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Update on SBT direct ageing using vertebrae, providing information on length classes targeted for gene-tagging

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1 Introduction

During the gene-tagging program SBT were tagged and released as 2-year-olds in the Great Australian Bight (GAB) in year 1 and the same cohort was re-sampled (during farm harvest) at age 3 in year 2, allowing at least 12 months for mixing. The initial target range for tagged fish was 70-85 cm length to caudal fork (LCF) and tissue samples were collected from harvested fish in the length range 98-109 cm. These ranges were assumed to represent 2 year-old and 3 year-old fish respectively. As SBT are spawned in summer, a theoretical birth date of January 1 is assigned, which is the middle of the spawning season. Given the theoretical birth date and that the harvest period is June-August, the fish sampled during the harvest would be close to 3.5 years-old.

Due to individual variability in growth rates there were possibly some uncertainties in the age classes of the fish sampled. To confirm that the length classes chosen to target 2 year-olds and 3 year-olds are accurate, direct ageing is needed. SBT are routinely aged using otoliths (Sulistyaningsih et al 2018). Otoliths can be sampled from mortalities during gene-tagging field trips in the GAB and from fish processed in Port Lincoln factories at harvest. However, to collect otoliths from SBT at harvest without damaging the external appearance of the fish, a drilling technique with a hole-saw is required to extract the otoliths from the base of the skull. Each fish sampled would need to be taken out of the processing line and the drilling can take several minutes.

An alternative direct ageing method has been considered that can provide information on the targeted age classes and does not interrupt the processing line. Tail stalks are removed as part of normal processing at harvest making vertebrae available, which can be used for direct ageing. The same tail stalks are used to collect the tissue samples for gene-tagging.

Vertebrae have been used previously for direct ageing of tunas (Farber and Lee 1981, Rodriguez-Marin et al. 2005, Uematsu et al. 2018) and specifically for ageing SBT (Gunn et al. 2008). Gunn et al. (2008) found that vertebrae provided accurate age estimates up to 10 years. Until that age, annual increment counts on vertebrae and otoliths were equivalent. Ages determined by counting growth zones (increments) on otoliths of SBT have been validated throughout the life of the fish (Clear et al. 2000, Kalish et al. 1996) so we can be confident that vertebrae ages from the targeted length classes would be reliable and accurate.

A trial to assess the feasibility of estimating ages from vertebrae using tail stalks collected during gene-tagging sampling was undertaken. Age estimates were made from vertebrae collected from tail stalks and the efficiency of collection, preparation and ageing method was considered. Following this trial a larger sample of vertebrae were examined to assist in refinement of the length classes to use in the gene-tagging program.

2 Methods

2.1 Trials for ageing vertebrae

Twenty tail stalks were chosen for the initial trials for ageing vertebrae: 10 from SBT mortalities during gene-tagging field trips in the GAB in 2018, and 10 from fish processed in Port Lincoln fish factories at harvest in 2017 (Table 1). Where possible vertebrae were chosen from fish that had had otoliths sampled as well.

Table 1. Vertebrae chosen for ageing trials.

Vertebrae number	Date Collected	LCF (cm)	gene-tagging batch	otoliths collected
1	26/02/2018	69	field mortality 2018	Yes
2	5/02/2018	80	field mortality 2018	Yes
3	28/03/2018	70	field mortality 2018	Yes
4	1/03/2018	85	field mortality 2018	Yes
5	5/03/2018	99	field mortality 2018	No
6	25/02/2018	93	field mortality 2018	Yes
7	10/03/2018	83	field mortality 2018	Yes
8	4/03/2018	96	field mortality 2018	Yes
9	16/03/2018	74	field mortality 2018	No
10	3/03/2018	73	field mortality 2018	Yes
11	16/07/2017	113	harvest 2017	Yes
12	12/07/2017	103	harvest 2017	Yes
13	11/07/2017	115	harvest 2017	Yes
14	10/07/2017	99	harvest 2017	Yes
15	13/07/2017	115	harvest 2017	Yes
16	10/07/2017	110	harvest 2017	Yes
17	10/07/2017	103	harvest 2017	Yes
18	10/07/2017	113	harvest 2017	Yes
19	17/07/2017	117	harvest 2017	Yes
20	7/07/2017	115	harvest 2017	Yes

Tuna have 39 vertebrae and the last 4 to 6 vertebrae were in the tail stalks discarded at harvest (Figure 1). All vertebrae available in a tail stalk were used for ageing as part of the trials. To preserve the quality of the inner surface of the vertebral cones, tail stalks were kept intact and frozen until just before processing because as the cone surface comes in contact with air, the surface dehydrates, doesn't take up stain and becomes more difficult to read (Ruiz et al. 2005).

Prior to staining, tail stalks were soaked in hot water and the outer flesh removed (Figure 2). The spine was cut transversely between the vertebrae to separate them and the internal cones were exposed and inter-vertebral jelly removed. The vertebrae were immediately immersed in alizarin stain before the cones dried out.

Figure 1. SBT tail stalks laid out in the factory for gene-tagging sampling.

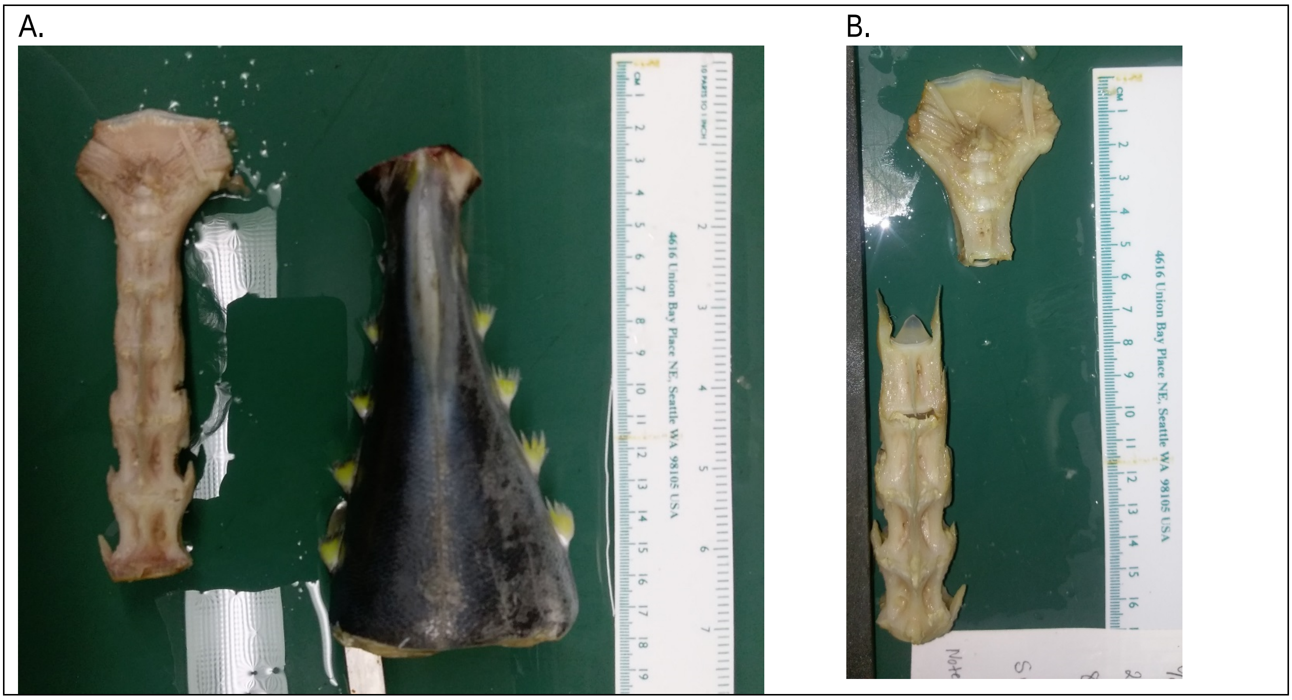

Figure 2. SBT tail stalk cleaned of flesh (A, left) and vertebrae separated exposing the cone jelly (B).

2.2 Staining of vertebrae

The alizarin stain recipe was modified from Berry et al. (1977):

Step one, make up alizarin stock solution

Mix 3.1 g alizarin red S, 50 cc distilled water and then 50 cc concentrated acetic acid, let cool for about 20 minutes.

Add the above mixture, slowly, to 200 cc glycerine and 1200 cc distilled water, discarding excess precipitate.

Step 2, make up alizarin stain solution ("the stain").

Potassium hydroxide (15% in distilled water) – 86%

Glycerine (lab grade) – 10%

Alizarin stock solution (from above) – 4%

Note: Make up the potassium hydroxide solution and let it cool. Add the glycerine and then the alizarin stock solution.

The vertebrae were soaked in alizarin stain for 1-6 hours. The smallest, from fish at around 70 cm LCF, needed at least 1 hour to stain the cones of the vertebrae, and the required time increased with the size of vertebrae. Vertebrae were rinsed in milli-Q water (Figure 3).

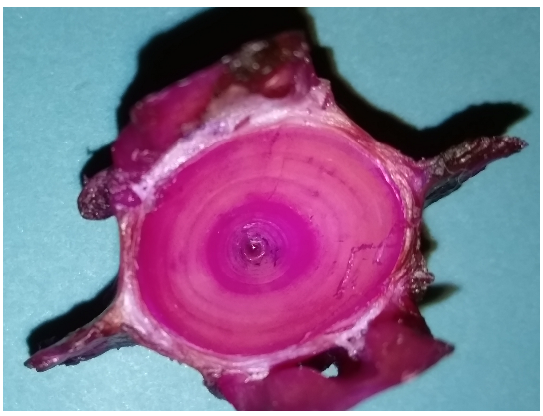

Figure 3. Vertebra from a 108 cm SBT, after 2 hours staining with alizarin red S.

2.3 Examining and interpreting vertebrae

Vertebrae were examined under low magnification and reflected light. A polarising filter on the light helped accentuate bands on the cone surface. The pattern and description of bands was recorded including width, staining effect and 3 dimensional appearance. A complete pattern was counted as 1 year's growth. All vertebrae from each fish were examined but a final count was made on either the 35th or 36th vertebrae.

Cone radius was measured from inner cone focus to the outer edge of the 36th vertebra to investigate the relationship between cone radius and fish length.

Age estimates from vertebrae were compared with age estimates from otoliths collected from the same fish, where they were available. Otoliths from the smaller fish were aged using a whole otolith method and the larger fish by reading sectioned otoliths.

2.4 Decimal age at length from a larger set of vertebrae

Another 80 vertebrae were chosen for ageing from SBT sampled during 2017 harvest, 2018 field tagging mortalities, 2018 farm mortalities and 2018 harvest, to examine length at age (to inform the length ranges used in the gene-tagging program).

3 Results

3.1 Trials for ageing vertebrae

Clear concentric bands were observed in all stained vertebrae on both anterior and posterior cones. The pattern of bands consisted of a darkly-stained narrow band; an unstained ridge; and a wider, lightly-stained band. The number of these complete patterns was counted to determine an age estimate.

For the size range analysed (69-117 cm LCF) there was a significant and positive linear correlation between fork length and the radius of the 36th vertebrae, which we refer to as the cone radius (r^2 = 0.948; Figure 4).

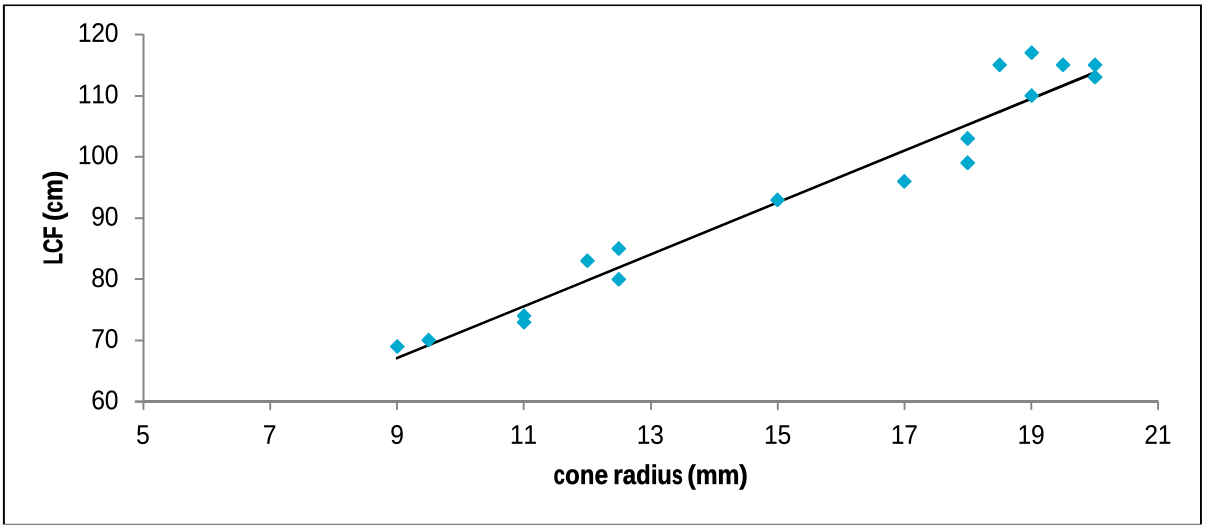

Figure 4. The relationship between fish length and vertebrae cone radius (N = 20).

The ages estimated from vertebrae were 1-4 years (Table 2 and Figure 5). The length range for fish of each age were discrete, i.e. there was no overlap between fish lengths of each age group: 1-year-olds were 69-74 cm, 2 year-olds were 80-85 cm, 3-year olds were 93-110 cm and 4 year-olds were 113-117 cm.

Table 2. Age estimates from vertebrae and otoliths from the vertebrae ageing trials.

Vertebra #	LCF (cm)	Age_ vertebra	Vertebra cone radius (mm)	Age_otolith	Otolith weight (g)	Readability otolith age	Difference vertebra age – otolith age
1	69	1	9	1	0.01715	3	0
2	80	2	12.5	2	0.025	3	0
3	70	1	9.5	1	0.02055	3	0
4	85	2	12.5	2	0.02435	3	0
5	99	3	18		na		
6	93	3	15	3	0.0397	2	0
7	83	2	12	2	0.0277	3	0
8	96	3	17	3	0.0413	2	0
9	74	1	11		0.0208		
10	73	1	11	1	0.02015	3	0
11	113	4	20	4	0.0575	3	0
12	103	3	18	3	0.0454	3	0
13	115	4	20	4	0.0558	4	0
14	99	3	18		na		
15	115	4	19.5	3	na	3	1
16	110	3	19	3	na	4	0
17	103	3	18	3	na	3	0
18	113	4	20	5	0.0583	5	-1
19	117	4	19	4	0.0654	3	0
20	115	4	18.5	4	0.0568	3	0

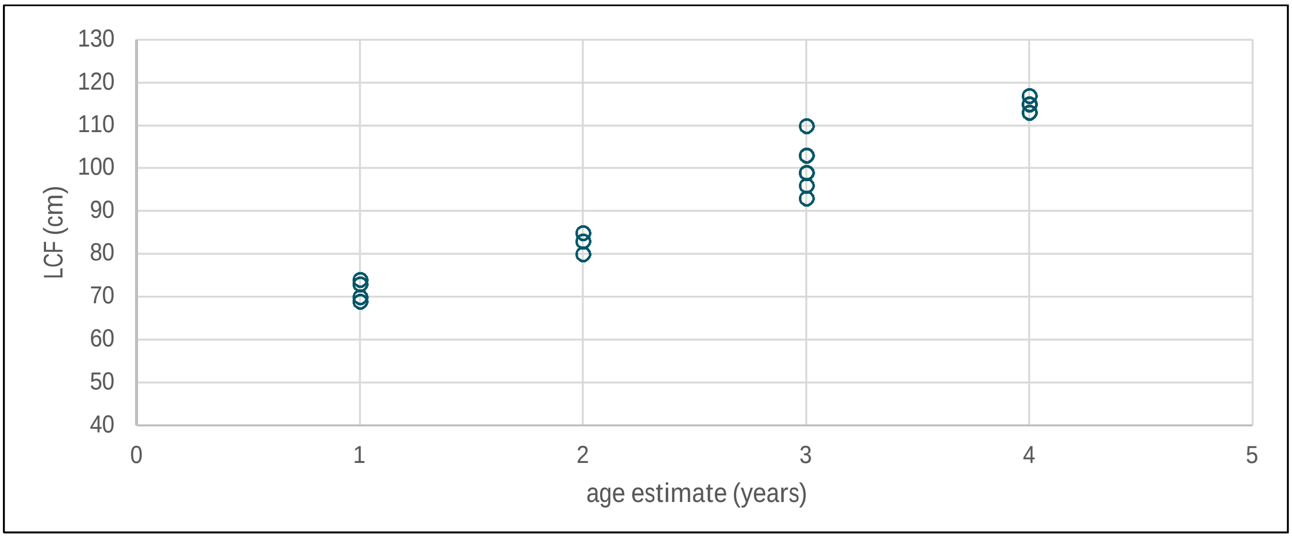

Figure 5. Ages estimated from SBT vertebrae by fish length (N = 20).

The appearance of the vertebral cone edge was recorded. For vertebrae #1 to 10, the summer-caught fish, the edge type was a wide, lightly-stained band. For vertebrae #11 to 20, the winter-caught fish, all but one fish had a narrow, darkly-stained band on the edge. The one other winter-caught fish had a wide, lightly-stained band on the edge. The timing of the narrow, darkly-stained band appears to be equivalent to the narrow zone that forms annually on SBT otoliths during the austral winter (Clear et al. 2000, Gunn et al. 2008).

For the winter-caught fish that had a narrow band on the cone edge, another year was added to the age estimate only if it was completely formed and growth beyond it was observed. If vertebrae growth is consistent with that of otoliths, there is likely to be some variation in the timing of the narrow band, and hence the month in which fish are aged another year older, can vary. Therefore it is possible that some winter-caught fish from a cohort can be estimated to be one year older than others from the same cohort. This can be corrected by calculating decimal age.

Age estimates from otoliths were available from 17 of the 20 fish included in the vertebrae trials (Table 2). Ages from vertebrae and otoliths were equivalent in 15 of the 17 cases. In the other two cases, the age estimates differed by 1 (Figure 6). In one case the age estimate from vertebrae was 4 and the otolith estimate was 5; and in the second case the vertebrae age was 3 and the otolith age was 4.

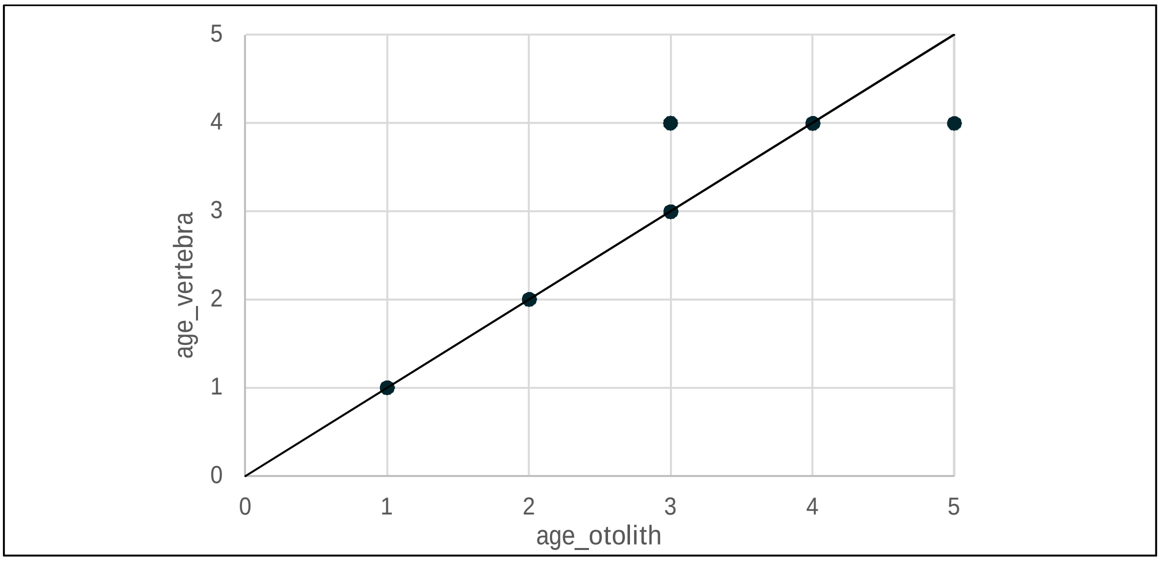

Figure 6. Age bias plot for vertebrae and otolith age estimates (N = 17).

The discrepancies between vertebrae and otolith age estimates could be due to several reasons including: the 1st increment was difficult to identify in one or other structure, the timing of increments deposited on otoliths and vertebrae is different or that the readers are interpreting the edge of the structure (newly-formed material) differently. To resolve the discrepancies, the otolith and vertebrae readers compared their counts and interpretations of each structure.

In the case where the age estimate from vertebrae was 4 and the otolith estimate was 5, the discrepancy was due to edge interpretation or a slight difference in the timing of the band deposition between vertebrae and otoliths. The vertebrae reader noted that the 5th narrow band could be seen on the cone edge but they had not seen any new growth beyond it, so the count was 4; whereas the otolith reader considered the 5th narrow band on the edge to have been completed and that there was new growth beyond, hence the count was 5. If a decimal age had been assigned, it would be equivalent for each structure.

In the second case, where the vertebrae age was 3 and the otolith age was 4, the discrepancy was due to a low confidence in the otolith count. The otolith reader gave a readability count of 3 (readability scale 0 to 5, with 5 being the highest confidence) but noted that he was uncertain whether to assign a count of 3 or 4. The same edge type was recorded for both hard part structures.

These two examples show the importance of understanding the timing of growth increments on hard part structures, interpreting edge type to determine decimal age and recording confidence (or readability) of age estimates.

3.2 Decimal age at length from a larger set of vertebrae

Age estimates made by counting growth increments on vertebrae and decimal ages from the 20 trial fish and the 80 fish aged subsequently, post-trials, are presented in Figure 7 and Appendix A. The ages estimated from vertebrae of fish with lengths 69–117 cm were 1-4 years.

The decimal (fractional) age was calculated using the vertebrae band count, the theoretical birth date of 1st January and catch date (Figure 8). The edge type was taken into account and used to assign fish caught around July to September to a cohort. This is the time of the year when a new dark band forms on the vertebra edge and coincides with SBT harvest in the farms. The exact time when new dark zones are deposited varies between fish so an extra band can be counted on some fish from the same cohort (Figure 9).

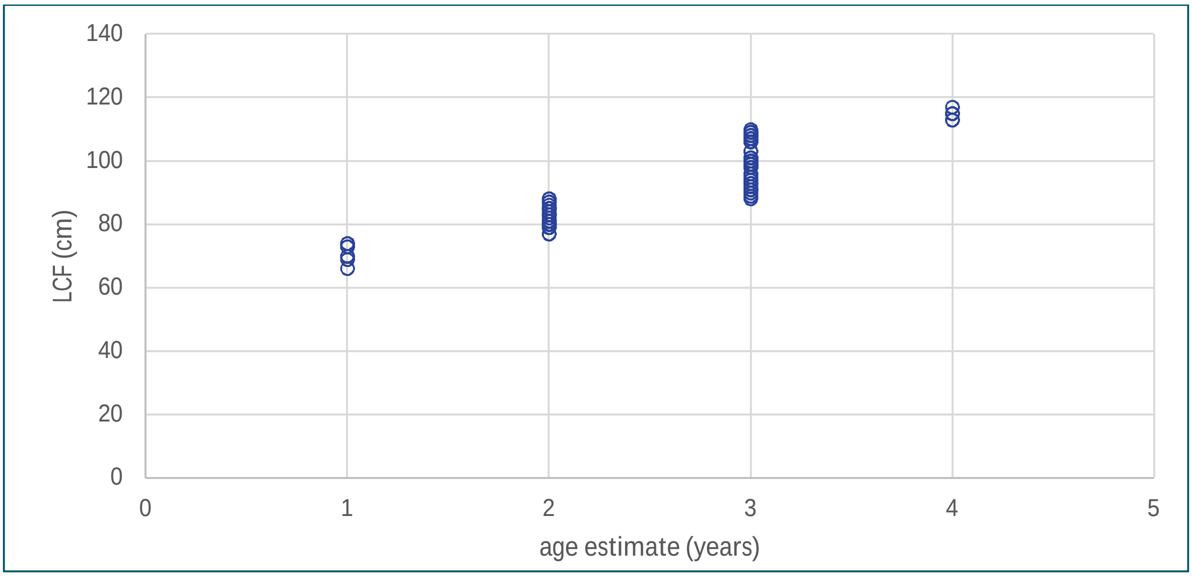

Figure 7. Ages estimated from SBT vertebrae by fish length (N = 100).

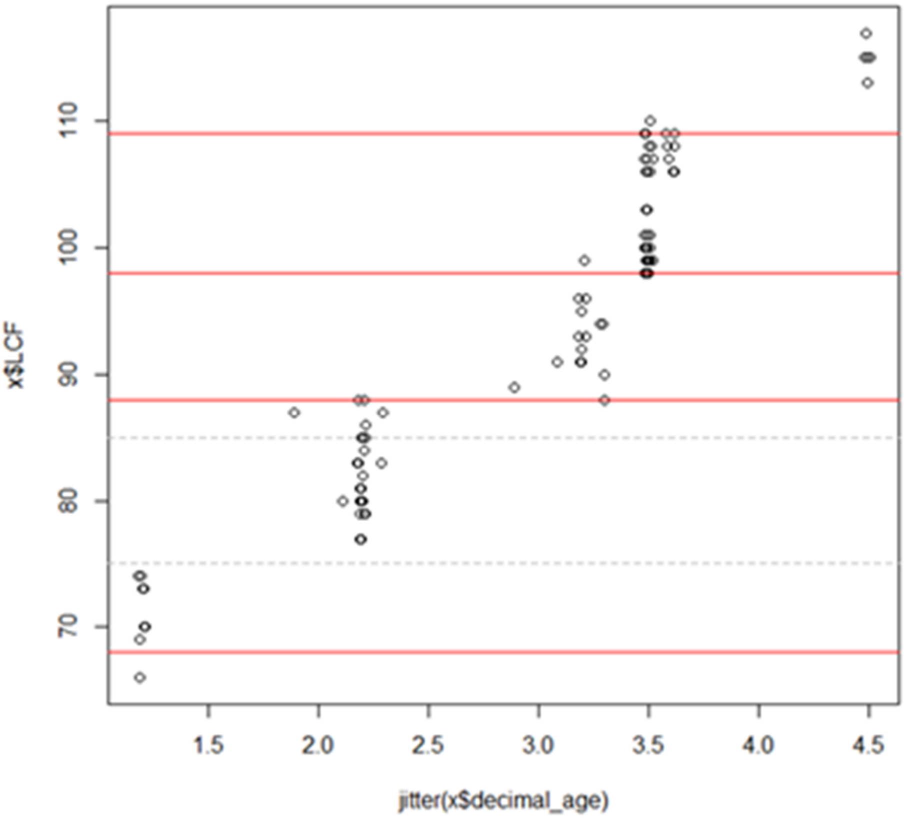

Figure 8. Decimal age versus length, where decimal age is the direct age estimate (vertebra band count) adjusted to decimal age using the known catch date and assigned birth date of 1st January. The lower red horizontal lines (68-88 cm) indicate the size range of fish tagged and released (in February/March), the grey dashed horizontal lines (75-85 cm) represent the revised length class for 2 year-olds in the gene-tagging analysis, and the upper red horizontal lines indicate the 98-109 cm length class used for selecting 3 year-old fish during harvesting (in July-August).

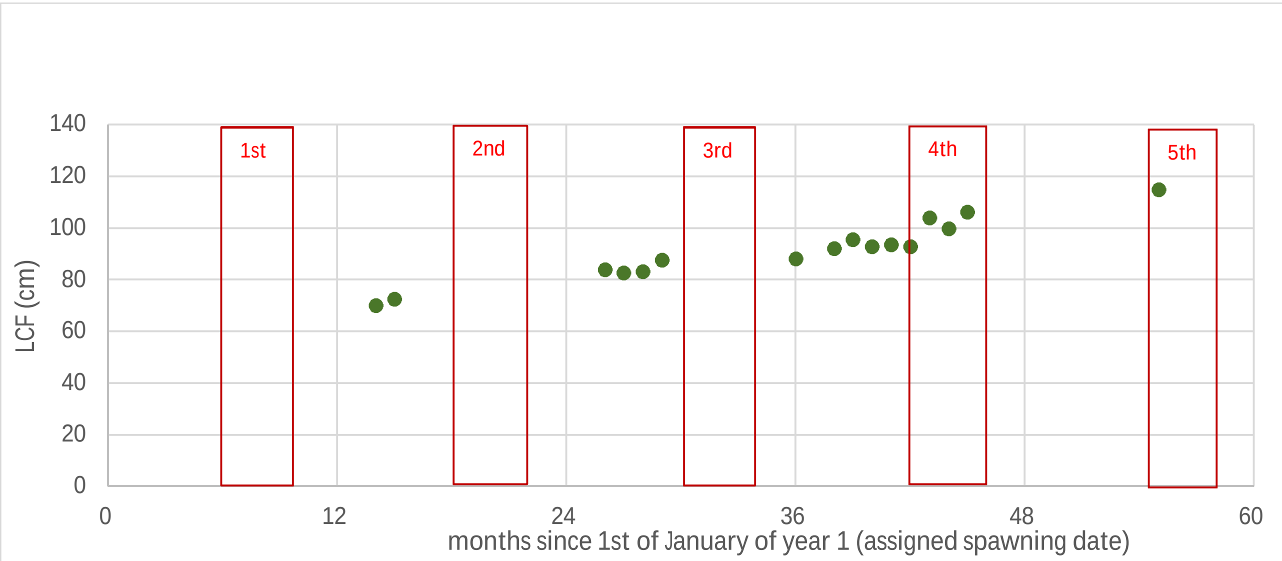

Figure 9. Mean length-at-age by month caught (green dots), estimated from SBT vertebrae (N = 100). Red columns indicate the approximate time of year when narrow growth zones are formed on SBT otoliths and vertebrae (July-September). Another year is added to the age estimate after the narrow “winter” zone is fully formed.

4 Discussion

This study has shown the feasibility of estimating ages from vertebrae using tail stalks to provide information on length classes for gene-tagging. Vertebrae increments are readable; clear concentric bands were observed in all stained vertebrae on both anterior and posterior cones. The timing of the narrow, darkly-stained band appears to be equivalent to the narrow zone that forms annually on SBT otoliths during the austral winter (Clear et al. 2000, Gunn et al. 2008) and the age estimates from vertebrae are reliable as they have been verified by otolith ages from the same fish.

Given that tail stalks are discarded during harvest and are stockpiled for gene-tagging tissue sampling, vertebrae sampling is simple and efficient.

The vertebrae age estimates have been used to refine the length classes for 2 year olds at the time of tagging. Age estimates indicated that the original lower end of the tagging range, 70 cm LCF, meant some 1 year old fish were tagged. Subsequently, fish outside the 75-85 cm length range have been excluded from gene-tag analyses (Preece et al. 2019).

In this study we examined the age estimates from vertebrae as they related to the target length ranges for 2 and 3 year-olds for the gene-tagging study. We hope to continue to analyse direct age from vertebrae and otoliths to examine annual variability in length at age. The comparison of age estimates from vertebrae and otoliths collected from the same fish will also enable detection of any shifts in method or interpretation of either structure.

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6 Appendix A

Table 3. Direct age estimates and decimal ages from 100 vertebrae

	catch date	LCF (cm)	age_vertebra (number of winters)	assigned birth date	decimal age (years)
1	26/02/2018	69	1	1/01/2017	1.2
2	5/02/2018	80	2	1/01/2016	2.1
3	28/03/2018	70	1	1/01/2017	1.2
4	1/03/2018	85	2	1/01/2016	2.2
5	5/03/2018	99	3	1/01/2015	3.2
6	25/02/2018	93	3	1/01/2015	3.2
7	10/03/2018	83	2	1/01/2016	2.2
8	4/03/2018	96	3	1/01/2015	3.2
9	16/03/2018	74	1	1/01/2017	1.2
10	3/03/2018	73	1	1/01/2017	1.2
11	16/07/2017	113	4	1/01/2013	4.5
12	12/07/2017	103	3	1/01/2014	3.5
13	11/07/2017	115	4	1/01/2013	4.5
14	10/07/2017	99	3	1/01/2014	3.5
15	13/07/2017	115	4	1/01/2013	4.5
16	10/07/2017	110	3	1/01/2014	3.5
17	10/07/2017	103	3	1/01/2014	3.5
18	10/07/2017	113	4	1/01/2013	4.5
19	17/07/2017	117	4	1/01/2013	4.5
20	7/07/2017	115	4	1/01/2013	4.5
21	3/03/2018	66	1	1/01/2017	1.2
22	26/02/2018	70	1	1/01/2017	1.2
23	14/03/2018	73	1	1/01/2017	1.2
24	14/03/2018	74	1	1/01/2017	1.2
25	8/03/2018	77	2	1/01/2016	2.2
26	8/03/2018	77	2	1/01/2016	2.2
27	4/03/2018	79	2	1/01/2016	2.2
28	8/03/2018	79	2	1/01/2016	2.2
29	13/03/2018	79	2	1/01/2016	2.2
30	2/03/2018	80	2	1/01/2016	2.2
31	2/03/2018	80	2	1/01/2016	2.2
32	8/03/2018	80	2	1/01/2016	2.2
33	3/03/2018	81	2	1/01/2016	2.2
34	4/03/2018	81	2	1/01/2016	2.2
35	19/03/2018	81	2	1/01/2016	2.2
36	10/03/2018	82	2	1/01/2016	2.2
37	2/03/2018	83	2	1/01/2016	2.2
38	3/03/2018	83	2	1/01/2016	2.2
39	26/04/2018	83	2	1/01/2016	2.3
40	9/03/2018	84	2	1/01/2016	2.2
41	25/02/2018	85	2	1/01/2016	2.2
42	9/03/2018	85	2	1/01/2016	2.2
43	14/03/2018	85	2	1/01/2016	2.2
44	7/03/2018	86	2	1/01/2016	2.2
45	2/05/2018	87	2	1/01/2016	2.3
46	1/12/2017	87	2	1/01/2016	1.9

47	13/03/2018	88	2	1/01/2016	2.2
48	16/03/2018	88	2	1/01/2016	2.2
49	3/05/2018	88	3	1/01/2015	3.3
50	10/12/2017	89	3	1/01/2015	2.9
51	23/04/2018	90	3	1/01/2015	3.3
52	25/02/2018	91	3	1/01/2015	3.2
53	7/02/2018	91	3	1/01/2015	3.1
54	9/03/2018	91	3	1/01/2015	3.2
55	15/03/2018	92	3	1/01/2015	3.2
56	13/03/2018	93	3	1/01/2015	3.2
57	5/04/2018	94	3	1/01/2015	3.3
58	30/04/2018	94	3	1/01/2015	3.3
59	28/03/2018	95	3	1/01/2015	3.2
60	5/03/2018	96	3	1/01/2015	3.2
61	23/06/2018	98	3	1/01/2015	3.5
62	27/06/2018	98	3	1/01/2015	3.5
63	27/06/2018	98	3	1/01/2015	3.5
64	30/06/2018	98	3	1/01/2015	3.5
65	5/07/2018	98	3	1/01/2015	3.5
66	23/06/2018	99	3	1/01/2015	3.5
67	27/06/2018	99	3	1/01/2015	3.5
68	27/06/2018	99	3	1/01/2015	3.5
69	30/06/2018	99	3	1/01/2015	3.5
70	2/07/2018	99	3	1/01/2015	3.5
71	23/06/2018	100	3	1/01/2015	3.5
72	27/06/2018	100	3	1/01/2015	3.5
73	27/06/2018	100	3	1/01/2015	3.5
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78	27/06/2018	101	3	1/01/2015	3.5
79	30/06/2018	101	3	1/01/2015	3.5
80	2/07/2018	101	3	1/01/2015	3.5
81	5/07/2018	106	3	1/01/2015	3.5
82	16/07/2018	106	3	1/01/2015	3.5
83	18/07/2018	106	3	1/01/2015	3.5
84	20/07/2018	106	3	1/01/2015	3.6
85	24/07/2018	106	3	1/01/2015	3.6
86	5/07/2018	107	3	1/01/2015	3.5
87	16/07/2018	107	3	1/01/2015	3.5
88	18/07/2018	107	3	1/01/2015	3.5
89	20/07/2018	107	3	1/01/2015	3.6
90	24/07/2018	107	3	1/01/2015	3.6
91	5/07/2018	108	3	1/01/2015	3.5
92	16/07/2018	108	3	1/01/2015	3.5
93	18/07/2018	108	3	1/01/2015	3.5
94	20/07/2018	108	3	1/01/2015	3.6
95	24/07/2018	108	3	1/01/2015	3.6
96	2/07/2018	109	3	1/01/2015	3.5
97	16/07/2018	109	3	1/01/2015	3.5
98	18/07/2018	109	3	1/01/2015	3.5

99	20/07/2018	109	3	1/01/2015	3.6
100	24/07/2018	109	3	1/01/2015	3.6

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AT CSIRO, WE DO THE EXTRAORDINARY EVERY DAY

We innovate for tomorrow and help improve today – for our customers, all Australians and the world.

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