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AGEING OF ALFONSINO (BERYX SPLENDENS) FOR THE SOUTHERN INDIAN OCEAN FISHERIES AGREEMENT (SIOFA)

Relate to agenda item: 6

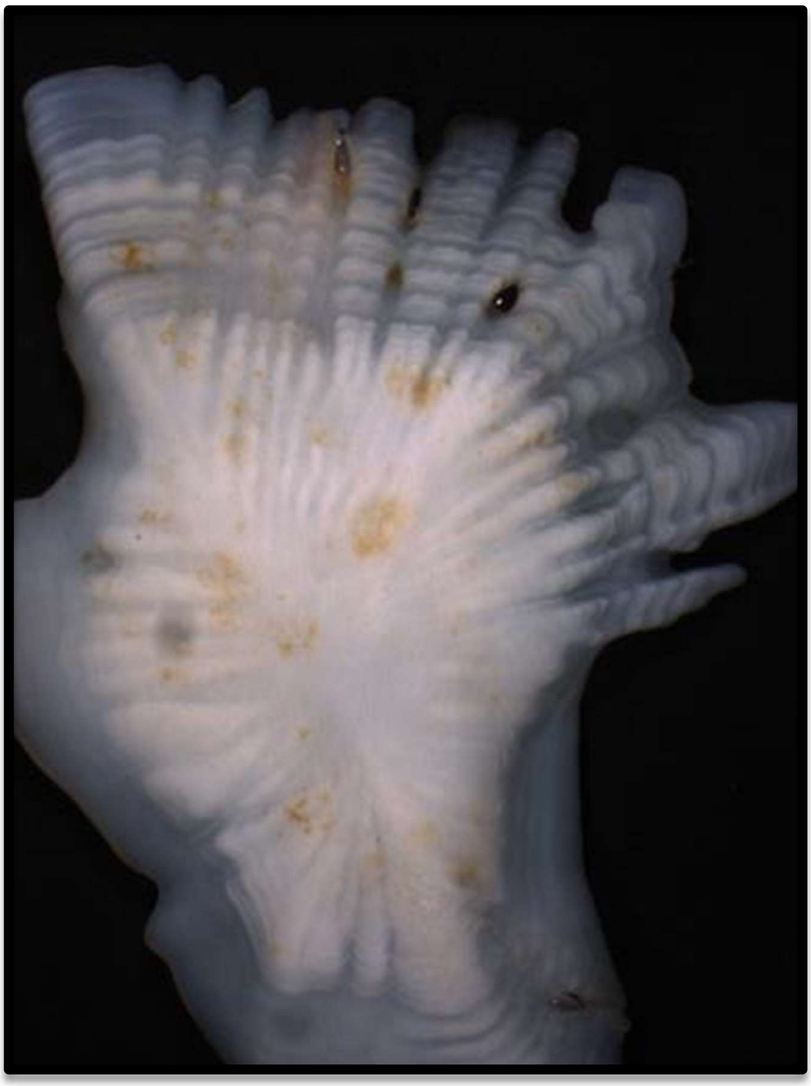
Working paper ☒ Info paper ☒ Restricted ☐

Fish Ageing Services Pty Ltd Consultant Report

Abstract

This report details the results from conducting age readings on 1031 Alfonsino otoliths from the Southern Indian Ocean based on whole otolith readings. Age estimates ranged from 0 to 25 years (18 – 54cm fork length) for the eastern samples and from 0 to 19 years (15 to 50cm fork length) the western samples. The precision estimates between the first and second reader (Eastern APE = 3.4% & Western APE = 4.8%) and the first and third reader (APE = 4.1%) on a subset of 10% of the total samples showed a high degree of agreement. The calculated APE values were well within acceptable limits and the comparison of age reading showed no evidence of between reader bias. This suggests that the age data produced in this study is adequate for estimating biological growth parameters. While the precision of the age estimates was considered high for these samples, we did note that the distribution of the length-at-age was much wider in the samples greater than nine years old. While the change in growth variability could be a result of biological changes such as the onset of maturity, it could also indicate a lack of precision in the whole ageing method. Even though the otoliths of smaller Alfonsino are considered relatively easy to age, it may be worth perusing direct validation for this species and thoroughly investigating the utility and precision of reading sectioned otoliths for this species. Consequently, we include a list of recommendations for the current ageing method using whole otoliths and future research.

AGEING OF ALFONSINO (*BERYX SPLENDENS*) FOR THE SOUTHERN INDIAN OCEAN FISHERIES AGREEMENT (SIOFA)



Fish Ageing Services Pty Ltd

Report No. 2020/008

Ageing of Alfonsino (*Beryx splendens*) for the Southern Indian Ocean Fisheries Agreement (SIOFA)

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Table of Contents

List of Tables

List of Figures

Non-Technical Summary

Age of Alfonsino (*Beryx splendens*) for the Southern Indian Ocean Fisheries Agreement (SIOFA)

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This report details the work carried out under contract for the Southern Indian Ocean Fisheries Agreement. Specifically, the objectives are as follows:

Objectives:

- Receive otoliths shipped by member countries and register the otoliths and associated biological data according to the Fish Ageing Services (FAS) internal coding system, ensuring at all times that the reference to the original sample ID is retained.
- Weigh one otolith from each sample (only if the otolith is complete).
- Provide 2 age estimates per sample using 2 different age readers.
- For each sample, capture two images, one with increments marked and the other without increment marks.
- For each sample measure the increment widths along a standardized transect starting at the primordium.
- Obtain a measure of inter-laboratory precision by engagement of an additional reader pertaining to another laboratory to complete age reading on 10% of the otoliths.
- Prepare a technical report detailing the methods and results.

Non-Technical Summary:

This report details the results from conducting age readings on 1031 Alfonsino otoliths from the Southern Indian Ocean based on whole otolith readings. Age estimates ranged from 0 to 25 years (18 – 54cm fork length) for the eastern samples and from 0 to 19 years (15 to 50cm fork length) the western samples. The precision estimates between the first and second reader (Eastern APE = 3.4% & Western APE = 4.8%) and the first and third reader (APE = 4.1%) on a subset of 10% of the total samples showed a high degree of agreement. The calculated APE values were well within acceptable limits and the comparison of age reading showed no evidence of between reader bias. This suggests that the age data produced in this study is adequate for estimating biological growth parameters. While the precision of the age estimates was considered high for these samples, we did note that the distribution of the length-at-age was much wider in the samples greater than nine years old.

While the change in growth variability could be a result of biological changes such as the onset of maturity, it could also indicate a lack of precision in the whole ageing method. Even though the otoliths of smaller Alfonsino are considered relatively easy to age, it may be worth perusing direct validation for this species and thoroughly investigating the utility and precision of reading sectioned otoliths for this species. Consequently, we include a list of recommendations for the current ageing method using whole otoliths and future research.

Keywords: Alfonsino, otolith, ageing, zone count, whole otolith, precision

Methods

In total 1031 otolith pairs were supplied to Fish Ageing Services (FAS) by Southern Indian Ocean Fisheries Agreement (SIOFA) member countries; Australia, Cook Islands and South Korea. Whole otoliths were provided in individual vials or envelopes that contained the corresponding original sample reference number and/or the biological and capture data.

All otoliths received by FAS were registered into a database. Otolith samples arriving at FAS were batched by country of origin and then by east and west (Table 1). Each sample within a batch was assigned a unique identification code (Sample ID) that is used by FAS for input into data systems. The sample consists of a 15-digit code, which is comprised of five sets of triplets, with each triplet being a category. An example of this would 103001706086015. This comprised of

1. The client code is the first three digits (103), SIOFA client code.
2. The job identification (001), the first job for this client
3. FAS species code (706), the species code for *B. splendens*
4. The batch ID (086), the 86th batch of *B. splendens* that we have processed at FAS
5. The fish ID, of fish number (015), fish number 15 from the 86th batch.

Using this method, all samples can be quickly and uniquely identified. Throughout this summary and in future reports, the client code and job code may be omitted for brevity, and underscores may be used between placeholders. Images (jpg's) collected as part of the annual ageing process use this code and are also appended by the reader and then reading number. Therefore, the image name 082002305011015_1_1.jpg would be the above sample, when aged by reader one, for the first time.

Table 1. Batch details for alfonsino otoliths registered at FAS.

Client ID	Job ID	Species	Batch ID	N	Sample type	Suppling Country	Sector
103	1	706	83	332	Otolith - Annual	Australia	EAST
103	1	706	84	14	Otolith - Annual	Australia	WEST
103	1	706	85	237	Otolith - Annual	South Korea	WEST
103	1	706	86	249	Otolith - Annual	Cook Islands	WEST
103	1	706	87	199	Otolith - Annual	Cook Islands	EAST

The biological and sampling data supplied by each of the countries supplying the otoliths was migrated into an internal FAS database ensuring that the original otolith ID code was retained at all stages. This data also included the unique codes that each country use for their internal sample archiving systems. One otolith from each pair was weighed on an electronic balance to the nearest 0.0001g only if the otolith was complete. Otolith weight is a useful diagnostic tool in assessing potential errors in age estimates and for examining patterns of otolith growth. Morison *et al* (1998) found that otolith weight has a strong relationship with fish size and age and in medium to long-lived species, the relationship of otolith weight against estimated age may show an increased slope if the ages have been underestimated. Large variation about the relationship may indicate a lack of precision in the estimates. Also, any outlying data points in the otolith weight/age and the fish length/otolith weight relationship may indicate an incorrect assignment of age or an issue with the length measurements.

Annual Age Estimation

It has been shown for some species that reading whole otoliths has led to an under ageing of older fish (Dwyer *et al*. 2003, Krusic-Golub *et al*. 2012). While no direct validation on Alfonsino age estimation has been completed, a small study (Anon. 2008) compared paired readings from whole and sectioned otoliths. This work indicated that the whole estimates were comparable to those from sectioned otoliths and perhaps whole otoliths may provide higher estimates of age for the larger samples than sectioned otoliths. Growth rings on whole otoliths have also been shown to be annuli by several studies (Adachi *et al*. 2000; Massey and Horn, 1990; Lehody and Grandperrin, 1996; Rico *et al*. 2001). Consequently, whole otoliths were considered suitable for this study

Ageing methods followed those closely outlined in Massey and Horn (1990). Whole otoliths were immersed in water and viewed against a black background with a Leica stereo dissecting microscope using reflected light. Age was estimated by counting completed opaque zones, with the first being the completion of the large opaque nucleus (Figure 1). Lehodey and Grandperrin (1996) determined through microincrement counts that the formation of the opaque nucleus takes approximately 10 months.

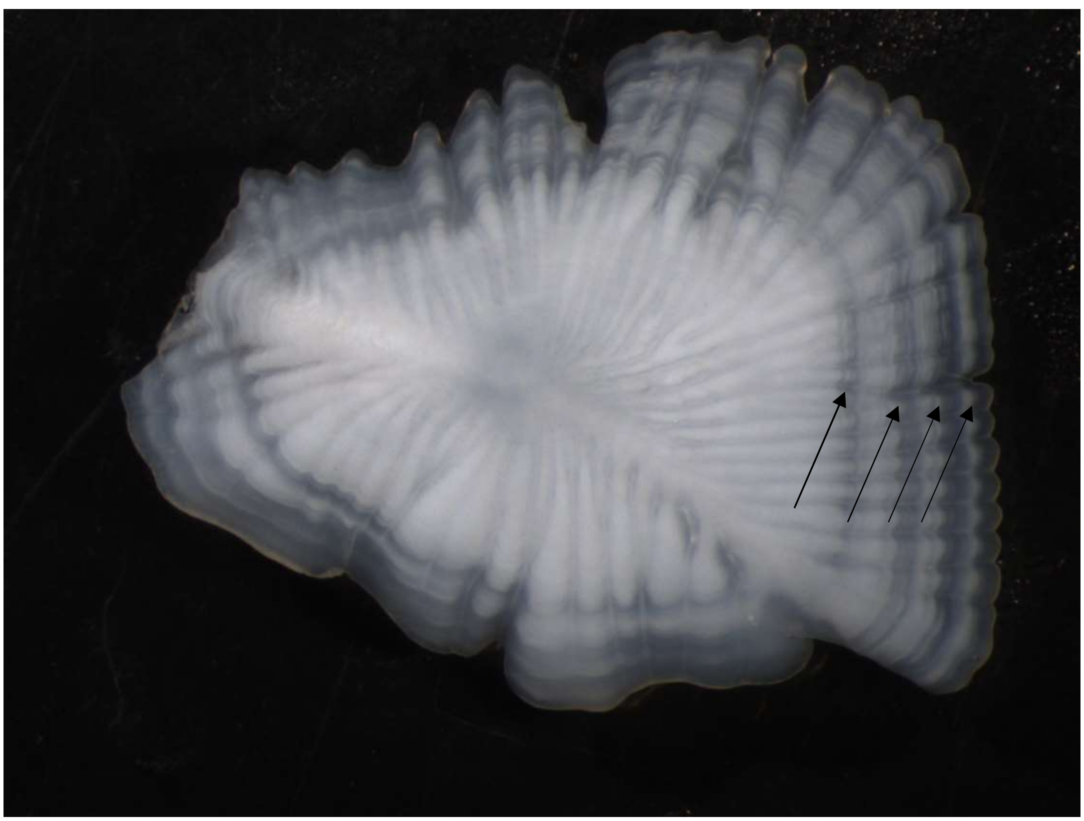

Figure 1. Example of an Alfonsino otolith with clear zone pattern. This sample was assigned a zone count of 4 with an opaque wide edge.

FAS protocol requires that before the reading of a set of otoliths begins, for each given species, a sub sample of otoliths is selected out from the reference set. The reference set consists of a sub-sample of previously read otoliths from that species. These samples are read and then compared against the originally assigned ages and ageing of the current sample only commences if an acceptable level of precision and accuracy has been achieved (species-specific indices). If acceptable levels are not achieved, then readers are retrained. Calibration was completed by both readers on a set of previously aged Alfonsino caught in the Australian East Coast Deepwater Fishery (Tasman Sea).

For the Indian Ocean samples all otoliths were read by two readers each reading the total sample once. To avoid the potential for biasing age estimates, all counts are made without knowledge of fish size, sex, and location of capture. To avoid the introduction of bias, the magnification at which otoliths are read remained consistent. Occasionally, readers will increase or decrease the magnification to resolve the structure of unclear otoliths. Along with a zone count, the reader recorded the edge margin type and a readability score for the otolith. The edge type classification and the readability scores are explained below.

Data acquisition

A custom image analysis system was used to measure zone distances and capture images. A CCD digital camera mounted onto the dissecting microscope (Leica MZ125) was used to display the live image on the monitor. Using the image analysis system, the primordia, the outer edge of each opaque zone and the edge of the otoliths was marked with a screen cursor along the preferred ageing path. The number of zones marked and the measurement from the primordium to each subsequent mark along the transect was automatically exported to a Microsoft database. The otolith image was automatically captured and exported into the database. Reader 1 captured the images with the zone marks positioned into the image and reader 2 capture the images without the zone markers.

In addition to the zone count data, the sample was assigned a readability score which rates the otoliths on a sale of 0 to 5 (Table 2Table 4) and the marginal edge type of the otolith section was recorded. The marginal edge type and the readability scores are described as follows:

Readability Score

The readability score used in this ageing work is consistent with that used routinely by FAS, however with slight modifications to more closely align with those used in other alfonsino ageing projects (Massey and Horn, 1990, Lehodey and Grandperrin, 1996).

Table 2. Readability Index.

Score	Interpretation
1	Unambiguous & clear to interpret
2	more difficult than 1, however little doubt
3	Slight uncertainty, possibly zone count might differ by 1
4	Some doubt, zone count could differ by 2 – 3 (usually +/-1 from zone count)
5	Unreadable/no sample

The readability score is just a relative index to indicate how easy or difficult the annual zone pattern of an otolith is to interpret. It is not intended to indicate solely whether that sample is suitable for including when using the age data for growth and other life history estimates.

Edge type

Followed the general protocol that Fish Ageing Services use to classify the edge of the otolith

- WT – Wide translucent
- NT – Narrow Translucent
- O – Opaque

These are the categories used routinely within FAS ageing methods and as such currently are the only edge types available to choose within the FAS image analysis software. While these edge classifications are slightly different to that used by Massey and Horn (1996), they were considered suitable for any zone formation adjustment that might be required.

Adjustment criteria to account for zone formation

Protocol for adjusting zone counts into age estimates followed the general methods outlined by Massey and Horn (1990). However, unlike their study that used a birthdate of 1st July and adjusted between the months of March and August a birthdate of the 1st August (Santamaria *et al*, 2006) and an adjustment period between May and October was considered more appropriate for these samples. The adjustment criteria are outlined in Table 3.

Table 3. Zone count adjustment criteria to account for zone formation.

Catch Month	January to March	March to August	July to October	November to December
Narrow translucent, (NT)	N	N-1	N	N
Wide translucent, (WT)	N	N	N	N
Opaque, (O)	N	N	N+1	N

All the eastern samples were collected outside of the nominated months for adjustment; therefore, zone count equalled age. All western samples collected between March to October were adjusted using the above criteria.

Zone count to age conversion (Decimal age)

If age estimates are to be used for growth calculations, then it is suggested that the integer age estimates should be converted to decimal ages. For Alfonsino, the following adjustment could be suitable. To convert ring counts to a decimal age, it is necessary to add the time for nucleus formation (10 months), the number of completed annuli, and the time between the nominated birthday and the date of capture. Therefore, a sample caught on the 1st January with a zone count of 4, would be 10 months + 3 years + 5 months and equal 4.254 years

While this conversion was suggested only month and year were supplied for the dates of capture, and some samples did not have a capture date, so we did not do the decimal age adjustment for the data presented in this report.

Precision and accuracy

Paired reading by either the same reader or a different reader provides the necessary data to determine the precision within the readings and to test for reader drift. Beamish and Fournier (1981) have developed an index of average percent error (IAPE), which has become a common method for quantifying this variation. The IAPE is calculated as:

$$IAPE = \frac{100}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij}-X_j|}{X_j}\right]$$

Where N is the number of fish aged, R is the number of times the fish are aged, X*ij* is the ith determination for the *j*th fish, and X*j* is the average estimated age of the *j*th fish. The index has the property that differences in age estimates for younger fish will contribute more to the final value than will the same absolute error for older fish (Anderson *et al.* 1992).

The IAPE was estimated for both the eastern and western sample separately. The IAPE was also converted to a coefficient of variation (CV) following methods in Kimura and Anderl (2005). The precision between readers was investigated by examining age difference tables and age bias plots. Re-reads do not validate the assigned ages but provide an indication of the magnitude of error expected within the set of age estimates. These differences are due to variations in interpretation of the otolith zones.

To provide an additional measure of quality control, a 10% sub-sample of otoliths (100) were selected to be read by an additional reader from another laboratory that was experienced with alfonsino ageing. This allowed for a measure of inter-laboratory precision to be calculated. Unfortunately, due to the current COVID-19 travel and work restrictions that are currently in place, this reading could not be completed on the actual samples. Instead, high-resolution images of each otolith were captured at 12.5 times magnification and provided to the additional reader for age estimation. For the small and medium sized otoliths generally only one image was captured for each sample, however for the larger and more difficult to read otoliths a second image was taken at higher magnification (16 times) which focused on the outer area of the otolith. The same methods described above were used to measure the precision between Reader 1 and Reader 3.

Additional analysis of sectioned otoliths

On the completion of the ageing process it was evident that these samples, particularly the eastern samples, contained a much higher proportion of samples greater than 40cm (FL) than the trial set used in the study to compare age estimates between whole and sectioned otoliths. In the SIOFA otolith set, 32% were larger than 40cm (FL), compared to just 8% for the method trial set. More importantly, the heaviest otolith in the SIOFA otolith set (0.7319g) was nearly twice as heavy as the heaviest otolith in the method comparison set (0.4110g). To extend the range of comparison between whole and sectioned otoliths, the samples containing the two heaviest otoliths were selected for further analysis. One otolith from each pair was prepared for transverse sectioning. Each otolith was cast in clear casting resin and a single transverse section (approx. 300µm) was cut from the nucleus of each otolith. The sections were mounted on glass microscope slides using more casting resin and covered with a single glass coverslip. As observed previously, the two sectioned otoliths proved difficult to interpret because of the presence of large amounts of fine growth zones throughout the section.

To help with the interpretation of the transverse sections, three smaller samples aged at 3, 7 and 13 from the whole age readings were selected for sectioning. These samples were selected because they were each assigned a readability of 1 and showed exceptionally clear zone pattern. Each sample was processed in the same way as used for the first two samples and the distance between the primordium and the proximal edge was measured (dorsal side only). These measurements were then overlaid on the otolith images of the two largest samples to provide the relative positions were the third, seventh and thirteenth zones should be expected. Using this method, reasonably reliable age estimates should be possible.

Results

Otoliths

In total, 1,031 otoliths sampled from 12 different years (2001-2017) were received and registered by FAS (Table 4Table 4). The numbers by each year for the eastern and western region are shown in Table 5.

For the eastern sample, otoliths were collected from seven months of the year (Table 6), however most of the samples were collected in December and January (89.8% of the samples). For the western sample, otoliths were sampled from all months in the calendar year (Table 7**Error! Reference source not found.**). The number of male and females in each 5cm length bin classes are shown in Table 8 and 9 for the eastern and western samples, respectively.

Table 4. Number of Alfonsino otoliths registered at FAS, separated by year of collection.

	Year of capture													
Batch	2001	2006	2007	2008	2010	2011	2012	2013	2014	2015	2016	2017	Unknown	N
83	1	61	87		17	166								332
84				8	1	5								14
85							86	151						237
86						1	34	82	45		30	54	3	249
87							2	22	14	51	17	88	5	199
N	1	61	87	8	18	172	122	255	59	51	47	142	8	1031

Table 5. Number of otoliths region and year of collection.

	Year of capture													
Region	2001	2006	2007	2008	2010	2011	2012	2013	2014	2015	2016	2017	Unknown	N
Eastern	1	61	87		17	166	2	22	14	51	17	88	5	531
Western				8	1	6	120	233	45		30	54	3	500
N	1	61	87	8	18	172	122	255	59	51	47	142	8	1031

Table 6. Number of otoliths collected for each month of the year – Eastern samples only.

	Month of capture								
Year	1	2	3	4	6	11	12	Unknown	N
2001	1								1
2006							61		61
2007	87								87
2010							17		17
2011	123	42			1				166
2012						2			2
2013	14		8						22
2014			1				13		14
2015	4	1				5	41		51
2016							17		17
2017	71		13	4					88
Unknown								5	5
N	300	43	22	4	1	7	149	5	531

Table 7. Number of otoliths collected for each month of the year – Western samples only.

	Month of Capture												
Year	1	2	3	4	5	6	7	8	9	10	11	12	Unknown	N
2008							1					7		8
2010	1													1
2011		2				3				1				6
2012			7	1	14		6	19	15	4	17	37		120
2013	5	8	11	48	3		8	7	17	48	44	34		233
2014			18					10	9	8				45
2016	30													30
2017				19	22		8	5						54
Unknown	1												2	3
N	37	10	36	68	39	3	23	41	41	61	61	78	2	500

Table 8. Number of Eastern samples within each 5cm bin length.

	Female		Male		
Row Labels	AUS	CI	AUS	CI	N
<20			2		2
20≤ <25	40		22		62
25≤ <30	35	2	46	1	84
30≤ <35	25	6	30	18	79
35≤ <40		48	47	3	98
40≤ <45		48	47	2	97
45≤	1	54	37	12	104
N	101	158	231	36	526

Table 9. Number of Western samples within each 5cm bin length.

	Female			Male			
Row Labels	AUS	CI	KOR	AUS	CI	KOR	N
<20			42			41	83
20≤ <25			42			41	83
25≤ <30			42	11		29	82
30≤ <35		15			16		31
35≤ <40		41			41		82
40≤ <45		42			41		83
45≤	3	17			33		53
N	3	115	126	11	131	111	497

Of the 1031 otoliths available for ageing, age estimates could be made on 989 samples. The modal readability was 3 for both the eastern and western otoliths (Table 10) and the readability distributions for both areas suggested little difference in the readability between otoliths from the eastern and western Indian Ocean.

Table 10. Sample readability score by index for eastern and western samples.

Readability Score	EAST	WEST	N
1	14	17	31
2	94	120	214
3	289	307	596
4	107	41	148
5	27	15	42
N	531	500	1031

Estimates of precision

Zone count data from reader 1 and reader 2 was compared separately for the eastern and western samples.

Eastern

The age difference distribution and the age difference table for the paired readings (Reader 1 vs Reader 2) are shown in **Error! Reference source not found.** Both show a mode of zero for the age difference and the comparison of age estimates from reader 1 and reader 2 produced an APE of 3.4% (CV 4.8%). Fifty four percent of the readings agreed and 98% were within one year. Examination of the age bias plots suggested no systematic bias between age difference distributions between Reader 1 and reader 2 for the eastern sample (Figure 3).

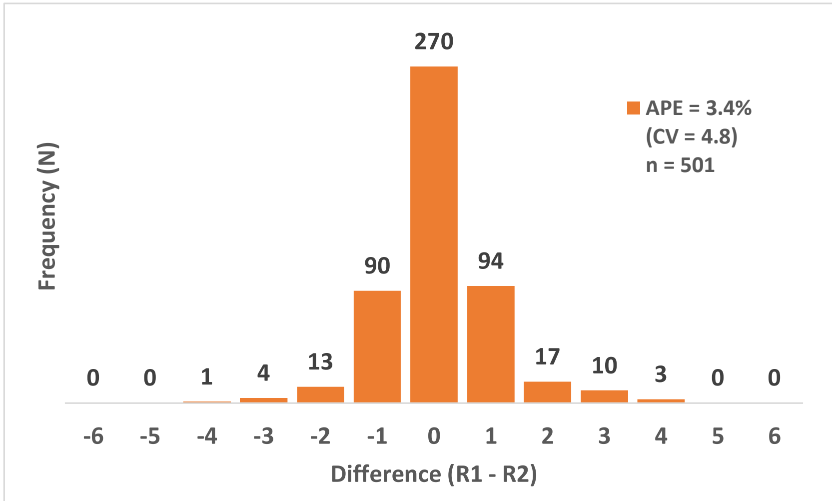

Figure 2. Zone count difference plot of the alfonsino age estimates, Reader 1 minus Reader 2 - Eastern sample.

Table 11. Age difference table between Reader 1 and Reader 2 – Eastern sample.

Difference	Zone count																									
(R1-R2)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	24	25	N	% Agreed	% ±1
-4									1																1		
-3									1		1						1	1							4		
-2										3	4	2	2		1			1							13		
-1		3	5	1	4	7	1	4	8	8	8	15	8	5	5	4	1		1		1			1	90		
0	1	29	23	19	35	24	10	9	20	27	23	17	12	9	3	6	1	1		1					270	54%	90%
1			2	4	4	4	3	3	3	7	6	7	18	11	7	8	4	2							93		
2										2	2		4	3	2	2	1			1					17		
3															2		3	1	1	1	1		1		10		
4																	2					1			3		
N	1	32	30	24	43	35	14	16	33	47	44	41	44	28	20	20	13	6	2	3	2	1	1	1	501		

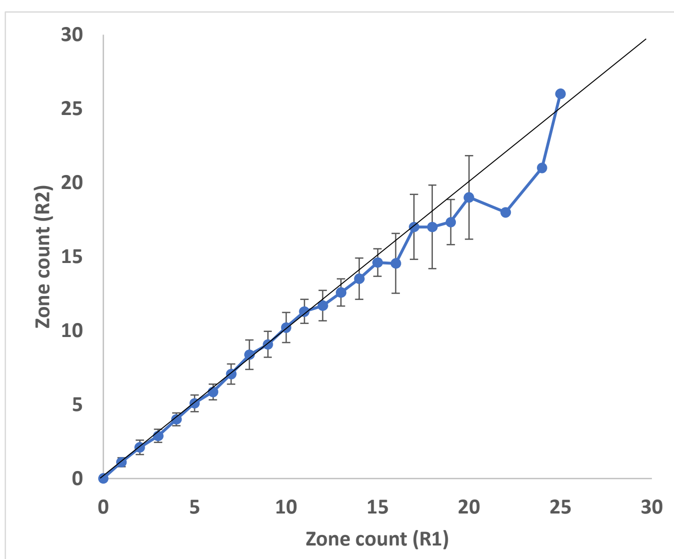

Figure 3. Age bias plot for the eastern samples comparing the age readings between Reader 1 and Reader 2. Error bars are one standard error. Black line indicates the line of agreement.

Western

The age difference distribution and the age difference table for the paired readings (Reader 1 vs Reader 2) are shown in Figure 4 and **Error! Reference source not found.** Both show a mode of one for the age difference and the comparison of age estimates from reader 1 and reader 2 produced an APE of 4.8% (CV 6.9%). Sixty six percent of the readings agreed and 98% were within one year. Examination of the age bias plots suggested no systematic bias between age difference distributions between Reader 1 and reader 2 for the western sample (Figure 5)

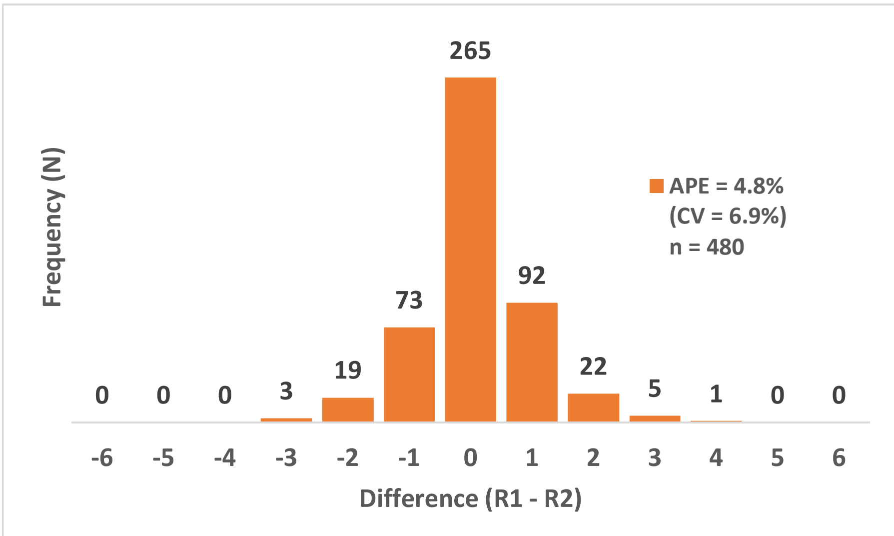

Figure 4. Zone count difference plot of the alfonsino age estimates, Reader 1 minus Reader 2 – Western sample.

Table 12. Age difference table between Reader 1 and Reader 2 – Western sample.

Difference (R1-R2)	Zone count 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	N	% Agreed	% ±
-4																					0		
-3											1				2						3		
-2										1	1	3	6	2	3	1		2			19		
-1		4	4	9	3	4	1		5	8	7	6	6	6	3	4	2			1	73		
0	6	99	26	34	12	5	4	7	5	7	12	16	11	5	7	6	1	1			264	55%	90%
1		2	12	6	9	12	3	1	5	7	2	6	7	9	3	4	2	1		1	92		
2						2				1	4	2	3	3	2	1	3	1			22		
3												1		2	2						5		
4																			1		1		
N	6	105	42	49	24	23	8	8	15	24	27	34	33	27	22	16	8	5	1	2	479		

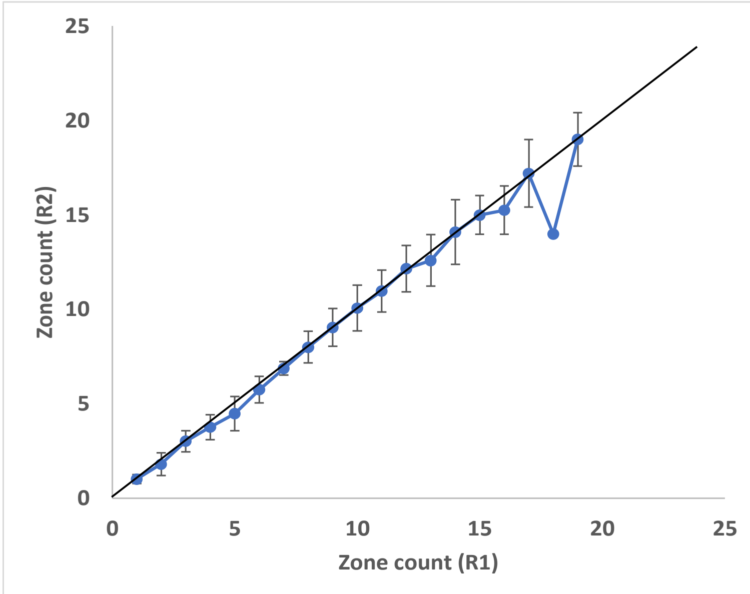

Figure 5. Age bias plot for the western Alfonsino samples comparing the age readings between Reader 1 and Reader 2. Error bars are one standard error. Black line indicates the line of agreement.

<u>10% re-read</u>

The comparison of the age estimates from the 10% subsampled for re-reading by an experienced reader (Reader 3) from another laboratory are shown below. To increase the sample number for analysis, both western and eastern samples have been combined. The age difference distribution and the age difference table are shown in Figure 6 and Table 13, respectively. The comparison of reading produced an APE of 4.1% and 5.8% (CV). No age bias plots were produced due to the small sample size of each sub-set Figure 7.

Figure 6. Zone count difference plot of the alfonsino age estimates, Reader 1 minus Reader 3 –10% subsample (east and west combined).

Table 13. Age difference table between Reader 1 and Reader 3 – 10% subsample.

Difference	Zone count																				
R1 -R3	1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	18	19	20	N	% Agreed	± 1
-4								1	1				1						3		
-3														1					1		
-2											3								3		
-1	1	2	3				2	1	1	6	1	2			2				21		
0	16	4	4	4	6	2	4	4	4	1	2		1	4	1		1	1	59	57%	90%
1		2	1		1				2	1	4		1	1		1			14		
2											1	1			1				3		
3																					
4																					
N	17	8	8	4	7	2	6	6	8	8	11	3	3	6	4	1	1	1	104		

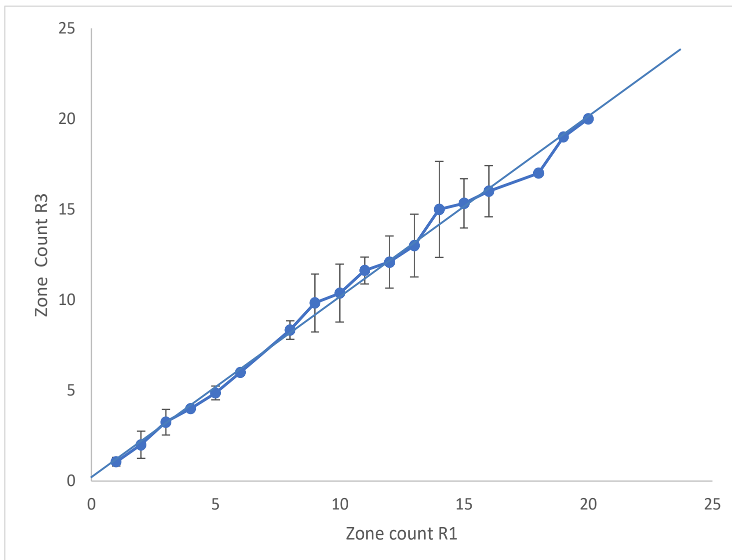

Figure 7. Age bias plot for the 10% alfonsino subsample comparing the age readings between Reader 1 and Reader 3. Error bars are one standard error. Black line indicates the line of agreement.

Age estimation

Age estimates were obtained from 989 from the 1031 samples available. The age-length relationship, length-otolith weight and the age-otolith weight relationship are shown in Figure 8 and Figure 9 for the eastern and western samples, respectively. The age composition for otoliths from both areas is shown in Figure 10. Age ranged from 0 to 25 for the eastern samples and 0 to 19 for the western samples.

Age-length keys for the eastern and western samples are shown in Table 14 and Table 15, respectively.

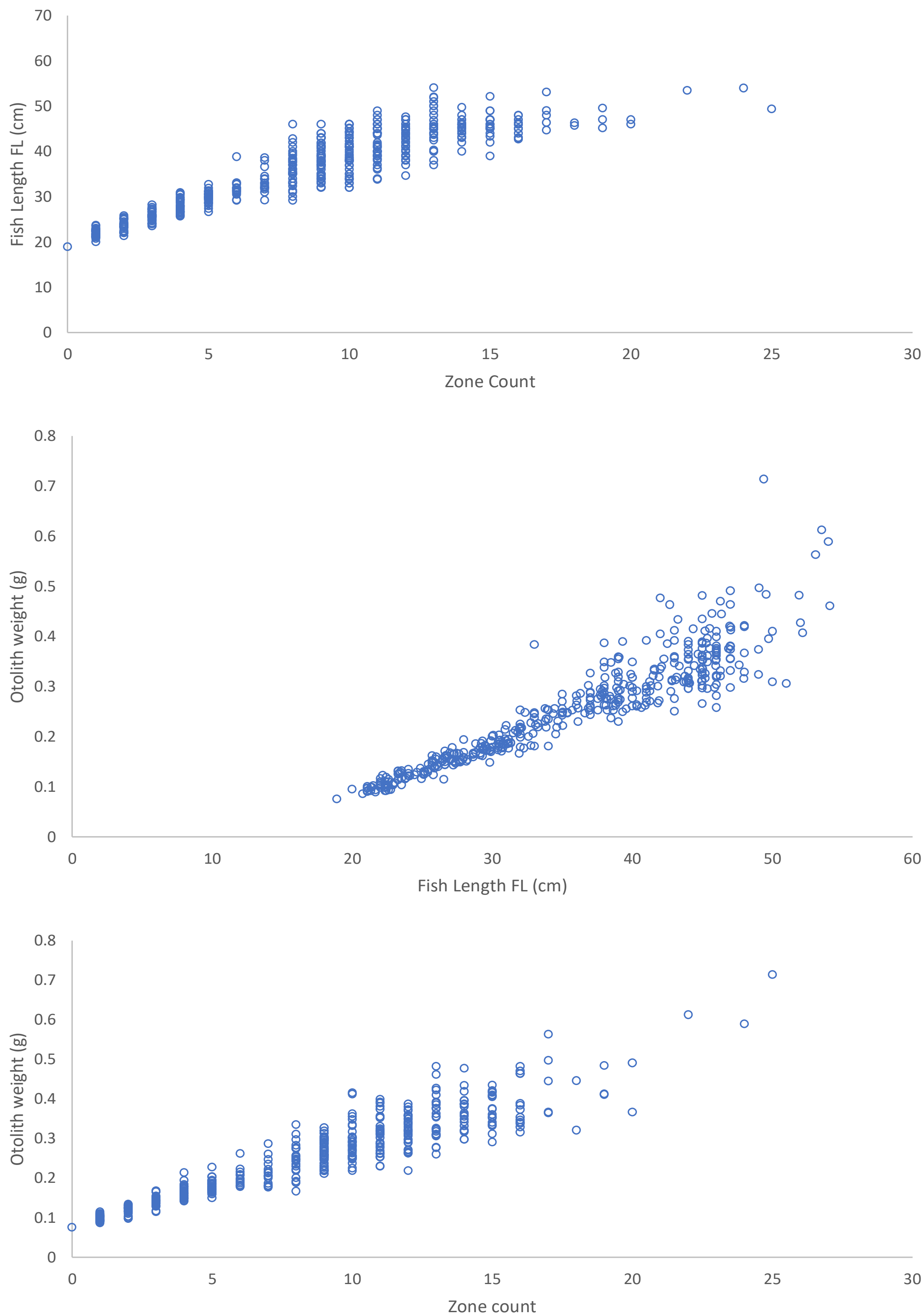

Figure 8. Age / length, length/otolith weight and age/otolith weight relationships for Alfonsino from the eastern Indian Ocean samples used in this study.

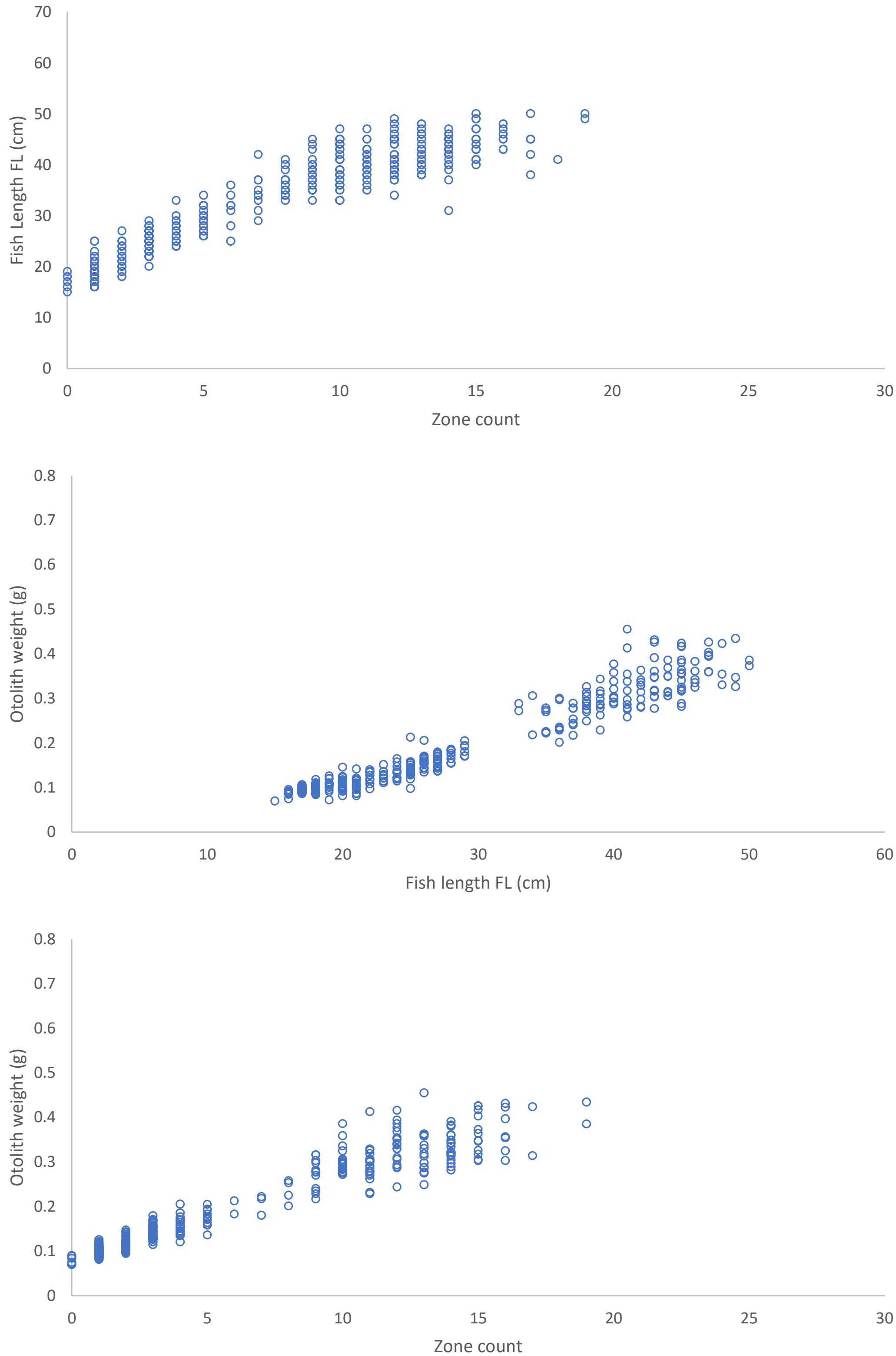

Figure 9. Age / length, length/otolith weight and age/otolith weight relationships for Alfonsino from the western Indian Ocean samples used in this study.

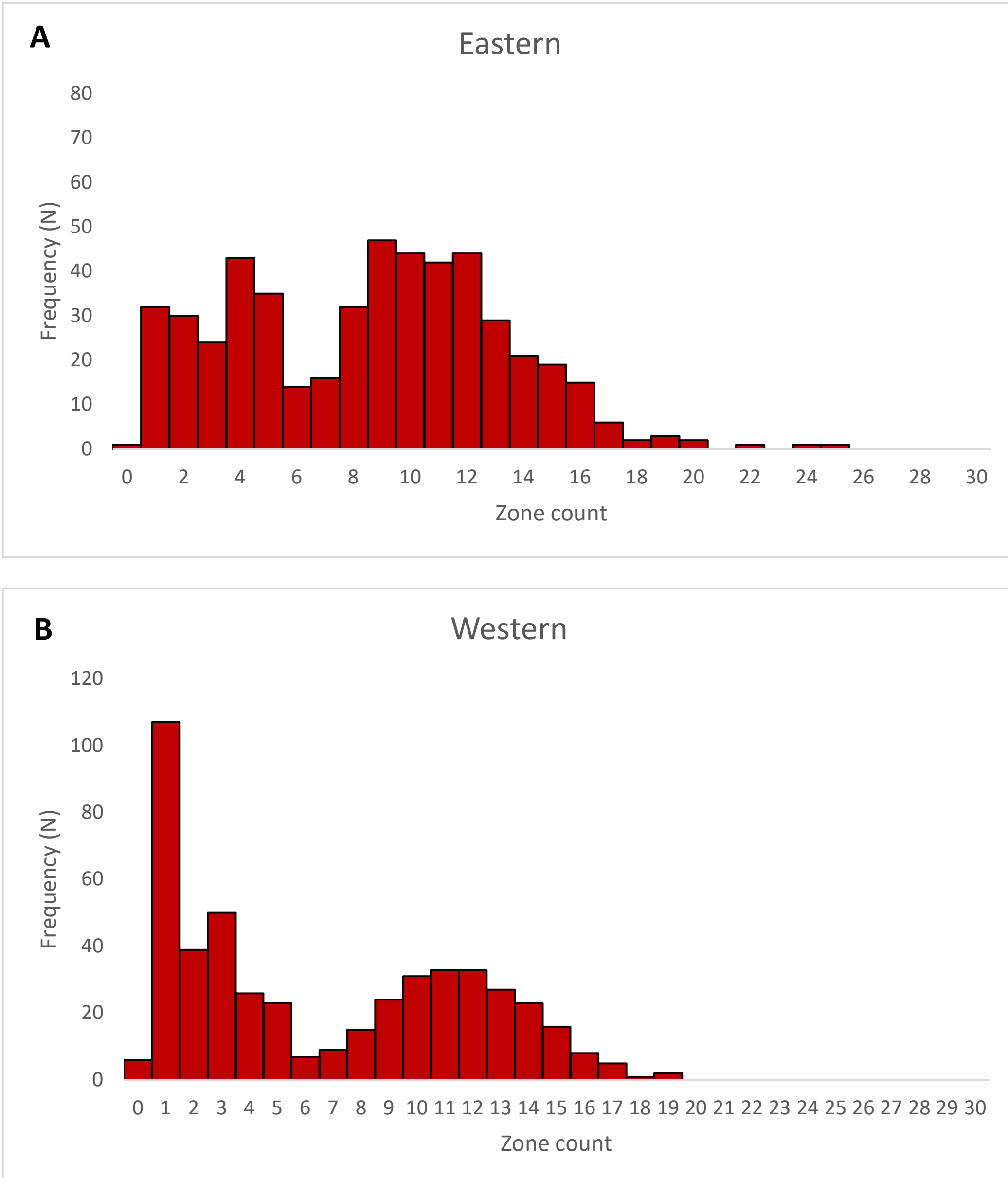

Figure 10. Age composition for combined years for A) eastern and B) western.

Sectioned otoliths

The age estimates from the largest and second largest otoliths were 26 and 24 years, respectively. These estimates were similar to those derived from the whole estimate and provided further weight of evidence that using whole otolith for the age estimation of this species should not lead to a significant underestimation of longevity. Various images from the comparison of methods for the two largest samples are shown in Figure 11 and Figure 12.

Table 14. Age-length-key for all years combined, binned into 5cm length classes – Eastern.

	Zone count																										
Length FL (cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	N
15																											
16																											
17																											
18	1																										1
19		1																									1
20		1																									1
21		13	1																								14
22		14	8																								22
23		3	11	2																							16
24			5	4																							9
25			5	8	4																						17
26				5	10	1																					16
27				4	12	2																					18
28				1	3	4																					8
29					9	10	2	1	1																		23
30					5	11	2	1	1																		20
31						6	5	1	2																		14
32						1	2	6	1	2	2																14
33							2	2	3	3	3	2															15
34								2	1	4	3	1	1														12
35									7	2	2																11
36								1	2	3	3	2															11
37									4	7	2	1	1	1													16
38							1	2	3	4	6	3	5	1													25
39									2	6	3	6	6			1											24
40									1	3	3	4	2	3	1												17
41									1	4	5	4	3														17
42									2		1		3	1	1	1	1										10
43										4	3	1	6	2	2	1	3										22
44										2	3	4	5	5	3	1	1	1									25
45											2	5	6	5	6	6	4		1	1							36
46									1	1	3	3	2	3	4	2	3	1	1		1						25
47												2	4		2	4	2			1	1						16
48												1		1	1		1	1									5
49												1		1	1	2		1		1						1	8
50														2													2
51														2													2
52														1		1											2
53																		1					1				2
54														1											1		2
55																											
56																											
57																											
58																											
59																											
60																											
N	1	32	30	24	43	35	14	16	32	47	44	42	44	29	21	19	15	6	2	3	2		1		1	1	504

Table 15. Age-length-key for all years combined, binned into 5cm length classes – Western.

	Zone count																										
Length (FL cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	N
15	1																										1
16	1	7																									8
17	1	21																									22
18	2	30	2																								34
19	1	14	2																								17
20		13	9	1																							23
21		17	10																								27
22		2	4	5																							11
23		1	2	5																							8
24			6	3	4																						13
25		2	3	12	8		1																				26
26				8	4	5																					17
27			1	12	3	3																					19
28				3	3	3	1																				10
29				1	2	3		1																			7
30					1	4																					5
31						1	1	1							1												4
32						3	2																				5
33					1			1	1	1	3																7
34						1	1	2	3				1														8
35								1	4	5	2	2															14
36							1		1	2	3	1															8
37								2	3	4	1	1	3		1												15
38										3	4	5	2	5				1									20
39									1	3	3	5	6	1	1												20
40									1	2		8	4	3	2	1											21
41									1	1	3	2	3	3	1	3			1								18
42								1			1	3	2	3	3			1									14
43										1	2	3		4	3	3	2										18
44										1	4		2	1	2	1											11
45										1	3	2	5	1	5	2	2	2									23
46													1	3	2		1										7
47											1	1	1	1	1	3	1										9
48													1	2			2										5
49													1			2				1							4
50																1		1		1							3
51																											
52																											
53																											
54																											
55																											
56																											
57																											
58																											
59																											
60																											
N	6	107	39	50	26	23	7	9	15	24	31	33	33	27	23	16	8	5	1	2							485

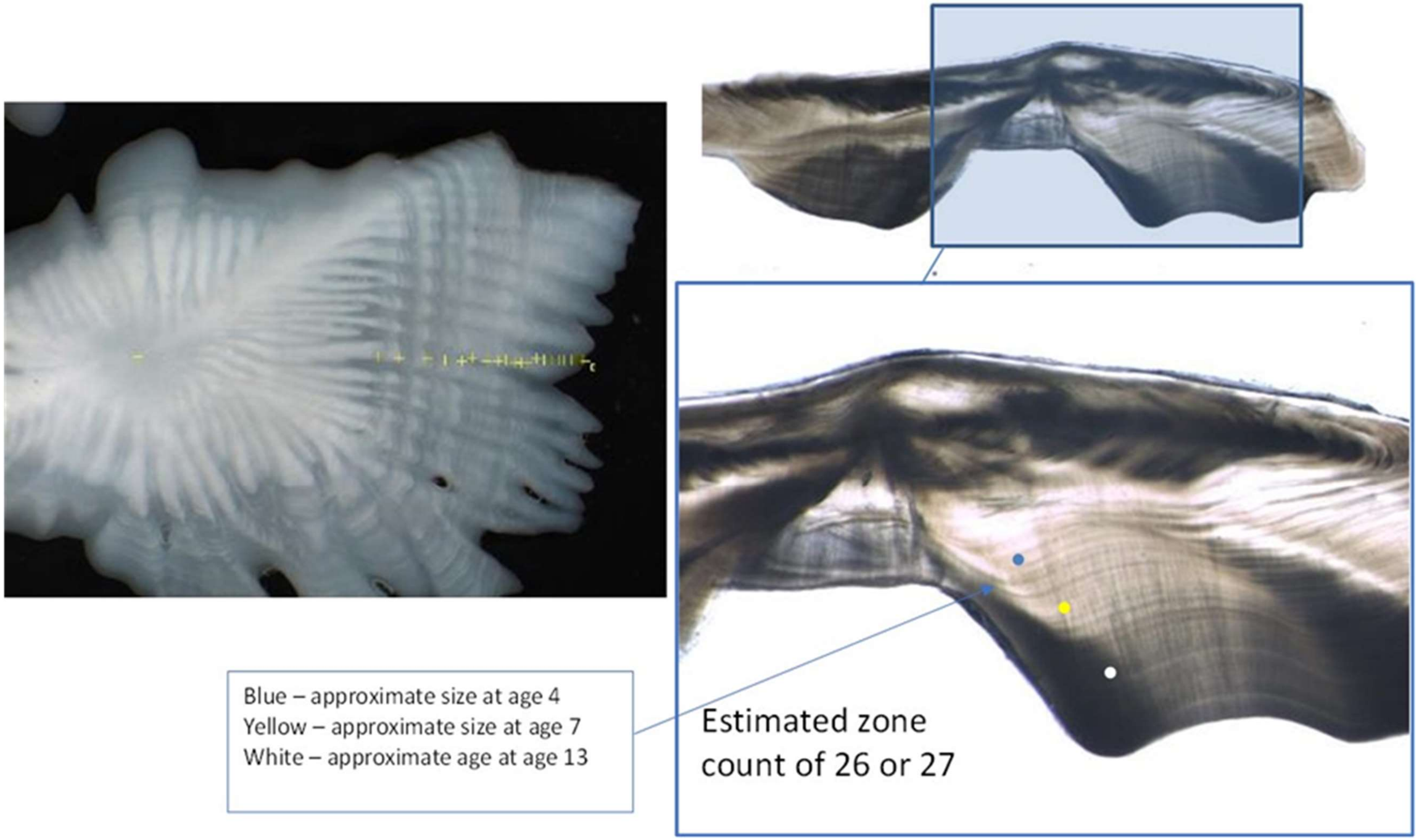

Figure 11 – Otolith images from a 49.4cm sample with an otolith weight of 0.7139g. Whole age reading provided a zone count of 25 while the corresponding transverse section provided a zone count of 26 or 27. The relative distance to the assumed third (blue circle), seventh (yellow circle) and the thirteenth annuli (white circle) are shown within the transverse section.

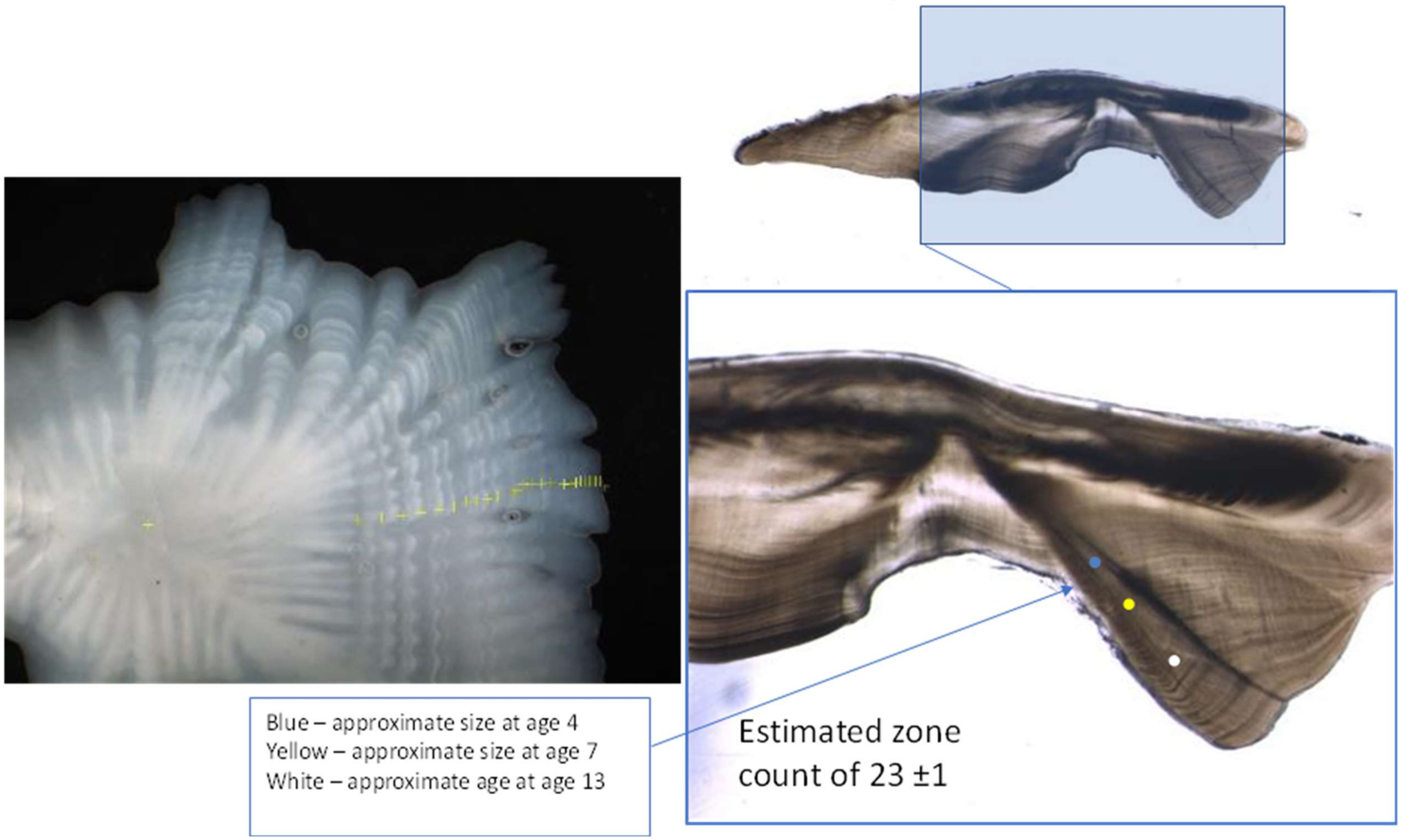

Figure 12. Otolith images from a 54cm sample with an otolith weight of 0.6122g. Whole age reading provided a zone count of 22 while the corresponding transverse section provided a zone count of 23 or 24. The relative distance to the assumed third (blue circle), seventh (yellow circle) and the thirteenth annuli (white circle) are shown within the transverse section.

Discussion

This report details the results from conducting age readings on 1031 Alfonsino otoliths from the Southern Indian Ocean. As noted by several other authors that have aged this species from reading whole otoliths (Lehodey and Grandperrin, 1996; Santamaria *et al*. 2006; Kozlov, 2014) the age readers in this study considered the otoliths of small Alfonsino to be relatively easy to interpret. While the presence of split rings and checks were apparent throughout otoliths from all age ranges and sizes, generally in the otoliths of smaller samples <40 cm (FL), the sub annuli were more easily distinguishable from the true annuli. This is likely because small otoliths are generally quite thin and relatively flat. For samples >40cm FL and approximately 9 years of age the whole otoliths become gradually thicker with size and age, the stacking of increments on the surface becomes more apparent and the otoliths become increasingly more difficult to age. While other studies (Dwyer *et al*. 2003; Krusic-Golub *et al.* 2012) have shown that ageing whole otoliths can lead to an underestimation of age after a certain age or size, the previous examination of a small number of samples using both reading methods (Anon, 2008) and the additional examination of the two largest otoliths in this set provided some weight of evidence that reading whole otoliths is not likely to lead to a major underestimation of longevity for this species.

The precision estimates between the first and second reader and the first and third reader on a subset of 10% of the samples showed a high degree of agreement. The calculated APE values were well within acceptable limits and the comparison of age reading showed no evidence of between reader bias. This suggests that the age data produced in this study is adequate for estimating biological growth parameters. While the precision of the age estimates was considered high for these samples, we did note that the distribution of the length at age was much wider in the samples greater than nine years old. While the change in growth variability could be a result of something like onset of maturity, it could also indicate a lack of precision in the whole ageing method, or perhaps is a mixture of both. Even though the otoliths of smaller Alfonsino are considered relatively easy to age it may be worth thoroughly investigating the utility and precision of reading sectioned otoliths for this species.

For both the eastern and western samples no zone adjustment was performed. For the eastern samples, all but 1 sample were collected in months outside of the proposed zone formation adjustment period (March to August) used by Massey and Horn (1990). For the western samples, while samples were available for most months of the year and even though the method was already slightly modified it was apparent that the adjustment criteria was not totally suitable for the SIOFA samples. The ageing method used in the current study counted completed opaque zones, however the age adjustment procedure largely ignored the months in which the otolith margin is likely to be changing from opaque to translucent (Jan– March).

While the age adjustment procedure may provide a suitable adjustment for those samples caught between March – August, errors will still exist without first adjusting around the months where opaque zones are completing their formation. For example, the majority of eastern samples were collected during December and January. For both months, more so in January, otoliths with opaque and translucent edge types were recorded. Therefore, two otoliths from the same cohort maybe assigned different ages if one otolith had a completed the opaque zone and the other had not. These errors can certainly be reduced by first considering an edge type adjustment criteria which perhaps is completed on samples caught between October to March for instance, before then applying a biological adjustment to bring each age estimate back to a biological year based on a birthdate of either July 1 or August 1.

Alternatively, it may be easier to modify the ageing protocol to allow for the zone formation adjustment period to be the same as the biological adjustment so that only one adjustment is necessary. Santamaria *et al*. (2006), after reviewing several other studies on Alfonsino, decided to count completed translucent zones and suggested the use of 1 August as a nominal birthdate adjustment for the southern Indian Ocean samples that were aged in their study. This date is only 1 month later than that used by Massey and Horn (1990) and seems reasonable since spawning in other oceans are thought to occur between June-September (Kozlov. 2014; Massey and Horn. 1990; Adachi *et al*. 2000). What was not apparent in their study was how the birthdate was applied, whether otolith edge type information was recorded or even if zone counts were converted to age estimates. However, using a nominated date of 1 August and our knowledge that translucent zone formation predominantly begins in May and is mostly complete by August/October (Massey and Horn. 1990; Adachi *et al*. 2000; Lehodey and Grandperrin. 1996) we can design a relatively straightforward adjustment protocol that would allow for both zone formation and birthday adjustment to be performed at the same time. The suggested method is presented in more detail in the next recommendations section. It should be noted however, that further marginal increment analysis examination is required to provide better information on the zone formation periodicity in southern Indian Ocean Alfonsino otoliths. It might be the case that the zone formation date for adjustment might needs to be modified from the suggested 1 August i.e. use 1st June or 1st July if translucent zones are completed earlier in the otoliths from the Indian Ocean than they are in otoliths from the Pacific Ocean.

Regardless of which approach is followed, assuming the zone count method is accurately described, and that zone count data and a descriptive otolith edge type classification is recorded, then theoretically all data should be comparable. In the case of this study the otolith zone count and the edge classification of each otolith was adequality recorded so the results should be able to be adjusted as necessary for different birthdates and/or different uses.

Recommendations

<u>Recommendation 1</u> – Direct validation.
Further direct validation should be explored through bomb radiocarbon age validation. This method could provide further information on maximum age and also provide information on the variability of length-at-age expected for various age classes throughout the species age range.

<u>Recommendation 2</u> – Modify the ageing method used by Santamaria study to be used for future ageing if required.
If further age estimation on Indian Ocean otolith samples is required, then it is recommended that the ageing methods follow the zone counting method used in Santamaria *et al.* 2006. This method counts completed translucent zones and considers the opaque nucleus and the subsequent first completed translucent zone as the first annuli. However, the following additions to that method should be implemented:

- The otolith edge should be classified according to the otolith margin type (opaque or translucent) and more importantly the relative width of the otolith margin compared to the width of the previously completed annuli using four edge types suggested; Translucent Narrow (TN), Translucent Wide (TW), Opaque Narrow (ON) and Opaque Wide (ON)
- Covert zone counts to age estimates based on the following tables:

Zone count adjustment criteria to account for zone formation & biological year

Catch Month	January to May	May to July	August to October	November to December
Translucent Narrow, (TN)	N	N	N+1	N
Translucent Wide, (TW)	N	N	N+1	N
Opaque Narrow, (ON)	N	N-1	N	N
Opaque wide, (OW)	N	N-1	N	N

Note: perhaps just optical classification (opaque or translucent) is required for this method and if the 1st August is considered the biological birthdate then this adjust would serve both purposes.

If the assessment requires calendar year age, then the following can be then applied. In the tables below, A = age adjusted for zone formation.

Table for calendar year adjustment criteria

Catch Month	January to July	July to December
Calendar year	A+1	A

<u>**Recommendation 3**</u> **- Adjust the current zone count to account for opaque zone formation allowing this data set to be comparable to future data sets if they are aged using the recommended modified Santamaria method.**
The age estimates in this study were provided as zone counts and the edge types were recorded. Since completed opaque zones were counted, it is necessary to first adjust the zone counts to account for the variation around the timing of opaque zone formation before considering any biological adjustment. For example, the following adjustment tables could be used:

Table for zone count adjustment criteria to account for zone formation

Catch Month	Dec to Feb	Mar to June	Aug- Nov
Translucent Narrow, (TN)	N-1	N	N
Translucent Wide, (TW)	N-1	N	N
Opaque Narrow, (ON)	N	N+1	N
Opaque wide, (OW)	N	N+1	N

Once zone counts have been adjusted for zone formation, the age estimates can then be adjusted to account for biological age and a calendar age.

Table for biological (1st August) adjustment criteria

Catch Month	Aug - Feb	March - July
Biological year	A	A -1

If the assessment requires a calendar year age, then the following can be then applied

Table for calendar year adjustment criteria

Catch Month	Dec to Jun	Aug to Nov
Calendar year	A - 1	A

Using the above zone count and the biological adjustment tables should provide age estimates that would be essentially the same, had the modified Santamaria method been used for these samples. Regardless, it might be good practise to re-examine the eastern and western otoliths and assign an age and edge type based on the recommended method. Given that the zone counts in this study were precise, it should be possible to reassign the edge type and adjust the zone count where necessary by just using the otolith images.

<u>Recommendation 4.</u> – Marginal increment information

It is suggested any future ageing conducted on Indian Ocean Alfonsino, should ensure the marginal increment measurement data is collected to allow for the accurate assessment of the translucent and opaque zone formation periodicity. Given that the interpretation of the zone pattern and the assignment of edge type becomes more difficult after age 9, perhaps it is only necessary to collect this data for the first 8 annuli.

Since completed opaque zones were counted in this study, the data collected here could not be combined with any new data which measures completed translucent zone. However, this data may be still quite useful in providing information on the annuli periodicity and which months relate to the completion and beginning of opaque and translucent zone formation, respectively.

<u>Recommendation 5.</u> – Reconsider the 2-reader model.

Moving away from the 2-reader model would potentially provide benefits in the cost and time required to collect age estimation data. Assuming that the primary age reading is being conducted by a reader with experience in interpreting Alfonsino otoliths, then possibly the model that is utilised in many routine ageing laboratories would be sufficient. Generally in production reading of a familiar species, all samples are read by the one reader (Reader 1) and a sub-sample (20-25%) is re-read by the same reader at the completion of ageing to assess precision and check for intra-reader drift. If the species is considered difficult to age or there is a requirement for an independent read, then a secondary reader (often from another laboratory) is employed to provide a secondary read. This is usually conducted on a sub-sample of otoliths, usually between 10-25%, rather than the whole sample set. This is however totally dependent on the number of otolith samples and the resources available at the time.

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Appendix 1: Intellectual Property

No intellectual property has arisen from the research that is likely to lead to significant commercial benefits, patents, or licences.

Appendix 2: Staff

Mr Kyne Krusic-Golub - Principal Investigator, age estimation (Reader 1) and report

Mr Simon Robertson – Age estimation (Reader 2)

Peter Horn (Pachyornis Science) – Age estimation 10% re-read (Reader 3)

Graham Porter -Registration, sample organisation and preparation

Appendix 3: Methods used in other Alfonsino ageing studies

Study	Study Area	Structure	Annuli count	MIA or Edge Type Analysis	Birthday	Adjusted for zone formation
Adachi *et al.* (2000)	Izu Islands - Pacific	Whole otoliths	completed opaque	Y	Arbitrary (1st Jan ??)	N
Massey and Horn (1990)	NZ - Southern Pacific	Whole otoliths	completed opaque	Y	1st August	Y
Anibal *et al.* (1998)	Azores - Atlantic	Whole otoliths	completed opaque	N	N	N
Kozolov (2014)	Azores - Atlantic	Whole otoliths	completed translucent	N?	June 1st	N
Lehodey and Grandperrin (1996)	New Caledonia - Pacific	Whole otoliths	completed translucent	Y	1st January	N
Rico *et al.* (2001)	Macaronesian archipelagos - Atlantic	Whole otoliths	completed translucent?	Y	?	N
Taniuchi *et al* . (2004)	Kato District Waters, Japan - Pacific	Sectioned otoliths	daily	N	N	N
Santamaria *et al.* (2006)	South West Indian Ocean	Whole otoliths	completed translucent?	N	1st August	N
Anon (2008)	East Coast Australia - Pacific	Whole otoliths	end of opaque	N	N	N