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Update on age and growth of bigeye tuna in the WCPO WCPFC Project 81

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1. Executive summary

This paper provides an update on a regional study of bigeye tuna age and growth in the western and central Pacific Ocean (WCPO) presented at the Western Central Pacific Fisheries Commission (WCPFC) Scientific Committee meeting in 2017. The objectives of this extension project are to (i) prepare and read an additional 125 otoliths from fish >130 cm fork length (FL) using the annual increment method identified in Farley et al. (2017); and (ii) revise and update the age and growth estimates provided in Farley et al. (2017) based on the additional new data.

Annual age estimates were obtained for an additional 237 bigeye tuna in the WCPO to strengthen the growth analysis reported by Farley et al. (2017). Of these, 188 were from fish >130 cm FL and 49 from fish 90-129 cm FL. Daily age was also estimated for an additional 11 very small bigeye (31-39 cm FL). The new annual and daily age estimates were combined with those of Project 35 and historic SPC daily age estimates to obtain new von Bertalanffy growth parameters for bigeye tuna. Fish caught east of the assessment area and daily age estimates >1 year were excluded from the analysis. The resulting L_∞ estimate was 156.9 cm FL, which is similar to that reported from Project 35 at SC13. The results of exploratory spatial analysis continue to indicate there are differences in the growth rates of bigeye tuna across the Pacific

The SPC Pre-assessment workshop in April 2018 recommended inter-laboratory comparison work be undertaken to standardise daily ageing methods between the WCPO and EPO. Since the age validation work completed in 2003 (Appendix A), an additional 30 $SrCl_2$ marked otoliths have been returned which may be useful for further age validation work.

2. Introduction

In 2014, the Western Central Pacific Fisheries Commission (WCPFC) endorsed the analysis phase of a regional project to estimate the age, growth and maturity of bigeye tuna (*Thunnus obesus*) in the western and central Pacific Ocean (WCPO), for application in regional stock assessment models (Project 35). The study was completed in 2017 and the results were reported at the 13th Regular Session of the WCPFC Scientific Committee (SC13) (Farley et al. 2017). Age-at-length estimates were obtained for 1039 fish, which ranged from 0.25 years for a 54 cm fork length (FL) fish to 13.7 years for a 133 cm FL fish. The majority of age estimates were for fish caught in the WCPO, supplemented by estimates for 68 fish caught just to the east of the assessment boundary in the eastern Pacific Ocean (EPO). The EPO ages were included in the analysis because many were from fish >130 cm FL, which were rare in the WCPO data set.

The study indicated that bigeye tuna in the WCPO are considerably smaller-at-age than assumed in previous stock assessments. Based on the age at length data provided by Farley et al. (2017), the estimated mean length at age 10 (Ln) was 152 cm FL compared to the Ln of 184 cm FL used in previous assessments (McKechnie et al. 2017b).

The inclusion of the new growth data in the 2017 WCPO bigeye tuna stock assessment (McKechnie et al. 2017a) contributed to a more optimistic view of the current status of the stock. It appeared that the stock is not in an overfished state and is not experiencing overfishing. Given the effect that the new growth estimates had on the assessment results, concerns were raised that large fish may be underrepresented in the data and, as a result, L_∞ may be higher than 158 cm FL estimated by Farley et al. (2017). SC13 requested that further work on bigeye tuna age and growth be undertaken and a new project (Project 81: Further work on bigeye tuna age and growth) was subsequently funded.

Here we present the results of Project 81. We initially provide background information on the methods used to estimate the age of bigeye tuna (annual and daily age) using otoliths, and the age validation work undertaken by CSIRO and SPC in the southwest Pacific. We then update the age and growth estimates based on the additional work undertaken in Project 81.

3. Background

3.1 Ageing methods for bigeye tuna in the WCPO

Otoliths are considered more accurate than other more metabolically active structures (e.g., spines, rays and vertebrae) for age estimation of fish, including tunas, because they are not susceptible to resorption. Otoliths can record:

1) **Annual increments**. These are records of seasonal variation in growth and are characterised by alternating opaque and translucent zones, which correspond to fast and slow growth. One annual increment comprises one opaque and one translucent zone.

2) **Daily increments**. These are records of daily fluctuations in growth and are also characterised by alternating opaque and translucent zones indicative of fast and slow growth. One micro-increment comprises one opaque and one translucent zone.

Age is estimated by interpreting (counting) the annual and/or daily increments on either whole or thin-sectioned otoliths. Reading thin-sections is generally preferred because differentiating the closely spaced increments near the otolith edge for long-lived tuna species can be difficult using whole otoliths. The frequency at which the zones form, however, must be validated before counts can be considered (true) age estimates. Estimating daily age is best suited to larvae, juveniles, fast growing species and many tropical species (Panella 1971; Brothers, et al. 1976). However, Morales-Nin (1988) showed that counts of daily growth increments in a tropical snapper were likely to underestimate age. Williams et al. (2013) has also indicated that it is likely that estimating annual age based on daily growth increments should be limited to the first 2 years in four species of tunas studied.

3.1.1 Annual ageing

To estimate the annual age of bigeye tuna, otoliths are sectioned on the transverse plane (Fig. 1) following the methods outlined in Anon. (2002) and Farley et al. (2017). By sectioning on the transverse plane, at least four serial sections can be prepared from each otolith. This 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, which reduces preparation time (Anon 2002). If otoliths were sectioned on the longitudinal plane (see daily ageing below) only one section can be prepared per otolith.

After sectioning, the opaque growth zones are counted from the primordium to the edge of the otolith along the ventral (long) arm (Fig. 2). All otoliths are read twice by the same reader, using a dissecting microscope and transmitted light, following the validated method developed by Clear et al. (2000) and Farley et al. (2006) (see section 3.2 below and Appendix A for details on the age validation work). Opaque zones at the terminal edge of the otolith are counted only if some translucent material is evident after the opaque zone. A final count is then assigned to the otolith and a readability score is given to the otolith (0-5). 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) and also whether the otolith edge is composed of opaque or translucent material. This helps determine the length of time between when the fish deposited the last opaque zone counted and when the fish was caught. For example, a narrow translucent edge type suggests the fish was caught soon after the last opaque zone was deposited, while an opaque edge type suggests the fish was caught a long time after the last opaque zone (counted) was deposited. The 'edge type' is used to estimate a decimal age (see section 3.1.3). The distance is also measured between the first inflection in the otolith to the distal edge of each opaque zone, and to the edge of the otolith.

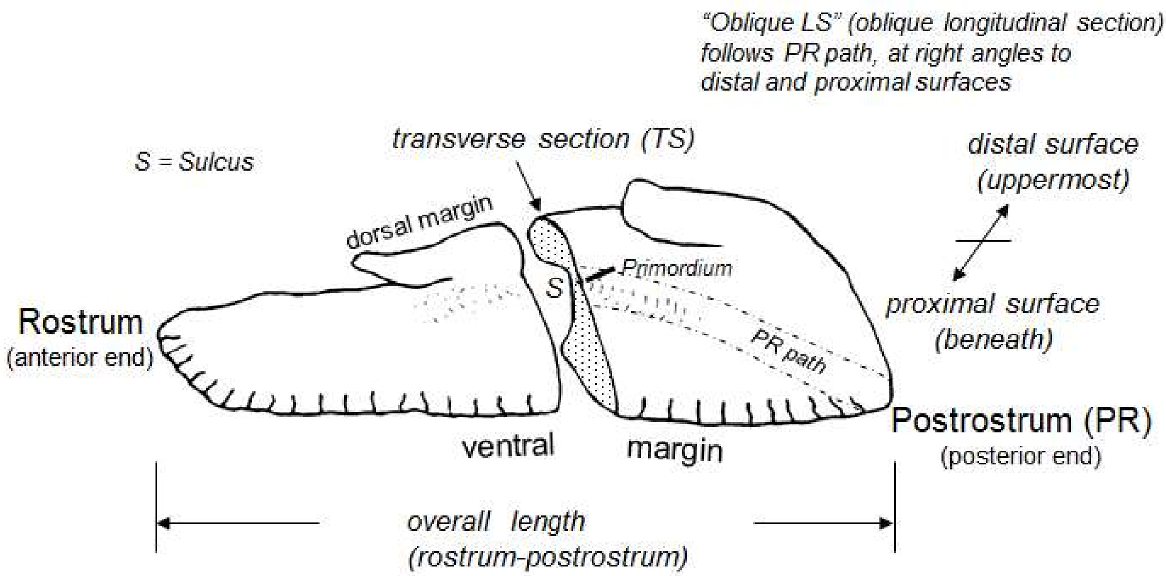

Figure 1. Generalised representation of a left hand sagittal otolith of a bigeye tuna with the transverse (TS) and longitudinal sectioning planes (LS).

Figure 2. Transverse sections of the otolith from a 169 cm FL bigeye tuna viewed under transmitted light. The yellow +'s mark the 12 opaque zones counted in this otolith.

3.1.2 Daily ageing

To estimate the daily age of bigeye tuna, otoliths can be sectioned on either of two planes (Fig. 1) following Williams et al. (2013):

1) Transverse section – from the dorsal edge to the ventral edge, through the primordium (Fig. 3a).
2) Longitudinal (frontal) section – from the primordium to the postrostral axis, through the primordium (Fig. 3b).

Williams et al. (2013) found that daily age estimates derived from counts in transverse and longitudinal sections were similar for young fish in the WCPO (Fig. 4). The longitudinal sections produce higher age estimates in fish older than two years possibly because they provide a longer reading plane (e.g., 4 mm versus 1.8 mm for the otoliths in Fig. 1). For this reason the longitudinal section is preferred when estimating daily age of older fish. However, as noted above, Williams et al. (2013) also suggested that daily age estimates were only accurate up to age two years for bigeye tuna in the WCPO. The counts of microincrements on the transverse sections can, however, be used to confirm the location of the first annual growth zone (before the 365th increment), as both ageing methods use the same sectioning plane.

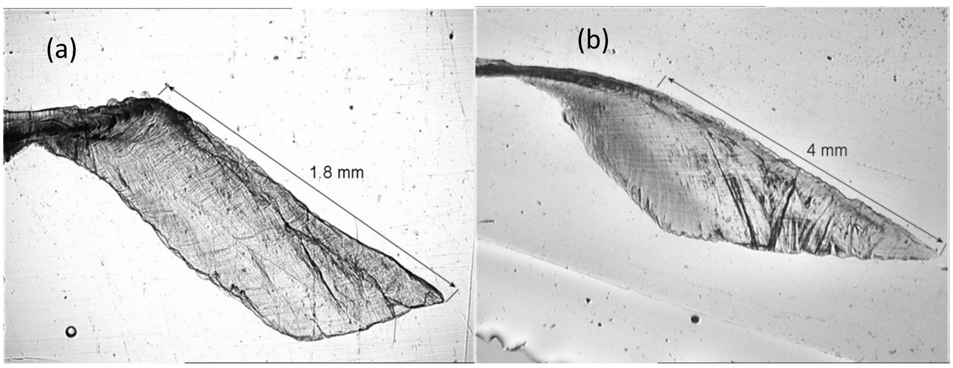

Figure 3. Transverse (a) and longitudinal (b) sections of the sagittae from a 97 cm FL bigeye tuna viewed under transmitted light showing the different length of the ventral arm in each section along which daily increments were counted. From Williams et al. (2013).

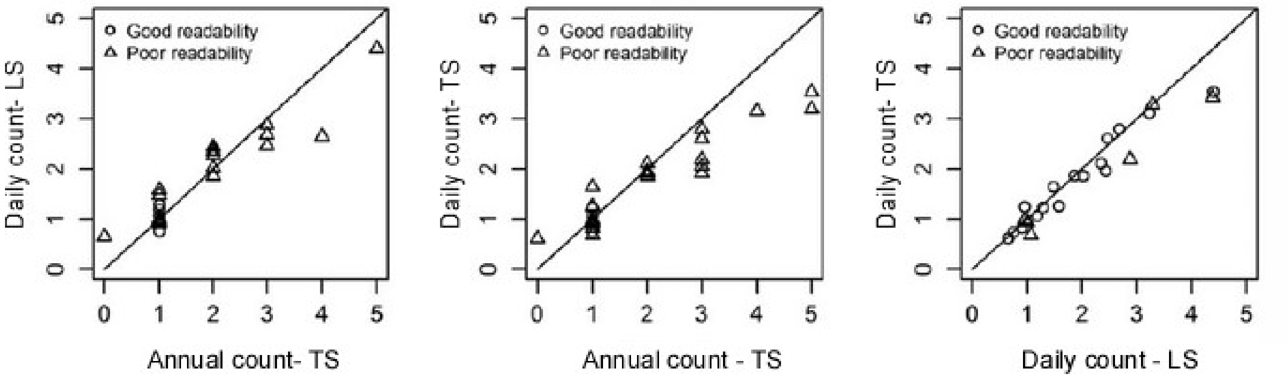

Figure 4. Age bias plot for bigeye tuna caught in the WCPO comparing annual and daily age estimates (in years) from longitudinal (LS) and transverse sectioned (TS) otoliths. Adapted from Williams et al. (2012).

3.1.3 Biological age – from annual age data

Because birth date, otolith zone formation date and catch date do not always coincide, the number of opaque zones counted in otoliths is not necessarily the fish's biological age. To estimate a biological age, it is necessary to convert the zone counts to a decimal (fractional) age using an algorithm that takes account of birth date, timing of zone formation during the year, edge type and catch date.

Age algorithm

For bigeye tuna in the WCPO, Farley et al. (2017) calculated decimal age 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 July; see below).

Table 1. Opaque zone count adjustment based on capture month (columns) and edge type (rows).

EDGE TYPE	OCTOBER TO MARCH	APRIL TO JUNE	JULY TO SEPTEMBER
Wide or Intermediate	0	0	+1
Narrow	0	-1	0

Farley et al. (2017) used marginal increment analysis to show that opaque zones in the otoliths examined formed between April and September (Fig. 5). For fish caught during these months, otolith 'edge type' was used 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. In short, the otolith edge type is an assessment of how much time had passed since the last opaque zone was deposited (narrow = short time; wide = long time). Table 2 provides examples of how this data is used to calculate decimal age for fish hatched on the same day but caught two months apart and with different numbers of opaque zones present/counted. Despite the differing numbers of zones and edge type, the algorithm provides the correct biological age from the individual's nominal date of hatching.

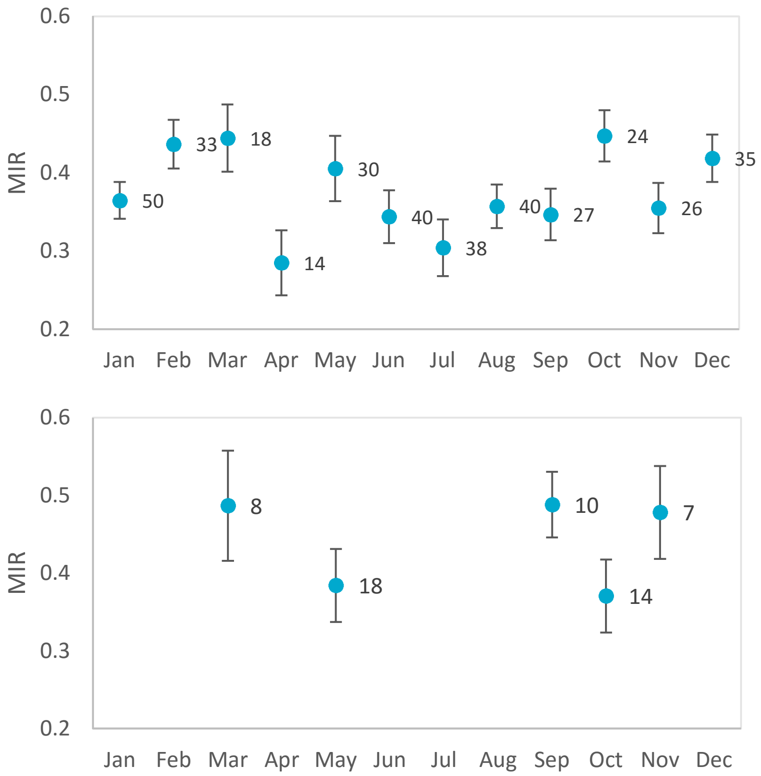

Figure 5. Mean (+/- SE) monthly marginal increment ratio (MIR) for bigeye tuna otoliths sampled north (top) and south (bottom) of 10°S. Sample size is shown next to the mean; only months where n ≥ 5 are shown. MIR data were restricted to age 2 and 3.

Table 2. Examples of decimal age calculations using the above algorithm and count adjustment (from Table 1).

Fish	1	2	3	4
Nominal birth date	1 July 2010	1 July 2010	1 July 2010	1 July 2010
Last birthday	1 July 2011	1 July 2011	1 July 2012	1 July 2012
Date caught	1 June 2012	1 June 2012	1 Aug 2012	1 Aug 2012
Day of capture after last birthday *(r)*	336	336	31	31
Zone count (*n*)	1	2	1	2
Edge type	Wide	Narrow	Wide	Narrow
Count adjustment (*b*)	0	-1	+1	0
Decimal age (*a*)	1.92	1.92	2.08	2.08

Nominal birth date

A nominal birth (hatch) date is needed when using a formula to adjust the otolith zone count to an age estimate, and the middle of the spawning season is often selected for a species and region. Clearly, not all individuals in a population will hatch on the same day, so fish that hatch earlier than the nominal birth date will be older than calculated (and vice versa). However, the growth curve estimated from the combined length-at-age data should not be biased as it is assumed that similar numbers of the fish will have hatched prior to and after the nominal birth date.

Deciding on a nominal birth date for bigeye tuna is difficult as they are capable of spawning year-round (Schaefer 2001; Farley et al. 2017) (Fig. 6). If daily age estimates are accurate, birthdate can be back calculated using capture date and age in days. In this project, only 58 daily ages were obtained for fish aged <1 year (from this project and daily ageing by SPC in the 1990s). The back-calculated birth dates were also spread across the year with possible peaks in December and April-June. Additional daily age data may assist to refine a nominal birthdate.

Morales-Nin (1992) suggested that if a birthdate is unknown, a standard birthdate of January 1 in the northern hemisphere and July 1 in the southern hemisphere can be applied. Given that bigeye tuna sampled in this project were caught in both hemispheres, and individuals can migrate between the two, picking one date over the other is difficult.

Farley et al. (2017) examined July 1 and January 1 birthdates in the age algorithm. Note that the count adjustments in Table 1 are only applicable to a July 1 birth date; different adjustments are used for a January 1 birth date. Figure 7 shows the difference in decimal age calculated in Project 35 between using a July 1 (Fig. 7a) and January 1 (Fig. 7b) birth date. The January birth date gives negative ages estimates and estimated length at age 1 (from a fitted VB growth curve) was 76 cm, higher than estimated from daily ageing. It was concluded that a nominal birth date of July 1 was the best option for bigeye tuna in the WCPO. Interestingly, the estimated L_∞ using both birthdates was ~158 cm FL using the annual age data available at the time.

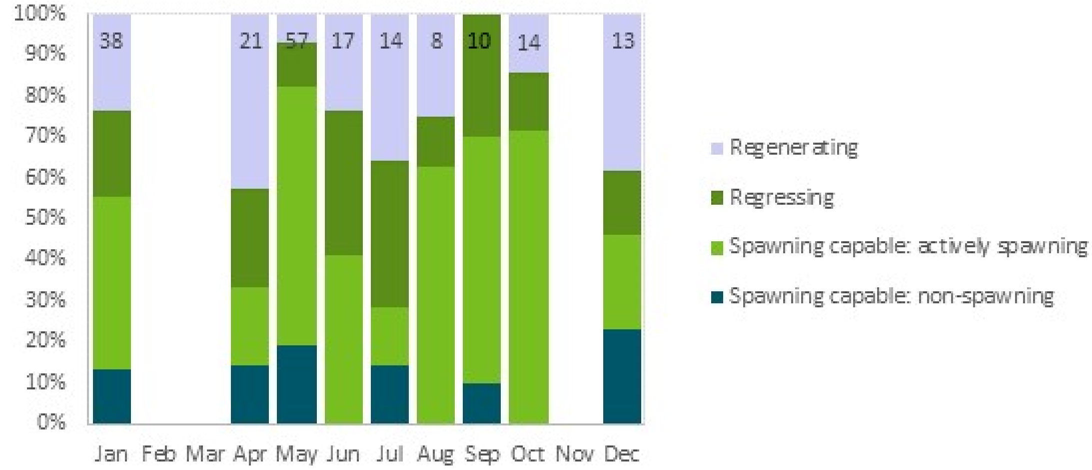

Figure 6. Percent of mature females by reproductive phase and month in the area north of 15°S in the WCPO (if n>5). Sample sizes shown at the top of the bars. Taken from Farley et al. (2017; Project 35)

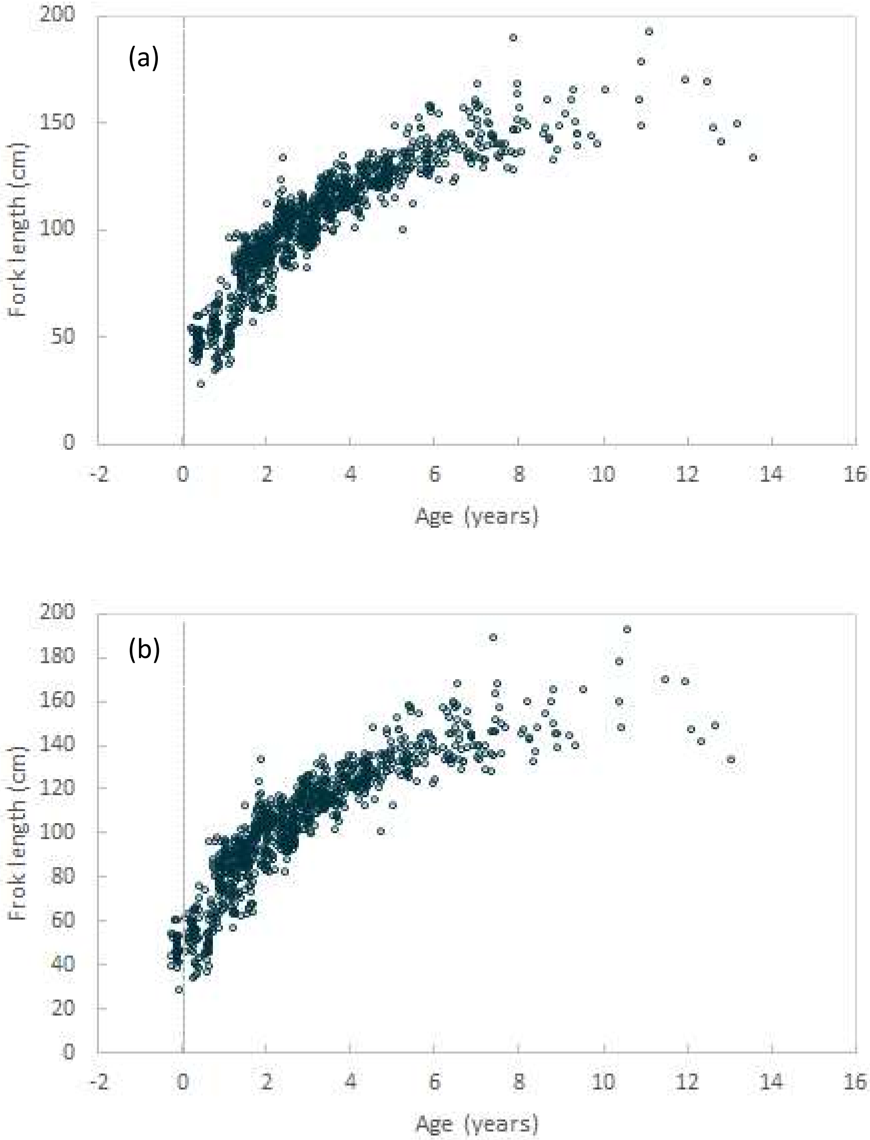

Figure 7. Estimated length at age for bigeye tuna analysed in Project 35 (Farley et al. 2017) using (a) July 1 and (b) January 1 as the assumed birth date for all fish.

3.2 Age validation in the WCPO

In the 1990s and early 2000s, three tagging programs were undertaken in the Coral Sea examining the exploitation and movement and bigeye and yellowfin tuna (Hampton and Gunn 1998, Farley et al. 2003). Fish were tagged and released, and a proportion were injected with strontium chloride ($SrCl_2$) for age validation. Otoliths from 34 recaptured fish were obtained and the strontium mark (Sr-mark) on the otolith provided a 'time-stamp' used to validate our daily and annual age estimate methods (Clear et al. 2000). Full details of the age validation work are provided in Appendix A (Chapter 7 of Farley et al. 2003) and the main results and conclusions are below.

3.2.1 Annual age validation

Otoliths from 11 bigeye tuna marked with $SrCl_2$ were analysed. The fish ranged from 72 to 125 cm FL at release and 85 to 159 cm FL at recapture. The time at liberty ranged from 207 days to over 6 years (Table 3). Transverse sections that included the primordium were prepared for each otolith and viewed under a light microscope. The number and position of each increment was recorded.

Each otolith was then prepared for examination using a scanning electronic microscope (SEM) coupled with a Robinson backscatter detector to determine the position of the Sr-mark. The Sr-mark was clearly visible in each otolith and its position was measured. Age estimates for these fish ranged from 2 to 9 years.

The number of increments expected after the Sr-mark was calculated from the time at liberty after tagging and injection with $SrCl_2$, and the number expected was compared with the number observed. There was some uncertainty in the number of increments "expected" after the Sr-mark because although our age estimates were in whole years, the periods at liberty were obviously not. When the days-at-liberty was closer to half years we were not able to predict if the increment for that year had been completely formed. Hence our expected number of increments could be one of two consecutive years (see Table 4). The age estimates were compared with ages predicted from a composite model (Hampton et al. 1998) derived from microincrement counts and tag-recapture data.

Of the 11 otolith sections analysed in the SEM, we were unable to obtain recapture information for one fish (#64; Table 3), hence we could not include it in the validation study. For the remaining 10 otolith sections there was agreement between the number of increments observed after the Sr-mark and the number expected, calculated from the time at liberty (Table 4). Thus the annual periodicity in formation of increments 2 to 9 has been validated for the otoliths analysed.

The first increment was not validated using this method because the appropriate otoliths had not been recovered. It would have required otoliths from a fish that was injected with $SrCl_2$ when it was young of the year (i.e. before the formation of the first complete increment) and subsequently recovered after the formation of the 1st increment. Although these young-of-the-year fish (0+) have been tagged and injected with $SrCl_2$, to date none have been recovered.

Table 3. bigeye tuna otoliths analysed for strontium marks in the scanning electronic microscope (SEM). (From Farley et al. 2003; see Appendix A),

Fish number	Release Date	Release fork length (cm)	Recapture Date	Recapture fork length (cm)	Growth (cm)	Days at Liberty
591	9/10/1995	80	2/11/1998	139	59	1120
37	13/11/1992	72	31/07/1993	85	13	260
57	6/10/1995	75	14/08/1997	128	53	678
59	12/11/1992	96	15/07/1998	159	63	2071
62	9/10/1995	109	3/05/1996	123	14	207
63	6/10/1995	83	10/06/1996	94	11	248
64	6/10/1995	79	unknown	unknown		
65	9/10/1995	78	26/01/1998	128	50	840
66	9/10/1995	84	18/12/1997	129	45	801
67	9/10/1995	78	4/11/1997			757
2820	9/10/1995	125	25/5/2002	157		32

Table 4. Analysis of bigeye tuna Sr-marked otoliths. The number of increments expected after the Sr-mark (determined from the time at liberty after tagging) was equal to the number observed, for all specimens analysed. (From Farley et al. 2003; see Appendix A). recap. = recapture, mths = months.

Fish number		37	57	59	62	63	64	65	66	67	591	2820
FL at tagging (cm)		72	75	96	109	83	79	78	84	78	80	125
FL at recapture (cm)		85	128	159	123	94	-	128	129	-	139	157
Time at liberty after tagging (days)		260 (8.5 mths)	678 (1 yr 10 mths)	2071 (5 yrs 8 mths)	207 (7 mths)	248 (8 mths)	recap. details not known	840 (2 yrs 3 mths)	801 (2 yrs 2 mths)	757 (2 yrs 1 mth)	1120 (3 yrs 1 mth)	2420 (6 yrs 7 mths)
Number of increments after Sr mark	**expected**	**0 or 1**	**1 or 2**	**5 or 6**	**0 or 1**	**0 or 1**		**2**	**2**	**2**	**3**	**6 or 7**
	observed	**1**	**1**	**5**	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**6**
Age estimate (this study) *		2	3	8	3	2	2	3	3	3	4	9
Age at tagging **		1.2	1.3	2.1	2.7	1.6	1.5	1.4	1.6	1.4	1.5	3.18
Age at recapture **		1.7	3.8	8.6	3.5	2.0	-	3.8	3.9	-	4.8	7.87
Month of recapture		July	Aug	July	May	June		Jan	Dec	Nov	Feb	May
distance from Sr mark to margin (cm)	Sr (O) -O	0.36	0.74	1.06	0.25	0.27	0.30	0.72	0.77	0.81	0.67	0.49
	Sr (I) -I	0.26	0.56	0.80	0.15	0.16	0.25	0.54	0.63	0.77	0.50	0.43

* Estimated by counting annual increments on sectioned sagittal otoliths.

** Estimated using results from a study of otolith microincrements and tagging data (Hampton et al. 1998).

3.2.2 Daily age validation

After the SEM analysis (above), the same transverse sections were examined by a reader experienced in counting microincrements of bigeye tuna and other closely related species. The otoliths were from fish larger than normally considered for microincrement counts in the WCPO but the Sr-mark on the otolith presented a potential opportunity to validate the counts of microincrements in larger (older) fish.

The otoliths were prepared for microincrement counts by removing the carbon coat that had been necessary to minimize charging in the SEM. The reader was told the position of the Sr-mark along the section but no other information about the fish or its time at liberty after tagging was given.

Two counts of the number of microincrements present after the position of the Sr-mark were made for each otolith by one reader. After the counts were made, the expected number of microincrements calculated from the period at liberty after tagging was compared with the microincrements counted after that position. A mean difference was calculated using

(Days at liberty – Mean of counts 1 and 2) / Days at liberty * 100

In five cases, both otoliths from the pair were recovered from tagged fish and these allowed a further opportunity for validating microincrement counts. For each "sister" otolith, a transverse section was cut and the microincrements were counted under a light microscope. It was assumed that the Sr-mark would be in the same position along the sister otolith.

All microincrement counts underestimated the days at liberty (Table 5). The mean difference of the replicate counts and the times at liberty ranged from 7.7% to 30.0%. Of the specimens analysed in the SEM, five were considered in good enough condition to make microincrement counts (two were damaged when the carbon coat was polished off the surface). Of these, three had sister otoliths from which replicate counts were made; the replicate counts were less than the counts made on the SEM-analysed fish.

Table 5. Results of microincrement counts on strontium-marked bigeye tuna otoliths (from Farley et al. 2003). FL = fork length

Fish no.	Release FL (cm)	Recapture FL (cm)	Days at Liberty	Otolith analysed in the SEM				Sister Otolith			
				Count 1	Count 2	Reading Score	% mean difference from days at liberty	Count 1	Count 2	Reading Score	% mean difference from days at liberty
37	72	85	260	218	216	A	-16.5				
57	75	128	678	587	570	B	-14.7	530	560	C	-19.6
62	109	123	207	155	137	C	-29.4	144	146	A	-30.0
63	83	94	248	230	228	B	-7.7	184	200	C	-22.6
65	78	128	840	597	666	B-	-24.7				
66	84	129	801			broken		567	582	C	-28.3
67	78	unknown	757					570	532	B	-27.2

A= count with high confidence, all areas have visible microincrements
B= count with medium confidence, most areas have visible microincrements but a few areas are unreadable
C= count with low confidence, many areas along the section are unreadable

3.2.3 Conclusions

In summary, the annual periodicity of increments in sectioned otoliths has been directly validated for bigeye tuna in the southwest Pacific Ocean for the age range 2 to 9 years. Additional indirect validation was undertaken by Farley et al. (2006; 2017) to confirm the location of the 1st opaque zones using counts of microincrements in otoliths sectioned on the transverse plane.

The large discrepancy between the days at liberty and the counts of microincrements deposited after the strontium mark indicate that age estimates in days using otoliths from bigeye tuna between 72 and 129 cm FL are not reliable. We considered whether tagging and injection could possibly have caused a growth check, meaning that daily increments weren't deposited for a period of time, producing a lower count. However the age discrepancy also occurred in fish that hadn't been tagged, where we had lower age estimates from daily counts than annual counts.

4. Objectives

The objectives of this extension project are to (i) prepare and read an additional 125 otoliths from bigeye tuna >130 cm FL using the annual increment method identified in Farley et al. (2017); and (ii) revise and update the Farley et al. (2017) age and growth estimates based on the additional new data.

5. Methods

5.1.1 Age estimation

During SC13, he Pacific Community (SPC) and the National Research Institute of Far Seas Fisheries (NRIFSF, Japan) identified additional otoliths from bigeye tuna >130 cm FL that had not yet been analysed and were available for the project. The majority were from fish 130 to 150 cm FL as fish >150 cm FL are uncommon in the WCPO catch. We selected all otoliths from fish >130 cm FL (n=136) and a subset of additional otoliths from fish 90 to 129 cm FL in the WCPFC specimen tissue bank (n=49). The latter otoliths were selected to obtain similar numbers across length classes 90-140 cm FL. All otoliths were weighed (if whole) and were prepared and read by Fish Ageing Services (FAS, Australia) using the methods described in Farley et al. (2017) and summarised in section 3.1 above. FAS provided a count of opaque zones, a readability score and an otolith edge type classification. A decimal age was calculated for each using the method described in section 3.1.3 above, and the data were combined with the age data from Project 35.

In addition to the above, age estimates were also obtained from fish >130 cm FL from a 2011 pilot project (Nicol et al. 2011). These otoliths were read by FAS but the otolith edge type was not recorded. Therefore, we only selected otoliths from fish caught outside the months that opaque zones form (October to March; n=52) so that decimal age could be calculated.

An additional 12 otoliths were selected from very small fish for daily ageing (n=12; 31-39 cm FL) by FAS.

After discussion at the SPC pre-assessment workshop in April 2018, it was agreed that only daily age estimates for bigeye tuna aged <1 year would be included in the growth analysis, and that annual ages from the same fish would be excluded if present (Pilling and Brouwer 2018). However, only ages <300 days were included from FAS as that was the maximum age they were confidence would provide an accurate estimate of actual age (Farley et al. 2017). Additional daily age data from earlier work in the 1990s by SPC for fish <1 year were also included (n=28).

5.1.2 Growth modelling

A von Bertalanffy (VB) growth model was fit to the age and length data following the methods described in Farley et al. (2017) (Project 35). The VB model has the form:

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

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.

Because no significant improvement was found by allowing for sex-specific growth parameters in Project 35, a single VB growth model was fit to all of the data. To determine if the growth curve was affected by otolith readability, separate VB models were also fit to the otolith data with low readability scores (<3) and high readability scores (≥3). Bigeye tuna caught in the EPO in Project 35 were not included in the growth analysis.

5.1.3 GAMs to investigate spatial effects

To investigate whether the spatial variation in growth presented at SC13 had changed after the inclusion of additional length at age data, we updated the analysis to include these new data. As before, we modelled the length data using a generalized additive model (GAM) with age and latitude x longitude as 1- and 2-dimensional smooth terms respectively, and assuming a Gaussian distribution. We fit the model in R (R Core Team 2013) using the gam function in the mgcv library (Wood 2011), using the default smoothing method (penalized regression splines) and allowing the degree of smoothness for each term to be estimated automatically. Age data obtained in Project 35 from the area just to the east of the assessment area were include in this analysis.

We also modelled the length data using a GAM with otolith weight (instead of age) and latitude x longitude as 1- and 2-dimensional smooth terms respectively (again assuming a Gaussian distribution). For this analysis, additional otolith weight data provided by NRIFSF for fish caught in the EPO were included.

6. Results and Discussion

6.1 Age estimates

The total of 1244 estimates were obtained from the WCPO. Ages ranged from 0.3 to 15.0 years. Figure 8 shows the size and age frequency of fish analysed in the current project compared to the previous project (Project 35). In accordance with the objectives of the current study, the number of fish >130 cm FL was increased substantially, particularly fish from 130 to 150 cm FL. As expected, this resulted in the number of fish aged >4 years also increasing. Fish >150 cm FL remained low as fish of this size are rare in the catch. Figure 9 shows the otolith sampling locations for all bigeye tuna with age estimated. Figure 10 shows the relationship between otolith weight and age. The goodness of fit (R^2) of the power function was 0.905.

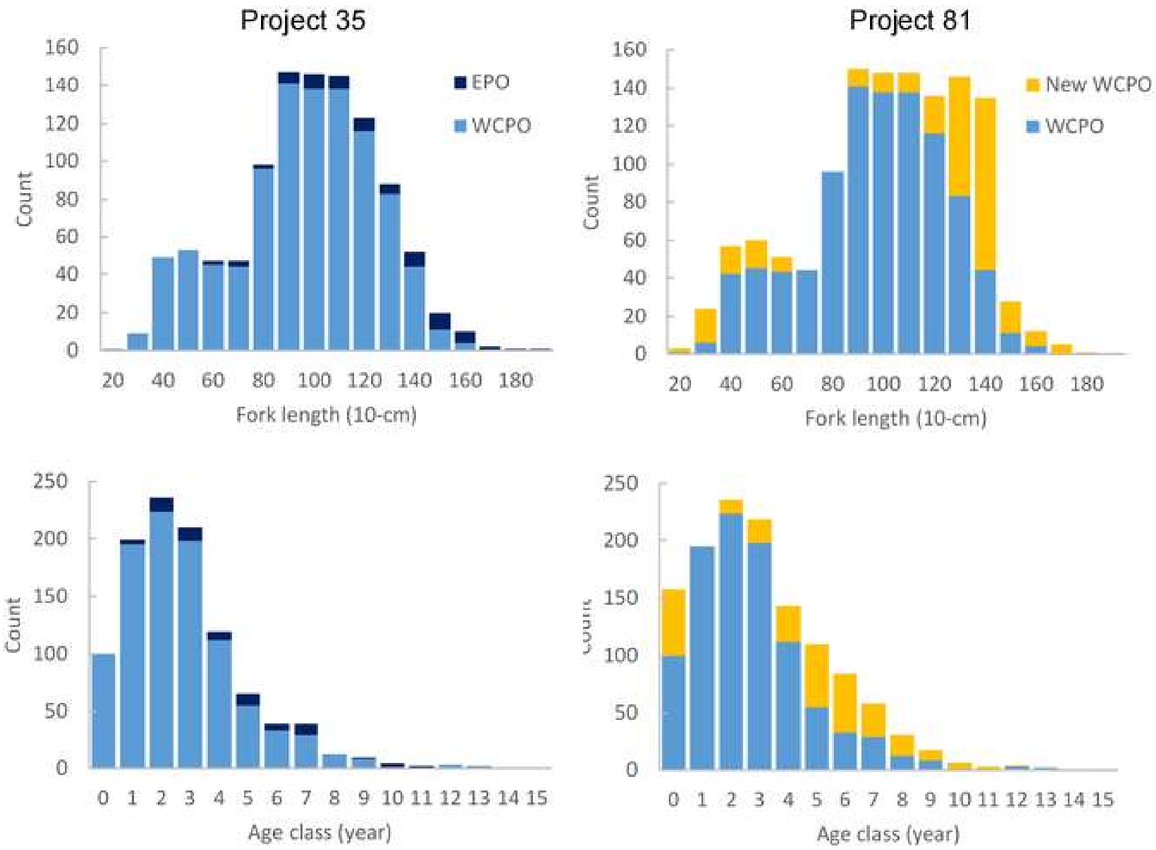

Figure 8. Length (top) and age (bottom) frequencies of bigeye tuna included in the growth analysis for Project 35 (original study) and the Project 81 (current study). The additional age estimates are shown in orange. The lower boundary length value of the bin is shown.

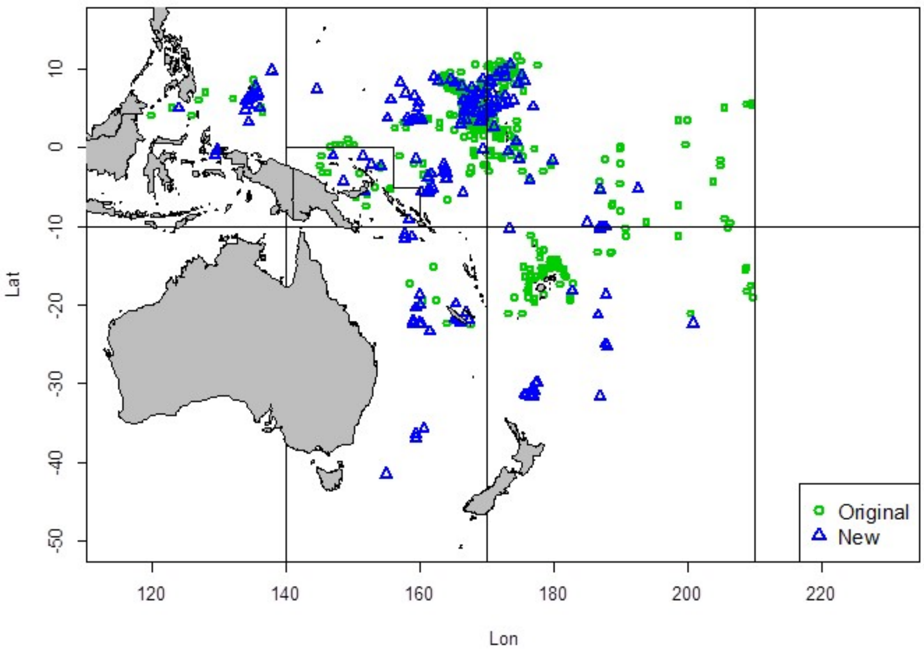

Figure 9. Map of the bigeye tuna otolith sampling locations where age estimates were obtained in the original study (Project 35; green circles) and the new ages obtained in the current study (blue triangles).

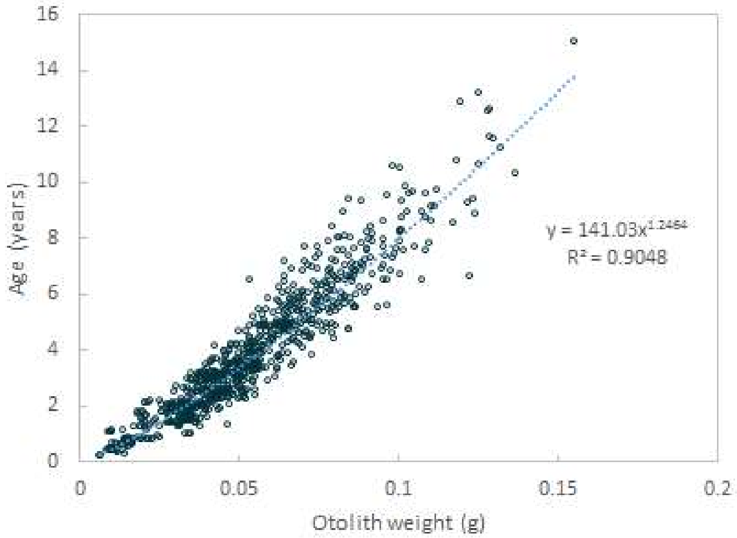

Figure 10. Otolith weight to age relationship and fitted power curve for bigeye tuna in the WCPO.

6.2 Growth modelling

The results of fitting a VB growth curve to the updated dataset are shown in Table 6 (VB1) and Figure 11. The VB model fit to the low and high otolith readability data (VB2 and VB3 in Table 6) are similar, suggesting no systematic bias in the readability of otoliths (Fig. 12). The growth parameters are also very similar to the parameters estimated in Project 35 and reported at SC13. The pre-assessment workshop agreed to include only the high readability annual age data in the new growth models.

Figure 13 shows a comparison of three growth curves for the WCPO:

1) the curve estimated in the 2014 BET stock assessment (MFCL 2014),
2) the "daily-integrated-VB" curve from McKechnie et al. (2017b), which was estimated using tagging increment and daily age data only, and
3) the updated curve from this study using high otolith readability age data (VB3).

The growth curve from the 2014 assessment had an estimated L_∞ of 200 cm FL but the mean length of the oldest fish (age 10 yrs) was fixed at 184 cm FL. Although this curve is consistent with growth information for bigeye tuna in the EPO, it is clearly not consistent with current information on the size of bigeye tuna in the WCPO as fish caught in this region are rarely >160 cm FL. Our exploratory investigation of spatial variation in growth also indicates that bigeye tuna in the EPO are larger at age than those in the WCPO (see section 6.3 below), supporting the use of a different growth curve.

The daily-integrated-VB curve is similar to the new growth curve using high otolith readability age data (VB3) (Fig. 13). However, slight differences are evident between ages ~2 and 7 years, where length at age from the daily-integrated-VB curve is higher than from the current study. This may be partly due to the daily age data used in the analysis, which included fish aged >1 year and are likely to be underestimated in the WCPO (see section 3.2 and Appendix A). The reliability of the tagging data included in the integrated model was discussed at the SPC pre-assessment workshop in Noumea in April 2018 (Pilling and Brouwer, 2018). Given the variability in estimates of individual growth curves (see Fig 6 in McKechnie et al. 2017), the workshop recognized that the data should be investigated further, possibly through a filtering process.

Table 6. Parameter estimates from fitting a von Bertalanffy (VB) growth model to the bigeye tuna age. Standard errors for the parameter estimates are given in parentheses. The sample size (n) are also presented. Daily age estimates were included in VB2 and VB3. Low otolith readability = <3; high otolith readability = ≥ 3

MODEL	Data	n	L_∞	k	t_0	σ
VB1	Project 81	1244	156.9 (1.7)	0.307 (0.010)	-0.69 (0.04)	9.3 (0.22)
VB2	Project 81 low readability	318	152.9 (1.6)	0.361 (0.015)	-0.47 (0.05)	8.0 (0.32)
VB3	Project 81 high readability	984	156.9 (1.7)	0.301 (0.010)	-0.71 (0.04)	9.4 (0.21)
VB4	Project 35	1039	158.1 (1.8)	0.292 (0.011)	-0.75 (0.05)	9.7 (0.21)

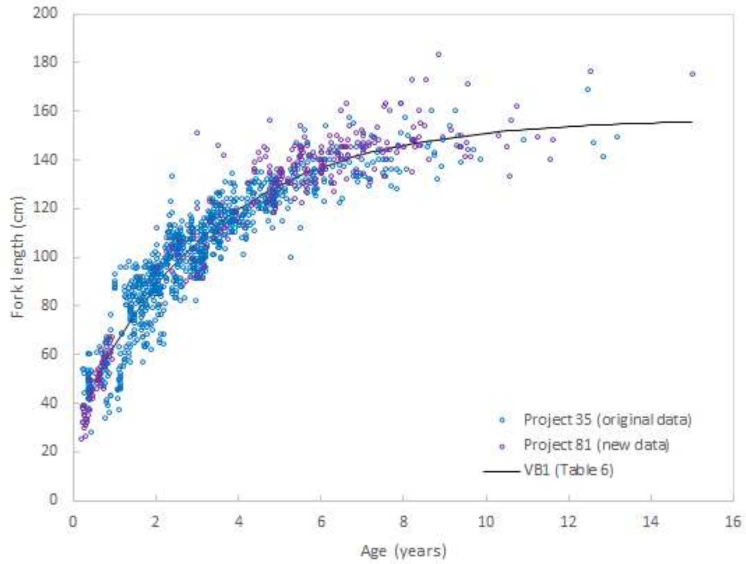

Figure 11.Bigeye tuna VB growth model fit to the combined length at age data from Project 35 (original data) and the new age estimates obtained in the current project.

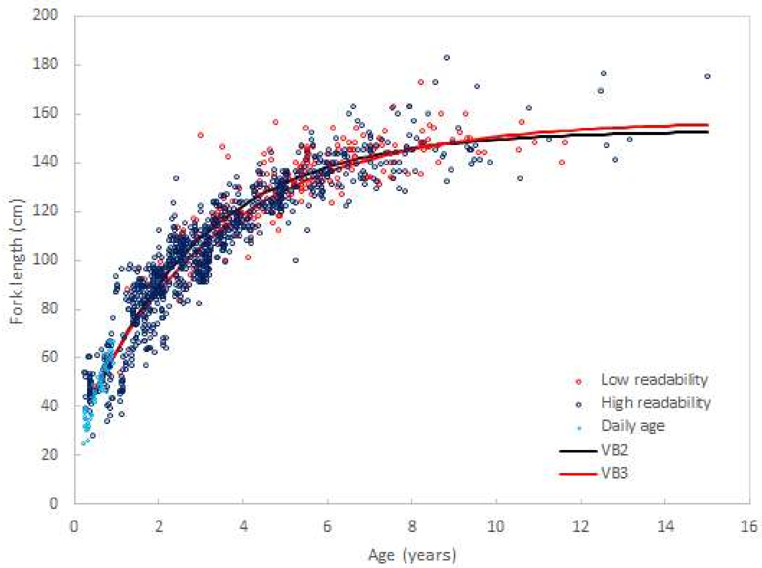

Figure 12. Bigeye tuna VB growth models fit to the updated length at age data by otolith readability score.

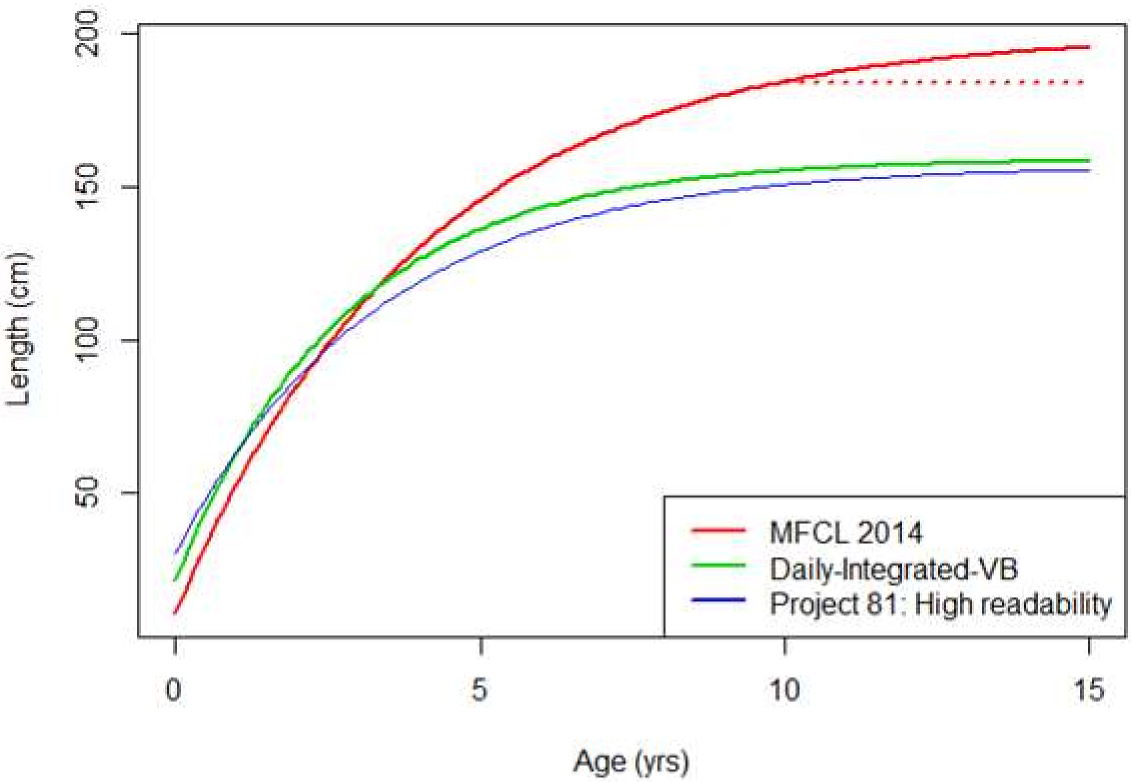

Figure 13. Bigeye tuna VB growth model fit to the high confidence age data compared to the curve estimated in the 2014 BET stock assessment (MFCL 2014) and the daily-integrated-VB curve from McKechnie et al. (2017b).

6.3 GAMs to investigate spatial effects

Figure 14 shows plots of the estimated spatial predictions of lengths from the GAMs. Fig. 14A shows a map of the predicted fish lengths from the GAM with age when age is fixed at the mean value in the dataset (3.3 years), and Fig. 14B shows the predicted fish lengths from the GAM with otolith weight when otolith weight is fixed at the mean value (0.06 g). Note that the colours in the two panels are not directly comparable as they are both scaled from white (max) to red (min).

The updated results continue to suggest there are differences in the growth rates of bigeye tuna across the Pacific, with greater length at age in the far east and far west of the area examined compared to the central longitudes. A similar pattern is present in the otolith growth data (i.e., otolith weight to fish length relationship) across the Pacific. The additional otolith weight data for fish caught in the eastern part of the EPO indicates that 'growth' in that region is particularly fast. As otolith weight data is relatively quick and inexpensive to obtain after the otoliths have been collected, it may be a useful additional tool to evaluate population structure of bigeye tuna in the Pacific. Analysis of additional age and otolith weight data from all areas and from the full size range of fish over a larger number of years is required to fully explore spatial variation in growth of bigeye tuna across the Pacific.

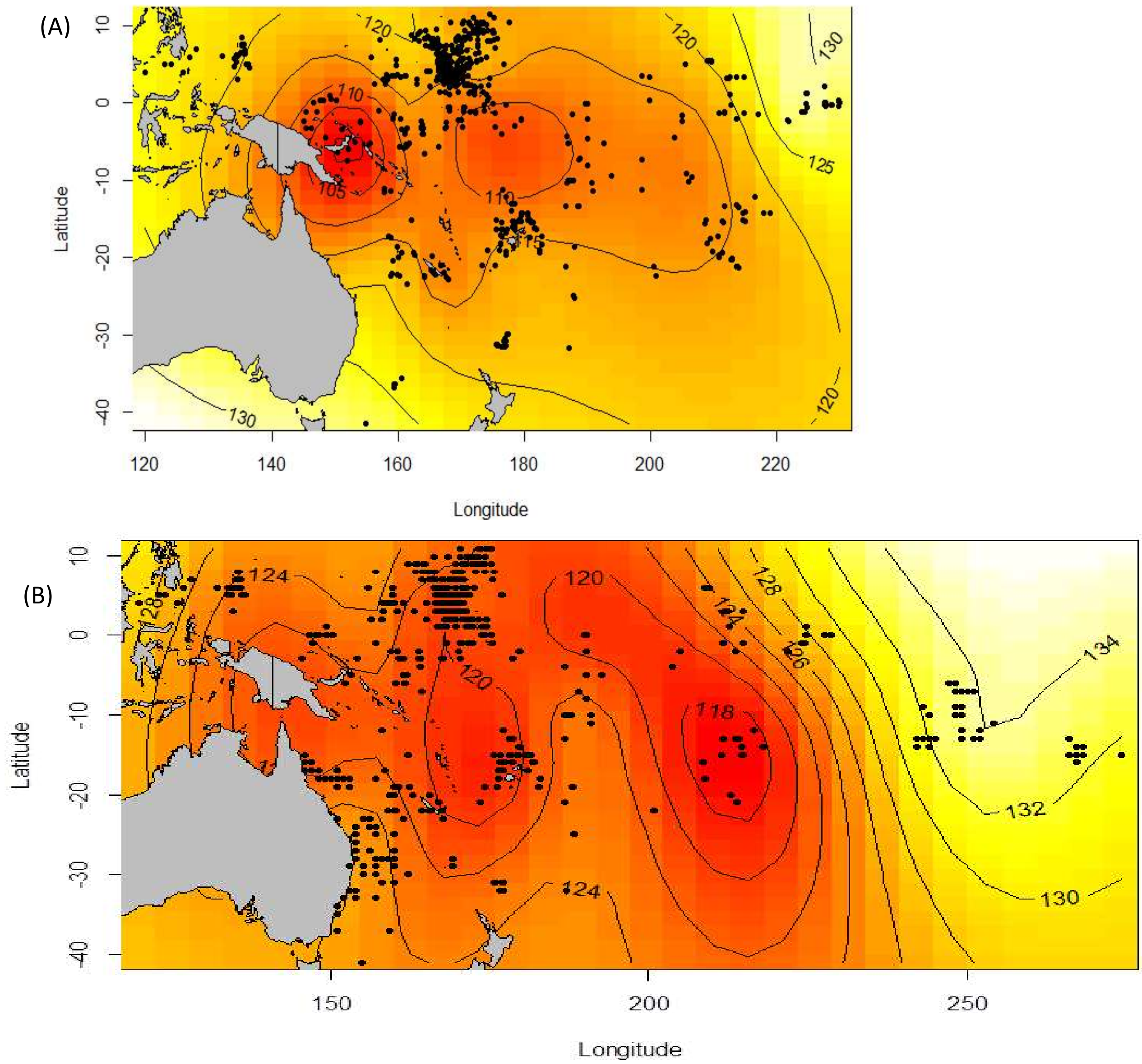

Figure 14. Results from fitting a GAM to length with age (A) and otolith weight (B) and longitude x latitude as a 2-dimensional smooth term. The predicted lengths at each spatial coordinate were calculated at the mean age (3.3 years) and mean otolith weight (0.06 g) in the datasets.

7. Summary

Annual age was estimated for an additional 237 bigeye tuna in the WCPO to strengthen the growth analysis reported by Farley et al. (2017). All otoliths were weighed (if whole) and were prepared and read by Fish Ageing Services (FAS) using the methods described in Farley et al. (2017). These age estimates were supplemented by 49 annual age estimates from fish >130 cm FL from a 2011 pilot study (Nicol et al. 2011) and 28 daily age data from earlier work by SPC for fish <1 year. After excluding data from fish caught in the EPO in Project 35, a total of 1244 age estimates were available for analysis.

The annual periodicity of increments in otoliths has been validated for bigeye tuna in the southwest Pacific Ocean for the age range 2 to 9 years. However, counts of microincrements underestimated daily age of fish between 72 and 129 cm FL. In the current study, daily age estimates were lower than annual age estimates for fish >1 year, and were also assumed to underestimate daily age. Only daily ages estimates for fish <1 year were included in the final analysis as recommended at the SPC Pre-assessment workshop in April 2018. The workshop also recommended inter-laboratory comparison work be undertaken to standardise daily ageing methods between the WCPO and EPO. Since the age validation work completed in 2003 (Appendix A), an additional 30 marked otoliths have been returned which may be useful for further daily and annual age validation.

A biological (decimal) age was estimated for each fish using an algorithm that accounted for birth date, time of zone formation, otolith edge type and catch date. However, assigning a nominal birth date for bigeye tuna is difficult and additional daily age data for bigeye <1 year may assist to refine the nominal birthdate (by back-calculating birth day).

All age data has been provided to SPC for further analysis.

The results of fitting a von Bertalanffy (VB) growth curve to the updated length at age data confirmed that bigeye tuna in the WCPO are considerably smaller-at-age than assumed in the 2014 stock assessment. Very little difference was detected in growth curves fitted to high and low otolith readability data, and it was agreed that only the high readability annual age data would be used to update growth estimates for the 2018 bigeye tuna assessment update. The nature of assigning readability scores, however, is interpretational and often related to the age or an age range. More thought may be warranted to ensure that by excluding hard to read otoliths we are not inadvertently reducing a higher proportion of otoliths in some areas of the growth curve compared to others.

The results of exploratory spatial analyses using the updated data set continue to indicate there are differences in the growth rates of bigeye tuna across the Pacific, with faster growth in the EPO relative to the WCPO.

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9. Appendix A: Direct validation of increments in otoliths of bigeye tuna injected with strontium chloride

Naomi Clear and Bruno Leroy

Chapter 7

In: Age and growth of bigeye tuna (*Thunnus obesus*), from the eastern and western AFZ. FRDC Final Report Project No. 2000/100, December 2003.

7.1 Introduction

An essential aspect of any age determination study is the validation of age estimates (Beamish and McFarlane 1983; Secor et al. 1995) as validation confirms the temporal meaning of the structures being counted. Other studies of bigeye age and growth have used dorsal spines (Sun et al. 2001) and vertebrae (Alves et al. 2002) to produce annual age estimates. However neither study included direct validation of the techniques so it is impossible to determine which structure, if any, is most reliable.

Previous age determination studies of other tunas have included direct validation of age estimates: Kalish et al. (1996) used bomb radiocarbon levels in otoliths to validate age estimates in southern bluefin tuna (SBT) *Thunnus maccoyii*; oxytetracyline has been used for several species including yellowfin tuna *Thunnus albacares* (Wild 1995), skipjack tuna *Katsuwanus pelamis* (Wild et al.1995) and albacore *Thunnus alalunga* (Ortiz de Zarate et al. 1994); and strontium chloride marking was used in SBT (Clear et al. 2000a). In this study of SBT, Clear *et al*. (2000a) examined strontium marks in three hard part structures: otoliths, vertebrae and scales. Growth zones (increments) were visible in each of the 3 structures but strontium marks were obvious only in the otoliths. Following these outcomes, Clear *et al*. (2000b) examined otoliths of bigeye tuna as part of a pilot study to establish validated techniques for estimating ages of bigeye tuna; they found that there were obvious increments in the otolith sections. Concomitantly, otoliths collected from bigeye tuna that had been injected with $SrCl_2$ were examined in the scanning electron microscope (SEM) — strontium marks were obvious in sections of bigeye otoliths and hence they were used to validate the counts of increments that were visible in the otoliths.

It has been impossible to validate the 1st increment using strontium chloride marking because the smallest fish tagged and released (assumed to be 0+) have not been recovered. Of the Sr-injected fish that have been recovered, the smallest at release had one increment visible on its otolith before the Sr-mark. However, we verified the counts of the 1st increment by counting microincrements (known to be deposited daily in other species) in the inner part of the otolith sections and identifying the expected position of the 1st increment. This analysis is presented in Chapter 8. In addition to the validation of annual increment formation, the Sr-marked otoliths were used in an attempt to validate the microincrement counts in larger (older) fish.

7.2 Methods

Specimens for validation were obtained from bigeye tuna that were tagged and injected with $SrCl_2$ solution (250 mg g^{-1}). The fish were released in 3 tagging programs conducted in the early and mid-1990s and early 2000s in the Coral Sea off north-eastern Australia. The incorporation of Sr into fish otoliths is not direct. After $SrCl_2$ is administered to the fish as an intramuscular injection, it is absorbed into the bloodstream and then incorporated into the endolymph, the fluid in which the otoliths form. Otoliths comprise in part an inorganic crystalline aragonite, which is largely calcium carbonate; it is in this part of the otolith that strontium atoms, at an increased concentration due to intramuscular injection, substitute for calcium atoms. From the 3 releases of tagged and Sr-injected bigeye, 34 sets of otoliths have been returned to CSIRO (Table 7.2.1).

In order to validate as many age-classes as possible during the pilot study, we analysed 10 specimens from a range of sizes-at-release (72-109 cm FL) and the periods at liberty (207 to 2071 days). See Figure 7.2.1 for details. The most recently analysed otolith was 125 cm at the time of release and at liberty for over 6 years (See Fig. 7.2.1 and Table 7.2.2). Its recovery and analysis has allowed us to extend the number of increments validated in bigeye tuna otoliths.

7.2.1 Annual Age Estimates

To prepare the otoliths for analysis, transverse sections that include the primordium were cut (Fig. 7.2.2) and then ground down until the primordium was exposed, following the methods of Gunn et al. (1992). The sections were then viewed under a light microscope to determine the number and position of increments that radiate out from the primordial area of the otolith, which is formed around the time of spawning. An age estimate was made for each fish (see Chapter 8 for details)

It was of course important to determine the position of the Sr-marks along the otoliths because they indicated the extent of the growth of the otolith on a known date. To prepare the otoliths for SEM analysis, they were polished and covered with a 25-30 nm thick carbon coat to minimize charging in the SEM. For analysis, the specimens were placed in a Philips 515 SEM coupled with a Robinson backscatter detector, which “visualises” differences in atomic weight. The greater the difference in atomic weight, the more obvious the appearance in the Robinson detector. This was significant for our analysis because strontium is almost twice the atomic weight of calcium, therefore the Sr-rich band in an otolith was obvious; it appeared as a weak to intense bright band across the growth axes (Fig. 7.2.3). The position of the Sr-mark was measured along the inner (I) and outer (O) margins of the ventral arm (Fig. 7.2.3).

In addition, the bright bands were examined using electron dispersive spectroscopy (EDS) while the specimens were loaded in the SEM, to verify that they were in fact areas of increased strontium.

The number of increments expected after the Sr-mark was calculated from the time at liberty after tagging and injection with $SrCl_2$, and the number expected was compared with the number observed. There was some uncertainty in the number of increments “expected” after the Sr-mark because although our age estimates were in whole years the periods at liberty were obviously not.

When the days at liberty was closer to half-years we were not able to predict if the increment for that year had been completely formed. Hence our expected number of increments could be one of two consecutive years (see Table 7.3.1). The age estimates were compared with ages predicted from a composite model (Hampton et al. 1998) derived from microincrement counts and tag-recapture data.

Table 7.2.1. Otoliths recovered from Sr-injected bigeye tuna from 3 tagging programs

Fish Number	Release fork length (cm)	Recapture fork length (cm)	Release Date	Recapture Date	Days at Liberty
37	72	85	13/11/1992	31/07/1993	260
59	96	159	12/11/1992	15/07/1998	2071
576	72	156	12/11/1992	6/09/1998	2124
64	79	unknown	6/10/1995	unknown	unknown
57	75	128	6/10/1995	14/08/1997	678
63	83	94	6/10/1995	10/06/1996	248
66	84	129	9/10/1995	18/12/1997	801
67	78		9/10/1995	4/11/1997	757
65	78	128	9/10/1995	26/01/1998	840
591	80	139	9/10/1995	2/11/1998	1120
62	109	123	9/10/1995	3/05/1996	207
2125	77	83	14/10/2001	29/10/2001	15
2126	78	80	14/10/2001	9/11/2001	26
2611	83	101	13/10/2001	30/06/2002	260
2131	80	80	13/10/2001	9/11/2001	27
2325	94	102	14/10/2001	27/04/2002	195
2326	83	97	13/10/2001	27/04/2002	196
2612	91	101	14/10/2001	25/05/2002	223
2613	84	94	13/10/2001	15/06/2002	245
2614	84	95	13/10/2001	25/06/2002	255
2819	95	113	13/10/2001	27/07/2002	287
2820	125	157	9/10/1995	25/05/2002	2420
3391	80	104	13/10/2001	29/09/2002	351
3392	80	104	13/10/2001	22/09/2002	344
3393	82	104	13/10/2001	29/09/2002	351
3394	81	103	13/10/2001	24/09/2002	346
3395	86	106	13/10/2001	24/09/2002	346
3396	83	116	13/10/2001	29/09/2002	351
3397	81	102	13/10/2001	23/09/2002	345
3398	85	103	13/10/2001	25/09/2002	347
3763	82	109	14/10/2001	15/12/2002	427
3764	78	98	14/10/2001	15/12/2002	427
3765	77	112	14/10/2001	15/12/2002	427
3766	93	118	14/10/2001	17/02/2003	491

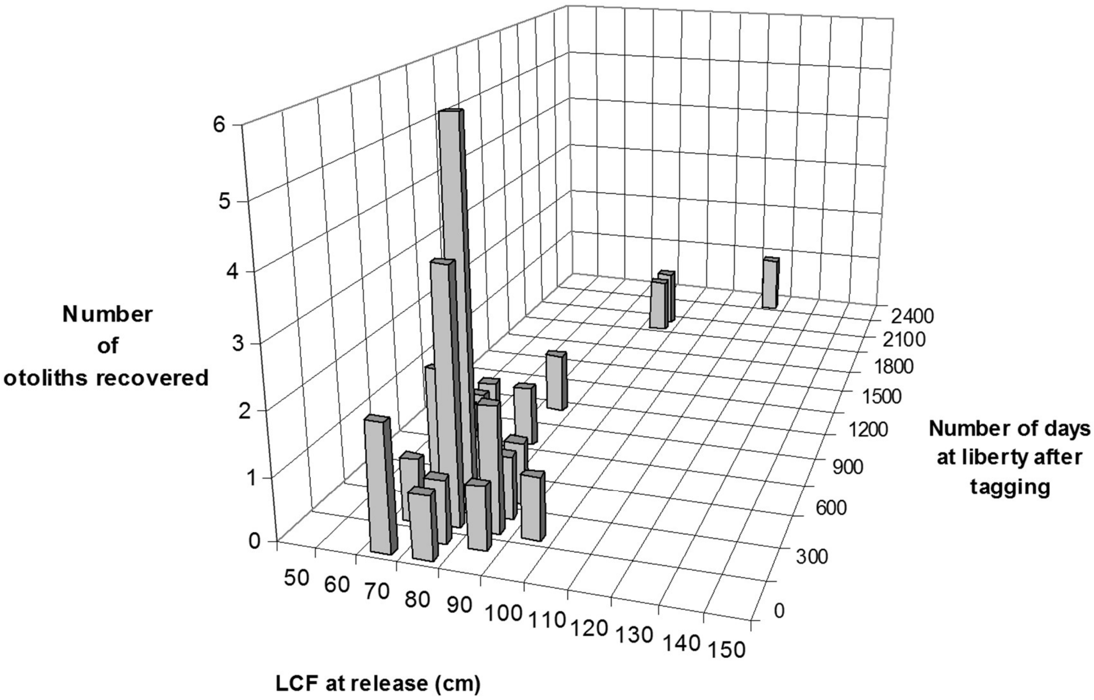

Figure 7.2.1. The size-at-release and period at liberty after tagging for the bigeye tuna from which Sr-marked otoliths were recovered.

Table 7.2.2. Otoliths analysed for strontium marks in the SEM.

Fish number	Release Date	Release fork length (cm)	Recapture Date	Recapture fork length (cm)	Growth (cm)	Days at Liberty
591	9/10/1995	80	2/11/1998	139	59	1120
37	13/11/1992	72	31/07/1993	85	13	260
57	6/10/1995	75	14/08/1997	128	53	678
59	12/11/1992	96	15/07/1998	159	63	2071
62	9/10/1995	109	3/05/1996	123	14	207
63	6/10/1995	83	10/06/1996	94	11	248
64	6/10/1995	79	unknown	unknown		
65	9/10/1995	78	26/01/1998	128	50	840
66	9/10/1995	84	18/12/1997	129	45	801
67	9/10/1995	78	4/11/1997			757
2820	9/10/1995	125	25/5/2002	157	32	2420

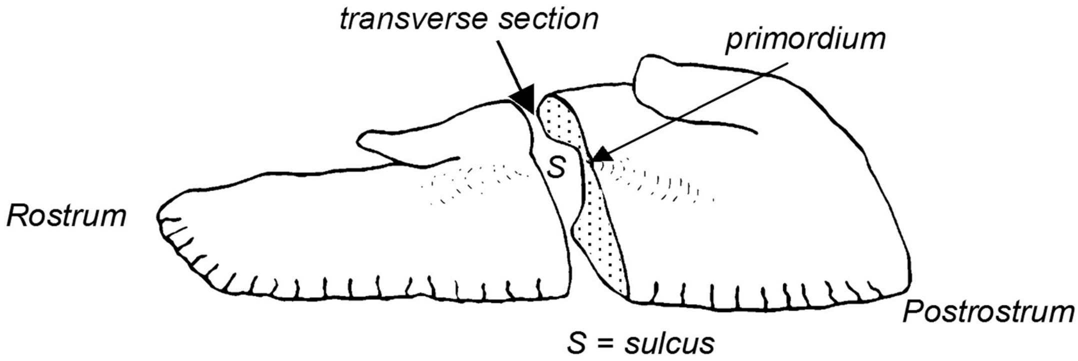

Figure 7.2.2. Diagram of a typical left-hand sagittal otolith. Transverse sections were prepared for SEM analysis of strontium marks.

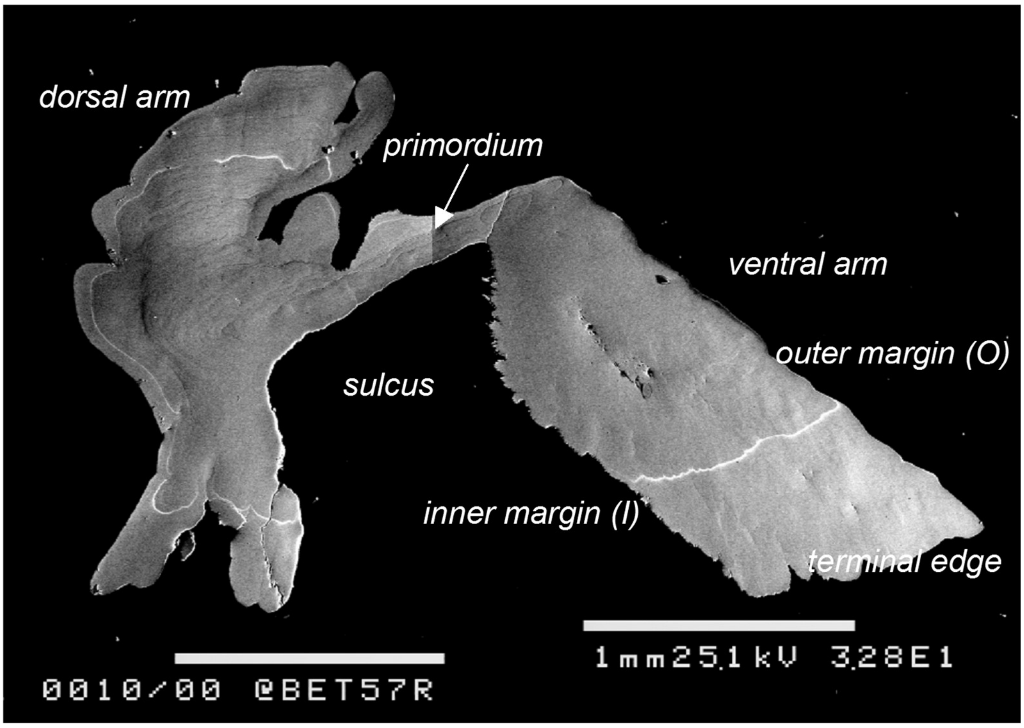

Figure 7.2.3. SEM micrograph of a longitudinal section (bigeye #57). Sr-marks were obvious as bright bands across the growth axes.

7.2.2 Daily Age Estimates

After SEM analysis, the otoliths were examined by a reader experienced in counting microincrements of bigeye tuna and other closely related species. The otoliths were from fish larger than normally considered for microincrement counts but the Sr-mark on the otolith presented a potential opportunity to validate the counts of microincrements in larger (older) fish.

The otoliths were prepared for microincrement counts by removing the carbon coat that had been necessary to minimize charging in the SEM. This involved polishing the surface with a 6 µm diamond paste, which also removed a small amount of the surface of the otolith (Fig. 7.2.4).

The reader was told the position of the Sr-mark along the section but no other information about the fish or its time at liberty after tagging was given. Two counts of the number of microincrements present after the position of the Sr-mark were made for each otolith. After the counts were made, the expected number of microincrements calculated from the period at liberty after tagging was compared with the microincrements counted after that position. A mean difference was calculated using:

(Days at Liberty) – (Mean of counts 1 and 2) / Days at Liberty * 100

In five cases, both otoliths from the pair were recovered from tagged fish and these allowed a further opportunity for validating microincrement counts. A comparison of the morphology of left and right sister otoliths was conducted during the pilot study and no significant differences were found. So although these specimens had not been analysed in the SEM to identify the position of the Sr-mark, it was assumed that the Sr-mark would be in the same position along the sister otolith.

Preparation of the otoliths was slightly different for these sister otoliths. They were cleaned with an ultra-sonic cleaner and dried, then embedded in polyester resin (Sody 33). A transverse section was cut with a low-speed Buehler Isomet saw to obtain a slice containing the primordium. The slice was attached to a glass slide with thermoplastic glue (Crystalbond), ground with wet sand paper (600 and 1200 grit) and polished with aluminium powder (3, 1 and 0.3 µm) until the primordium was reached. Next, the section was turned on a hot plate and polished on the other side until a thin section of 50-75 µm maximum was obtained. The surface of the section was partially decalcified with 5 % EDTA (pH 7.4) to emphasize the increments.

The microincrements observed on the thin section were counted under a light microscope (x 1000) with a Leica DMLB 10 with a x100 dry objective. A 3-CCD colour video camera (Sony DXC-950P) mounted on the microscope and linked to a 20p computer screen via a frame grabber card Matrox Meteor and the analysing software Kheops from Noesis. The image on the screen was magnified up to 4000X with good resolution.

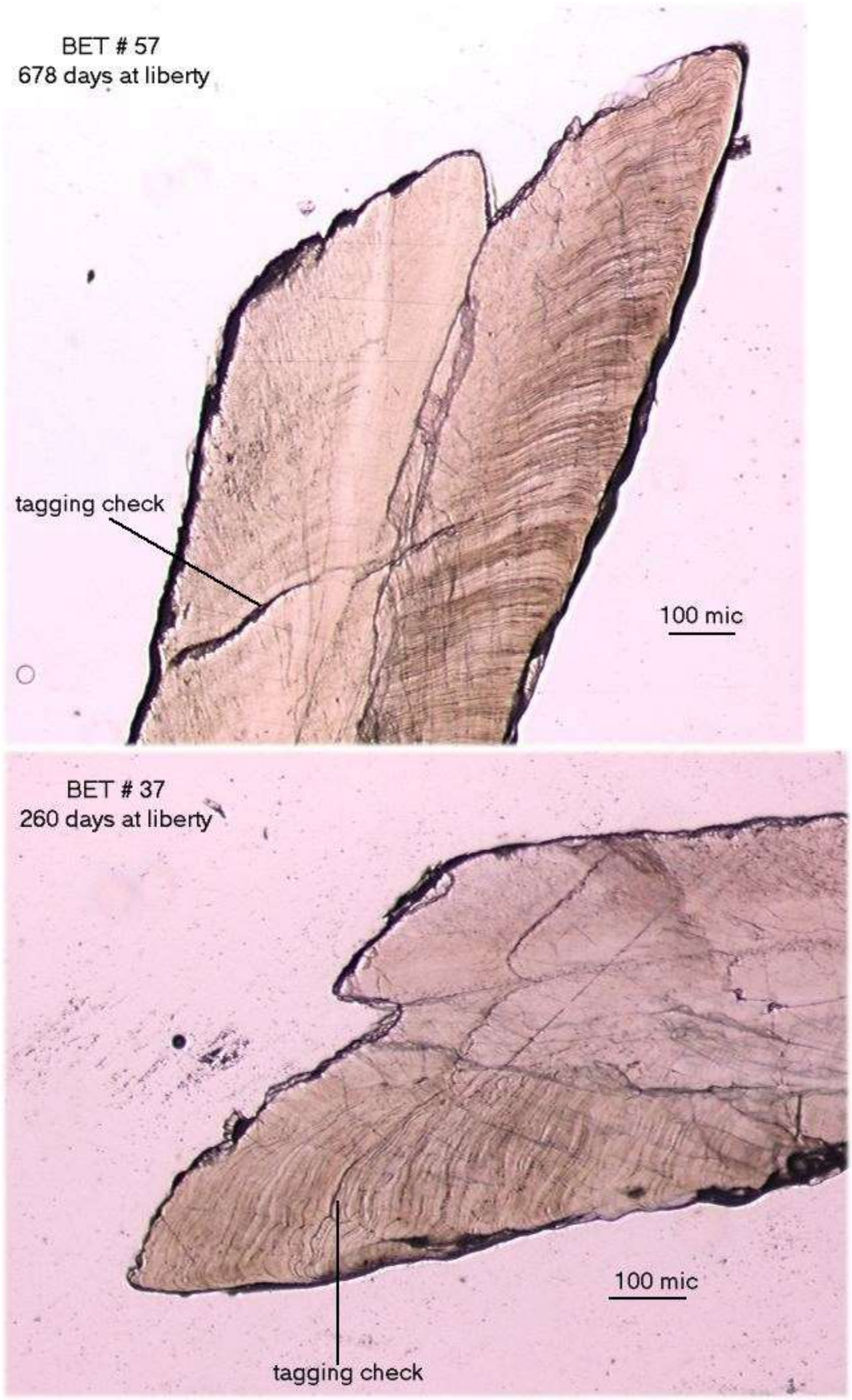

Figure 7.2.4. Images of thin transverse sections used to count microincrements. The reader was told the position of the strontium mark along the section, coinciding with a tagging ‘check’ that was usually visible at the high magnifications used for microincrement counts.

7.3 Results

7.3.1 Annual Age Estimates

The 11 fish that were analysed in the SEM (in the pilot and current study) ranged from 85 to 159 cm LCF at recapture and had been at liberty since tagging from 207 to 2420 days (about 6 years and 7 months). Age estimates for these fish ranged from 2 to 9 years. The polished sections of all otoliths had obvious Sr-marks when viewed in the SEM. The Sr-mark was visible as a weak to intense, bright band across the growth axes when viewed as backscattered electron images using the Robinson detector (light microscope and SEM images are shown in Appendix 1).

EDS spectra showed a strong peak of strontium Lα x-rays when the electron beam was directed to the Sr-mark and, in contrast, very low (background) levels in the regions of the otolith preceding the mark (Fig. 7.3.1). There was no evidence of increased chlorine incorporation associated with the injection of $SrCl_2$ into the fish.

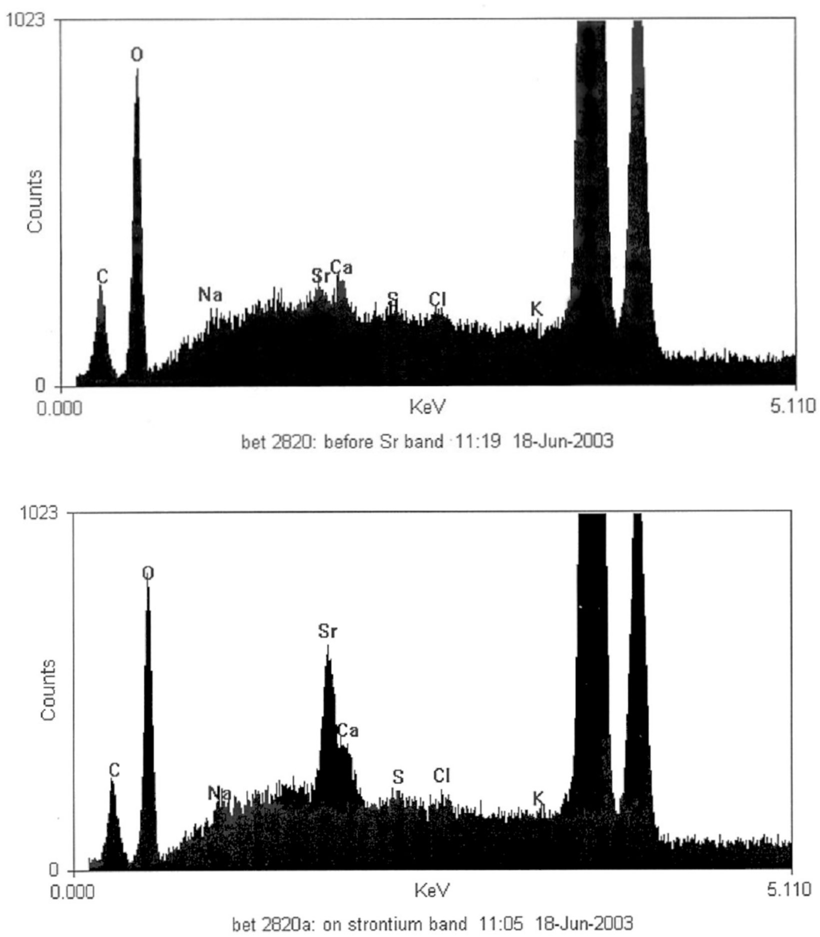

Figure 7.3.1. Examples of EDS spectra from a sectioned bigeye tuna otolith (bigeye #2820) showing peaks due to background levels of strontium (A) and enhanced Sr levels associated with the strontium mark (B). The enhanced peak was used to positively identify the position of the strontium mark.

Of the 11 otolith sections analysed in the SEM, we were unable to obtain recapture information about bigeye #64 hence we could not include it in the validation study. For the remaining 10 otolith sections there was agreement between the number of increments observed after the Sr-mark and the number expected, calculated from the time at liberty (Table 7.3.1). Thus the annual periodicity in formation of increments 2 to 9 has been validated for the otoliths analysed. The first increment was not validated using this method because, as previously stated, the appropriate otoliths had not been recovered. It would have required otoliths from a fish that was injected with $SrCl_2$ when it was young of the year, i.e. before the formation of the first complete increment, and was subsequently recovered after the formation of the 1st increment. Although these young-of-the-year fish (0+) have been tagged and injected with $SrCl_2$, to date none have been recovered.

Table 7.3.1. Analysis of BET Sr-marked otoliths. The number of increments expected after the Sr-mark (determined from the time at liberty after tagging) was equal to the number observed, for all specimens analysed.

BET specimen #		37	57	59	62	63	64	65	66	67	591	2820
FL at tagging (cm)		72	75	96	109	83	79	78	84	78	80	125
FL at recapture (cm)		85	128	159	123	94	-	128	129	-	139	157
Time at liberty after tagging (days)		260 (8.5 mths)	678 (1 yr 10 mths)	2071 (5 yrs 8 mths)	207 (7 mths)	248 (8 mths)	recap. details not known	840 (2 yrs 3 mths)	801 (2 yrs 2 mths)	757 (2 yrs 1 mth)	1120 (3 yrs 1 mth)	2420 (6 yrs 7 mths)
Number of increments after Sr mark	**expected**	**0 or 1**	**1 or 2**	**5 or 6**	**0 or 1**	**0 or 1**		**2**	**2**	**2**	**3**	**6 or 7**
	observed	**1**	**1**	**5**	**1**	**1**	**1**	**2**	**2**	**2**	**3**	**6**
Age estimate (this study) *		2	3	8	3	2	2	3	3	3	4	9
Age at tagging **		1.2	1.3	2.1	2.7	1.6	1.5	1.4	1.6	1.4	1.5	3.18
Age at recapture **		1.7	3.8	8.6	3.5	2.0	-	3.8	3.9	-	4.8	7.87
Month of recapture		July	Aug	July	May	June		Jan	Dec	Nov	Feb	May
distance from Sr mark to margin (cm)	Sr (O) -O	0.36	0.74	1.06	0.25	0.27	0.30	0.72	0.77	0.81	0.67	0.49
	Sr (I) -I	0.26	0.56	0.80	0.15	0.16	0.25	0.54	0.63	0.77	0.50	0.43

* Estimated by counting annual increments on sectioned sagittal otoliths

** Estimated using results from a study of otolith microincrements and tagging data (Hampton et al. 1998).

We compared our age estimates with the age-at-recapture estimated using the growth curve derived from otolith microincrement and tagging data (Hampton et al. 1998) and found reasonable agreement (see Table 7.3.1). In all cases, except the largest fish, the discrepancy is less than 1 year and can be explained possibly by three aspects of our technique:

1. Our age estimates are in whole (integer) years; the counts do not give an indication of how much of the marginal increment has formed. Hence, for example, 6 months growth on the margin of an otolith would not be counted as an increment and the resulting age estimate would be 0.5 year less than the true age.
2. In some cases the number of increments observed after the Sr-mark over estimated the 'time at liberty' (the period between tagging and recapture). This was because the increment being deposited at the time of tagging and injection was counted as 'an increment after the Sr-mark'.
3. There is some uncertainty in the counts of increments before the Sr-mark. Only the number of increments after the strontium mark could be validated from knowing the period at liberty after tagging and injection.

In the case of the largest fish the discrepancy between our age estimate and the estimate based on the composite model is just over 1 year, our age estimate being higher. The 3 points listed above could in part explain this difference but in addition, for larger fish, we might expect more scatter around mean age-at-length. Hampton et al. (1998) found that the ages from otolith microincrement counts for fish > 110 cm didn't fit the composite model very well and possibly underestimated the age, so for fish >110 cm the model was refitted using only the tagging data. In contrast, our age estimate was higher than the age predicted by the model.

The visibility of the Sr-mark (intensity in the backscattered electron image) was highest in fish that had been relatively small at the time of injection (e.g. less than 90 cm LCF). An example is otolith from bigeye #57 (Fig. 7.2.3), which measured 75 cm at time of release. In contrast, #2820 was 125 cm at the time of tagging and the Sr-mark was weak in the backscattered image (Fig. 7.3.1). In this case the EDS system did not just verify the identity of a bright band in the SEM, is was essential for testing the identity of several weak bands when it was not clear from the backscattered imaging which, if any, was a strontium mark.

7.3.2 Daily Age Estimates

All microincrements counts underestimated the days at liberty (Table 7.3.2). The mean difference of the replicate counts and the times at liberty ranged from 7.7% to 30.0%. Of the specimens analysed in the SEM, five were considered in good enough condition to make microincrement counts (2 were damaged when the carbon coat was polished off the surface). Of these, 3 had sister otoliths from which replicate counts were made; the replicate counts were less than the counts made on the SEM-analysed fish.

A. B.

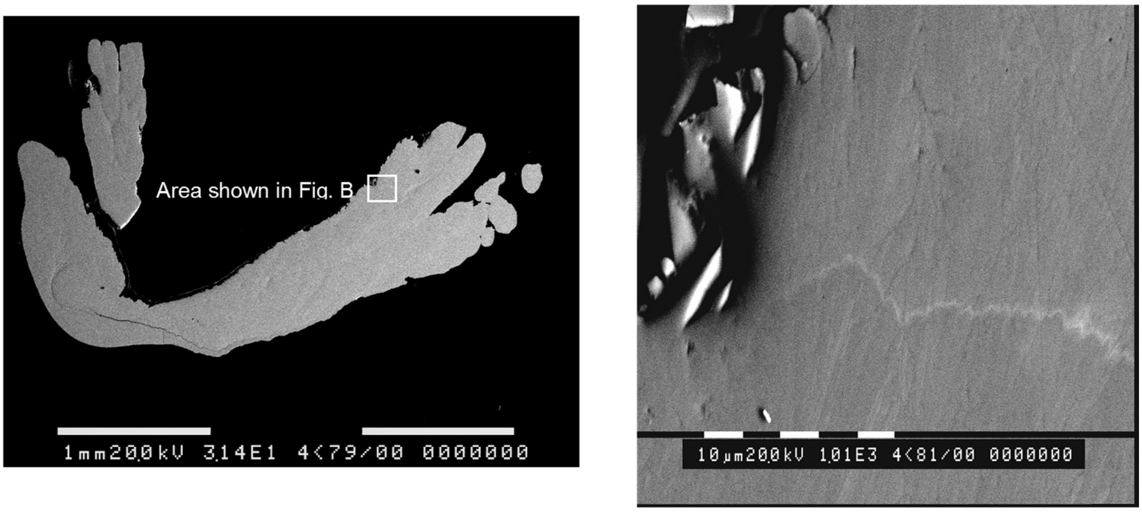

Figure 7.3.2. An example of a section in which the Sr-mark was weak and was positively identified using EDS.

Table 7.3.2. Results of microincrement counts on strontium-marked otoliths

Fish no.	Release fork length (cm)	Recapture fork length (cm)	Days at Liberty	Otolith analysed in the SEM				Sister Otolith			
				Count 1	Count 2	Reading Score	% mean difference from days at liberty	Count 1	Count 2	Reading Score	% mean difference from days at liberty
37	72	85	260	218	216	A	-16.5				
57	75	128	678	587	570	B	-14.7	530	560	C	-19.6
62	109	123	207	155	137	C	-29.4	144	146	A	-30.0
63	83	94	248	230	228	B	-7.7	184	200	C	-22.6
65	78	128	840	597	666	B-	-24.7				
66	84	129	801			broken		567	582	C	-28.3
67	78	unknown	757					570	532	B	-27.2

A= count with high confidence, all areas have visible microincrements
B= count with confidence, most areas have visible microincrements but a few areas are unreadable
C= count with low confidence, many areas along the section are unreadable

7.4 Discussion

Otoliths from bigeye tuna tagged and injected with $SrCl_2$ have provided the means to validate the annual formation of the 2nd to 9th increments. This is independent of the size of the fish when it was tagged or how long it was at liberty after tagging. It has not been possible to validate the 1st increment using strontium chloride marking because the smallest fish tagged and released (assumed to be 0+) have not been recaptured.

Results from this study have shown that an intramuscular injection of $SrCl_2$ leaves a mark on the otolith that is visible in the backscatter image from a Robinson detector. 100% of the bigeye tuna otoliths, once suitably sectioned and coated, had Sr-marks visible on the growth axes. EDS analysis confirmed that the mark was in fact an area of increased strontium uptake and also showed that there wasn't a corresponding increase in the incorporation of chlorine associated with the injection of $SrCl_2$.

Although 100% of the bigeye otoliths examined in the SEM had visible Sr-marks, the Sr-marks in otoliths from the fish that were tagged as larger animals were much less intense than those from fish tagged as smaller fish. An example is the comparison of Sr-marks of bigeye #57 and bigeye #2820 (Fig. 7.2.3 and 7.3.1). In fact, for 2 specimens the EDS proved essential to identify which of the bands visible across the growth axes was in fact the Sr-mark. To avoid the possibility of Sr-marks not being detected in the SEM, we recommend increasing the dosage of $SrCl_2$ for larger fish in any future tagging programs.

Clear *et al*. (2000b) quantitatively analysed the Sr-mark in otoliths of southern bluefin tuna (SBT), running a quantitative line-scan across the bright band visible in the Robinson detector images. Although absolute values were slightly different between SBT specimens, using one sample (OB 96) as an example, they found that there was an increase in measured Sr concentration of around 7.1% and a fall in measured Ca concentration from 39–40% before the bright band to a minimum of 35.5% on the band—a decrease of 3.5–4.5% in absolute value or 10% relative. The weight fraction of Sr and Ca combined did not change. Clear *et al.* (2000b) suggest that this supports the theory that Ca atoms are replaced by Sr atoms in the atomic structure on a 1:1 basis, each Sr atom being approximately twice as heavy as a Ca atom.

In the current study we found the EDS an extremely useful tool for testing the identity of weak bands in sectioned specimens when it was not clear from the backscattered imaging which, if any, was the strontium mark. From their analysis of SBT otoliths Clear *et al.* (2000b) measured the elevated levels of strontium and noted the concentration at which they were no longer visible by backscatter imaging. In the sample (OB 96) the Sr-mark was an intense bright band easily visible in the Robinson detector. The 7% increase in Sr and 3.5% decrease in Ca in the Sr-mark gives a atomic value of approximately 104 compared with 100 for the unaltered $CaCO_3$ — a difference resolvable with backscattered electron imaging on the SEM. However, they found that once the elevated levels of Sr fell to 0.5–5% beyond the Sr-mark they were not detectable by the Robinson detector, i.e. they were no longer visible in the backscatter images. Similarly, weak Sr-marks may not be visible in the Robinson detector but still detectable by EDS.

Much of the information gained from the study of southern bluefin tuna can be used as a basis for understanding bigeye tuna because the two species are very closely related. However, there were some differences in the otoliths — the increments comprising opaque and translucent zones were obvious only in the sections of bigeye otoliths whereas increments were obvious in whole and sectioned otoliths of juvenile SBT. Strontium-marks were also detectable in both whole and sectioned SBT otoliths hence validation of the annual formation of increments was possible in both whole and sectioned otoliths. Not having to section otoliths saved a great deal of time and hence it was possible to analyse more specimens over time. Another obvious difference between the otoliths of the 2 species was that although in both species there were regular, narrower increments towards the terminal edge of an otolith section these began much closer to the primordium in bigeye tuna, perhaps indicating an earlier onset of a significant event in the life history.

In summary, $SrCl_2$ has proved an effective marker for the validation of annual increments in bigeye tuna otoliths. One important consideration for any mark-recapture program that involves wild-caught fish is the potential hazards to humans consuming a fish that has been injected with a marking agent. Oxytetracycline, previously used in other age validation studies, has been known to cause allergic reactions, leading the U.S. Food and Drug Authority to ban their use in commercial fisheries. $SrCl_2$ does not cause such allergic reactions. In fact $SrCl_2$ is considered safe for human consumption and is a component of 'Sensodyne' and other toothpastes.

It is possible that otoliths will be recovered from larger (older) fish in the future and the validation of annual increments will be extended beyond the 9th increment. A large number of fish in this study have been estimated to be older than this (see Chapter 8) so every further age class (increment) validated will be significant to the understanding of age and growth of bigeye tuna. It is also possible that otoliths will be recovered from fish that were 0+ at the time of tagging, i.e. before any increments had formed completely. As the smallest fish tagged was less than 50 cm FL this is a possibility that could extend the validation downwards, enabling direct validation of increments in the youngest fish.

The large discrepancy between the days at liberty and the counts of microincrements deposited after the strontium mark indicate that age estimates in days using otoliths from bigeye tuna of this size are not reliable. Except for two fish, #37 and #63, all were larger than 120 cm FL at recapture. This is considered to be above the limit of readability, i.e. the outer microincrements on the otoliths of fish larger than 120 cm FL are deposited so closely that they are difficult to resolve under a light microscope. It is likely that the underestimates are due in part to a temporary interruption in daily otolith growth caused by tagging. However, in southern bluefin tuna, the growth 'check' has been estimated to be only 1-4 weeks (Rees et al. 1996; Hearn and Polacheck 2003). In the current study, the microincrement count closest to the known days at liberty was from a fish that was 94 cm FL at recapture — the mean difference between the days at liberty and the microincrement count was 7.7% (19 days); this underestimate could be explained by an interruption in growth after tagging. However the much greater underestimates and low confidence assigned to the counts from otoliths of bigger fish indicate that using the larger Sr-marked bigeye tuna for validating daily age estimates has limited value.

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