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Progress on splendid alfonsino age determination works using otoliths from the SIOFA member countries

Relates to agenda item: 3 Info paper

Part I (Preparatory works)

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Part II (Age determination works)

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ABSTRACT

This document describes progress on splendid alfonsino age determination works (2019-2020) using otoliths from the SIOFA member countries by two parts, i.e., Part I: preparatory works and Part II: age determination works.

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Part I: Preparatory works

1. Introduction

The last SC4 (2019) recommended to estimate splendid alfonsino age by otoliths collected by member countries in the SIOFA CA, as a part of its work plan. Results plan to be used to estimate the growth equations for stock assessments conducted by the Consultant for SERA-WG2, March 2020. MoP6 (2019) approved the budget (EURO 16,000) for this work. This document describes progress in two parts, i.e., Part I (preparatory works) and Part II (age determination). March 2020. During the working period, SC Head of Delegations intersessionally discussed relevant issues such as inventory of otolith, selection of otoliths, methods and other important matters to decide. Table 1 is the summary of relevant activities (2019-2020).

Table 1 Summary of activities on splendid alfonsino age determination works by otolith reading (2019-2020)

Year	Mo	Activities
2019	3	SC4 recommendations for age determination as one of its work plans
	7	MoP6 approved the budget EURO 16,000 for aging work
	9	inventory of available otolith (Australia, Cook Islands, Japan and Korea) was completed.
		FAS was selected as the contacted company for ageing works
	11	All otoliths from Australia, Cook Islands and Korea were delivered to FAS.
2020	1	FAS completed age determination work for EAST
	2	Cook Islands estimated the growth equation (EAST) using the ages estimated by FAS and submitted the working paper to SERA-WG2
		Japan estimated the growth equation (WEST) based on the age estimated by otoliths collected by Japan and submitted the working paper to SERA-WG2
	3-4	SERA-WG2 and SC5: Cook Islands and Japan will present their papers.
	4	FAS will complete otolith reading for WEST by April 30
	5	FAS will submit the final report by May 31

2. Collection of otoliths

After MoP6 (2019) approved the budget, an inventory of otolith collections template (Table 2) was constructed to determine the numbers of otoliths that had been collected over time by Australia, Cook Islands, Japan and Korea. Total number of otoliths are n=2,532 (male) and n=3,123(female) (total n=5,655) collected in 12 years (2006-2017). Please well note that majority otoliths are paired (2 otoliths) which are counted as one in the total. Hence if we count it as a single, the total sample number will be more than 11,000. Table 3 is the summary of the inventory by MoP, year, management unit and sex.

As Japan completed ageing (WEST) by that time using their otoliths and planned to provide the growth equation to SERA-WG2, the rest of three countries (Australia, Cook Islands and Korea) decided to provide otoliths for ageing works. Within the budget (EURO 16,000), EURO 4,000 will need for the final report, thus the rest of EURO, 12,000 is available for otolith reading works.

Table 2 Template to collect otoliths from member countries

SIOFA ALFONSINO OTOLITH INVENTORY:

INVENTED BY:

AGENCY/INSTITUTE:

DATE:

sample ID number	Area (locations) Collected (rough) Lat/long or indicate areas in the map next page	Year and month collected		How were the samples collected? R: Randomly S: basis of size O: others (specify)	Fishing depth (m or range)	Number (n=) of otoliths collected						Number (n=) of Length and Weight data collected		How have the otoliths been stored? D: dry P: stuck to paper (dry) S: In preservative solution (thymol, alcohol, etc.) O: other (specify)	Can you release your otolith to the company for ageing ? YES, NO or specify
						Male		Female		Unknown					
		Year	Mo			single	paired	single	paired	single	paired	Length	Weight		

Note:

Table 3 Summary of available otoliths by country, year, management area (WEST and EAST) and sex (total n=5,655) collected in 12 years (2006-2017)

		Total/ Male							Total / Female						
		EAST	WEST	WEST	WEST	WEST	WEST	WEST	EAST	WEST	WEST	WEST	WEST	WEST	WEST
	year	4	1	2	6	2+3a	3a	3b	4	1	2	6	2+3a	3a	3b
AUS	2006	107							94						
AUS	2007	97							97						
AUS	2008						18	36						12	26
AUS	2010	23		41			6	11	27		41			3	10
AUS	2011	156		31			15	14	193		31			18	8
CI	2011	36		16	12		69	7	66		26	18		105	13
CI	2012	87		111			268	75	122		139			340	117
CI	2013	64	6	75			94	71	85	4	75			106	119
CI	2014	20		14			53	36	20		16			59	54
CI	2015						7	8						3	12
CI	2017	58					5	13	59					4	11
JPN	2012			72				55			89				44
JPN	2016			11			28	14			19			30	42
JPN	2017	11		54			53	143	18		63			67	200
JPN	2018						27	43						58	46
KOR	2012					93							125		
KOR	2013					168							189		
Total		659	6	425	12	261	643	526	781	4	499	18	314	805	702

3. Contracting the company to work otolith readings

Three experienced companies (2-Australian and 1-Japanese) were nominated by SC HoD as likely candidates to complete the ageing work. For otolith reading works, HoD requested cross readings to be completed (inter-reader comparison) on the same splendid alfonsino otoliths by two experienced readers. After SC HoD evaluated three companies, one company from Australia (FAS) was selected because FAS satisfied these conditions and were able to provide the services at a reasonable price and adhere to the relatively short delivery times.

4. Selections of otolith

Within the available budget for otolith age determination works (EURO 12,000), maximum number of otoliths are assigned by management unit (WEST and EAST), sex and size bins. Tables 3-6 shows the results.

Table 3 Target number of otoliths to read (yellow marker) and relevant information (EAST MALE)

EAST MALE							
bin code	size range FL (cm)	# sent to FAS				Target to read	Spare
		AUS (FL converted	CI(FL)	Total	Total (actual number) (pair counted as 2)		
		pair	pair	pair			
EM-1	< 25	24	1	25	25	25	0
EM-2	25≤ <30	46	2	48	96	42	54
EM-3	30≤ <35	30	19	49	98	42	56
EM-4	35≤ <40	47	2	49	98	42	56
EM-5	40≤ <45	47	2	49	98	42	56
EM-6	45≤	37	12	49	98	42	56
	Total	231	38	269	538	235	278

Table 4 Target number of otoliths to read (yellow marker) and relevant information (EAST FEMALE)

EAST FEMALE							
bin code	size range (FL) (cm)	# sent to FAS				Target to read	Spare
		AUS (FL conveted from TL)		CI(FL)	Total (actual number) (pair counted as 2)		
		Pair	Single	Pair			
EF-1	< 25	39	1		40	40	0
EF-2	25≤ <30	35		2	74	40	34
EF-3	30≤ <35	25		6	62	40	22
EF-4	35≤ <40			49	98	40	58
EF-5	40≤ <45			49	98	40	58
EF-6	45≤ <50			49	98	40	58
EF-7	50≤	1		5	12	12	0
	Total	100	1	160	482	252	230

Table 5 Target number of otoliths to read (yellow marker) and relevant information (WEST MALE)

WEST MALE								
bin code	size range FL (cm)	# sent to FAS				Total (actual number) (pair counted as 2)	Traget to read	Spare
		AUS (FL converted from TL)	CI (FL)	KOR (FL converted from TL)				
		pair	pair	pair	single			
WM-1	< 20			41		82	37	45
WM-2	20≤ <25			38	3	79	37	42
WM-3	25≤ <30	11		25	4	76	37	39
WM-4	30≤ <35		16	0		32	32	0
WM-5	35≤ <40		41	0		82	37	45
WM-6	40≤ <45		41	0		82	37	45
WM-7	45≤		41	0		82	37	45
	Total	11	139	104		515	254	261

Table 6 Target number of otoliths to read (yellow marker) and relevant information (WEST FEMALE)

WEST FEMALE								
bin code	size range FL (cm)	# To send				Total (actual number) (pair counted as 2)	Traget to read	Spare
		AUS (FL converted from TL)	CI (FL)	KOR (FL converted from TL)				
		Pair	Pair	Pair	Single			
WF-1	< 20			42		84	36	48
WF-2	20≤ <25			38	4	80	36	44
WF-3	25≤ <30			35	7	77	36	41
WF-4	30≤ <35		15			30	30	0
WF-5	35≤ <40		42			84	36	48
WF-6	40≤ <45		42			84	36	48
WF-7	45≤	3	18			42	36	6
Total		3	117	115		481	246	235

5. Reviews and future considerations

5.1 Problems

According to Brouwer et al (2020) estimating the growth equation in EAST, there are two problems on estimated ages as below

- Not enough sample size of otoliths for females (EAST)

This is observed in Table 4, i.e., the sample size of otolith > 50cm is only 12 (red rectangle box). As this is the maximum number in Australia and Cook Islands, we need to increase samples in the future

- 25% of samples are not good quality of estimated ages

Brouwer et al (SERAWG-02-07 REV_1, 2020) used 531 ages (male and female together) determined by FAS to estimate the growth equation (EAST). FAS rated the reliability of the age determinations by 5 ranks (R1-R5). The lower is more reliable and Rank 1-3 is acceptable range. Brouwer et al used 397 samples rated as R1-R3, which is 75% of the total. As we have many otolith samples except very younger and older ages, we can do more ageing to get more reliable results in the future if the budgets are available and SC wants to do so.

5.2 Merits

According to the document on comparisons of four growth curves (SERAWG-02- INFO-01 REV_1), samples of this project cover almost all age classes, major fishing grounds, many years (12 years) and wide depth ranges, thus sample coverages are far better than other three cases. This is because samples are from three member countries and were selected from an archive of approximately 5,600 otoliths. We selected just over 1,000 otoliths for this time due to the budget constraint. As described above (problems), about 25% are not good quality, but in the future if we get more funds, we can use more samples and can get more reliable age estimations to estimate robust growth equations.

6. Storages of otoliths and the results (excel file)

SIOFA SERAWG2 and SC5 need to discuss where 5,600 otoliths delivered to FAS need to be kept. It is not the good idea to return to member countries. One of the options is that FAS keeps it until we decide the next works if FAS is OK. Or other ideas need to be discussed during these meetings. The property of these samples is certainly for each member contributing its otoliths.

Regarding results of age determination (excel file), they belong to three member countries. But they should be stored in the SIOFA data deposit. If someone (from other countries) needs to use the data, they need permissions from the data owners (three counties). In addition, the final ageing report to be ready in May this year by FAS, should be available for the public and could be available on the web site if agreeable. We need to discuss this issue in SIOFA SERAWG2 and SC5 meetings in 2020.

Part II: Age determination works

Alfonsino Ageing Brief (FAS)

In total 1,031 otolith pairs were supplied to Fish Ageing Services (FAS). These were provided by scientists from Australia, New Zealand and 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. 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 7. 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	New Zealand	WEST
103	1	706	87	199	Otolith - Annual	New Zealand	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. Whole otoliths were immersed in water against a black background and viewed with reflected light.

All samples available from the EAST were aged as the 1st work by Jan 15, 2020 and the 2nd work for WEST will be completed by April 30, 2020. Age was estimated by counting completed opaque zones, with the first being the completion of the large opaque nucleus (Figure 1). 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.

Figure 1. Example of a clear to read Alfonsino otolith.
This sample was assigned as 4 with an opaque edge.

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

Note 1: often the translucent zone is quite narrow so perhaps the only the optical properties Translucent and Opaque should only be used in any adjustment of edge type.

Note 2: The edge assignment on older samples >8 years is very difficult due to the spacing of the zones being very small and the use of reflected light as the illumination source.

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 alight with those used in other alfonsino ageing projects (Lehoday and Grandperrin, 1996, Massey and Horn, 1990)

- 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

Note 3: 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. The exclusion based on just a readability score needs to be carefully considered because the readability score can also be correlated to the age of a samples. For example, one would expect that the zone count uncertainty would likely be greater in a 50-year-old sample compared to a 2-year-old sample.

Since each sample was aged by two readers, perhaps a better indication of suitability may be to exclude any samples that differed by a zone count by more than one. If this criterion were used, only 10% of the samples would be rejected, compared to the 25% if all samples with a readability ≥4 were excluded. The readability score could also be used in sensitivity estimates. i.e. to determine the effect that including or excluding estimates based on their readability score.

Adjustment criteria to account for zone formation

Massey and Horn (1990) adjusted for edge type during the months between April – August for their samples. All the EAST samples we collected outside of these months therefore zone count = adjusted zone count.

Zone count to Age conversion (Decimal age)

- Approx. 10 months for the completion of the opaque nucleus counted as the first zone (from Lehoday and Grandperrin, 1996)
- Used 1st August as the birthdate (as per Santamaria *et al*, 2006)
    - Example 1. a sample caught on the 1st Jan with a zone count of 4 would be 10 months + 3 years + 5 months /12 = 4.254 years

Note: 3 Only month and year were supplied for the dates of capture, and there are some unknown dates, so I did not do the conversion within the data sheet. If required, then the samples with no data could be ignored and the date of capture can just be assumed to be the first day of each capture month.

References:

Lehodey, P., Grandperrin, R. (1996) Age and Growth of the alfonsino *Beryx splendens* over the seamounts off New Caledonia. Marine Biology 125: 249-258

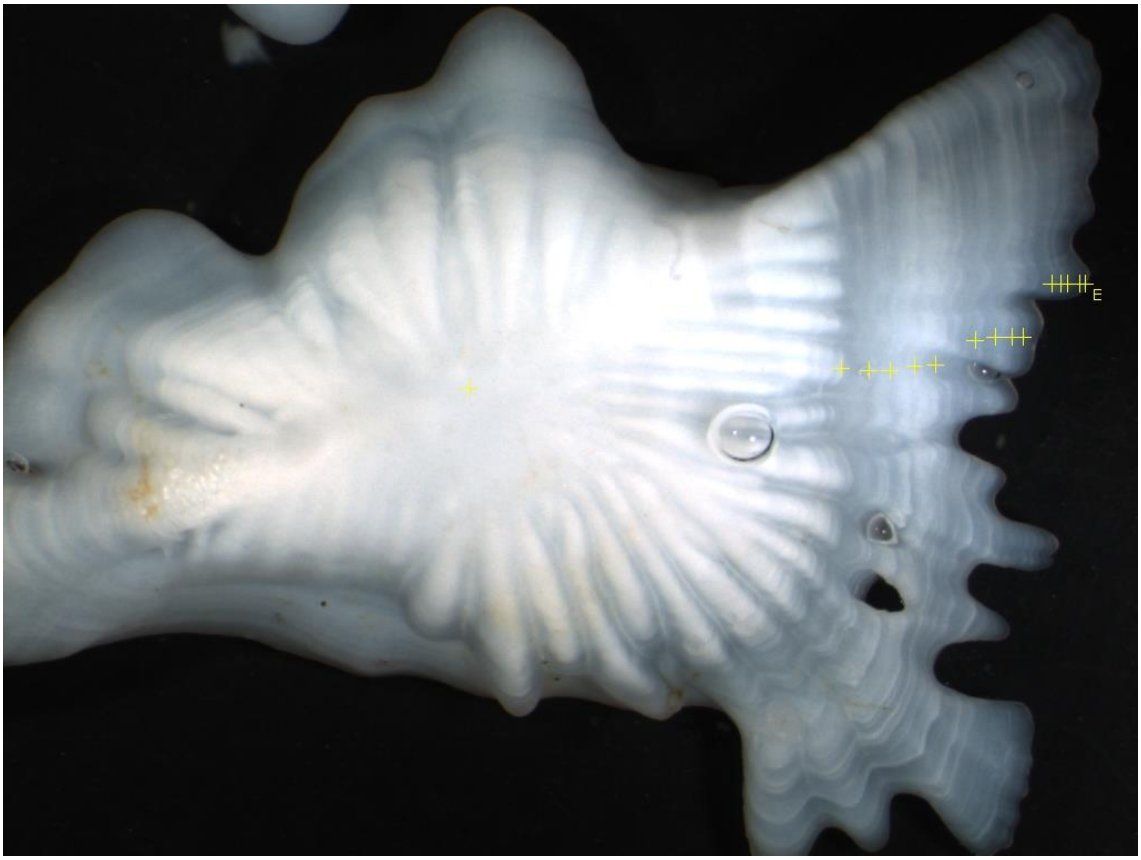
Massey B.R., Horn P.L. (1990) Growth and age structure of alfonsino (*Beryx splendens)* from the lower east coast, North Island, New Zealand. NZ J mar Freshwater Res 24:121-136

Santamaria, MTG, Lopez Abellan LJ, Gonzalez (2006). Growth of alfonsino *Beryx splendens* Lowe 1834 in the South-West Indian Ocean. African Journal of marine Science 2006, 28(1): 33-40.

Additional information (by e-mail from Kyne Krusic-Golub to Tom Nishida) (dated Jan 16, 2020) relating the presentation of the data within the supplied ageing spreadsheets.

In the excel document that FAS supplied there are 4 worksheets:

1) "EAST Age and sample COMB"- This contains the Age data and the sample data (as supplied in the original formats). I have some diagnostic plots to the right of the data that I used for error checking.
2) "EAST Zone count & increment data" - This is the raw zone count file and includes the counts and the measurements taken (our image software only does the measurements for the first 15 annuli). Because the counting path (and measurement path) for alfonsino doesn't always follow a straight line I would consider only the first 5 or 6 zones to be useful for comparative purposes (see image below as an example).



3) “Re-reading data R1 vs R2” - This is the comparison of Reader 1 (myself) against Reader 2 (Simon Robertson) for the first 332 samples (all the AUS samples). The precision within these samples look very good and I was very pleased with the results. Simon will be completing the remaining 199 CI samples over the coming days. Once completed I will send an updated version of this worksheet separately.

4) “Re-reading data PH 10%” - This compares R1 vs the reads that Peter Horn completed on 53 images that I supplied him. While the fit between these reads is not quite a close as the fits between R1 and R2, it is still very much acceptable. It is also much more difficult to age and classify margins from images than it is using a live image or the microscope. Looking closely at the results I think that some differences in individuals can be attributed to different edge classification and whether the opaque zone on the edge was counted or not.