,2], D. Rosa[1], R. Muñoz-Lechuga[1] and R. Coelho[1,3]

[1]Portuguese Institute for the Ocean and Atmosphere (IPMA), Avenida 5 de Outubro s/n, 8700-305 Olhão, Portugal.

[2] University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

[3] Centre of Marine Sciences (CCMAR), University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Running headline: Age and growth of the blue shark

[*] Corresponding author. Tel.: +(351)289700504; email: inesfcandrade@gmail.com

ABSTRACT

Since there is still a lack of biological information regarding *Prionace glauca* in the Indian Ocean, specifically in terms of age estimation and growth modelling, the age and growth of this species was studied by analysing vertebral samples. All samples were collected from specimens captured by pelagic longliners between March 2013 and September 2016, with sizes ranging from 82 to 301 cm fork length (L_F). Two growth models were fitted to the age data, a three-parameter von Bertalanffy growth function (VBGF) re-parameterized to calculate L_0 (size at birth) and a two-parameter VBGF with a fixed L_0. The latter was considered the most adequate to describe the growth of the species, with the estimated parameters being L_∞= 283.8 cm L_F, k = 0.13 year^{-1} for males and L_∞= 290.6 cm L_F, k = 0.12 year^{-1} for females. These results suggest that females have a slower growth than males. The maximum age estimated was 25 years, representing the oldest attributed age to this species so far. Further work is needed regarding *P. glauca* in the Indian Ocean, but this study adds important life-history information that can contribute for the management and conservation of the species.

Keywords: bycatch, elasmobranchs, fisheries, growth modelling, pelagic longline

1. Introduction

The blue shark *Prionace glauca* (L. 1758) is the only species belonging to the genus *Prionace* which belongs to the Carcharhinidae Family (Order Carcharhiniformes). *Prionace glauca* is a pelagic and oceanic species with a worldwide distribution, including both temperate and tropical waters (Compagno, 1984). In addition, it is considered by many authors as the most abundant of pelagic sharks (Compagno, 1984; McKenzie & Tibbo, 1964; Nakano & Seki, 2003; Nakano & Stevens, 2008). Therefore, these apex predators are a highly important component of pelagic ecosystems globally (IOTC, 2007). Regarding the distribution of *P. glauca* in the Indian Ocean, results of a recent study by Coelho *et al.* (2018) suggest that larger individuals are found in equatorial and tropical parts of this ocean, while smaller specimens seem to prefer higher latitudes. Sharks belonging to this species can be longer than 300 cm in total length (L_T) (Pratt, 1979) and have been suggested to reach as much as 380 cm L_T (Compagno, 1984). They are placental viviparous sharks with a gestation period going from 9 to 12 months after which females give birth to 4 to 135 pups per litter (Castro & Mejuto, 1995; Compagno, 1984; IOTC, 2007; Nakano, 1994; Pratt, 1979). The young are born in the Spring and Summer (Compagno, 1984; Nakano, 1994; Pratt, 1979). Both sexes attain sexual maturity at a similar body length and age, the latter being between 4 and 6 years for males and from 5 to 7 years for females (Cailliet *et al.,* 1983; Lessa *et al.,* 2004; Nakano, 1994; Pratt, 1979; Vas, 1990). Also regarding age, the longevity of *P. glauca* is thought to be of about 20 to 23 years (Cailliet *et al*., 1983; Manning & Francis, 2005;; Stevens, 2009).

Despite being considered the most abundant of pelagic sharks (McKenzie & Tibbo, 1964; Nakano & Seki, 2003; Nakano & Stevens, 2008), *P. glauca* still faces some threats that could compromise their current populations. Specimens of this species are the most frequently caught pelagic sharks as bycatch by fisheries worldwide, in particular by pelagic longline fisheries targeting tuna and swordfish (Anderson, 1980; Bailey *et al*., 1996; Campana *et al*., 2009; Carruthers, *et al.,* 2011; Francis *et al.,* 2001; IOTC, 2016; Pratt, 1979; Stevens, 1992). When it comes to sports fishing, *P. glauca* is one of the preferred species of sharks by who practices this activity, being one of the main targets (Anderson, 1980; Casey & Hoey, 1985; Compagno, 1984; Skomal & Natanson, 2003; Stevens, 1984, 2009; Vas, 1990). In terms of commercial fisheries, *P. glauca* was rarely a targeted species in the past (Nakano & Stevens, 2008; Stevens, 2009). However, there has been an increasing commercial interest on this species in the recent years, both as a food source and also for its fins (Aires-da-Silva *et al.,* 2008; Dent & Clarke, 2015; Eriksson & Clarke, 2015). In the Indian Ocean, *P. glauca* is the most caught species of shark by Portuguese pelagic longlines, and it is the second most caught species following the main target (Muñoz-Lechuga *et al.,* 2016). *P. glauca* is considered globally as "Near Threatened" by the International Union for Conservation of Nature (IUCN) Red List (Stevens, 2009). Regionally, it has been considered "Near Threatened" in the Northeast Atlantic (Sims *et al*., 2015) and "Critically Endangered" in the Mediterranean (Sims *et al.,* 2016).

The inter-governmental organization responsible for the management of *P. glauca* in the Indian Ocean is the *Indian Ocean Tuna Commission* (*IOTC*). In 2015, such organization carried out the first stock assessment for this species in the Indian Ocean, however the condition of the stock remained uncertain due to the conflicting results obtained. When it comes to stock assessment, age and growth of organisms are very important parameters to estimate growth rates, mortality rates, longevity, and other relevant aspects to evaluate the condition of stocks (Campana, 2001, 2014; Goldman, *et* *al.*, 2012). Since *P. glauca* is very common in pelagic ecosystems, the biology of this species has been well studied over the years, including age and growth studies. However, of the studies performed to date, most have focused on the populations of the Atlantic and Pacific Oceans with only two studies for the Indian Ocean (Jolly *et al., 2013;* Rabehagasoa *et al*., 2014).

Considering the lack of biological information about *P. glauca* in the Indian Ocean, specifically regarding age and growth, the present work aims to 1) estimate the age of *P. glauca* individuals through the reading of growth bands in vertebrae; 2) obtain growth models for both sexes in the South Indian Ocean; and finally 3) provide age and growth data of this species to IOTC for stock assessment purposes and management advice.

2. Materials and methods

2.1. Sampling

All the samples used in this study were collected by scientific fishery observers from the *Instituto Português do Mar e da Atmosfera* (IPMA) on board of Portuguese commercial longline vessels that target swordfish (*Xiphias gladius*) in the Indian Ocean. A total of 818 vertebrae were collected from March 2013 to September 2016. Vertebral samples were collected in the South Indian Ocean between 23.75°S and 34.85°S (latitude) and from 40.70°E to 92.97°E (longitude) (**Fig. 1**).

While on board of the fishing vessels, the sex of all individuals as well as the fork length (L_F) were recorded. Fork lengths were measured in a straight line to the nearest lower cm. Vertebrae were removed from the region below the anterior part of the first dorsal fin of each individual. All vertebral samples were kept frozen from extraction until they were cleaned and then preserved.

2.2. Sample processing

All vertebrae were first cleaned and then sectioned. The cleaning process started by manually removing all the organic tissues around each vertebra using scalpels and tweezers. After that, they were immersed in a solution of 4–6% sodium hypochlorite (commercial bleach) during approximately 5 to 10 minutes (depending on the size of each vertebra) to remove any remaining soft tissues, and finally placed in water for a few minutes to eliminate all the sodium hypochlorite. Once cleaned, all vertebrae were stored in ethanol at 70% until further use.

To prepare the vertebrae for the sectioning process, they were first air-dried from the storing ethanol during approximately 30 minutes and then mounted on microscope slides, using a synthetic polymer glue (Pattex; Henkel, Düsseldorf, Germany). They were left during 24 hours for the glue to air dry completely. Once the glue was fully dried, each slide was placed in a sectioning cutter, a Buehler Isomet 1000 precision low-speed saw, with two diamond waffering blades, to produce 0.5 mm longitudinal sections. The sections were cut through the centre of the vertebrae to reveal the *corpus calcareum* (bow-tie) and banding structure used to estimate age.

To enhance the band pattern, the sections obtained were stained with crystal violet solution (Sigma-Aldrich Co., St. Louis, MO), previously used in other shark ageing studies (e.g., Coelho *et al.,* 2011; Fernandez-Carvalho *et al.,* 2011; Fernandez-Carvalho *et al*., 2015; Rosa *et al*., 2017), during 2 (for small sections) or 3 minutes (for bigger sections) and then turned upside down and left for another 2 or 3 minutes to guarantee that the staining is even on both sides. Only one of the two sections obtained from each vertebra was stained, to later compare the visibility of the stained versus the non stained bow-ties of the vertebrae. After staining, both sections of each sample were covered with paper and tightly wrapped between two microscope slides, in order to maintain the original shape once fully dried. They remained wrapped for 24h until dry.

The completed sections of each vertebra were mounted onto glass microscope slides using Neo-Mount and observed for growth band structure under a Nikon dissecting microscope with a mounted high resolution digital camera, using transmitted white light. Photographs of each observed sample were recorded and then digitally enhanced using the ImageJ software (Schindelin *et al.*, 2015) by adjusting the contrast and brightness. The same software was used to mark the growth bands, as well as the focus and the outer edge of the *corpus calcareum* of each vertebral sample (**Fig. 2**).

2.3. Age estimation and precision analysis

Age was estimated by counting the number of band pairs visible along the *corpus calcareum* in each sample using the enhanced photographs, with a band pair consisting of one wide band and one narrow band. Annual deposition of growth bands was assumed and the first distinct band (usually associated to an angle change in the outer edge of the *corpus calcareum*) was considered to be the birthmark (e.g., Francis & Maolagáin, 2016; Hsu *et al.,* Liu, 2015; Jolly *et al.,* 2013; Rabehagasoa *et al.*, 2014).

To develop the age reading protocol a reference set of 50 specimens (25 male and 25 female) representing the length range available was selected to be used among the age readers. These were selected to contain approximately the same number of samples corresponding to individuals of each body length class (size classes of 10 cm), in order to be representative of the total sample size. Once the reference set was complete, growth bands in these 50 vertebrae were then read by the main reader together with two other readers, reaching a consensus for the age of all of them. Then each reader carried out an independent reading. When the results from the three readers had two or three counts differing from the initial agreed age for a certain sample, those vertebrae were analysed again by the three readers together to reach a new consensus/agreed age.

From the total sample size (n=818), 793 samples were used for age readings, with the remaining 25 being initially excluded because of inconsistent band patterns. All 793 vertebrae were read three times and without previous knowledge of the length or sex of each specimen in order to prevent bias while counting the growth bands. To calibrate the readings (i.e., making sure the same criteria were always used when marking the growth zones), a reading of the reference set was carried out before the start of each reading. Also, to prevent familiarity with any particular vertebra, each reading was finished before starting the following one. Additionally, a fourth reading was carried out for the samples whose first three readings produced three different attributed ages, but with two of the three differing only by one year. After all the readings, only vertebrae whose band pair counts obtained three or two out of three equal readings were considered for the age and growth analysis.

In order to compare the precision between the three initial readings, the coefficient of variation (CV) (Chang, 1982), the average percent error (APE) (Beamish & Fournier, 1981), the percentage of agreement (PA) (Beamish & Fournier, 1981) and percentage of agreement within one growth band, and two growth bands (PA ± 1 year, PA ± 2 years) were calculated and compared among the readings. Additionally, age bias plots were also used to graphically compare the accuracy of the three readings (Campana, 2001). Each of the three readings (with 95% CI) was plotted for the agreed age. The agreed age was attributed when between the three readings, at least two were identical. The precision analysis was carried out using the R statistical language (R Core Team, 2018).

2.4. Growth modelling

In order to obtain the vertebral radius (VR) of each vertebra, the distance between the focus of vertebrae and the outer edge of the *corpus calcareum* was digitally measured in the photographs of each vertebral sample using the "Measure Cumulative Distances [1]" macro in the ImageJ software. This macro measures cumulative distances along a segmented line selection or between the points of a point selection. The distances were measured to the nearest pixel. Three different scales were used when taking the photos, depending on the size of the vertebrae, and all of them were adjusted to pixels in ImageJ (resulting in 1 mm = 298 pixels, 1 mm = 157 pixels or 1 mm = 99 pixels). Since in some of the vertebral sections the tips were broken, thus not showing the focus or the complete outer edge of the *corpus calcareum*, those were not used to calculate VRs, only 714 out of the 818 were used. The relationship between the vertebral radius and fork length (L_F) of each specimen was then obtained using a linear model following the equation (1):

$$L_F = a + b\text{VR}, \quad (1)$$

where, b is the slope and a is the intercept. In order to test differences in the L_F to VR relationship between sexes an ANOVA test was performed.

To obtain growth curves for the studied species, two growth models were used, both of them applied to males and females separately and to the two sexes combined. The first model used was a three-parameter von Bertalanffy growth function (VBGF) re-parameterized to estimate L_0 (size at birth) instead of t_0 (theoretical age at which the expected length is zero) (Cailliet *et al.*, 2006) following equation (2):

$$L_t = L_\infty - (L_\infty - L_0) \times \exp^{(-kt)}, \quad (2)$$

where L_t is the mean size (L_F, cm) at age t (year), L_∞ is the maximum asymptotic size (L_F), L_0 is the size (L_F, cm) at birth and k is the growth coefficient. The second model used was a two-parameter VBGF, following equation (2), with L_0 fixed to the medium size at birth of 39.5 cm L_F described for this species considering the 35 to 44 cm interval (Pratt, 1979; IOTC, 2007).

Both models were fitted to the age data, corrected to the midpoint of each age class by adding 0.5 years to the estimated ages, using nonlinear least squares (nls function in R) and all plots were created with the package "ggplot2" (Wickham, 2009) in R (R Core Team, 2018). For each of the fitted models, the growth parameters were estimated, along with standard error (SE) and 95% confidence intervals (CIs).

In order to test the null hypothesis that there was no difference in growth parameters between both sexes, a likelihood ratio test (LRT) (Kimura, 1980) was performed on both the three and two-parameter VBGF. Additionally, the model goodness-of-fit was compared with the Akaike Information Criterion (AIC), as well as with the Bayesian Information Criterion (BIC) values. The model with the smallest AIC and BIC values is considered the best fit to the data.

3. Results

3.1. Sample characteristics

A total of 818 vertebrae of *P. glauca* specimens were collected for the present study, of which 491 (60%) were from male sharks and 327 (40%) were from females. The size distribution of specimens ranged from 93 to 301 cm L_F for males (mean ± SD: 203 ± 50 cm) and the females ranged from 82 to 284 cm L_F (mean ± SD: 204 ± 41 cm) (**Fig. 3**).

Of the 818 samples, 793 were used for age readings, with 133 of these having three different readings but at least two of them differing only by 1 year, thus a fourth reading was carried out for these 133 samples. After all readings were completed, 679 (85.6%)

vertebrae (421 males and 267 females) were considered to have a valid estimated age (at least two identical readings) and were thus considered for the age and growth analysis.

3.2. Age estimation and precision analysis

The PA between the three readings, first and the second, first and third and the second and third was 29%, 37%, 44% and 54%, respectively, suggesting a progressive improvement in the consistency of readings. The same pattern was observed with PA ± 1 and PA ± 2 years. The CV and APE between the three readings were 8.95% and 6.72%, respectively. The age bias plots (**Fig. 4**) between each reading and the agreed age between the three reveal a high agreement with no systematic bias.

3.3. Growth modelling

Regarding the relationship between vertebral radius (mm) of each vertebra and the L_F (cm) of the respective specimen (**Fig. 5**), the main effect of sex was not significant (ANOVA: F(1, 710) = 1.78, P > 0.05) but the interaction term between VR and sex is significant (ANOVA: F(1, 710) = 21.62, P < 0.05;.. Therefore, the regression equations between VR and L_F were calculated for females (L_F = 17.45 VR + 13.26; r^2 = 0.91) and males (L_F = 15.82 VR + 29.82; r^2 = 0.95) separately.

A total of 679 *P. glauca* specimens were given a final agreed estimated age, with ages ranging between 1 to 20 years for females and between 1 to 25 years for males. The LRT test revealed differences between males and females growth parameters for both the three-parameter VBGF (LRT: X^2 (3, *N*= 679) = 11.85, *P* < 0.05) and two-parameter VBGF (LRT: X^2 (2, *N*= 679) = 15.99, *P* < 0.05). Therefore, both the three-parameter VBGF and the VBGF with a fixed L_0 were fitted for females and males separately (**Fig. 6**).

The estimates for growth parameters are displayed in **Table 1**. The estimated values of L_∞ were greater for both sexes when using the three-parameter VBGF instead of VBGF with fixed L_0. For both models, L_∞ was greater for females. The values for k were slightly greater when using VBGF with a fixed L_0. The estimates for L_0 with the three-parameter VBGF were similar for males (L_0 = 61.9 cm L_F) and females (L_0 = 64.1 cm L_F) (**Table 1**). The three-parameter model presented a lower AIC and BIC than the model with a fixed L_0, suggesting that the first model represents a better fit to the data. However, more biologically reasonable values are likely produced with the two-parameter model with fixed L_0 *(see discussion section for details*). The results obtained with the recommended final model suggest females reach a greater asymptotic length (L_∞) than males, and males have a greater growth coefficient (k), indicating a slower growth for females (males: L_∞= 283.8 cm L_F, k = 0.13 year^{-1}; females: L_∞ = 290.6 cm L_F, k = 0.12 year^{-1}).

4. Discussion

In the present work, two new growth curves were obtained for *P. glauca* in the Indian Ocean, one for males and the other for females, with new estimated growth parameters. This new information can now be used in future stock assessments, to provide more robust scientific advice on the exploitation of this stock. In addition, the maximum estimated age of 25 years that we report is the highest attributed age to this species so far.

Vertebrae of *P. glauca* are known to be difficult to read due to a poor growth band contrast (Skomal & Natanson, 2003; Manning & Francis, 2005; Rabehagasoa *et al.*, 2014). In the present study, 25 vertebral samples of the total 818 collected were initially excluded because no band pattern was visible that could be quantified. The low number of rejections in the present study may exemplify the fact that other studies have had significant difficulties in the age reading of vertebrae. These represent a very low percentage of rejection, however, in studies with much smaller sample sizes, discarding considerable amounts of samples can represent significant age estimation problems. Some studies of other shark species have used crystal violet as a staining solution to enhance growth band structure in vertebrae (e.g., Coelho *et al.,* 2011; Fernandez-Carvalho *et al.,* 2011; Fernandez-Carvalho *et al*., 2015; Rosa *et al*., 2017), but this approach had not previously been applied to *P. glauca.* In the current study, a comparison was made between unstained and stained vertebral sections, the latter providing a significant improvement to the band structure contrast. Thus, staining sections with crystal violet as well as digitally enhancing the contrast of the growth bands seems to be a better solution to address the difficulties found with age reading of *P. glauca.*

In terms of precision analysis, CV and APE are both widely used in ageing studies. Campana (2001, 2014) suggested values of less than 7.6% for CV and 5.5% for APE, although mentioning that most shark age studies have a CV exceeding 10%. In the present study, CV and APE were of 8.95% and 6.72%, respectively, which is higher than the values suggested by Campana (2001, 2014) but lower than most shark age studies CV reported by the same author. The precision analysis together with the age bias plots, supports the consistency of age estimations and their adequacy for the studied species.

The longevity of *P. glauca* was previously thought to be of about 20 to 23 years (Cailliet *et al*., 1983; Manning & Francis, 2005; Stevens, 2009). These values are close to the maximum estimated ages in this study which were 20 and 25 years for females and males, respectively. The oldest individual was a 25 year old male with 301 cm L_F (**Fig. 2C**). The age estimates here obtained for both sexes are greater than any of the previously estimated ages of *P. glauca*.

Age validation was not performed in the current study, but other studies have verified or validated an annual periodicity of growth band deposition for *P. glauca* that could be applicable to the age readings performed in the current study (Skomal & Natanson, 2003; Lessa *et al*., 2004; Hsu *et al*., 2015; Wells *et al*., 2016). A preliminary age validation study using bomb radiocarbon dating on two male blue sharks from the Indian Ocean provides support for the age reading protocol used in the current study with ages of 18 and 22 years for lengths of 273 cm L_F and 270 cm L_F, respectively (E.V. Romanov, 2018, pers. comm.[1]). Although these results are preliminary, these specimens presented different ages despite being of the same sex and almost the same size. Therefore, age validation should not be overlooked, particularly for the largest, and presumably older, individuals. As an example, discrepancies have been observed in longevity estimates of other large pelagic sharks (e.g. sand tiger shark, Passerotti *et al*., 2014 and dusky shark, Natanson *et al*., 2014). Bomb radiocarbon dating of these large pelagic shark species have revealed a similar pattern of underestimated age for the largest sharks. In each study, the growth of the vertebrae appears to have growth bands that are missing and cannot be quantified beyond a certain size or age. In addition, another study has challenged the notion that vertebrae can reliably reflect annual growth by showing close ties to somatic growth (Natanson *et al*., 2018). If this is the case, then vertebrae may stop growing, as evidenced in bomb radiocarbon studies, once maximum size is approached or reached and therefore any estimates of age would be underestimated for a shark that has lived beyond this point in size and age. For *P. glauca*, the ages estimated here are supported to the early to mid 20s, based on the findings of E.V. Romanov (2018, pers. comm.), but do not preclude the possibility of greater longevity.

[1] E. Romanov: Centre technique d'appui à la pêche réunionnaise – CAP RUN - NEXA, Le Port, Île de la Réunion.

Regarding the VBGF used in this study, and specifically for the three parameter equation, the model was re-parameterized to estimate L_0 (size at birth) instead of t_0 (theoretical age at which the expected length is zero). This was based on the fact that t_0 lacks biological meaning making L_0 a more robust approach with an immediate interpretation (Goosen & Smale, (1979)1997; Carlson *et al.,* 2003; Cailliet *et al.,* 2006; Goldman *et al.,* 2012). This is particularly relevant in the case of elasmobranchs, since size at birth is usually well defined (Goldman *et al.*, 2012). The estimated L_0 from our study were 61.9 cm L_F for males, 64.1 cm L_F for females and 64.9 cm L_F for the combined sexes. These values are substantially greater than the 35 - 44 cm L_F size at birth range described by Pratt (1979), and by IOTC (2007) in the Indian Ocean. Estimates of L_0 in other *P. glauca* studies fall in this range (Cailliet *et al.*, 1983), while others estimated slightly lower values (Megalofonou *et al.*, 2009; Rabehagasoa *et al.*, 2014). In the present study, the greater estimates for size at birth could be explained by the lack of samples of younger ages when comparing with the remaining ages within the total sample size. This may be due to the smaller individuals of the younger age classes not being fully selected by this gear and therefore the length-at-age for these age classes might be biased. Another reason could be that there is a limited overlap between the area operated by the fishery and juvenile aggregation areas of the studied species. A recent paper by Coelho et al. (2018) showed that juvenile blue sharks tend to be present mainly in high latitudes, which in this specific case of the southwest Indian Ocean does not fully overlap with the fishery where the samples were collected, specifically swordfish targeting longlines that operate in temperate waters but slightly in more northern waters, not fully in those southern juveniles dominated areas.

In terms of model comparison, The AIC and BIC values for both models used in this study suggest that the three-parameter VBGF has a better statistical fit to the age data

(**Table 1**). However, while the fits are better from a statistical perspective, in biological terms it might be more adequate to use the two-parameter VBGF with a fixed L_0, since the birth size of the blue shark is already known (Pratt, 1979; IOTC, 2007) and the observed length-at-age might be biased (see discussion above), therefore the three-parameter model is projected towards an unrealistic length at t_0. As such, the inclusion of a well-known parameter in the model as a fixed value, rather than allowing for its estimation with the associated uncertainties, might be more adequate even at the expense of a somewhat poorer overall fit to the data. Therefore, the VBGF with fixed L_0 was the model selected for this study and was applied to separate sexes due to significant differences in growth characteristics. Additionally, it is relevant to mention that the choice of model makes no practical difference for the majority of the data, as both the three and two parameter VBGF are nearly identical for the 4-17 years old.

When considering previous studies as well as this study (**Tables 2a-c**), there are no evident trends in growth between the Atlantic, the Pacific and the Indian oceans, suggesting a similar growth for *P. glauca* among different world regions. This has been previously mentioned by Nakano and Seki (2003) and Tanaka *et al.* (1990), who reported that variations in the estimates between different studies are most likely due to differences in techniques used to prepare the samples, different criteria for growth zones ageing and reader precision and bias, which compromises a realistic comparison of growth between different areas, and even between studies of the same area. The L_∞ estimates in this study are slightly greater than the ones obtained by other authors in the Indian Ocean and *k* values are very similar, noting however the issue previously mentioned that using a 3-parameter model with size/age data truncated at the lower range can produce very low *k* values (**Table 2a-c**). In our study the results suggest that females reach a greater asymptotic length than males, and males have a greater growth coefficient, indicating a slower growth for females. It is of noted, however, that differences in *Linf* seem to have little impact in this case, as growth and length-at-age are relatively similar over the range of observed data. The same was found in some of the previous studies done all over the world (**Tables 2a-c**), while in others the opposite results were obtained. The L_∞ values estimated in our study are within the range of values estimated by authors in the three oceans, which range from 198.8 cm L_F *[2] to 353 cm L_F *. The same happens for the *k* estimates of our study, which are within the range of 0.10 year^{-1} to 0.18 year^{-1} observed in nearly all the other studies.

Overall, the present study provides an improved age reading protocol and is indirectly supported by bomb radiocarbon validation data. Thus, it may provide some of the most reliable life history results to date for this species in the Indian Ocean. The maximum observed age is greater than what was previously described. These results support the fact that *P. glauca* is a long-lived, slow growth species, and provide important additional knowledge to its biology in the Indian Ocean. Nonetheless, the longevity of *P. glauca* could be even greater than estimated since as previously discussed, shark vertebrae may stop growing in some large pelagic species. If that was the case for *P. glauca*, growth parameter values could potentially change. Therefore, more work regarding age and growth of this species in the Indian Ocean should be carried out, with age validation being a priority topic that needs further exploration.

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[2] All L_F * (fork length) measures were obtained by converting original L_T (total length) measures using the equation by Kohler *et al.* (1995) for *P. glauca*: $L_F = 0.8313 \times L_T + 1.39$.

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Figure captions

Fig. 1. Map of the area of collection of *Prionace glauca* samples (females and males represented) in the South Indian Oceann. The plots are represented in 5x5 degree grids, with the sizes of the plots proportional to sample size (N).

Fig. 2. Microphotographs of three vertebral samples of *Prionace glauca* specimens collected for the present study with identification of the birthmark (b) and the growth bands (indicated by numbers), as well as the focus and the outer edge of the corpus calcareum (OE). The individual on the top left (A) has an estimated age of 3 years and the one on the top right (B) has an estimated age of 13 years. The individual on the bottom

(C) has an estimated age of 25 years, corresponding to the oldest attributed age in this study.

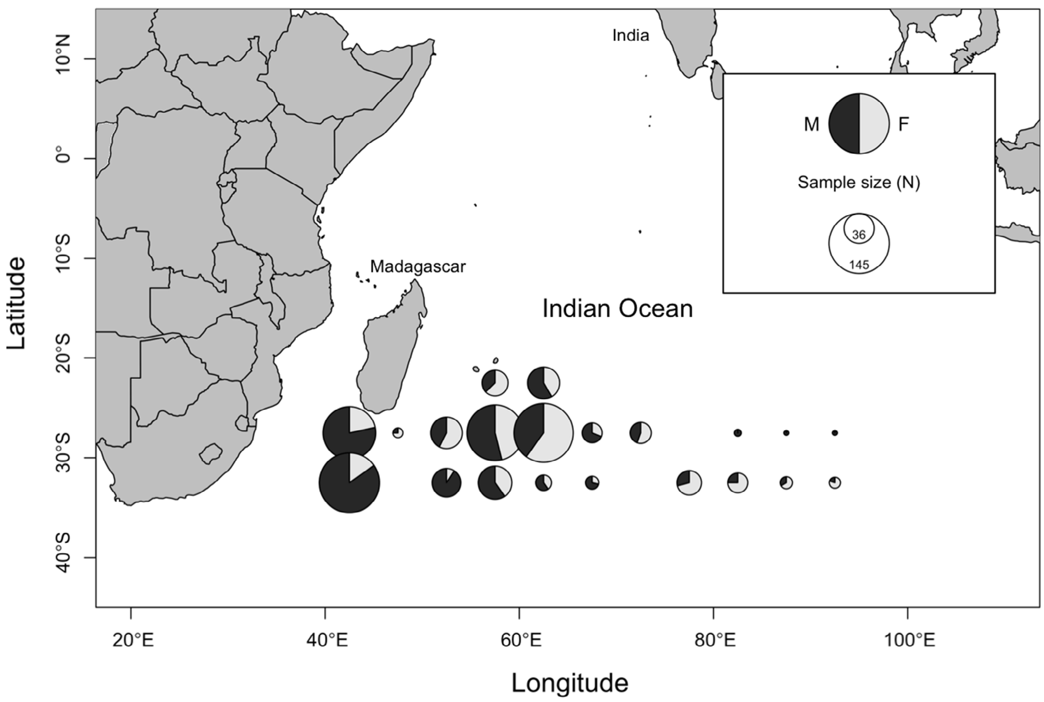
Fig. 3. Size (L_F, cm) frequency distribution of males (n=491) and females (n=327) vertebral samples of *Prionace glauca* individuals collected in the South Indian Ocean between March 2013 and September 2016 (n=818).

Fig. 4. Age-bias plots of pairwise age comparisons (n=793) between reading 1 (A), reading 2 (B), reading 3 (C) and the accepted band pair count, for vertebral samples from *Prionace glauca* collected from the South Indian Ocean.

Fig. 5. Relationship between the fork length (cm) and the vertebral centrum radius (mm) for *Prionace glauca* males (M) and females (F) from the South Indian Ocean (n=727). Dots represent individual observations and the solid lines represent the linear regressions where L_F = 15.82 VR + 29.82 for males and L_F = 17.45 VR + 13.26 for females. L_F = fork length and VR = vertebral radius.

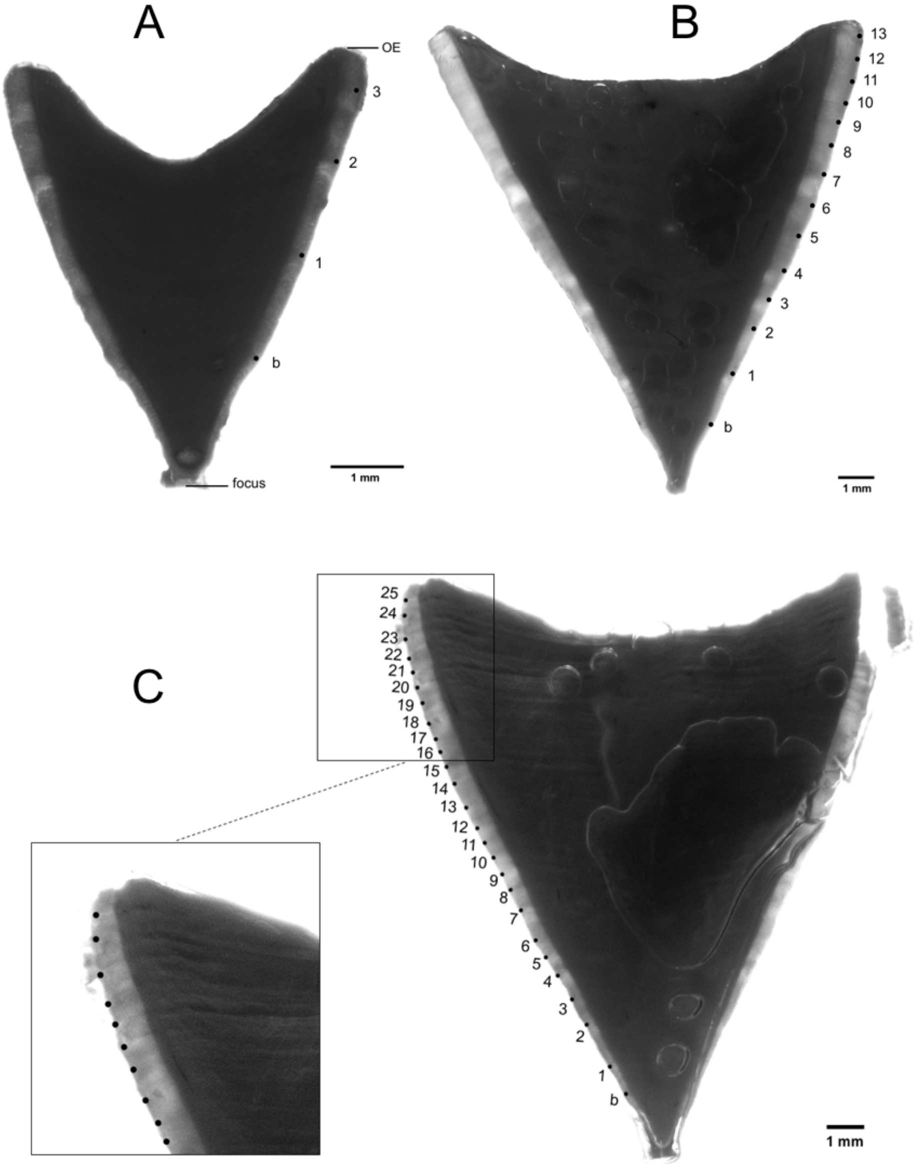
Fig. 6. The von Bertalanffy growth function (VBGF) for *Prionace glauca* based on age estimations through counting of vertebrae growth bands (n=679). Circles represent observed data and the lines represent the VBGF (three-parameters VBGF and VBGF with fixed L_0) for males (A), females (B) and combined sexes (C).

Figure 1



India
Madagascar
Indian Ocean
M
F
Sample size (N)
36
145
10°N
0°
10°S
20°S
30°S
40°S
Latitude
20°E
40°E
60°E
80°E
100°E
Longitude

Figure 2



A
OE
3
2
1
b
focus
1 mm
B
13
12
11
10
9
8
7
6
5
4
3
2
1
b
1 mm
C
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
b
1 mm

Figure 3

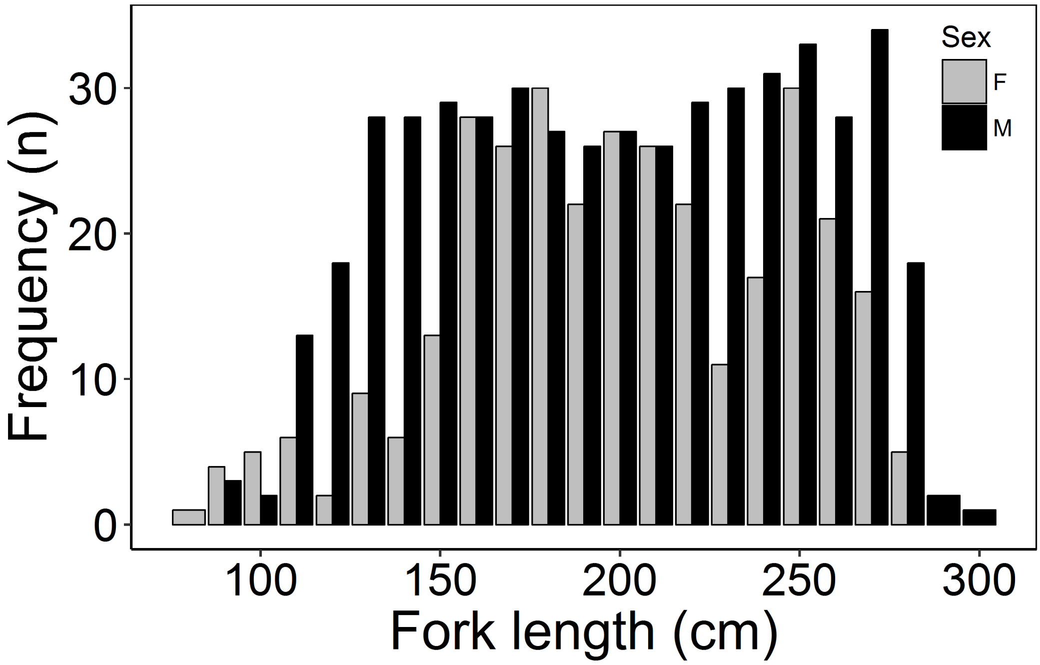
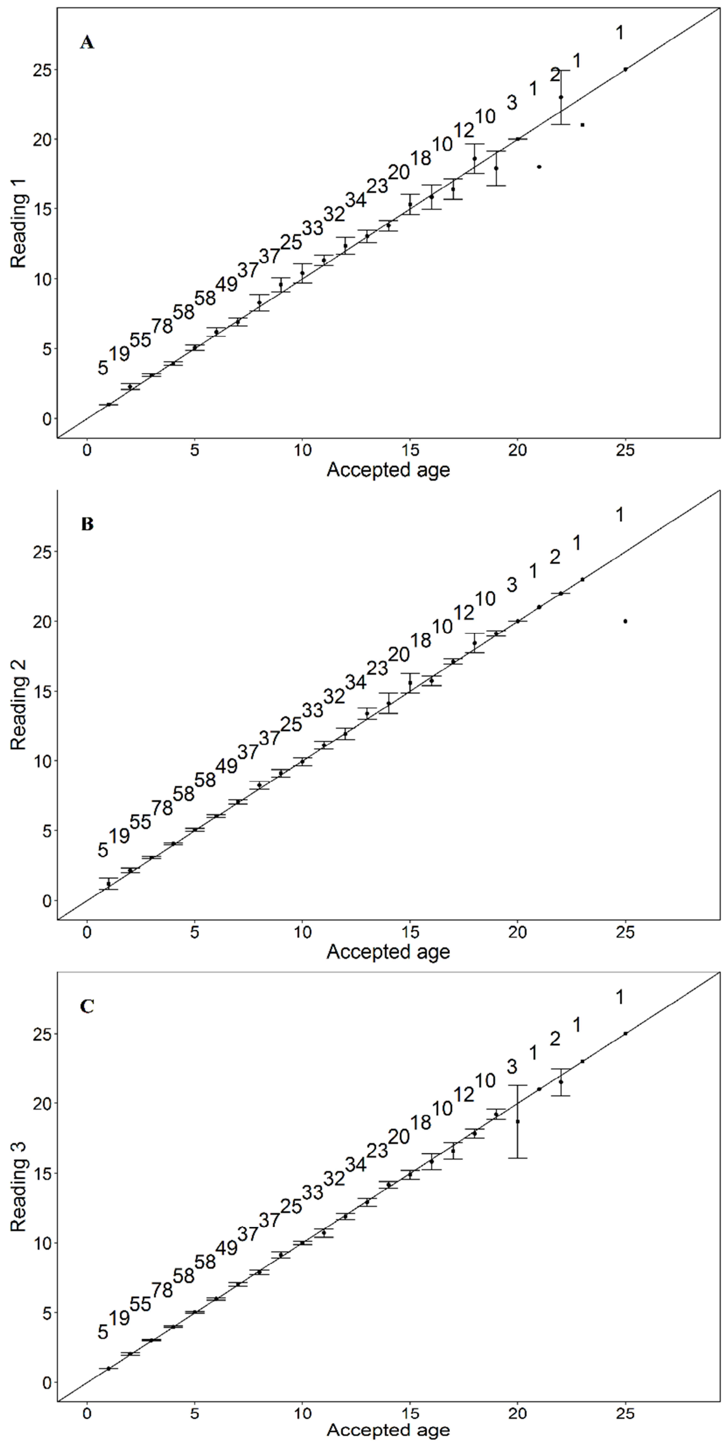
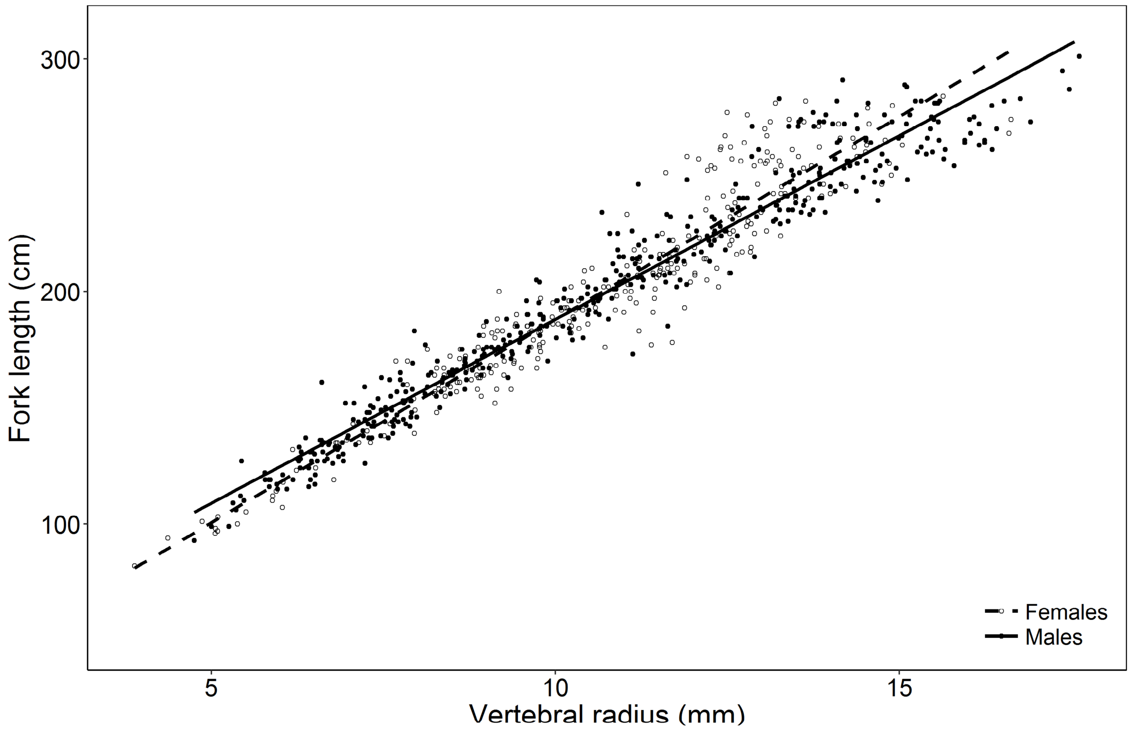


Figure 4



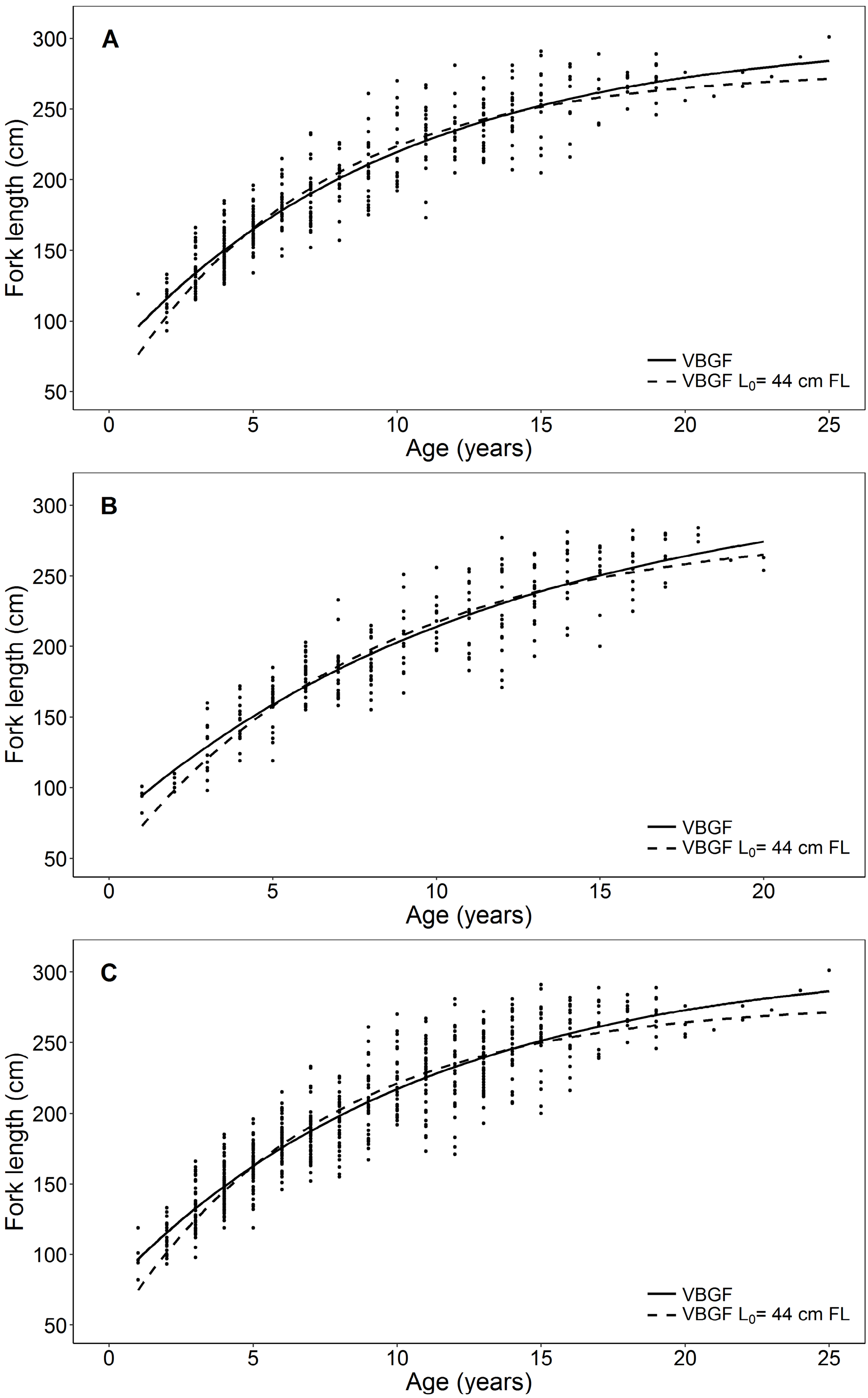
A
Reading 1
Accepted age
B
Reading 2
Accepted age
C
Reading 3
Accepted age

Figure 5



Fork length (cm)
300
200
100
5
10
15
Vertebral radius (mm)
Females
Males

Figure 6


A
B
C
Fork length (cm)
Age (years)
VBGF
VBGF L0= 44 cm FL
300
250
200
150
100
50
0
5
10
15
20
25