Growth of albacore tuna (*Thunnus alalunga*) in the western Indian Ocean using direct age estimates

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Summary

This paper describes a study to estimate the age and growth of albacore tuna in the western Indian Ocean using otoliths. A total of 600 otoliths were selected for analysis. Females ranged in length from 74 to 108 cm fork length (FL) and males from 67 to 115 cm FL. Otolith morphometric data indicate sex and regional (ocean) differences in otolith growth.

Annual age was estimated following protocols developed and validated for South Pacific albacore. Decimal (fractional) age was estimated using count of opaque zones in the otolith, an assumed birth date, capture date and the state of completion of the marginal increment (otolith edge classification). A final age was estimated for 574 fish, ranging from 2.0 to 16.0 years for females and 2.4 to 14.0 years for males. Growth differed between the sexes with males growing faster than females after ~85 cm FL and reaching larger mean asymptotic length. Albacore in the western Indian Ocean appear to grow slightly faster than in the Pacific. However, this may be partly due to the absence of small fish in our samples resulting in higher estimated length-at-age for young fish. Additional age data for small fish (particularly <75 cm FL in temperate latitudes) and from the south and eastern Indian oceans would improve this study. Further work is required to examine the timing of increment formation and refine the age algorithm. Direct validation of the age estimation methods for the Indian Ocean are also recommended.

A preliminary estimate of the proportion of females mature at age was also estimated, although the result should be considered preliminary given the lack of data on small/young fish in the growth and maturity analysis. Age at 50% maturity was estimated at 3.2 years.

Introduction

Albacore tuna in the Indian Ocean is considered a single stock for assessment purposes. Knowledge of the stock structure, however, is uncertain as larval, genetic and morphometric studies suggest two populations may exist, separated by 90°E longitude (Stequert & Marsac 1989, Penney et al. 1998; Yeh et al. 1996). Catches of albacore in the Indian Ocean have fluctuated between ~30,000 to 40,000 tonnes per year since it peaked at ~ 43,000 tonnes in 2010 (IOTC 2016). The most recent stock assessment indicated that the stock is not overfished and that overfishing is not occurring (Langley and Hoyle 2016). However, several different models were run in the assessment and some indicated an overfished state and that overfishing was occurring.

Uncertainty about the growth curve for albacore in the Indian Ocean is a primary source of uncertainty in the stock assessment (Hoyle et al. 2014). It is recognised that analysis of otoliths provides the most reliable age and growth information for fish species, including tunas. As there are no published studies estimating growth using direct ageing methods (e.g., otoliths) for albacore in the Indian Ocean, the assessment currently uses otolith-based growth information from Chen et al (2012) for the North Pacific stock. In addition, the assessment uses maturity information from Farley et al. (2013, 2014) for the South Pacific. The suitability of these parameters is unknown, introducing considerable uncertainty into the assessment results.

The IOTC Working Party on Temperate Tunas (WPTmT) identified the need to improve the understanding of life-history parameters for albacore in the Indian Ocean. In particular, they noted that age and growth research was required and recommended it as a high priority in the WPTmT Program of Work in 2014 and 2016 (Anon 2014, 2016). In 2017, the IOTC secretariat developed and funded a research project to estimate the age of Indian Ocean albacore. This report summarises the results of that project.

Terms of reference – Outputs

1) Growth estimates from direct aging of 600 albacore sagittal otoliths
2) One IOTC working paper describing key results of otolith aging study and growth modelling, to be submitted to the relevant IOTC Temperate tuna Working Party.

Methods

Study material

Sagittal otoliths were obtained from 686 albacore caught in the western Indian Ocean in 2013 and 2014, between latitudes 0-40°S and longitudes 15-65°E (Fig. 1). The majority of fish were caught east of Madagascar by longline or off South Africa by pole and line while a small number were collected from purse seine between 0-10°S. Otoliths were not available from albacore caught east of 70°E.

Otoliths were extracted and soft tissues were removed as much as possible. The majority of otoliths were first immersed for 24 h in a 15% hydrogen peroxide solution buffered with sodium hydroxide and rinsed with purified water to finish cleaning. They were then dried using a blotting paper and placed under a hood for a whole night. Both concave and convex sides of otoliths were scanned using a high resolution scanner to be able to perform shape analyses in the future. The remaining otoliths were cleaned with deionised water and dried, but not scanned.

To be useful for ageing purposes, otoliths must continue to grow throughout the life of the fish. One otolith from each pair was weighed to the nearest 0.001 g (if not broken). The growth of otoliths was assessed by examining the relationship between fish length and age against otolith weight.

Forty three fish were removed from further analysis because they either had (i) no fish length information (n=5), (ii) the otolith was broken and not suitable for ageing (n=34), or (iii) the fish length was likely to be incorrect given the otolith weight (n=5; see outliers in Fig 4 in the Results section).

Of the otoliths remaining, 600 were selected for ageing based on fish length and sex. All otoliths from small fish (<98 cm FL) and large fish (>106 cm FL) were selected to obtain as many age estimates from length classes where sample sizes were small. Additional otoliths were selected randomly from the remaining 1-cm length classes to reach 600 otoliths. The size frequency of fish sampled by sex is shown in Figure 2. Females ranged in length from 74 to 108 cm FL and males from 67 to 115 cm FL. Otoliths from smaller fish were not available for analysis.

Otolith preparation

The otoliths were sent to Fish Ageing Services (FAS) for sectioning. Transverse sections of the otoliths were prepared following the methods outlined in Anon (2002) and Farley et al. (2013). Otoliths were embedded in clear casting polyester resin and four or five serial sections 300 μm thick were cut from each otolith (around the primordium). Sections from each sample were cleaned, dried and mounted on glass microscope slides (50 × 76 mm) with resin. Sections were then covered with further resin and two glass coverslips (22 × 60 mm) were placed side by side.

Serial sectioning on the transverse plane provides a greater chance that at least one section will be clear and the precision of cutting is reduced as one section will always include the primordium (Anon 2002).

Otolith reading

Each otolith section was read by a primary otolith reader (CSIRO; reader 1) and secondary otolith reader (FAS; reader 2) following validated annual ageing protocols developed for South Pacific albacore (Farley et al. 2013). Direct validation of the ageing method (i.e., via a mark-recapture experiment) was not possible for Indian Ocean albacore in this project.

All readings were conducted without reference to the size of the fish, date of capture, or to previous readings. Opaque zones were counted along a transect that ran from the first inflection point on the otolith to the edge of the otolith. An opaque zone on the margin was only counted if it was fully formed with a new (narrow) translucent zone forming on the otolith edge. A confidence score was assigned to each otolith as 1-5 (poor to good). Otoliths with a confidence score of 1 could not be aged and were excluded. An otolith edge type (narrow translucent, wide translucent or opaque) is assigned subjectively based on the distance between the terminal edge of the last opaque growth zone and the otolith edge (relative to the distance between the previous two opaque zones).

FAS used a customised image analysis system to manually marked opaque zones during the reading process. A single jpg image of the section was captured which indicated the positions of the zones.

A final count of opaque zones was assigned to each fish by CSIRO with knowledge of the two previous readings. To examine precision, 10% of the otoliths were re-read by the Reader 1 using the protocols described above. The precision of readings (intra- and inter-reader consistency) was assumed using the coefficient of variation (CV) (Chang 1982). Age bias and age difference plots were also used to examine differences between readers.

Biological age

A decimal (fractional) age was calculated using an algorithm similar to that developed by Farley et al (2013) for South Pacific albacore. The method uses the count of opaque zones, an assumed (nominal) birth date, otolith edge classification, and date of capture using the following algorithm:

$a = (n + b) + r/365$

where a is the decimal age, n the count of opaque zones, b the count adjustment based on otolith edge type and month of capture (from Table 1), and r the catch date (expressed as number of days since the nominal birth date of 1 December; see below).

A nominal birth (hatch) date of December 1 was selected as the middle of the spawning season for albacore in the western Indian Ocean (Dhurmeea et al. 2016). The same birth date was used for South Pacific albacore by Farley et al (2013).

It was not possible to fully examine the timing of increment formation in the current project. However, Farley et al. (2013) used marginal increment analysis to show that opaque zones in the otoliths of South Pacific albacore formed between April and August, and a preliminary examination suggests that increments may form at a similar time of the year in otolith from the Indian Ocean. For fish caught during these months, we used otolith ‘edge type’ assigned to each reading to determine whether a zone had recently formed in the otolith (and was counted) or was not yet complete (and was not counted), so that biological age could be estimated. As noted above, the counts of opaque zones were adjusted following the rules in Table 1 to estimate a biological age.

Figure 3 provides an example of otoliths from two fish caught in July: otolith 407 had four opaque zones and a 5^{th} was almost complete. Otolith 384 had 5 complete opaque zones and a narrow translucent zone on the edge. The age algorithm accounts for the different count and edge type, and gives a similar age for both fish (4.65 and 4.61 years respectively).

Growth analysis

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

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

where L_t is the fork length at age t, L_∞ is the mean asymptotic length, k is a relative growth rate parameter (year^{-1}), and t_0 is the age at which fish have a theoretical length of zero. We used maximum likelihood estimation assuming a Gaussian error structure with mean 0 and variance σ^2.

For comparison, a Richards growth model was also fit to all of the data. The Richards growth curve was parameterized as:

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

where all parameters are defined as for the VB model except the additional parameter b, which allows for more flexibility in the shape of the curve. We again used maximum likelihood estimation assuming a Gaussian error structure with mean 0 and variance σ^2.

Akaike's information criterion (AIC) (Akaike 1974) was used to compare the model fits.

Age at maturity

The preliminary growth curve allowed us to examine the proportion mature at age for females using the length and maturity data from Dhurmeea et al. (2016), using oocyte stage vitellogenic 3 and onwards as mature. The age of each fish in Dhurmeea et al. (2016) was estimated from length using a transformation of the VB growth function (for females). A logistic model was then fit to the length- and age-maturity data and length/age at 50% maturity estimated.

Results and Discussion

Otolith metrics

Otolith weight increased with fish size for both males and females (Fig. 4a). A significant difference was detected in this relationship between the sexes where females had larger otoliths for their size relative to males (ANCOVA; P<0.001). The difference was apparent in fish larger than ~85 cm FL.

Inter-Ocean differences in otolith weight-fish length relationships were also detected between fish caught in the western Indian and South Pacific Oceans (Fig. 5). Additional otolith weight data for fish smaller than 75 cm FL in the Indian Ocean would improve this comparison, however, since otolith weight is related to fish age (Fig. 4b), the results suggest that differences in albacore growth rates may also exist among oceans.

Otolith reading and age estimates

The primary reader assigned a count of opaque zones to 574 otoliths and the remaining 26 (4.3%) were considered unreadable. No bias was detected in replicate readings. The CV of counts between readers was low (5.47%). The percent agreement was 56.0% and when counts differed, 95% were only by +/- 1 (Fig. 6). However, a slight bias was detected where counts by reader 1 were slightly (but significantly) lower than counts by reader 2 (paired t-test, d.f. = 572, P< 0.001) (Fig. 6). The mean differences was -0.156 (+/- 0.065 95% CI). A similar bias was detected between these two readers in the South Pacific albacore study (Farley et al. 2013). In that study, the bias was not present when the otolith edge classification associated with the zone count was accounted for. For example, reader 2 was more likely to count an opaque zone near the edge with a narrow edge type to indicate the zone was only recently completed. Reader 1 often did not count this zone and recorded a wide edge type to indicate that a zone had not recently completed. Since this difference in age interpretation is more likely to occur during the months that opaque zones are forming, and as the age algorithm accounts for this, the estimated biological age will be the same for both readers. We assume the same bias is occurring in the current study, and the age algorithm can account for it.

Estimates of biological age ranged from 2.0 to 16.0 years for females and 2.4 to 14.0 years for males. However, very few small/young fish were included in the study; only 44 fish (7.7%) were aged ≤ 3 years. A large variation in length-at-age was found for both sexes (Fig. 7). For example, 105 cm males ranged from ~6.0 to 11.6 years (Fig 8).

Growth analysis

The results of fitting VB and Richards growth curves to the dataset are shown in Table 2 and Figures 7 and 9. Growth differed between the sexes, with males growing faster than females after ~85 cm FL and reaching larger mean asymptotic length. This corresponds with the estimated length at 50% maturity of females in the western Indian Ocean (Dhurmeea et al. 2016). Sexual dimorphism in growth has also been reported in the Pacific Ocean (Chen et al. 2012; Williams et al., 2012) North Atlantic (Santiago and Arrizabalaga 2005) and the Mediterranean Sea (Megalofonou 2000).

The mean growth curves estimated from the Richards model is virtually identical to the mean growth curve from the VB model for both males and females (Fig. 9). The Richards curve is slightly preferred based on AIC, however for practical purposes, the VB model might be preferred since it involves one less parameter. However, until there is more data available on the smallest/youngest fish, we can’t say which curve is preferred because the inflection (that the Richards curve allows for) often happens at a young age, so perhaps the Richards curve will turn out to be the better model.

Figure 10 shows the growth curves for the western Indian Ocean and otolith-based growth curves from the Pacific. Albacore in the western Indian Ocean appear to grow slightly faster than in the Pacific. However, this may be partly due to the absence of small fish in our samples resulting in higher estimated length-at-age for young fish.

Age at maturity

Figure 10 shows the maturity ogive for albacore in the western Pacific by length and age. Length at 50% maturity (L_{50}) was estimated at 84.9 cm FL, which is slightly lower than estimated by Dhurmeea et al. (2016); most likely because 1-cm length classes were used in the current analysis rather than 3-cm classes. Age at 50% maturity (A_{50}) was estimated at 3.2 years. These results should be considered preliminary due to the lack of comprehensive data on small/young fish in both the growth and maturity analysis (Fig. 10) and the current analysis does not account for spatial and temporal variation in the proportion mature at length/age (see Farley et al. 2014).

Summary and recommendations

The project met its objectives to read the otoliths of 600 fish caught in the western Indian Ocean. A final age was estimated for 574 of the fish, which ranged from 2.0 to 16.0 years for females and 2.4 to 14.0 years for males. Although direct validation of the ageing method was not possible in this project, direct validation has been undertaken for albacore in the South Pacific (Farley et al. 2013) and the same otolith readers and reading methods were used in both studies.

The growth parameters for albacore in the western Indian Ocean appear to differ from those estimated for the North and South Pacific and we recommend that the new age estimates/growth parameters are included in future assessments. However, very few otoliths were available from fish ≤75 cm FL and no otoliths were available from fish caught east of 70°E. For these reasons, a growth curve encompassing the full range of ages could not be obtained, and spatial variation in growth could not be examined.

In addition, we examined the proportion mature at age for females using the length and maturity data from Dhurmeea et al. (2016). Again, the results should be considered preliminary given the lack of data on small/young fish in the analysis.

Given this, we recommend that:

- Otoliths from small fish (particularly <75 cm FL) are collected and analysed to improve the growth curves.
- Ovaries and otoliths from small fish (particularly <90 cm FL) are collected and analysed to improve the maturity at length/age analysis.

- Otolith and ovary sampling is expanded across the Indian Ocean to examine spatial variation in growth and maturity.
- If otoliths from fish aged ≤ 1 year are collected, undertake daily ageing on (i) the longitudinal (frontal) section to estimate length-at-age of very small fish and (ii) the transverse section to confirm the location of the first opaque growth zone (validation).
- Further work is undertaken to examine the timing of increment formation and refine the age algorithm.
- Direct validation of the age estimation methods is undertaken in the Indian Ocean.

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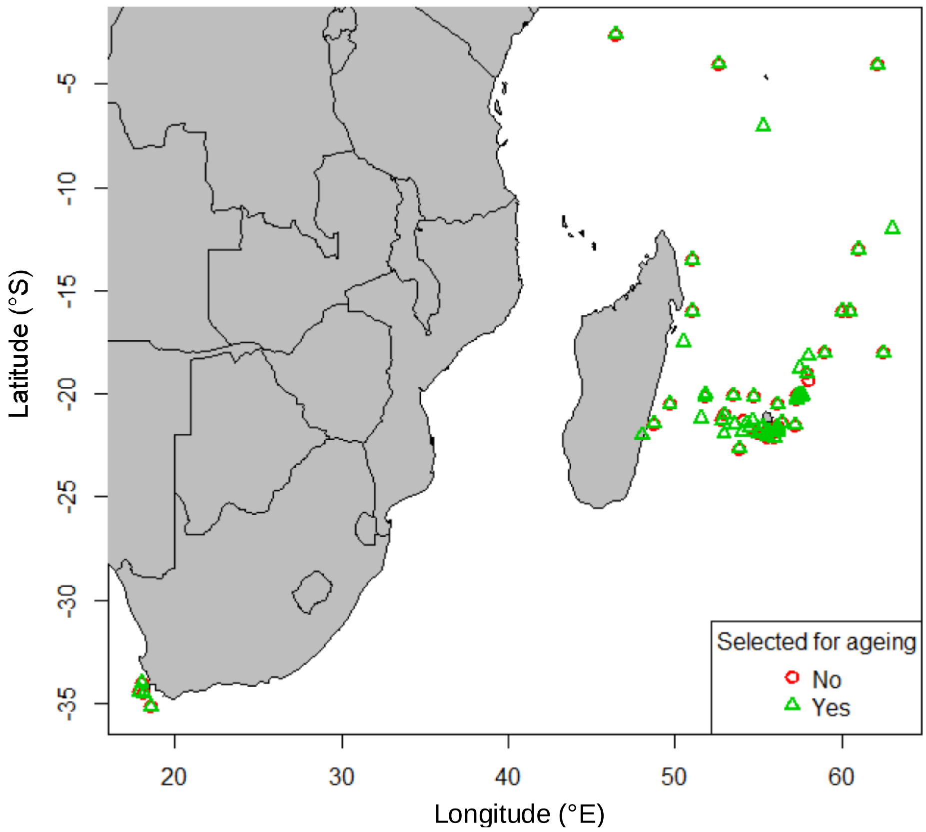

Figure 1. Map of the otolith sampling locations. Different colours represent if the fish was selected for ageing or not.

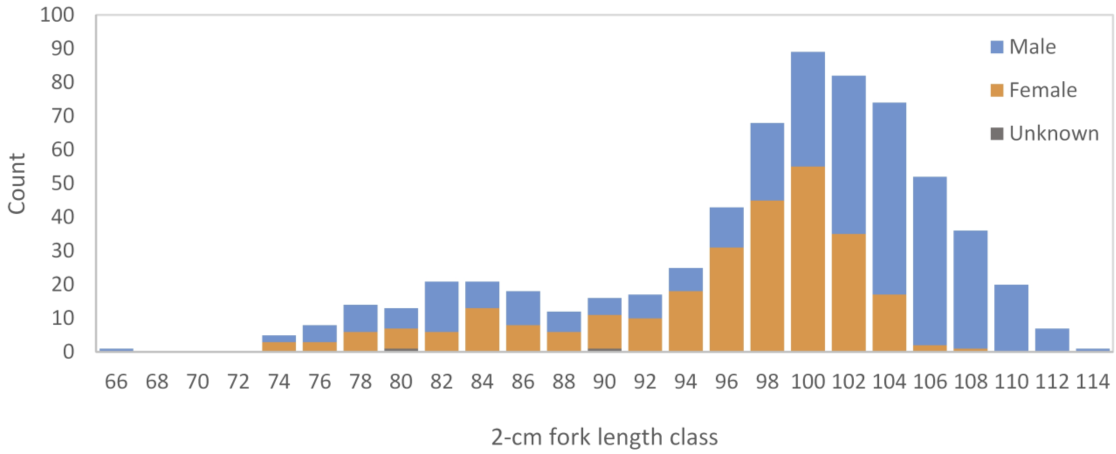

Figure 2. Length frequency (2-cm) of albacore selected for age estimation by sex.

#407: 95 cm FL, 4 opaque zones completed (red circles) and a 5th opaque zone almost complete on the edge (black circle). Catch date 26 July.

Age 4.65 years.

#384: 90 cm FL, 5 opaque zones completed (red circle) and a narrow translucent zone on the edge. Catch date 11 July.

Age 4.61 years.

Figure 3. Examples of transverse sections of sagittal otoliths under transmitted light showing clear opaque (red circles) and translucent growth zones.

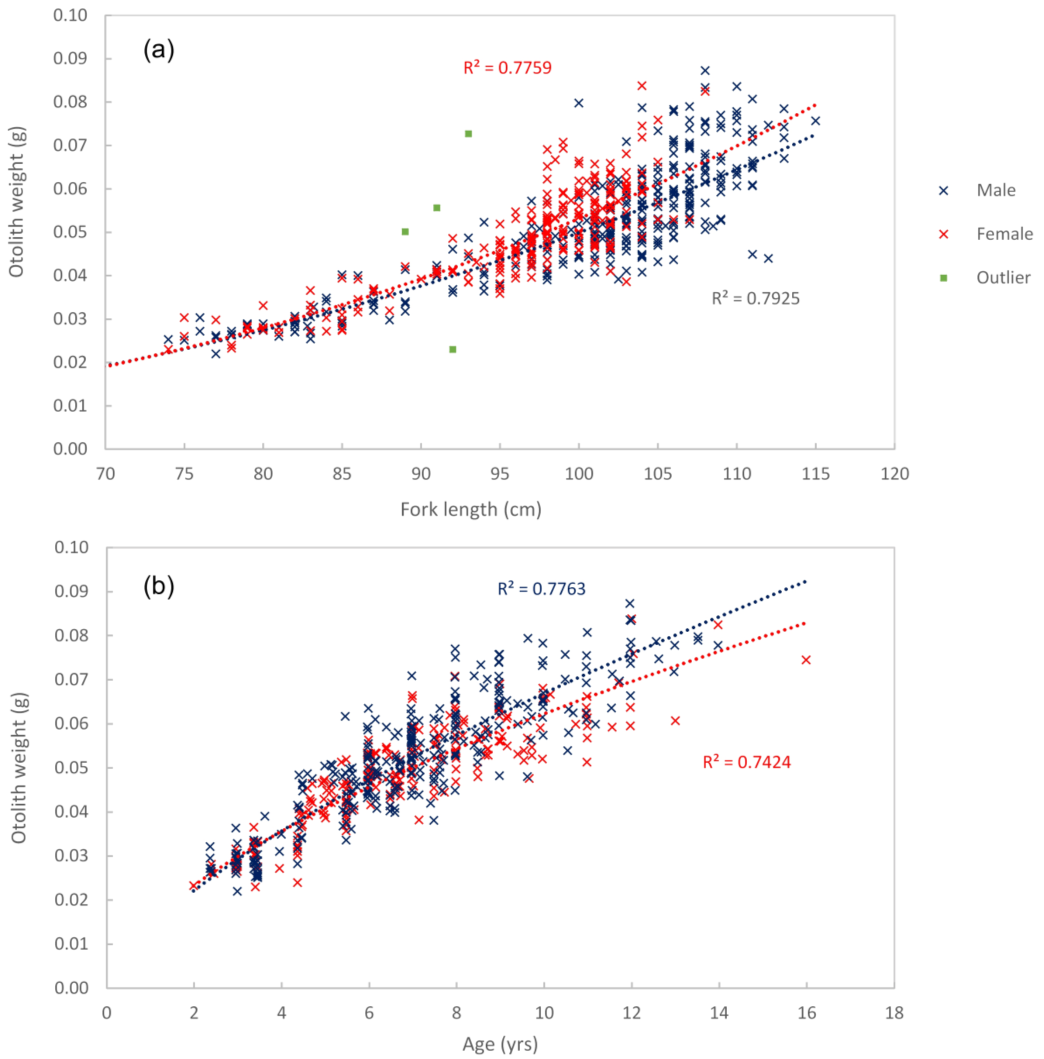

Figure 4. Relationship between otolith weight and fish length (a) and age (b) by sex. Power curves were fitted to data. Four outliers are identified in the weight versus length data and were excluded from the analysis.

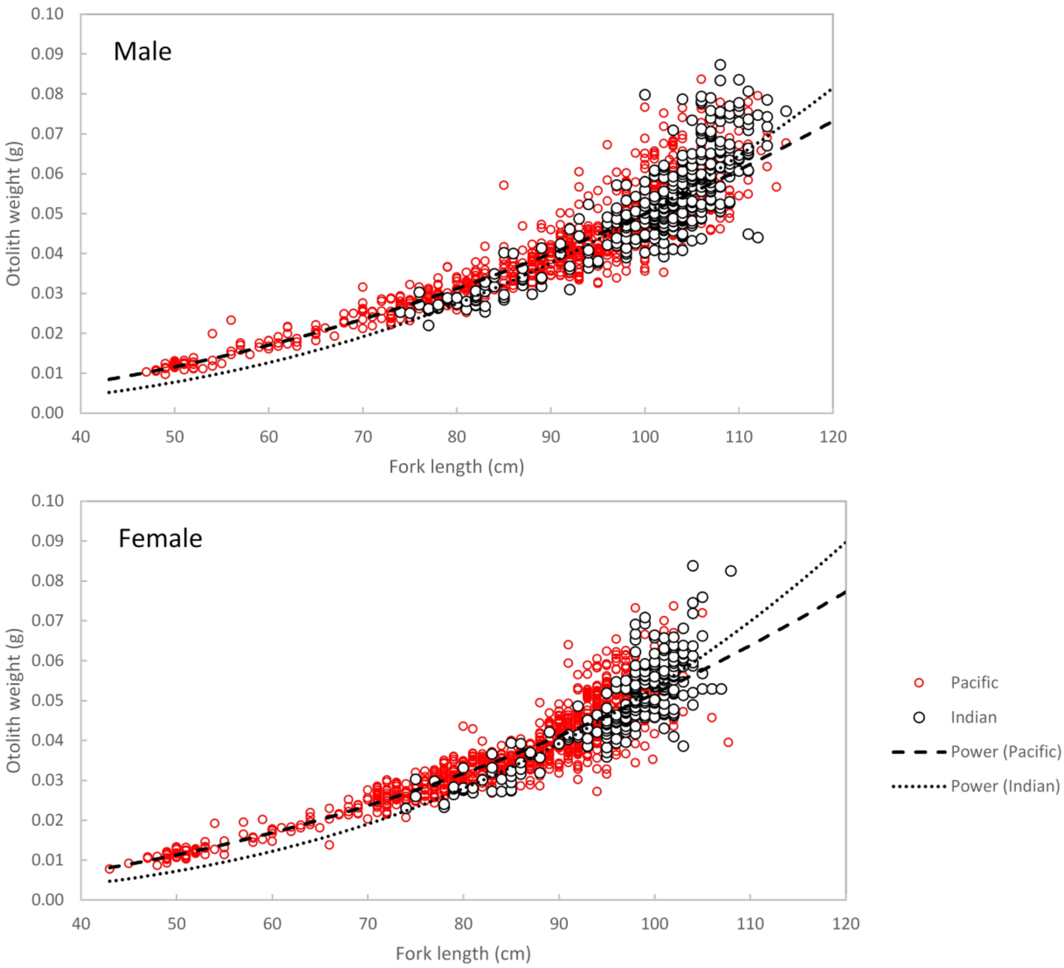

Figure 5. Albacore otolith weight versus fish length for males (top) and females (bottom) caught in the Indian and Pacific Oceans. A power curve was fitted to the data by ocean. Otolith weight-length data for the Pacific was obtained from a study by Farley et al. (2013).

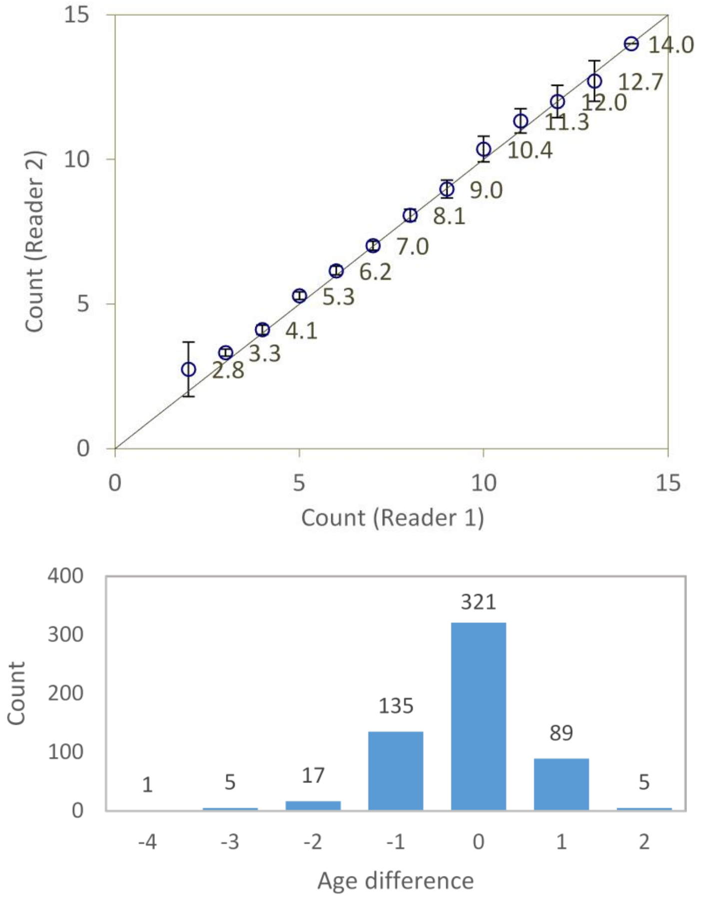

Figure 6. Age bias (top) and age difference (reader 1 – reader 2; bottom) plots comparing final counts by two experienced readers.

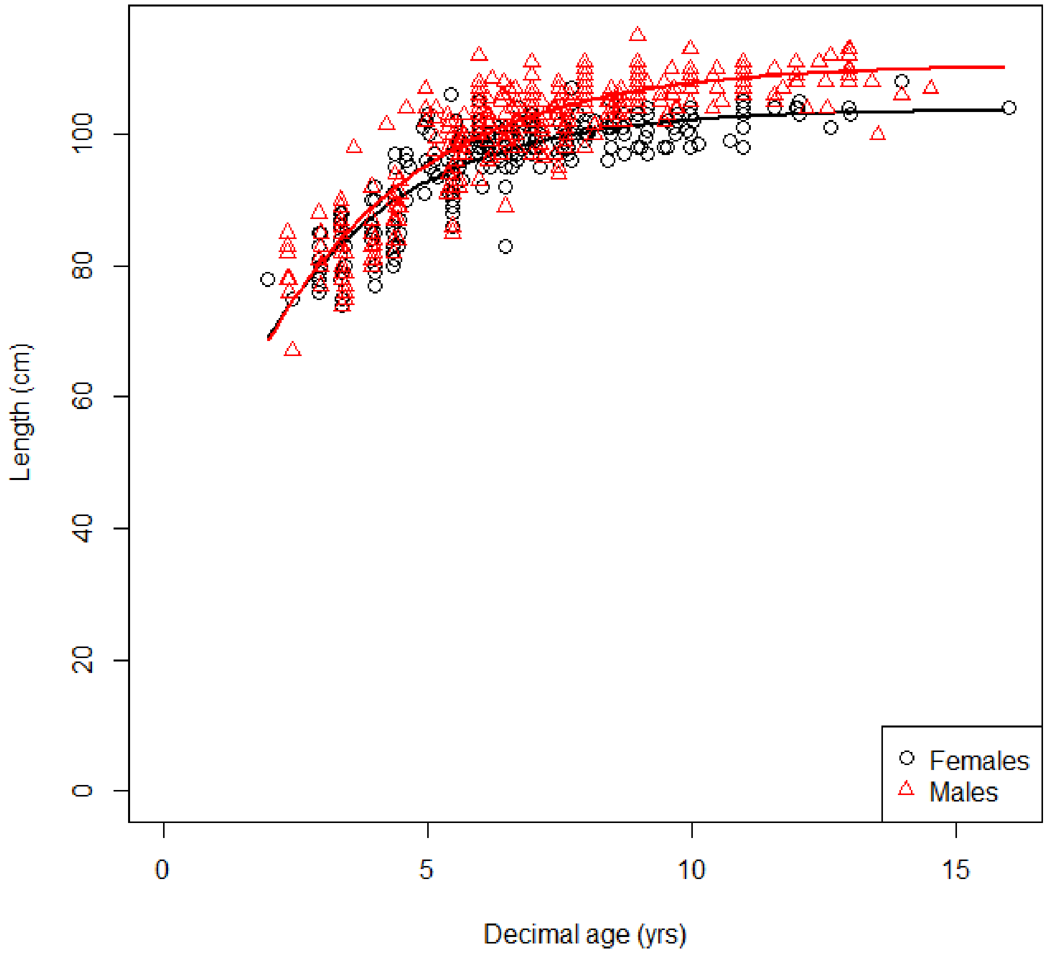

Figure 7. VB growth model fit to male and female data separately. A significant difference was present between sexes based on AIC.

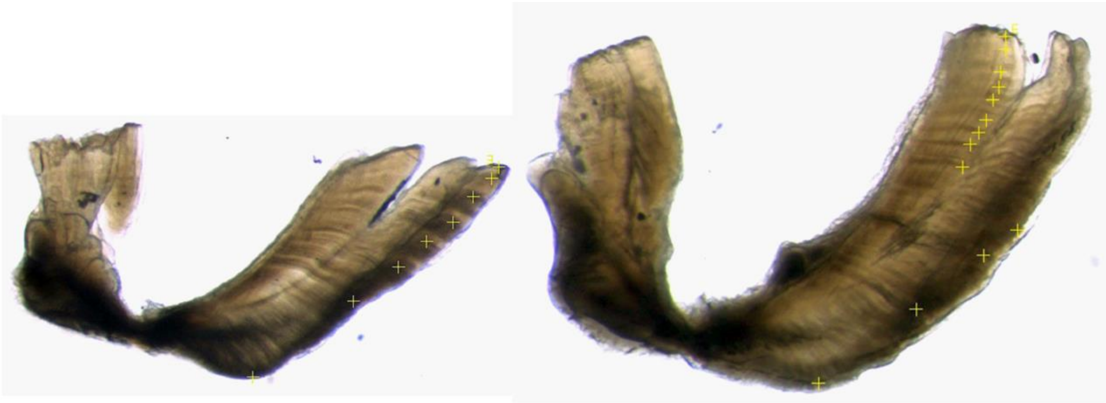

Figure. 8. Sectioned otoliths from two male albacore 105 cm FL aged 5.96 years (left) and 11.55 years (right). Opaque zones are marked by +.

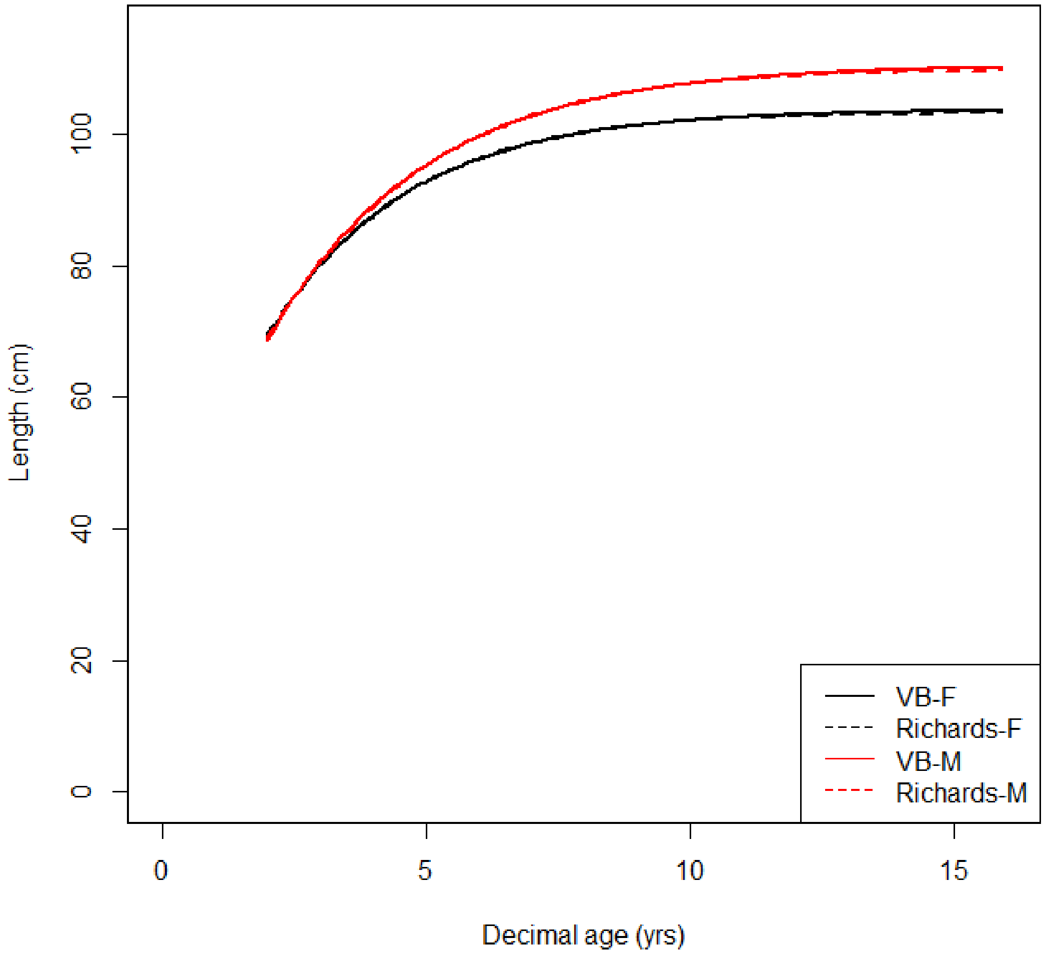

Figure 9. Comparison of the mean growth curves estimated from the von Bertalanffy (VB) and Richards growth models fit to all of the age and length data.

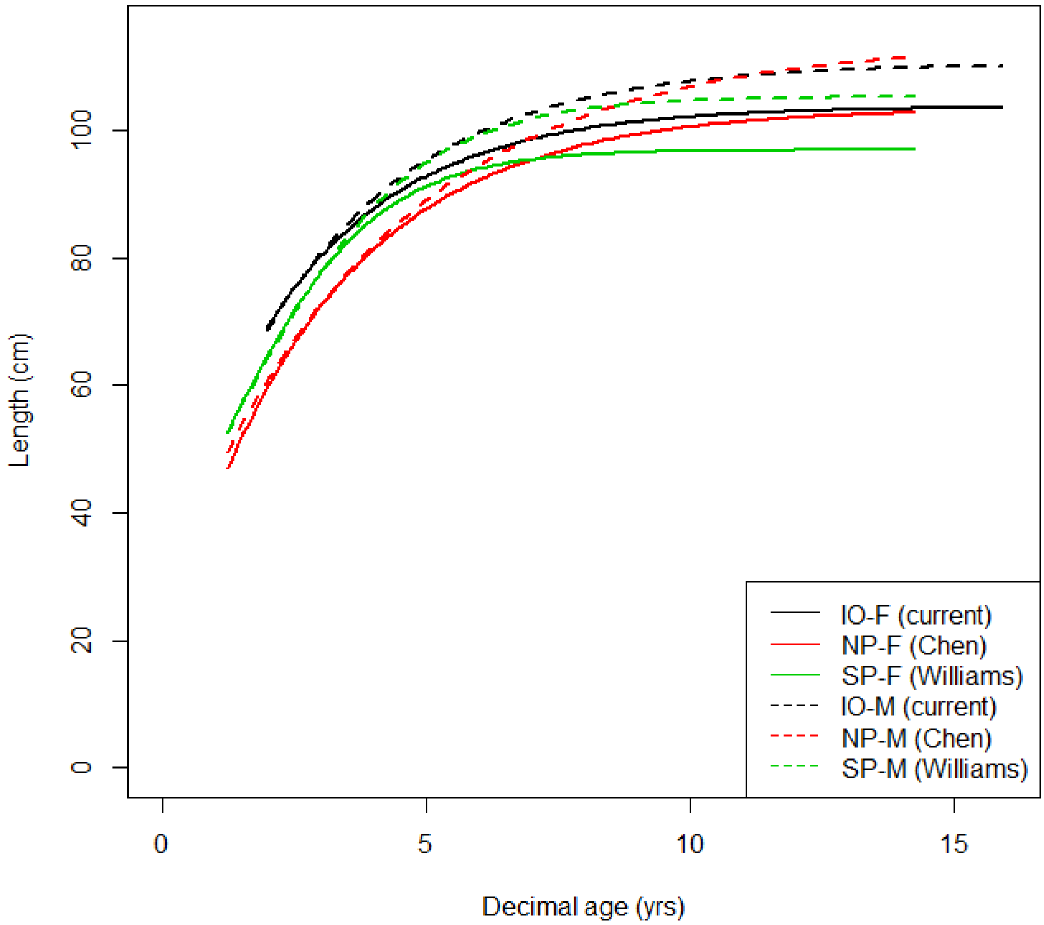

Figure 10. Comparison of the current von Bertalanffy growth curve by sex for the western Indian Ocean (IO) with otolith-based growth curves for the North Pacific (NP) and South Pacific (SP) (Chen et al. 2012, Williams et al. 2012). F = female, M = male.

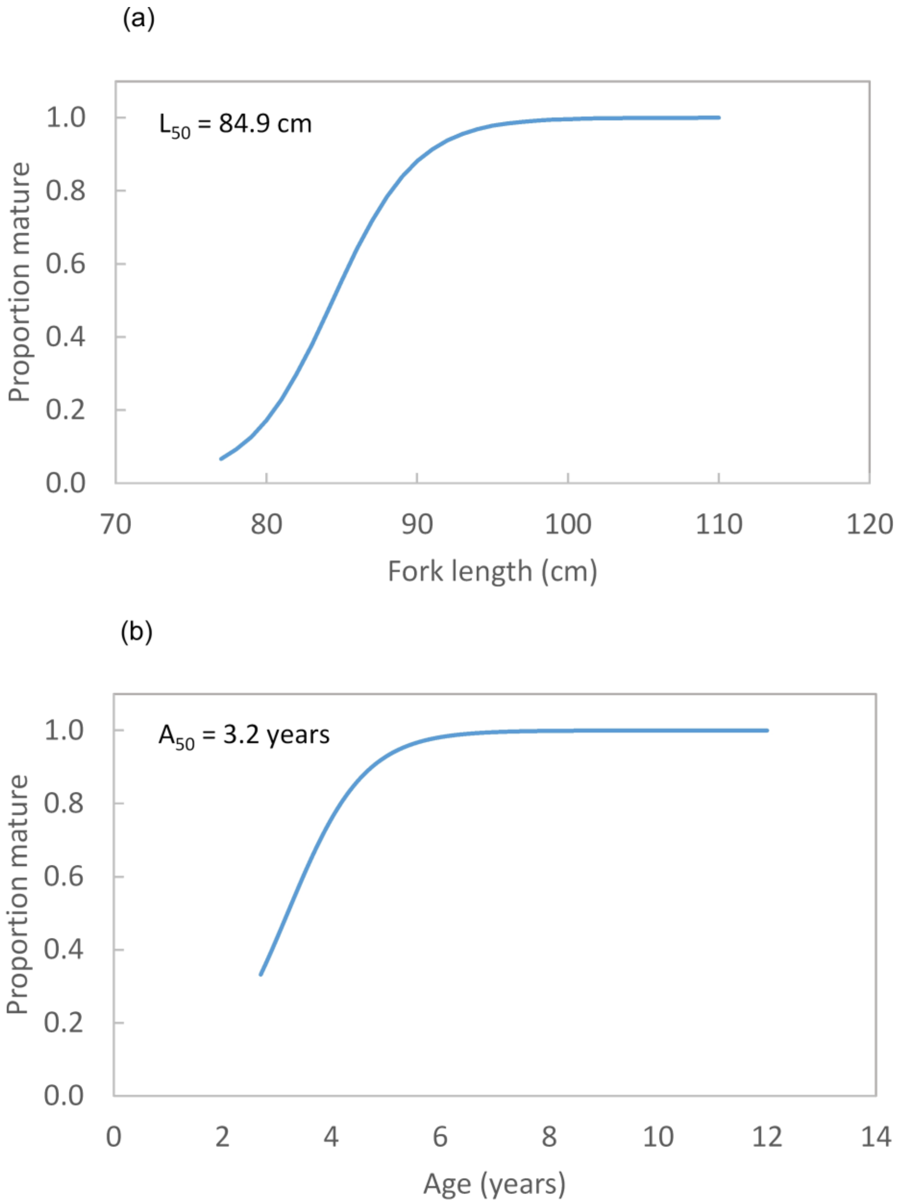

Figure 10. Estimated proportion of mature females by (a) length and (b) age. Length and age at 50% maturity are given (L_{50} and A_{50}). Only the size/age range that maturity data are available for are shown.

Table 1. Opaque zone count adjustment based on capture month (columns) and edge type (rows). Wide = wide translucent and opaque edge. Narrow = narrow translucent.

EDGE TYPE	DECEMBER TO MARCH	APRIL TO AUGUST	SEPTEMBER TO NOVEMBER
Wide	0	0	-1
Narrow	0	-1	-1

Table 2. Parameter estimates from fitting a von Bertalanffy (VB) and Richards growth models to the albacore age and length data for males (M), females (F). Standard errors for the parameter estimates are given in parentheses. The sample size (n) and AIC value for each model are also presented. Note that two fish of unknown sex were not included.

MODEL	SEX	n	L_∞	k	b	t_0	σ	AIC
VB	F	251	103.8 (0.77)	0.38 (0.03)	-	-0.86 (0.33)	3.76 (0.17)	1386.5
Richards	F	334	103.4 (0.71)	0.43 (0.04)	3784.4 (NA)	-0.16 (0.27)	3.74 (0.17)	1384.5
VB	M	251	110.6 (0.81)	0.34 (0.02)		-0.87 (0.25)	4.48 (0.17)	1958.1
Richards	M	334	110.0 (0.73)	0.39 (0.03)	1494.1 (339.7)	0.02 (0.19)	4.45 (0.17)	1954.8