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Developing techniques to estimate the age of bigeye tuna and broadbill swordfish off eastern Australia: A pilot project

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TABLE OF CONTENTS

GLOSSARY

AFMA	Australian Fisheries Management Authority
AFZ	Australian Fishing Zone
APE	average percent error
BBL	broadbill swordfish
BET	bigeye tuna
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Eastern Tuna MAC	Eastern Tuna Management Advisory Committee
ETBF	Eastern Tuna and Billfish Fishery
FAE	final age estimate
FL	length to caudal fork
FRDC	Fisheries Research and Development Corporation
LJFL	lower jaw to fork length
NMFS	National Marine Fisheries Service
OFL	orbit (eye) to fork length
SBT	southern bluefin tuna
SBTMAC	Southern Bluefin Tuna Management Advisory Committee
SEM	scanning electron microscope
SPC	Secretariat of the Pacific Community (formerly South Pacific Commission)
S&W Tuna MAC	Southern and Western Tuna Management Advisory Committee
WPO	Western Pacific Ocean

NON-TECHNICAL SUMMARY

98/113 Developing techniques to estimate the age of bigeye tuna and broadbill swordfish off eastern Australia: a pilot project

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OBJECTIVES:

1. To collect hard parts — otoliths and anal fin rays — from bigeye tuna (BET) and from broadbill swordfish (BBL).
2. Assess a range of standard age estimation techniques using otoliths, vertebrate and fin rays of BET and BBL, in collaboration with Secretariat of the Pacific Community (SPC) and National Marine Fisheries Service (NMFS).
3. Compare age estimates derived using these techniques with those from other studies, including research by the SPC and NMFS.
4. Using marked (strontium chloride) BET otoliths from previous SPC/CSIRO tagging studies, as well as tag release/recapture growth data from other tagging programs, attempt to validate our age estimation methods and techniques.
5. Make recommendations to Eastern Tuna Management Advisory Committee on the feasibility of an extended age and growth project to provide biological information to stock assessment studies.

NON TECHNICAL SUMMARY:

The fishery for bigeye tuna (BET) and broadbill swordfish (BBL) is expanding rapidly off the east coast of Australia and there is an urgent need to gain an understanding of the stock structure of this fishery. Grave concerns are held about the possibility of over-exploitation of these species, within both the Pacific and the Indian Oceans. It is essential that research addresses the key uncertainties in the population biology and stock dynamics of the species, so that rigorous population assessments can be completed and scientific advice provided in support of management.

The determination of age in fishes is crucial to understanding their population dynamics. Age composition data is used in determining stock productivity, growth, mortality and recruitment and as such is an integral component of any stock assessment analysis. To date only limited age

assessment studies have been carried out on BET and BBL, and most of these have lacked substantive validation. There is a need to develop verifiable age determination techniques for both species for stock assessment studies.

The objectives of this pilot study were to collect and archive hard parts (otoliths and anal fin rays) from BBL and BET. From the 772 BET and 310 BBL hard part samples archived, we used samples from 50 BET and 50 BBL to assess a range of standard age estimation techniques on these structures. We considered 50 samples to be adequate to fully assess potential age estimation techniques. Also, we planned to examine BET otoliths that were previously marked with strontium chloride during a SPC/CSIRO tagging program in an attempt to validate our age estimation methods and techniques.

We found that the otoliths of BET were similar in appearance and structure to otoliths of southern bluefin tuna (SBT). The techniques used to determine and validate the age of SBT also worked successfully on BET. We found good precision and reproducibility between age estimates. However, we found there was some difficulty in detecting the first annulus in BET. Age estimates (in days) by SPC have confirmed the location of the first annulus so this should not present a problem in a full-scale age and growth study. The age of older fish was validated through strontium chloride marking experiments. Nine BET that were injected with strontium chloride at tagging and at liberty for 207 to 2,071 days had laid down an increment for each successive year at liberty. This confirms the annual nature of increment formation for fish two- to eight-years-old. We have therefore established a reliable technique for determining the age of BET through counting annual increments. We recommend that these techniques now be applied in a comprehensive age and growth project on this species to assist in stock assessment studies.

We found that BBL otoliths were too small and fragile and lacked the structure needed for making annual age estimates. However, sections taken from the second ray of the first anal fin had a clear structure of bands comprising alternating translucent and opaque zones. We were able to estimate age of BBL, presuming that these bands were laid down annually. We found acceptable precision and reproducibility between age estimates, and there was little evidence of bias in age estimates over the age range of BBL examined. However, previous work on BBL in the Mediterranean has suggested that there is difficulty in locating the first annulus as it becomes obscured as fish get larger. As we did not have samples of fish smaller than 100 cm in length we could not confirm difficulty in detecting the first annulus. However, more recent work by NMFS on Pacific BBL produced an estimate, from counts of microincrements on sagittal otoliths, that fish of 114 cm are 1-year-old. This is very close to our estimate of mean size at age 1 — 109 cm. The differences between the results would need to be explored more thoroughly in any larger scale study.

We attempted to validate the annual nature of growth patterns in BBL fin rays using marginal increment analysis. For a complete marginal increment analysis it is necessary to examine samples collected year-round. Although the samples available for this study were adequate to develop techniques to estimate ages, our marginal increment analysis was limited because no samples had been collected during some months. However, our initial examination of marginal increments in the samples that were available suggests that marginal increment analysis may be a promising method of validating age estimates. We recommend that any age determination study on BBL in the Australian Fishing Zone would need to address validation by marginal increment analysis as one of its first objectives.

1. BACKGROUND

A rapid expansion of longline effort off Mooloolaba and northern New South Wales, significant investment in new and larger boats capable of fishing further offshore, shifts in targeting practices of longliners from yellowfin tuna to bigeye tuna (BET) and broadbill swordfish (BBL), and advances in the efficiency of gear and methods for targeting these species have resulted in significant increases in the catch of high quality BET and BBL in recent years. BET and BBL are being targeted from Cairns to southern New South Wales; they are now valuable components of the Eastern Tuna and Billfish Fishery (ETBF).

The situation of increasing catches of BET and BBL in the western Pacific Ocean (WPO), and the realisation that baseline data on the life history, stock structure and population size of the species in our region are grossly inadequate, have prompted the Forum Fisheries Agency to call for a review of management arrangements in the WPO. While it is likely that this review will recommend a precautionary approach, it is essential that research is initiated to address the key uncertainties in the population biology and stock dynamics of the species, so that rigorous population assessments can be completed and scientific advice provided in support of management.

The determination of age in fishes is crucial to understanding their population dynamics. Age composition data is used in determining stock productivity, growth, mortality and recruitment and as such is an integral component of any stock assessment analysis.

To date, only limited age assessment studies have been carried out on both BET and BBL. Most of the previous BET studies have used scales (Tankevich, 1982; Yukinawa and Yabuta, 1963), which are fairly difficult to analyse due to regeneration and lack of distinct ‘marks’. Some studies have used modal progression and length frequency techniques (Yukinawa and Yabuta, 1963). Again there has been little validation in these studies. Previous growth studies in the literature seem to indicate that BET have a maximum life expectancy of six to eight years (Iverson, 1955; Tankevich, 1982), yet fish tagged by the joint SPC/CSIRO Coral sea tagging project are still being recovered at around 150 cm, eight years after being tagged as one- or two-year-olds. The maximum length of BET caught in the Australian Fishing Zone (AFZ) is around 187 cm. This seems to indicate that previous age and growth studies may have been underestimating maximum age, which has implications for stock assessments.

Attempts at estimating ages of BBL have been made using several techniques, including modal analysis of length frequencies and examination of hard parts such as vertebrate, otoliths and fin rays. Unfortunately many discrepancies exist due to differing methods. Swordfish otoliths have not been widely used for age estimation due to their small size and fragility, however, with the proper techniques, it reportedly is practical to examine otoliths for incremental growth patterns, both daily and annual.

There are other international organisations that have initiated studies on the age and growth of BET and BBL. It is intended to examine the results of these studies and incorporate them where applicable. The other studies, which are mainly directed at analysing microincrements producing estimates of age in days, may assist in verifying age estimates of small fish and for the first years of life in older fish.

CSIRO has developed a world-renowned expertise in the age determination of pelagic species. It was intended for this project to utilise our expertise and methodologies to ascertain the feasibility of making annual age estimates using direct methods for both BET and BBL. Aspects of the project — sample collection and analysis of hard parts — were planned to be done in collaboration with the SPC, which had begun a study on age and growth of BET using microincrements.

If this feasibility study is successful it would be intended to apply for further funding, either from Eastern Tuna Management Advisory Committee (Eastern Tuna MAC) or Fisheries Research and Development Corporation (FRDC) to carry out a comprehensive age and growth study on these species to assist in stock assessment studies.

2. NEED

The BET and BBL fishery is expanding rapidly off the east coast of Australia; the warning ‘trigger’, set by Australian Fisheries Management Authority (AFMA), of 800 t of BBL caught in 1 year has been exceeded. There is now an urgent need to gain an understanding of the stock structure of this fishery; there are grave concerns, both within the Pacific and the Indian Ocean, about the possibility of over-exploitation of these species.

Very little is known scientifically about these species (Whitelaw and Unnithan, 1997). Critically there is no verifiable information on the size-at-maturity and age-structure of populations of BET and BBL in the western Pacific. Stock assessments require knowledge of size-at-maturity, size-at-age, growth rate and estimates of the variability in these parameters within the stock.

For management plans to be devised and implemented it is necessary to expand our biological knowledge. A first step in a structured stock assessment of a fishery is to determine whether ages of individuals can be estimated from their bony parts (otoliths and fin rays). There is a need to develop verifiable age estimation techniques for stock assessment studies.

3. OBJECTIVES

1. To collect hard parts — otoliths and anal fin rays — from bigeye tuna (BET) and from broadbill swordfish (BBL).
2. Assess a range of standard age estimation techniques using otoliths, vertebrate and fin rays of BET and BBL, in collaboration with Secretariat of the Pacific Community (SPC) and National Marine Fisheries Service (NMFS).
3. Compare age estimates derived using these techniques with those from other studies, including research by the SPC and NMFS.
4. Using marked (strontium chloride) BET otoliths from previous SPC/CSIRO tagging studies, as well as tag release/recapture growth data from other tagging programs, attempt to validate our age estimation methods and techniques.
5. Make recommendations to the Eastern Tuna MAC on the feasibility of an extended age and growth project to provide biological information to stock assessment studies.

4. METHODS

4.1 Collection of hard parts

The first objective of this project was to collect hard parts — sagittal otoliths (ear bones) and fin rays — from BBL and BET and include them in the CSIRO hard part archives. These archives hold collections of otoliths, scales, fin rays and vertebrae from species of tuna and billfish. The hard part structures provide valuable information about the fish from which they were collected and hence the archives support a range of studies including research on age determination, age structure of fish populations, historical changes in growth and mortality and stock delineation. Maintenance of the hard part archives is funded by Eastern Tuna MAC, Southern Bluefin Tuna Management Advisory Committee (SBTMAC), Southern and Western Tuna MAC.

The otoliths and fin rays collected from BBL and BET were cleaned, dried and catalogued, ready for analysis. A relational database was established for both of the species sampled. The databases hold collection details about the fishing operations in which the samples were collected and details about each fish, such as length, weight and sex. The key benefit of storing data this way are that it ensures choosing appropriate samples for analysis is an efficient process: information about specimens is easily accessible and records can be cross-referenced with information from other databases.

There are 772 otolith samples from BET in the CSIRO hard parts archives. Otoliths were collected from the Eastern Tuna and Billfish Fishery for this project but the archives hold additional bigeye samples that were collected over a number of years and from a range of areas where bigeye is targeted or is a by-catch species. These areas include Western Australia, Indonesia, New Zealand, Solomon Islands and Philippines. The size range of samples reflects the size of fish in the exploited population (see Figure 1).

The otoliths have been collected not only by CSIRO staff but also by Commonwealth observers, external contractors and commercial and recreational fishers. Otoliths from BET caught on commercial vessels are normally extracted using a drill and holesaw to ensure that the external appearance of the fish is not affected.

As part of a tagging program in the early 1990s, BET were caught in the Coral Sea, injected with strontium chloride (a harmless salt that marked their otoliths) and tagged with a distinctive orange tag. The tagging program was run jointly by CSIRO and the SPC. Now the otoliths from orange-tagged bigeye are being returned to CSIRO and, marked with a strontium-induced 'time-stamp', they form the basis of the validation for our age estimates. The CSIRO archives currently hold 11 sets of strontium-marked otoliths, collected from fish that have been at liberty from 207 to 2,071 days.

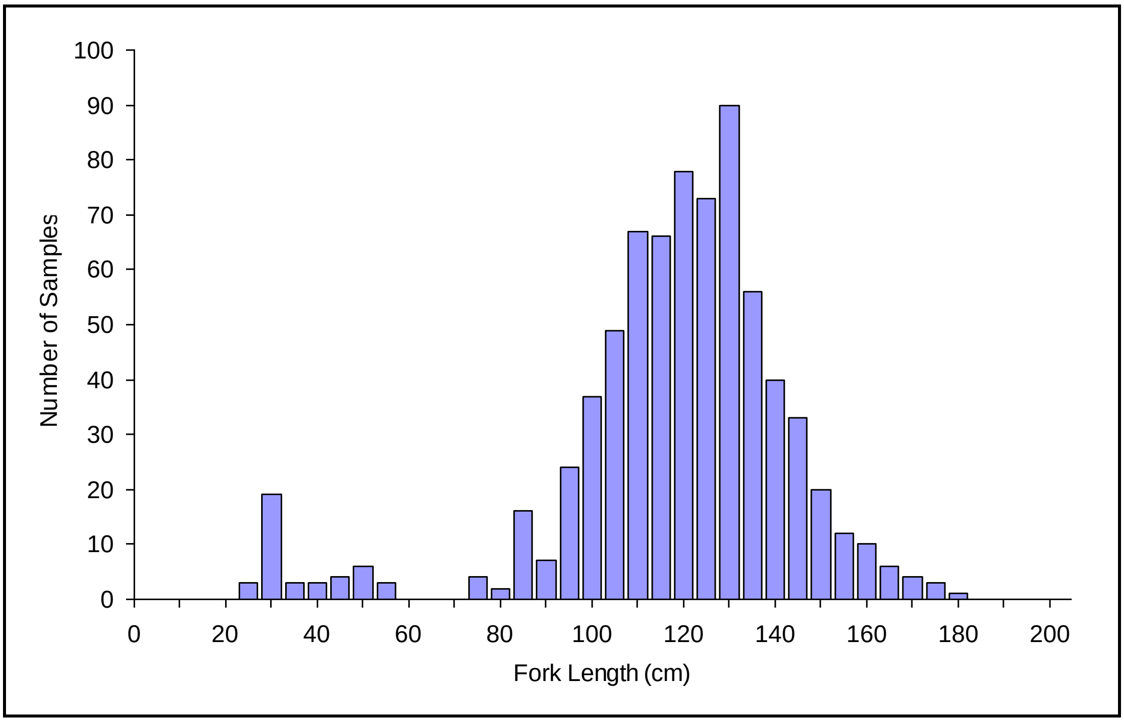

Figure 1. Size of bigeye tuna from which samples have been collected and archived (*N = 772*).

When this project began, the CSIRO archives held some BBL scales and fin rays that had been collected previously by Commonwealth observers but no otolith samples had been collected. It was only when samples were specifically required for this pilot project that a procedure for collecting the broadbill swordfish otoliths was established. A structured sampling plan was implemented that aimed to collect a representative sample of hard parts from the exploited population.

Otoliths from BBL are difficult to sample; they are smaller than a match head and extremely fragile and hence difficult to extract from the skull, especially while at sea. However, as broadbill heads are normally removed during processing, our collectors were able to sample the otoliths without having to use the difficult drilling technique required for BET. Some of the sampling was shore-based and for this we asked that vessels kept some of the broadbill heads they normally discarded and also record information such as length, weight and sex and retain this with the fish.

Sampling commenced in August 1998 and since BBL are generally targeted around the time of the full moon, this was when most samples were collected. By May 2000, 310 fish had been sampled for hard parts (see Fig. 2). Due to the difficulties encountered in using vertebrae for age determination in SBT and the lack of success in their use in other studies on BBL, we did not make collections of vertebrae in this study.

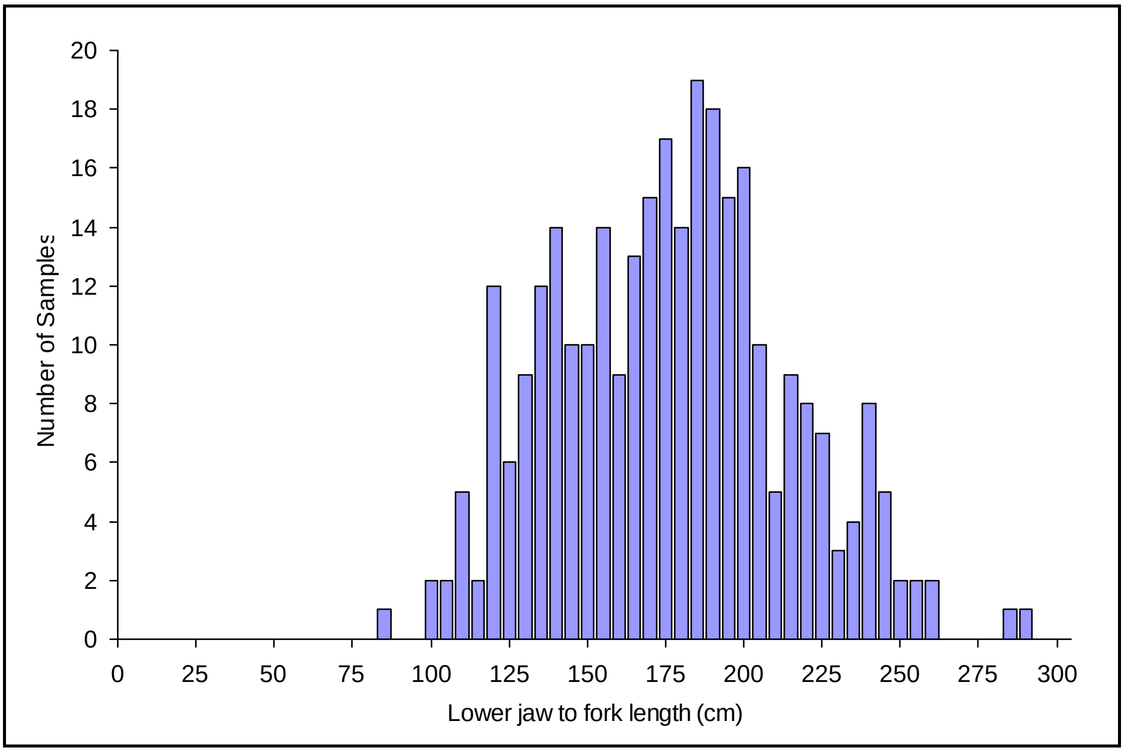

Figure 2. Size of broadbill swordfish from which samples have been collected and archived (*N = 310*).

4.2 Age determination and validation

The second objective of this project was to assess a range of age estimation techniques for use with hard parts of BET and BBL. It has been demonstrated that environmental and physiological factors that affect the growth of fish over a year may be reflected in their otoliths or other hard parts, producing a pattern that can be counted, like growth rings in a tree cross-section. Our first task was to determine if such a pattern was evident in the hard parts of BET and BBL. Techniques that have been successfully used to estimate the age in other species were trialed. However, there are uncertainties in doing this: techniques useful for estimating age in one species are not necessarily transferable and we found that we needed to modify the techniques so that they were more suitable for the species and size of fish being aged.

4.2.1 Bigeye tuna

Over the last seven years, CSIRO has established expertise in age determination of SBT using direct methods. SBT and BET are closely related species, reflected in the many similarities apparent in their otoliths. Hence, our first step in attempting to estimate ages of BET was evaluating two methods that were successful in determining age of bluefin as possible techniques for use with BET.

Fifty otoliths were selected from the 772 in the CSIRO archives; the fish from which the 50 otoliths were sampled were from the full size range in the archives. This number of otoliths was adequate to trial relevant age estimation techniques and determine the most appropriate for the species.

4.2.1.1 Estimating age

Bigeye tuna otoliths were examined for annual increments that, in other species, are evident as opaque and translucent banding. To observe the increments we prepared paired otoliths using two techniques and compared the results; this was possible as fish have two sets of otoliths. We burnt one otolith from each pair to accentuate the banding pattern on the surface: otoliths were burnt on a 400°C hotplate (following the method of Thorogood [1987]) until they turned brown. The matching otolith from the pair was sectioned to determine if the increments were visible internally. Both techniques involve counting the broad zones of opaque and translucent material deposited alternately; one opaque zone and one subsequent translucent zone are considered to be one increment.

Counts of increments in whole and sectioned otoliths from the same fish were made blind (without knowledge of the size of the fish or any previous estimates), by a reader who has extensive experience from previous age and growth studies. Replicate readings were made, separated by a period of not less than one month. A third reading was made to determine a final age estimate (FAE) for each fish. This reading was made with the knowledge of the two previous readings.

4.2.1.2 Validation of age estimates

To validate that the increments in otoliths were annual, i.e. to prove that the structures counted as 'years' were, in fact, deposited annually we used chemically-marked otoliths that had a 'time-stamp' induced by injecting fish with strontium chloride ($SrCl_2$). Bigeye tuna were tagged and injected with $SrCl_2$ as part of the SPC/CSIRO tagging program in the Coral Sea between 1992 and 1995. The returned otoliths were prepared according to the methods described in Clear et al. (2000).

The time at liberty was calculated from the date of tagging and injection, and the date of recapture (Table 1). From the time at liberty we determined the number of increments expected after the position of the strontium mark on the otolith; if the time at liberty after tagging was, for example 3 years, we would expect to count 3 increments on the otolith after the strontium mark.

Sectioned otoliths were viewed firstly using light microscopy to determine the number and position of increments. Images were then obtained using a scanning electron microscope (SEM) which visualized the band produced by an uptake of strontium immediately after injection of $SrCl_2$. Once we had measured the position of the Sr mark on the otolith section, we then compared the number of increments formed since tagging with the number expected.

Knowing the period at liberty after tagging and Sr-injection validated the number of increments counted after the strontium mark but it was also necessary to confirm the counts of increments before the strontium mark. Scientists from SPC have been studying the microstructure of bigeye otoliths to determine age in days. So far, they have been able to confidently estimate age of bigeye up to about three years using microincrements in otoliths. We used these estimates to verify our counts of presumed annual increments deposited in the first years of life, i.e. those increments deposited before tagging and Sr-injection.

4.2.2 Broadbill swordfish

To determine the best method for estimating age in this species we examined two hard part structures: otoliths and anal fin rays. Fifty samples were selected from the 310 in the CSIRO archives; these came from the full size range of fish sampled. This number was adequate to trial relevant age estimation techniques and determine the most appropriate for the species.

4.2.2.1 Estimating age

The fifty fin rays collected from BBL were prepared for examination by cleaning the soft tissue from around the fin ray, dividing the bilaterally-paired rays in half, and drying them completely at 28°C. The second ray from the anal fin has produced good results in previous studies of swordfish from other parts of the world (Berkley and Houde,1983; Esteves, 1995; Tserpes and Tsimenides, 1995; Sun et al., 1999), so we performed trials to establish the best techniques to section these fin rays. The results of the trials indicate that the optimum section varies with the size of the fish. However, for a fish of about 140 cm orbit (eye) to fork length, a cross-section cut 10 mm away from the condyle (at the base of the ray) and approximately 1 mm thick is best. On such sections, we could count translucent and opaque zones that were evident as light and dark bands when viewed with transmitted light.

Unfortunately, there are uncertainties in counts from fin rays. Resorption of calcium may occur when the animal is under stress and the centre of fin ray sections from bigger fish can be obscured. Both of these problems may result in underestimating the age of the fish. To examine the possibility that we were underestimating age of larger fish we compared measurements made on the sections from fish of all sizes. Measurements were made from the central core (or focus) to the margin and to each annulus.

We examined twenty-five sets of BBL otoliths, both whole and sectioned. To find the optimum section for age estimates, the otoliths were cut in several planes, and ground and polished to a range of thicknesses.

4.2.2.2 Validation of age estimates

We attempted to apply the technique of marginal increment analysis to look for seasonal patterns in increment formation. Basically, we are measuring the amount of growth in the ray since the last increment was completed. As we have a large size range of fish, we expect considerable differences in the width of the last increment due to fish size/age. So we standardized the marginal increment measurement by expressing it as a proportion of the size of the previous increment.

Table 1. Release and recapture details of recaptured bigeye tuna injected with $SrCl_2$.

BET #	Release			Recapture			Days at Liberty	Growth (cm/ month)
	FL (cm)	Date	Position	FL (cm)	Date	Position		
37	72	13/11/92	16.58°S 146.73°E	85	31/07/93	16.5°S 146.38°E	260	13
57	75	06/10/95	17.15°S 147.97°E	128	14/08/97	18.25°S 151.91°E	678	53
59	96	12/11/92	16.48°S 147.77°E	159	15/07/98	16.96°S 146.89°E	2071	63
62	109	09/10/95	17.15°S 147.97°E	123	03/05/96	17.28°S 147.16°E	207	14
63	83	06/10/95	17.15°S 147.97°E	94	10/06/96	17.61°S 147.94°E	248	11
64	79	06/10/95	17.15°S 147.97°E	*	*	*	-	-
65	78	09/10/95	17.15°S 147.97°E	128	26/01/98	16.66°S 146.93°E	840	50
66	84	09/10/95	17.15°S 147.97°E	129	18/12/97	16.96°S 147.16°E	801	45
67	78	09/10/95	17.15°S 147.97°E	*	04/11/97	16.16°S 146.41°E	757	-
576	72	12/11/92	16.48°S 146.77°E	156	06/09/98	16.21°S 146.33°E	2124	84
591	80	09/10/95	17.15°S 147.97°E	139	02/11/98	17.10°S 146.80°E	1120	59

*Recapture details not known

5. RESULTS AND DISCUSSION

We have determined from examining the hard parts of BET and BBL that there are patterns of increments evident in the otoliths of both species and in the fin rays of BBL.

5.1 Bigeye tuna

5.1.1 Otolith measurements

The sagittal otoliths of BET have essentially the same appearance and structure as those from SBT. Otolith weight and various lengths of lines on whole and sectioned otoliths (shown in Figures 3 and 4) were measured to determine the relationship between otolith size and fish length.

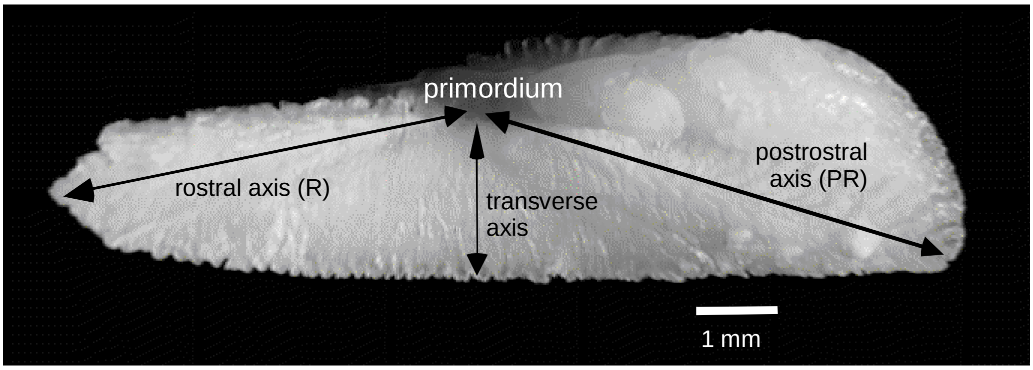

Figure 3. Whole sagittal otolith of a bigeye tuna and the axes that were measured.

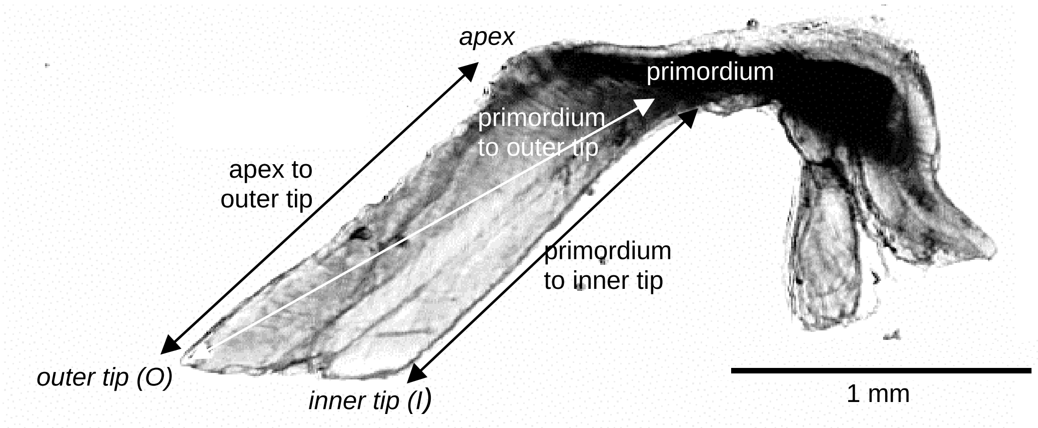

Figure 4. The whole otolith was sectioned along the transverse axis to obtain a cross-section that was used to count increments.

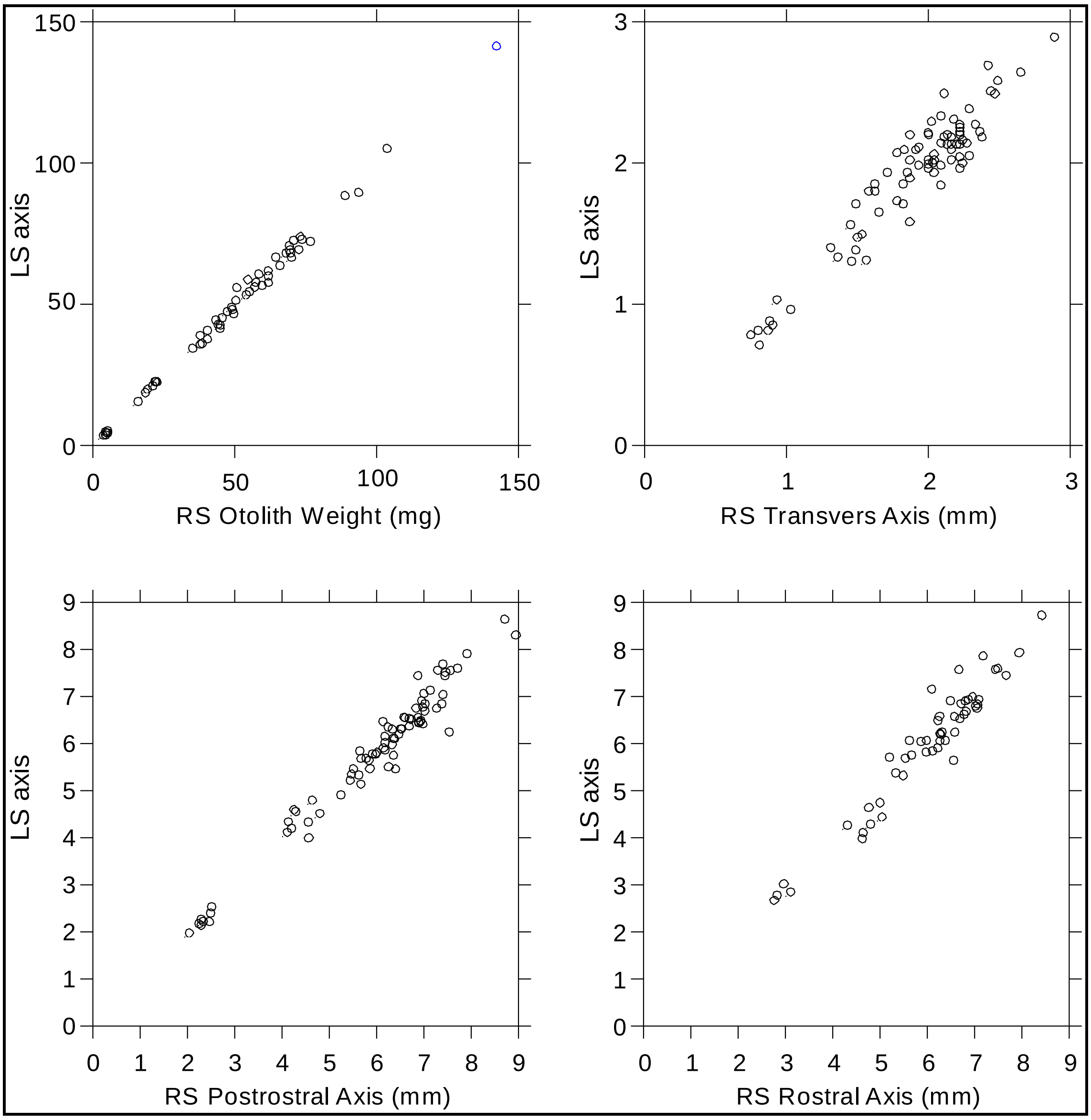

Figure 5. Comparison of left and right sagittal otoliths: otolith weight (*N = 65*), transverse axis (*N = 80*), rostral axis (*N = 60*) and postrostral axis (*N = 79*). LS = left sagitta, RS = right sagitta.

The length of the transverse, rostral and postrostral axes and otolith weight did not differ between left and right sagitta (Figure 5), so otoliths from either side could be interchanged for measurements. The relationship between whole and sectioned otolith measurements and fish length are presented in Table 2 and Figures 6 and 7, respectively. The best relationship was found to be a power curve of the form:

$$L = a * Y^b$$

where L = fish length (FL) in cm
Y = otolith axis measurement in mm
a and b are parameters

Table 2. Parameters and R-square for relationship between otolith measurements and fish length (cm) in bigeye tuna.

Otolith measurement (mm)	a	b	R-square
Whole otolith			
Transverse axis	0.161	0.543	0.871
Postrostral axis	0.365	0.604	0.908
Rostral axis	0.524	0.535	0.9
Sectioned otolith			
Primordium to inner tip	0.104	0.601	0.876
Primordium to outer tip	0.176	0.556	0.887
Apex to outer tip	0.063	0.727	0.89

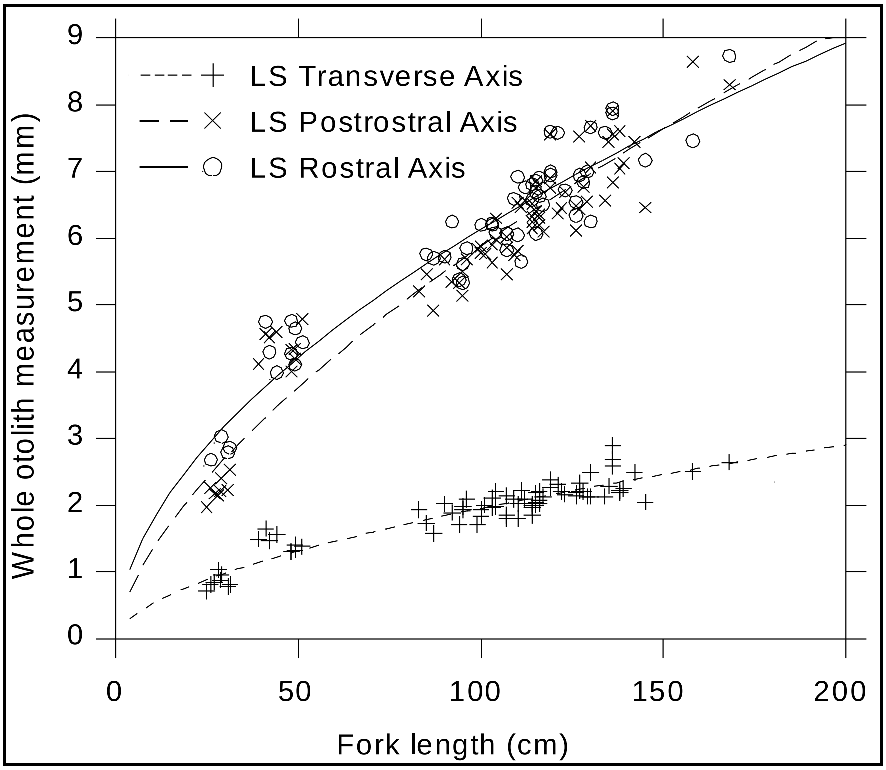

Figure 6. Relationship between length of transverse, postrostral and rostral axis in sagittal otoliths and fish length in bigeye tuna.

While there were no small fish to determine the intercept of the curves it is clear that a straight line would have been a poor fit of the data. All otolith measurements provided about the same R-squared and could be used to predict fish length.

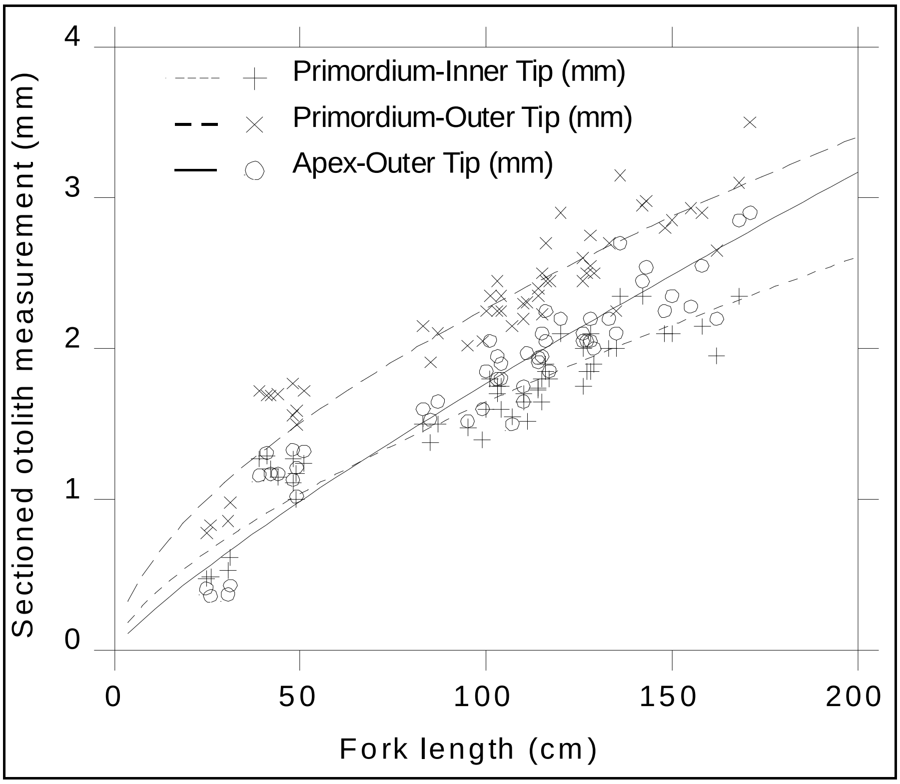

Figure 7. Relationship between length of transverse, postrostral and rostral axes in otoliths and fish length in bigeye tuna.

5.1.2 Measure of precision/reproducibility in age estimates

We used the estimates made on the sectioned otoliths in our analyses; the whole otolith method did not provide reproducible results, i.e. there were large differences between replicate readings and the reader assigned every reading a low confidence level. The otolith sections used for estimates were those made along the transverse axis (Figures 3 and 4) – it was the quickest section to prepare (rostral and postrostral sections required more time) and, along the cross-section of the transverse axis, increments were visible and clear enough to count. One section was damaged during preparation and therefore excluded but we were able to make age estimates from all other sections prepared.

The average percent error (APE) of Beamish and Fournier (1981) was determined to compare age estimates between subsequent blind readings. The index provides a measure of the precision or reproducibility of age estimates. The APE for all readings was 19.7%, which exceeds the acceptable level of 10%. However, exclusion of readings of 0 age fish reduces the APE to 5.5%. This occurs because the APE uses the difference between all age estimates and divides by the mean. So, if the first estimate is 1 and the next is 0 (i.e. first increment not complete), this has a large effect. From Table 3 it can be seen that a number of the first age estimates were 0 years whereas they were aged one year the second time. If age estimates are 19 and then 20 it doesn't affect the APE very much. Our results, however, do highlight the difficulty of detecting the first annulus. Results from the age and growth study by SPC produced age estimates in days for young fish and these confirmed the location of our first annulus. Hence, we do not expect detection of the first annulus to present a problem in a full-scale age study of BET in the future.

Table 3. Comparison of age estimates by reading 1 with the average age estimates of readings 1 and 2.

Reading 1 Age (years)	Mean reading Age (years)	Number of fish
0	0.0	6
1	0.4	10
2	2.5	11
3	2.8	5
4	3.9	7
5	4.6	7
6	6.0	2
7	8.0	1
Total		**49**

An age bias graph was plotted to look for bias in determining age over the range of ages estimated (Figure 8).

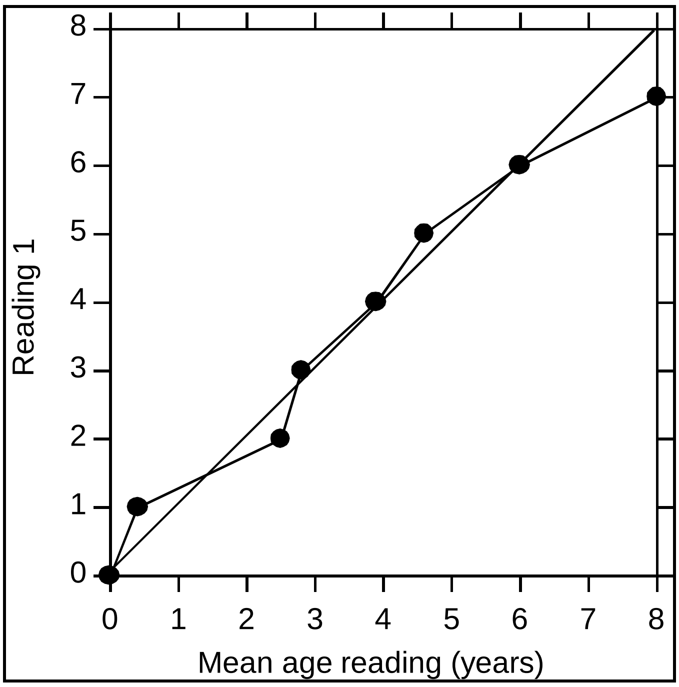

Figure 8. Age bias graph for two blind age determinations of bigeye otoliths. The 1:1 equivalence line (straight) is also plotted.

There is no marked bias in age estimates apart from that mentioned previously regarding 0+ age fish. It would be expected that there would be less apparent bias if estimates of a large sample of fish had been made (beyond the scope of this pilot study).

5.1.3 Validation of age estimates from Sr marks

Sectioned otoliths were viewed firstly using light microscopy to determine the number and position of increments. Images were then obtained using an SEM, which visualized the band produced by uptake of strontium immediately after injection of $SrCl_2$. Strontium marks were obvious as bright bands in the 10 sagittal otoliths examined in the SEM (SEM images are shown in Appendix 1).

The nine fish for which we had recapture information were at liberty from 207 to 2,071 days (details in Table 1). 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 (see Table 4).

The positions of the $SrCl_2$ bands in all marked fish indicated that an increment was deposited each successive year at liberty after marking. This confirms the periodicity of increment formation: increments are deposited annually. An example is BET #59 (Figure 8): five increments were observed after the $SrCl_2$ band, which equals the number of years at liberty and hence the number of increments expected.

Table 4. 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
FL at tagging (cm)		72	75	96	109	83	79	78	84	78	80
FL at recapture (cm)		85	128	159	123	94	-	128	129	-	139
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)
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**
	observed	**1**	**1**	**5**	**1**	**1**	**1**	**2**	**2**	**2**	**3**
Age estimate (this study) *		2	3	8	3	2	2	3	3	3	4
Age at tagging **		1.2	1.3	2.1	2.7	1.6	1.5	1.4	1.6	1.4	1.5
Age at recapture **		1.7	3.8	8.6	3.5	2.0	-	3.8	3.9	-	4.8
Month of recapture		July	Aug	July	May	June		Jan	Dec	Nov	Feb
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
	Sr (I) -I	0.26	0.56	0.80	0.15	0.16	0.25	0.54	0.63	0.77	0.50

* 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).

Figure 9A. Light microscope view.

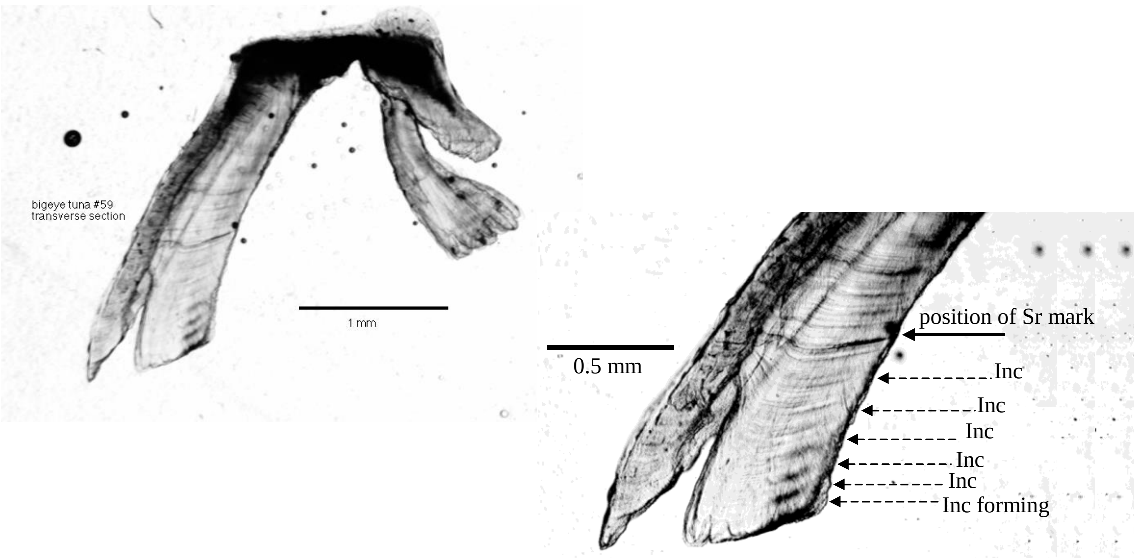

Figure 9B. Scanning electron microscope view.

Figure 9. Examples of the two views of an otolith section (BET #59). Firstly, sections were viewed under light microscope (Fig. 9A) to estimate the age of a fish, then the strontium mark was revealed in the SEM (Fig. 9B). Inc = the position along the otolith section where a completed increment was counted.

We compared our age estimate with the age at recapture estimated using the growth curve derived from otolith microincrement data (Hampton et al., 1998) and found reasonable agreement (see Table 4). In all cases 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 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 overestimated 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 by knowing the period at liberty after tagging and injection.

5.1.4 Age estimates

The ages of the BET examined were estimated to range from 0 to 10 years. Growth curves are usually generated from larger sample sizes than used in this pilot study. However, it is useful to see whether our preliminary age estimates generate meaningful growth curves for this species. The age/length data and fitted von Bertalanffy growth curve have been plotted in Figure 10 and the parameters of the curve are in Table 5. The growth curve falls within the range determined from tag and length frequency data in the Pacific region (Kume and Joseph, 1966; Hampton et al., 1998).

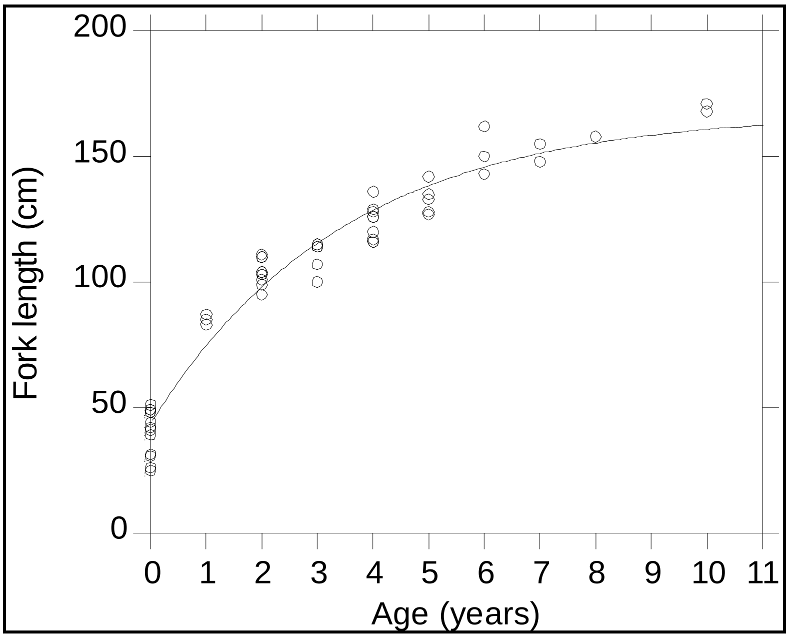

Figure 10. Age estimates for bigeye tuna and fitted growth curve (*N = 55*).

Table 5. Parameters of von Bertalanffy growth curve for bigeye tuna.

Parameter	Estimate	S.E.	Lower 95% CL	Upper 95% CL
L_∞	167.9	6.177	155.5	180.3
k	0.29	0.031	0.229	0.352
t_0	-1.010	0.113	-1.237	-0.783

Results indicate that the techniques we have developed to age BET using otoliths are appropriate and reliable. Strontium-marked otoliths (with a chemically-induced 'time-stamp') have enabled us to validate our age estimates; the results prove that increments counted on the bigeye otoliths are, in fact, deposited annually. These results, together with results from the recent SPC study of microincrements in otoliths from fish up to three years, make us confident that we can determine ages of BET accurately.

5.2 Broadbill swordfish

5.2.1 Examination of hard parts

The otoliths of BBL are extremely small and fragile. The sagitta is the largest of the three otoliths and has a complex structure (Figure 11). To determine if the otoliths contained meaningful structure that could be used for age estimates, otoliths were sectioned along the rostral-postral axis and examined under a light microscope. An example is presented in Figure 12.

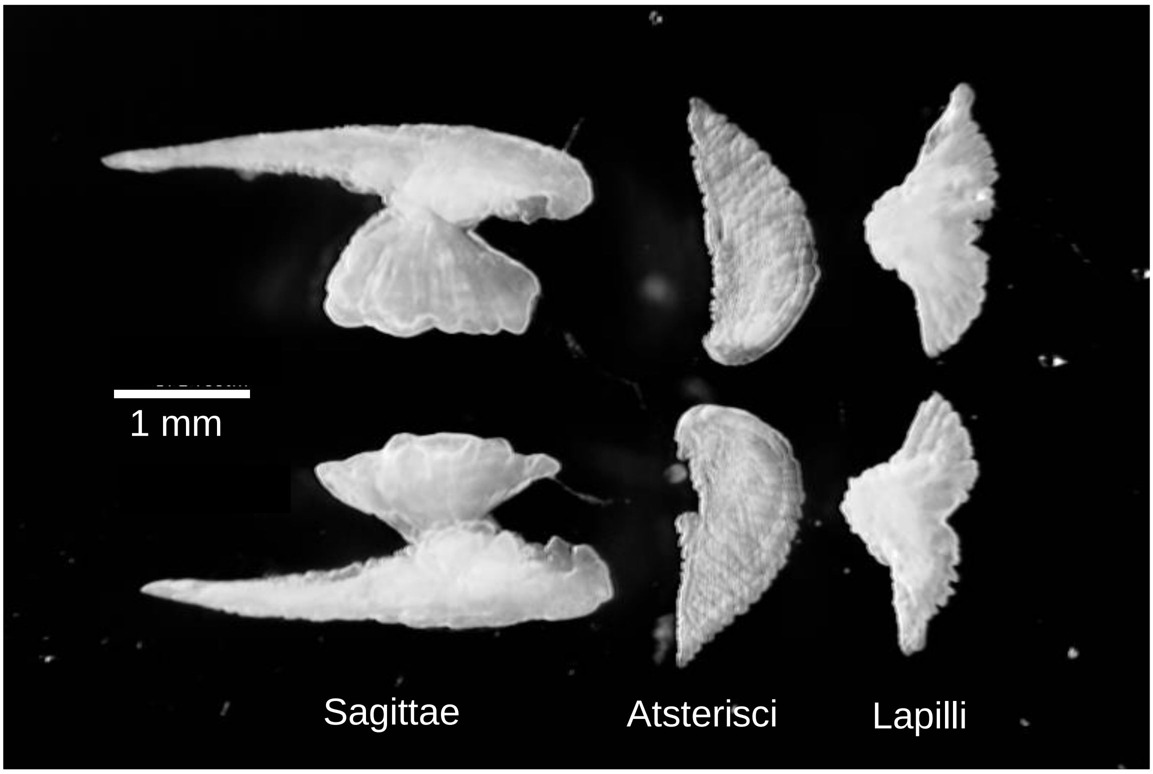

Figure 11. A set of otoliths from a 138 cm eye-fork length (OFL) broadbill swordfish.

We found obvious microincrements (Figure 12) known to be deposited daily in other species. Castro-Longoria and Sosa-Nishizaki (1998) counted these presumed daily increments and used the counts to verify the number of annuli counted on the fin rays from the same fish. They report close agreement between the otolith and fin ray counts in fish less than 2 years. Similar to their study, we did not seen patterns of growth in the otoliths that equate to seasonal or annual growth so, because of the lack of obvious annual structure, we did not pursue age determination using otoliths any further.

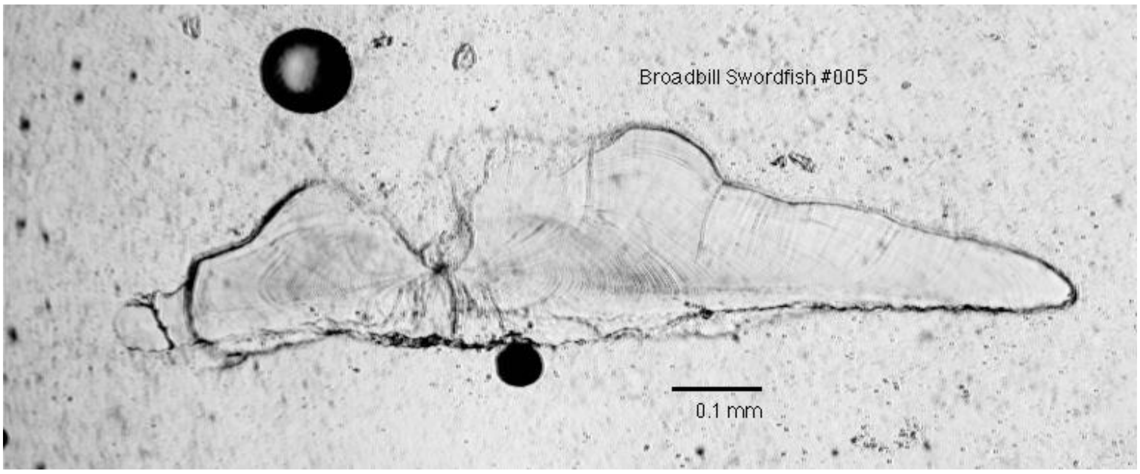

Figure 12(a). Longitudinal rostral-postral section of a broadbill swordfish sagittal otolith.

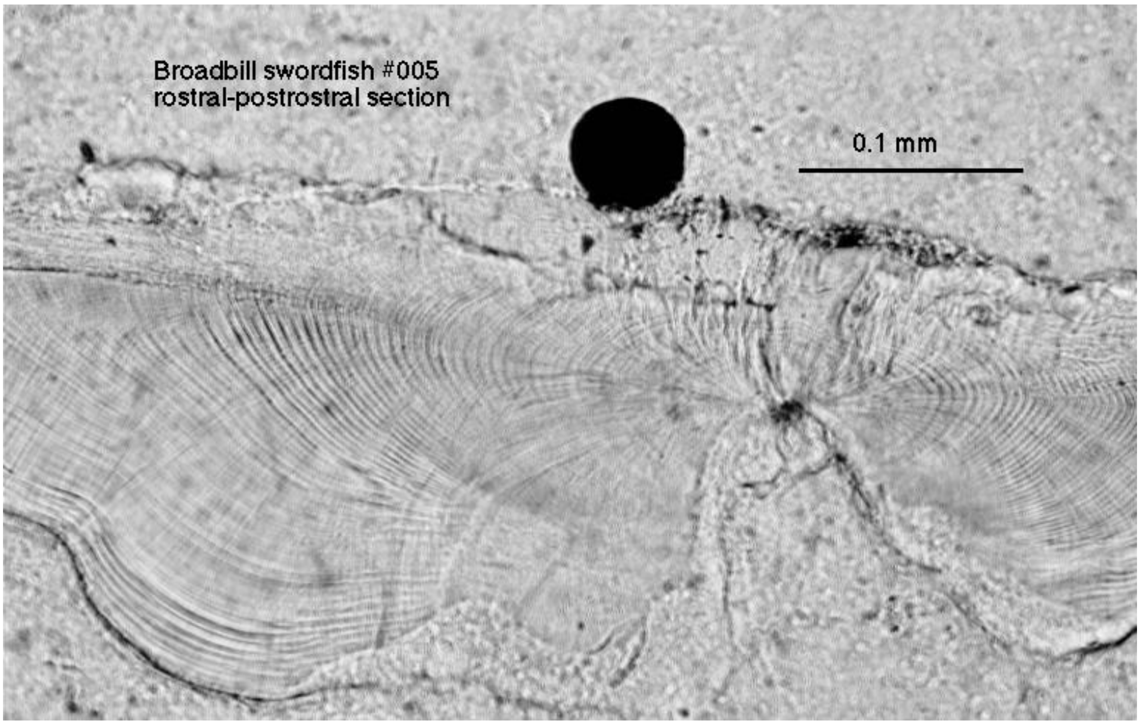

Figure 12(b). Increased magnification of same specimen. Note the increments around the primordial area.

Sections taken from the second anal fin rays of BBL had a clear structure of alternating opaque and translucent zones (Figure13); typically the translucent zone was much narrower. The thinner, translucent zones radiating out from the centre of the ray are known as annuli (meaning 'ring', i.e. not necessarily indicating an 'annual' event). We were able to count annuli on all fifty fin ray sections examined.

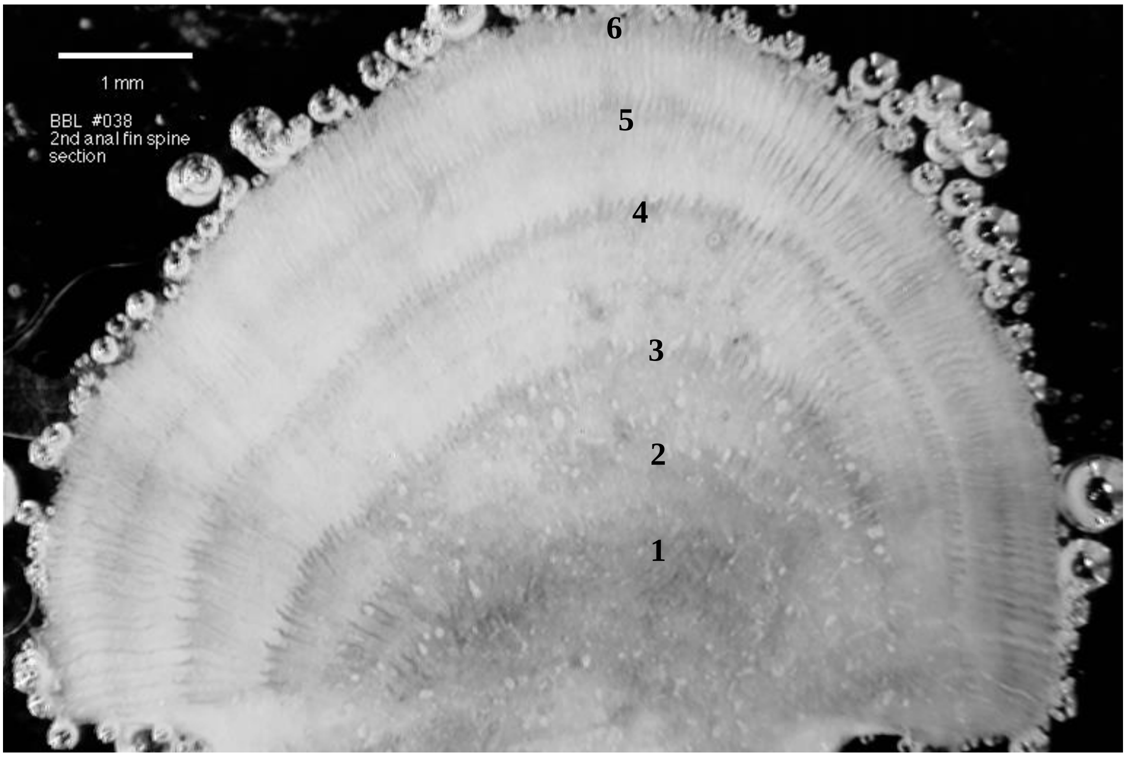

Figure 13. Section of a broadbill swordfish second anal fin ray. Six annuli were counted on this section.

5.2.2 Anal fin ray measurements

We determined the relationship between fin ray radius and fish length in BBL. There was a good relationship (Figure 14) and we fitted a power curve to the data as we did for BET. While we did not have measurements on small fish to define the intercept of the line it is clear that a straight line would not have passed any where near the origin of the x and y axes. The relationship was:

$$L = 78.8 * S^{0.535}$$

where L = fish length (LJFL) in cm
S = fin ray radius in mm
R-square = 0.811

5.2.3 Measure of precision/reproducibility in age estimates

The APE of Beamish and Fournier (1981) was determined to compare age estimates between subsequent blind readings. The index provides measure of the precision or reproducibility of age determinations. The APE for all readings was 9.77 % and this is within the acceptable level of 10%. The age bias plots indicate that there is little or no bias over the age range of BBL that was examined (Table 6 and Figure 15).

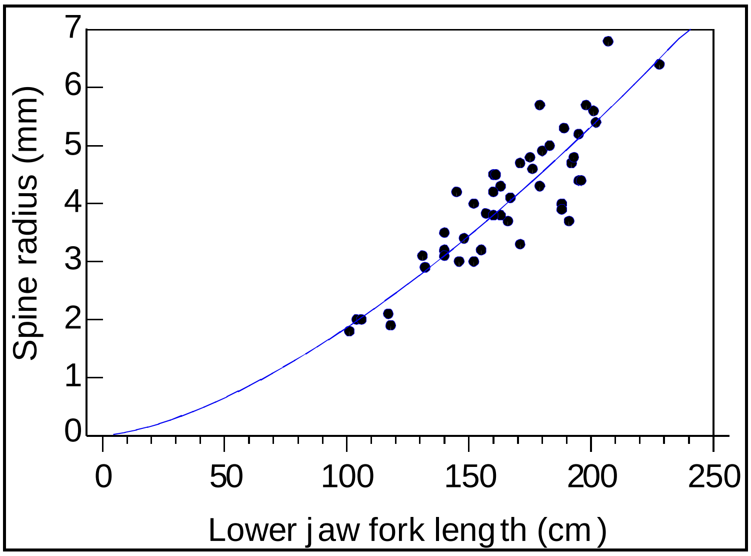

Figure 14. Relationship between fin ray radius and length in broadbill swordfish (*N = 50*).

Table 6. Comparison of age estimates by reading 1 with average age estimates of both readings.

Reading 1 Age (years)	Mean reading Age (years)	Number of fish
1	1.2	8
2	2.4	5
3	3.1	12
4	4.0	5
5	5.0	11
6	5.9	5
7	6.8	2
9	8.0	1
11	11.5	1
Total		**50**

5.2.4 Detection of increments

While we obtained good reproducibility between readings there are suggestions of problems in measuring the position of individual annuli with increasing fish size. The radius of annulus 4 and greater appear to be dependent on fish size (Figure 16). The radius of increments 4-5 increase with fish size. This does not appear to be a problem for increments 1-3 and none of the regression lines in Figure 16 are significant. The trend is probably caused by the way the fin ray grows and is something that any future age determination study would need to address.

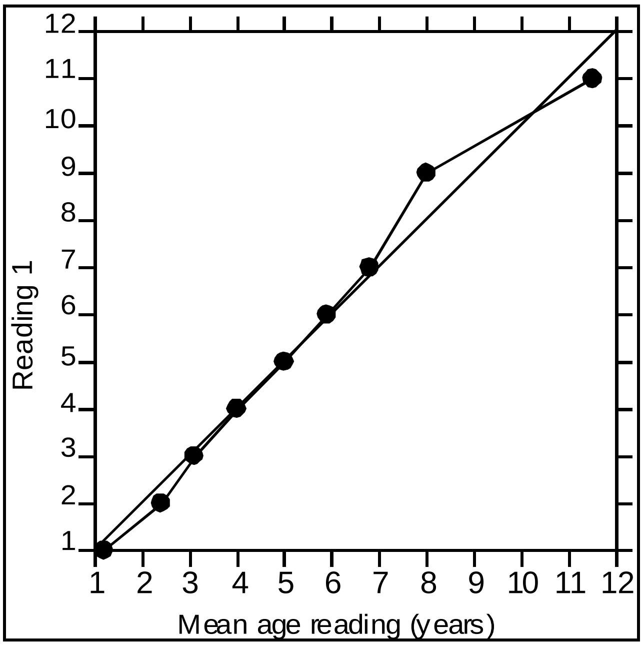

Figure 15. Age bias graph for two blind age determinations of broadbill swordfish fin rays. The 1:1 equivalence line (straight) is plotted.

5.2.5 Validation of age estimates by marginal increment analysis

The marginal increment of individual fish has been plotted against time of year (Figure 17), indicating that there is a possibility that the annulus might form around September/October. It is during these months that we see maximum and minimum marginal increments, whereas earlier in the year there are only small increments. If the annulus is close to the edge of the fin ray it may not be possible to identify it until growth has occurred outside it so, around the time of increment formation, you are likely to see fin rays with small increments and fin rays with large increments. We have only a limited seasonal coverage of samples. The limitations of the small data set do not enable us to see anything more than a suggestion of a seasonal change in the size of the marginal increment. Using marginal increment analysis Ehrhardt (1992) and Ehrhardt et al. (1996) reported that marginal increment formation is at a maximum in summer and is reduced during winter in the northwest Atlantic. An annual check in growth has been corroborated by Tserpes and Tsimenides (1995) for annuli 1-4 in broadbill swordfish from the Mediterranean, but they showed that the annuli are formed in spring to early summer. Tserpes and Tsimenides (1995) also caution against assuming that annuli are formed annually in fish older than this until marginal increment analysis has been carried out on these age groups. Any age determination study in the AFZ would need to address validation by marginal increment analysis as one of its first objectives.

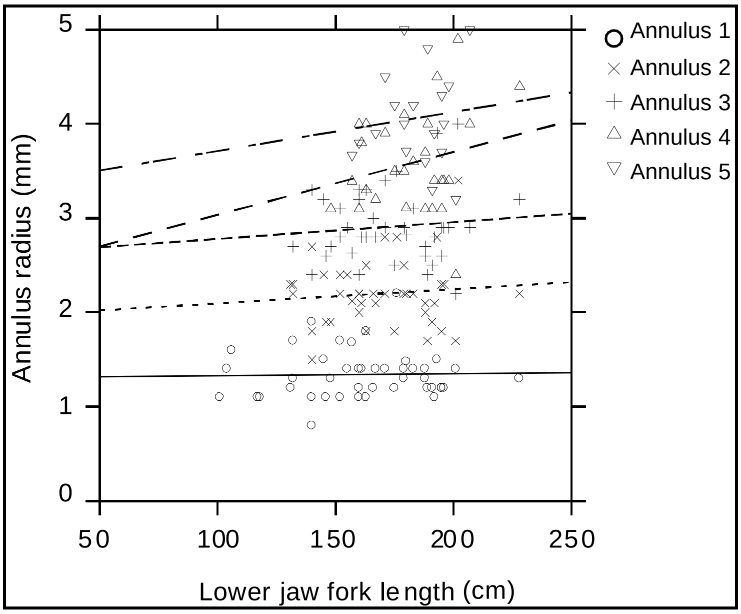

Figure 16. Radius of annulus 1-5 (from center of the fin ray to the annulus) by fish length in broadbill swordfish.

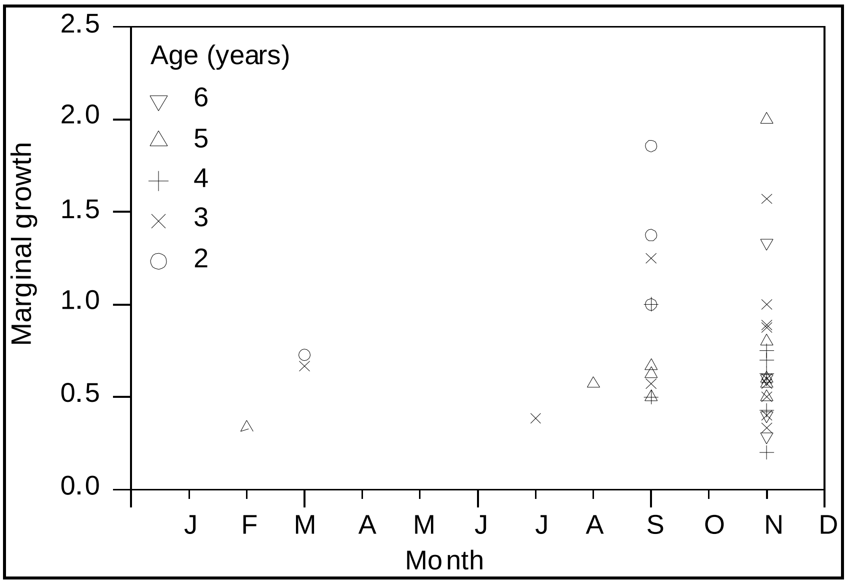

Figure 17. Marginal increment (standardized against width of previous increment) plotted against time of year for broadbill swordfish (*N = 36*).

5.1.4 Age estimates

The ages of 50 BBL were estimated to range from one to 12 years. Growth curves are usually generated from larger sample sizes than used in this pilot study. However, it is useful to see whether our preliminary age estimates generate meaningful growth curves for this species. The age/length data and fitted von Bertalanffy growth curve have been plotted in Figure 18 and the parameters of the curve are in Table 7.

Our estimate of L_∞ = 216.5 cm falls within the value of 203 cm for males and 226.5 cm for females that Tserpes and Tsimenides (1995) calculated using the standard von Bertalanffy growth equation. As our sample of 50 fish included both males and females, we would expect L_∞ somewhere in between the two.

Tserpes and Tsimenides (1995) had difficulty locating the first annulus in BBL fin rays and this got progressively more difficult in larger fish. They estimated the mean size of 1-year-old fish to be 89.7 cm. This is smaller than the smallest fish examined in our study. Our mean size at 1 year was 109.2 cm and 2 years was 134 cm, whereas Tserpes and Tsimenides (1995) estimated them to be 89.7 and 114.9, respectively. This suggests that we may have missed the first annulus. However, the results of studies conducted by NMFS produced a size at age 1 much closer to our own: using counts of microincrements on sagittal otoliths they estimated bigeye tuna to be 1 year at 114 cm LJFL (Sosa-Nishizaki, 1999). The methods of otolith preparation and examination may explain the differences between these results; they would need to be explored more thoroughly in any larger scale study of BBL age and growth. The NMFS research is focused currently on determining sex-specific size at age 1, using counts of microincrements on otoliths from fish less than 125 cm, which may provide further insight into age and growth of BBL during the first years of life.

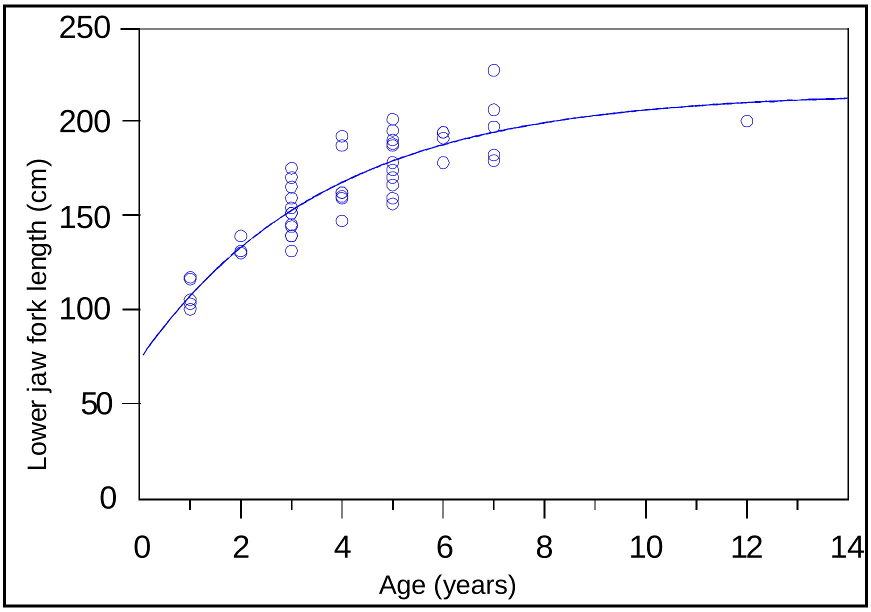

Figure 18. Age estimates for broadbill swordfish and fitted growth curve (*N = 50*).

Table 7. Parameters of von Bertalanffy growth curve for broadbill swordfish.

Parameter	Estimate	S.E.	Lower 95% CL	Upper 95% CL
L_∞	216.5	13.2	189.9	243.2
k	0.27	0.067	0.135	0.408
t_0	-1.552	0.564	-2.689	-0.415

6. CONCLUSION

6.1 Bigeye tuna

We found that the otoliths of BET were similar in appearance and structure to otoliths of SBT. The techniques using sectioned otoliths that were developed to determine and validate the age of SBT also worked successfully on BET. The average percent error of repeated blind readings was acceptable at 5.5% for fish greater than one-year-old. However, we found there was some difficulty in detecting the first annulus. Age estimates by SPC have confirmed the location of the first annulus so this should not present a problem in a full-scale age determination study. The age of older fish was validated through strontium marking experiments. Nine BET that were marked at tagging with strontium and at liberty for 207 to 2,071 days had laid down an increment for each successive year at liberty. This confirms the annual nature of increment formation for fish 2-8 years old. We have therefore established a reliable technique for determining the age of BET through counting annual increments. This will enable us to estimate ages of adequate numbers of BET to determine the age structure of fished populations, investigate sexual differentiation in growth, and determine mean age at first maturity. We recommend that these techniques now be applied in a comprehensive age and growth program on this species to assist in stock assessment studies.

6.2 Broadbill swordfish

We found that BBL otoliths were too small and fragile and lacked the annual structure needed for direct annual age estimates. However, sections taken from the second ray from the anal fin had a clear structure of alternating translucent and opaque zones. We were able to estimates age of BBL presuming that these bands were laid down annually. The average percent error of repeated blind readings was acceptable at 9.77% and there was little evidence of bias in age determinations over the age range of BBL examined. Previous work by Tserpes and Tsimendes (1995) indicated difficulty in locating the first annulus as it becomes obscured as fish get larger. As we did not have samples of fish smaller than 100 cm we could not confirm difficulty in detecting the first annulus. However, our estimate of mean size-at-age 1 is close to that of a study conducted by NMFS using otolith microincrements, in which fish of 114 cm were considered to be 1-year-old. We attempted to validate the annual nature of annulus formation using marginal increment analysis. For this, samples collected year-round are required however, there were some months of the year during which no fin rays had been collected for the archives. We recommend that any age determination study in the AFZ would need to address validation by marginal increment analysis as one of its first objectives.

7. ACKNOWLEDGEMENTS

This project was originally developed by Wade Whitelaw as principal investigator until he was seconded to the SPC. We gratefully acknowledge those who assisted in collecting samples, including Mooloolaba fishing vessel owners, skippers and crew, Mooloolaba Seafood Processors, recreational fishers, Commonwealth observers and CSIRO staff. We also thank

Jessica Farley for her help in preparing the final report. Validation of bigeye age estimates was only possible because of the tagging and strontium marking of BET by CSIRO and SPC in a joint tagging program in the Coral Sea during 1992-95.

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9. REFERENCE LIST

Beamish, Richard J. and Fournier, David A. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences. 38(8): 982-983.

Berkeley, S.A., and Houde, E.D. 1983. Age determination of broadbill swordfish, *Xiphias gladius*, from the Straits of Florida, using anal fin spine sections. In E.D. Prince and L.M. Pulos (eds.), Proceedings of the international workshop on age determination of oceanic pelagic fishes: tunas, billfishes and sharks. U.S. Dep. Commer., NOAA, Tech. Rep. NMFS 8: 137-143.

Castro-Longoria, R and Sosa-Nishizaki, O. Age determination of swordfish, *Xiphias gladius* L., from waters off Baja California, using anal fin rays and otoliths. In, Barrett, I, Sosa-Nishizaki, O and Bartoo, N. (eds). 1998. Biology and fisheries of swordfish, *Xiphias gladius.* Papers from the international symposium on Pacific swordfish, Ensenada, Mexico, 11-14 Dec. 1994. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 142, 276 p.

Clear, N. P., Gunn, J. S. and Rees, A. J. Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, through a large scale mark and recapture experiment using strontium chloride. Fish. Bull. 98:25-40.

Esteves, E., Simoes, P., Da Silva, H. and Andrade, J.P. 1995. Ageing of swordfish, *Xiphias gladius* Linnaeus, 1758, from the Azores, using sagittae, anal-fin spines and vertebrae. Cienc. Biol. Mar./Life Mar. Sci. 13A: 39-51.

Ehrhardt, N.M. 1992. Age and growth of swordfish, *Xiphias gladius*, in the northwestern Atlantic. Bull. Mar. Sci. 50: 292-301.

Ehrhardt, N.M., Robbins, R. J., and Arocha, F. 1996. Age validation and growth of swordfish, *Xiphias gladius*, in the northwestern Atlantic (SCRS/95/99). Collect. Vol. Sci. Pap. ICCAT., vol. 45(2): 358-367.

Hampton, J., Bigelow, K.A. and Labelle, M. 1998. A summary of current information on the biology, fisheries and stock assessment of bigeye tuna (*Thunnus obesus*) in the Pacific Ocean, with recommendations for data requirements and future research. Noumea, New Caledonia, Secretariat of the Pacific Community. Tech. Rep. 36: 1-46.

Iverson, E.S. 1955. Size frequencies and growth of central and western Pacific bigeye tuna. U.S. Fish. Wildl. Serv. Spec. Sci. Rep. Fish. 162.

Kume, S. and Joseph, J. 1966. Size composition, growth and sexual maturity of bigeye tuna, *Thunnus obesus* (Lowe), from the Japanese long-line fishery in the eastern Pacific Ocean. Int. Am. Trop. Tun. Com. Bull. 11(2): 47-99.

Sosa-Nishizaki, O. Ageing swordfish using otoliths. In, DiNardo, Gerad T. (ed). 1999. Proceedings of the second international Pacific swordfish symposium. U.S. Dep. Commer., NOAA Tech. Memo., NOAA-TM-NMFS-SWFSC-263, 246 p.

Sun, C-L., Wang, S-P., and Yeh, S-Z. 1999. Age and growth of the swordfish *Xiphias gladius* L. in the waters around Taiwan using anal fin spines. Standing Committee on Tuna and Billfish Working paper, 16-23 June 1999, Tahiti. SCTB 12, BBRG-7: 1-17.

Tankevich, P.B. 1982. Age and growth of bigeye tuna in the India ocean. J. lethyol. 22(4): 26-31.

Tserpes, G. and Tsimenides, N. 1995. Determination of age and growth of swordfish, *Xiphias gladius* L., 1758, in the eastern Mediterranean using anal-fin spines. Fish. Bull. 93: 594-602.

Thorogood, J. 1987. Age and growth rate determination of southern bluefin tuna, *Thunnus maccoyii* , using otolith banding. Journal of Fish Biology 30(1): 7-14.

Yukinawa, M and Yabuta, Y. 1963. Age and growth of bigeye tuna. Rep. Nankai Reg. Fish. res. Lab. 19: 103- 1 03.

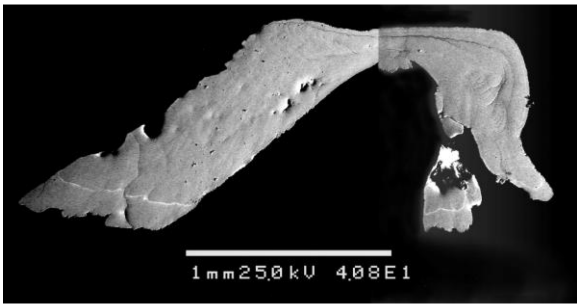
Whitelaw, A.W. and Unnithan, V.K. 1997. Synopsis of the distribution, biology and fisheries of the bigeye tuna, with a bibliography. CSIRO Marine Laboratories Report 228. 62pp.

APPENDIX 1. IMAGES OF STRONITUM-MARKED OTOLITHS

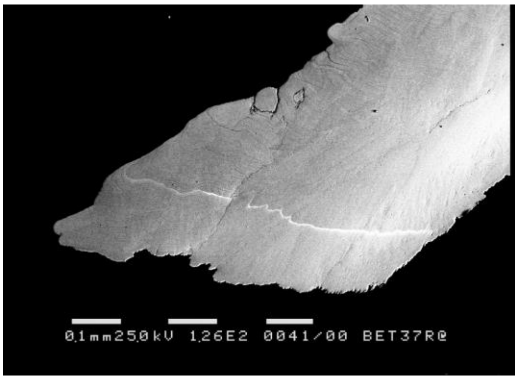
Scanning electron microscope images of otoliths from bigeye tuna (BET) injected with strontium chloride (A and B): the strontium mark is obvious as a bright band on the transverse otolith sections. Increments observed after the position of the strontium mark are indicated (broken lines) on images from the light microscope (C).

BET#37

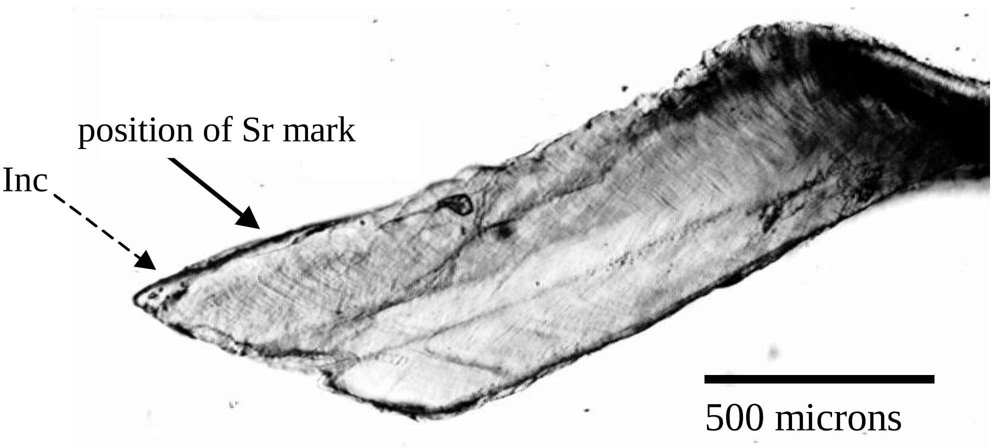
A.



B.

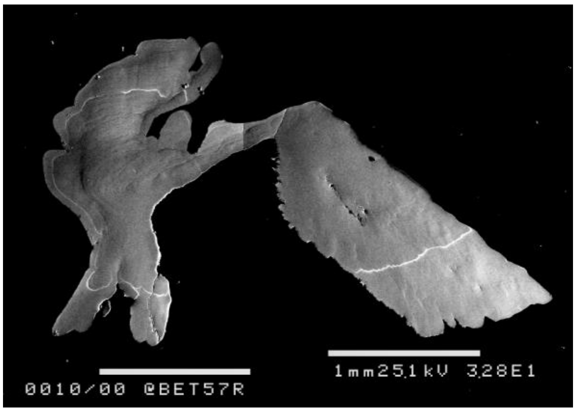


C.

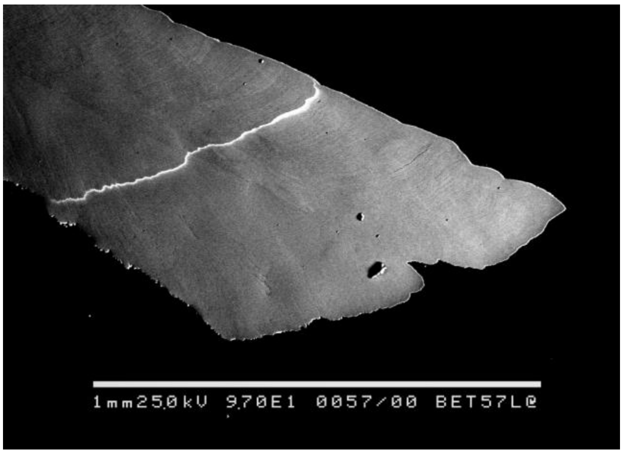


BET#57

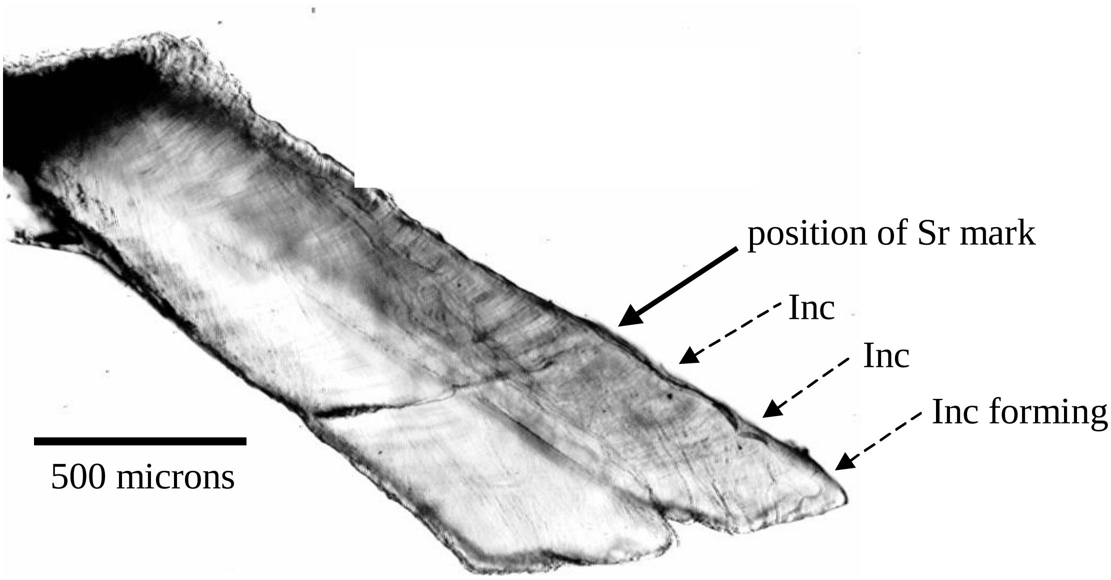
A.


1mm25.1kV 3.28E1
0010/00 @BET57R

B.


1mm25.0kV 9.70E1 0057/00 BET57L@

C.

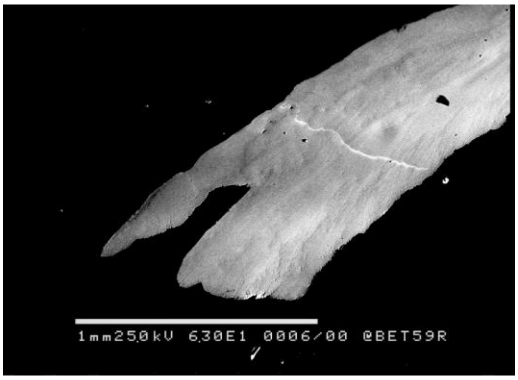

position of Sr mark
Inc
Inc
Inc forming
500 microns

BET#59

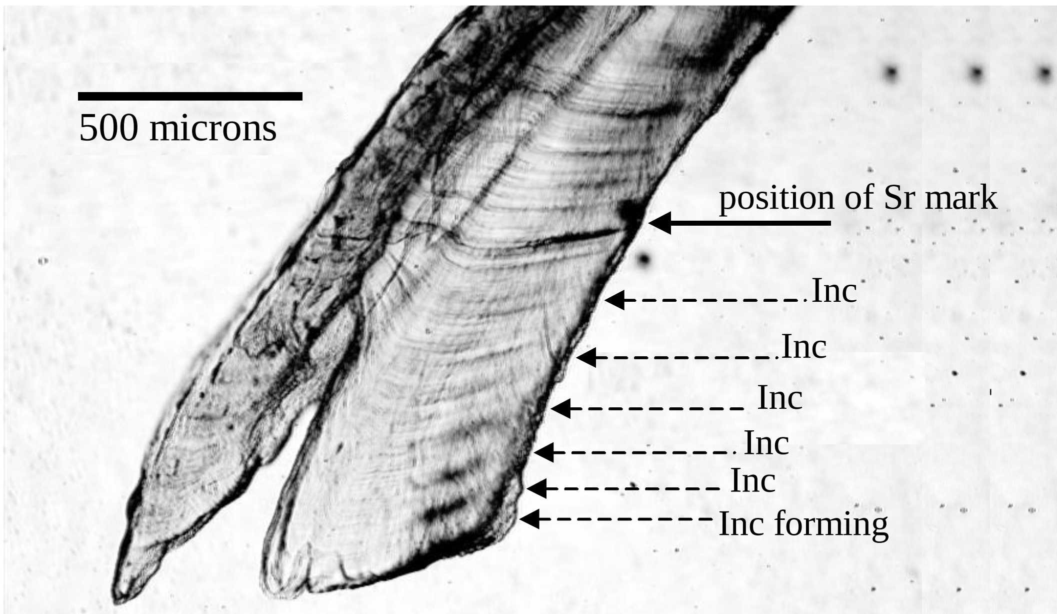
A.


1mm25.0kV 3.14E1 0020/00 @BET59R

B.


1mm25.0kV 6.30E1 0006/00 @BET59R

C.

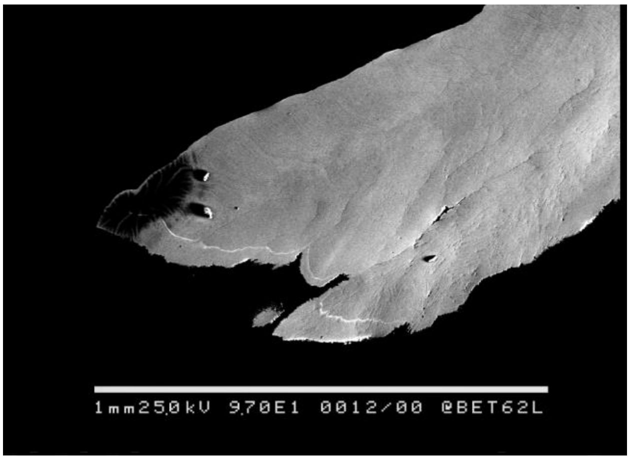

500 microns
position of Sr mark
Inc
Inc
Inc
Inc
Inc
Inc forming

BET#62

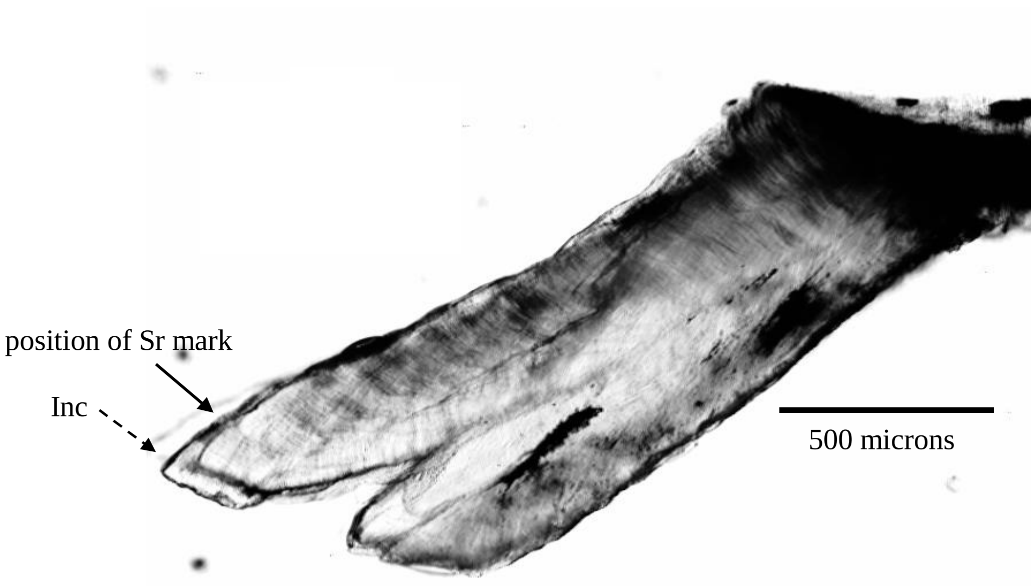
A.


1mm25.0kV 4.86E1 0012/00 @BET62L

B.

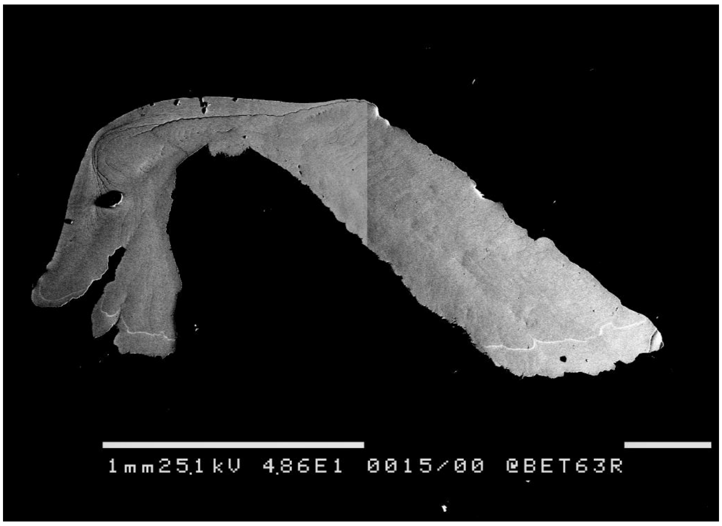

1mm25.0kV 9.70E1 0012/00 @BET62L

C.

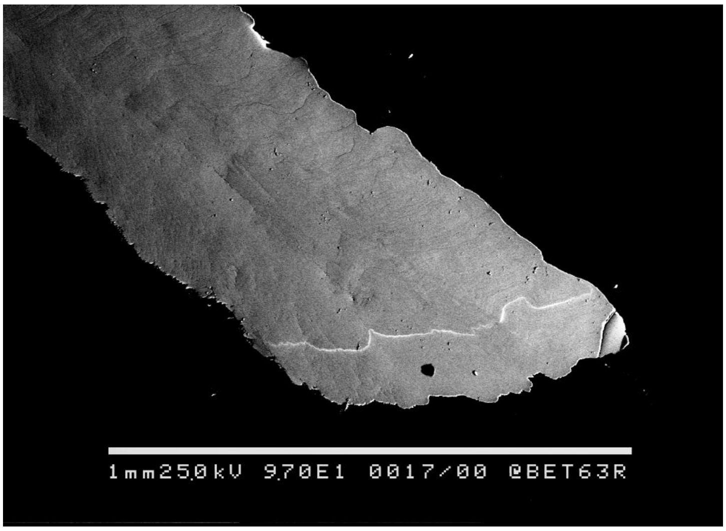

position of Sr mark
Inc
500 microns

BET#63

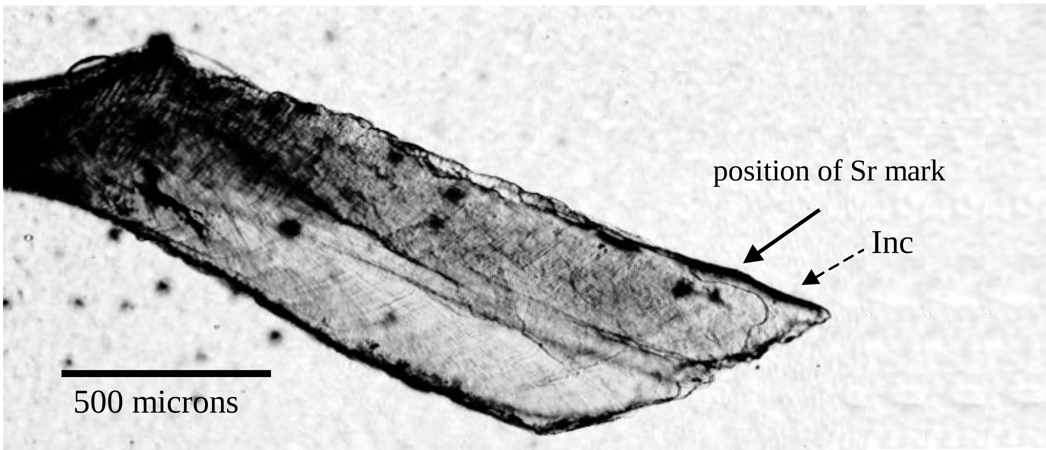
A.


1mm25.1kV 4.86E1 0015/00 @BET63R

B.

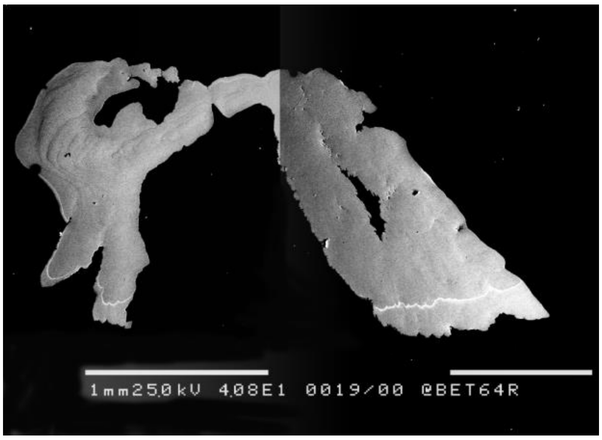

1mm25.0kV 9.70E1 0017/00 @BET63R

C.

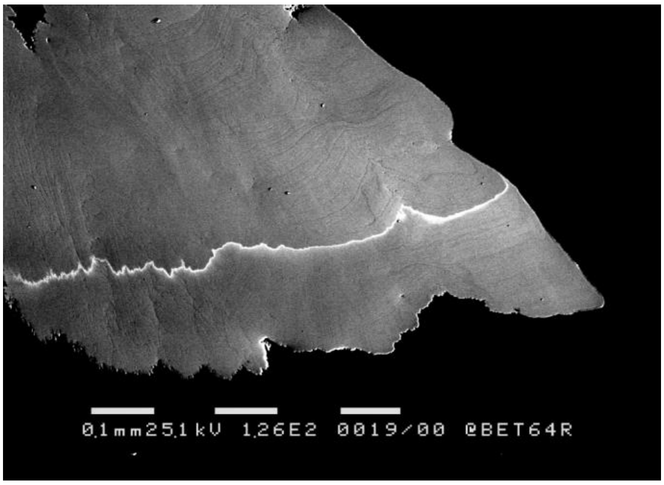

position of Sr mark
Inc
500 microns

BET#64

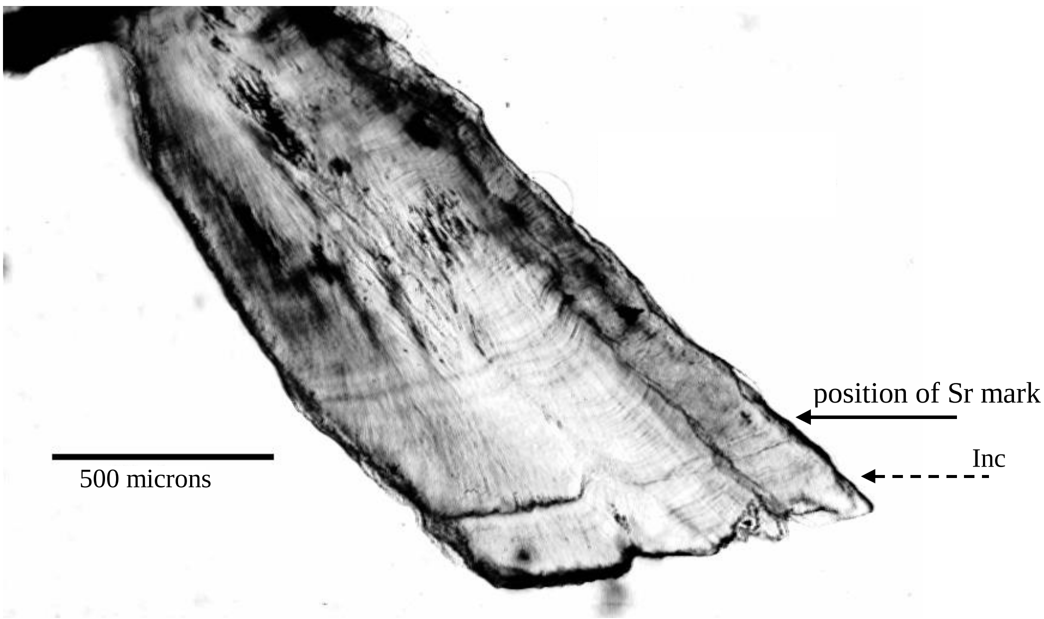
A.


1mm25.0kV 4.08E1 0019/00 @BET64R

B.


0.1mm25.1kV 1.26E2 0019/00 @BET64R

C.

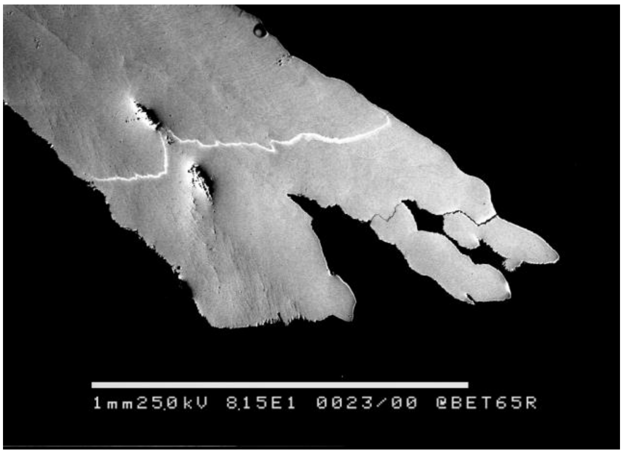

position of Sr mark
Inc
500 microns

BET#65

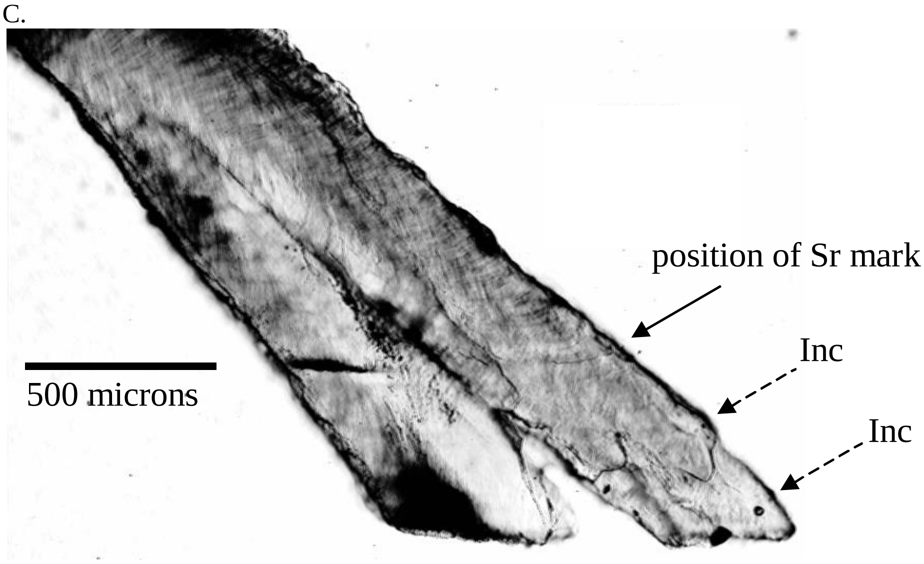
A.


1mm25.0kV 3.44E1 0020/00 @BET65R

B.

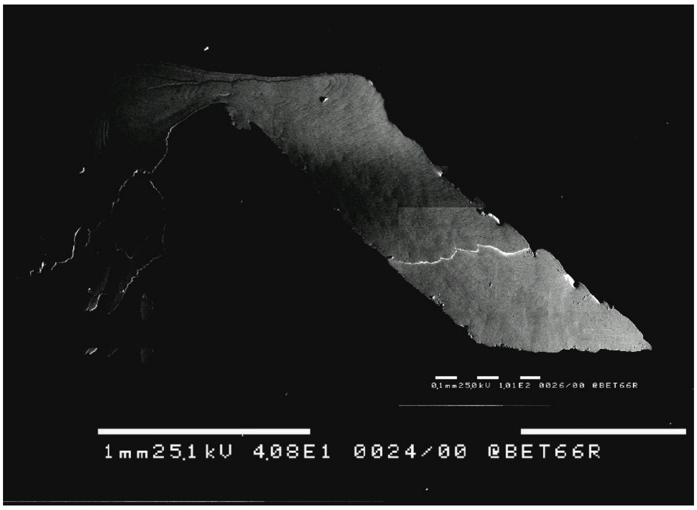

1mm25.0kV 8.15E1 0023/00 @BET65R

C.

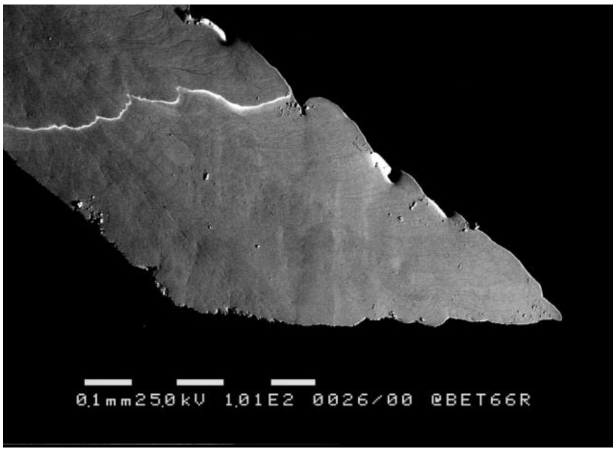

position of Sr mark
Inc
Inc
500 microns

BET#66

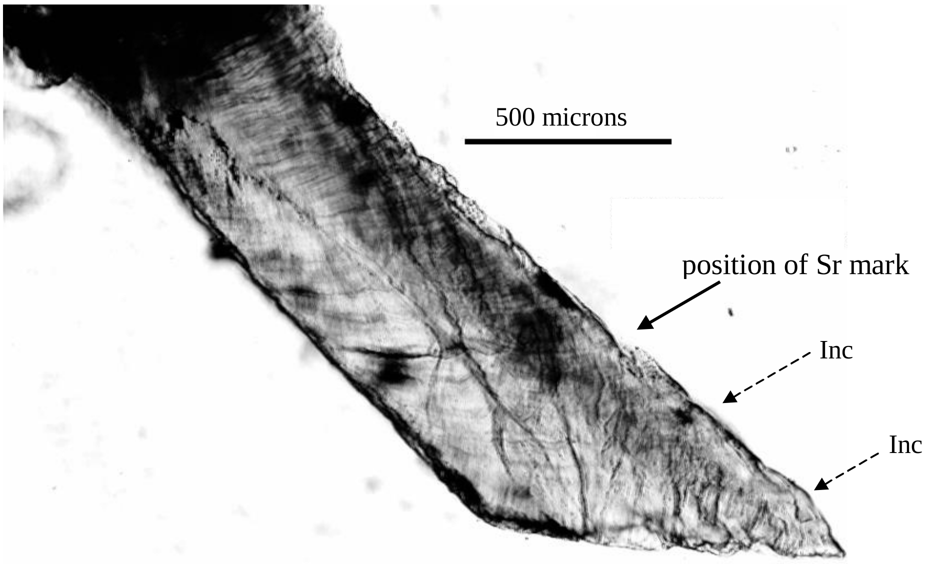
A.


1mm25.1kV 4.08E1 0024/00 @BET66R

B.


0.1mm25.0kV 1.01E2 0026/00 @BET66R

C.

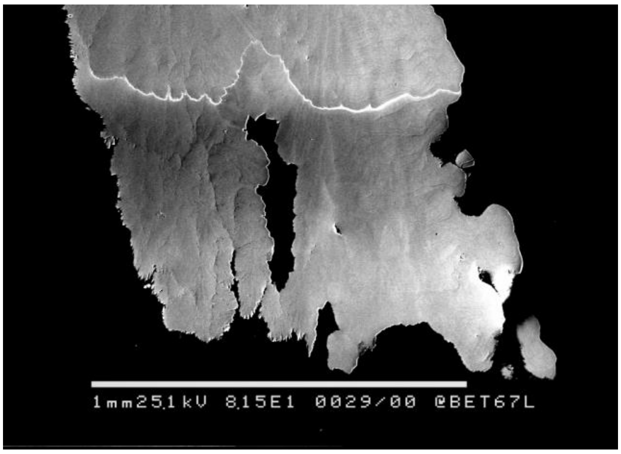

500 microns
position of Sr mark
Inc
Inc

BET#67

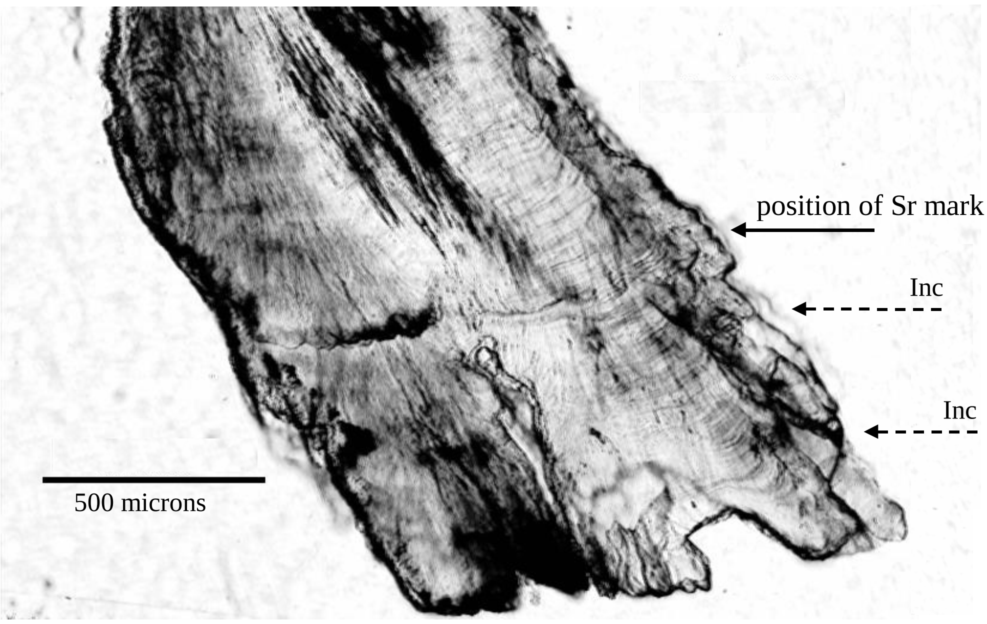
A.


1mm25.0kV 2.88E1
@BET67L

B.


1mm25.1kV 8.15E1 0029/00 @BET67L

C.

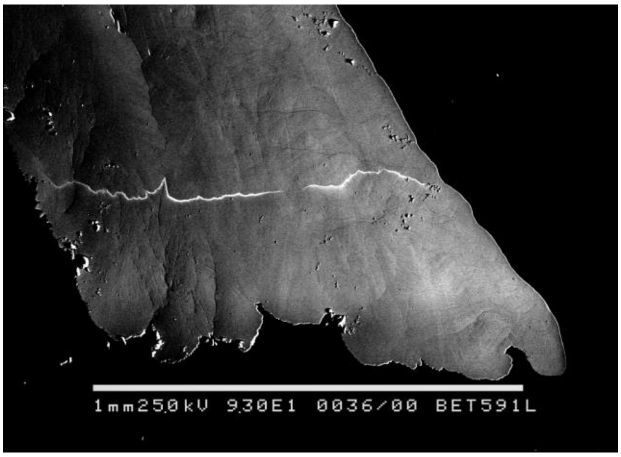

position of Sr mark
Inc
Inc
500 microns

BET#591

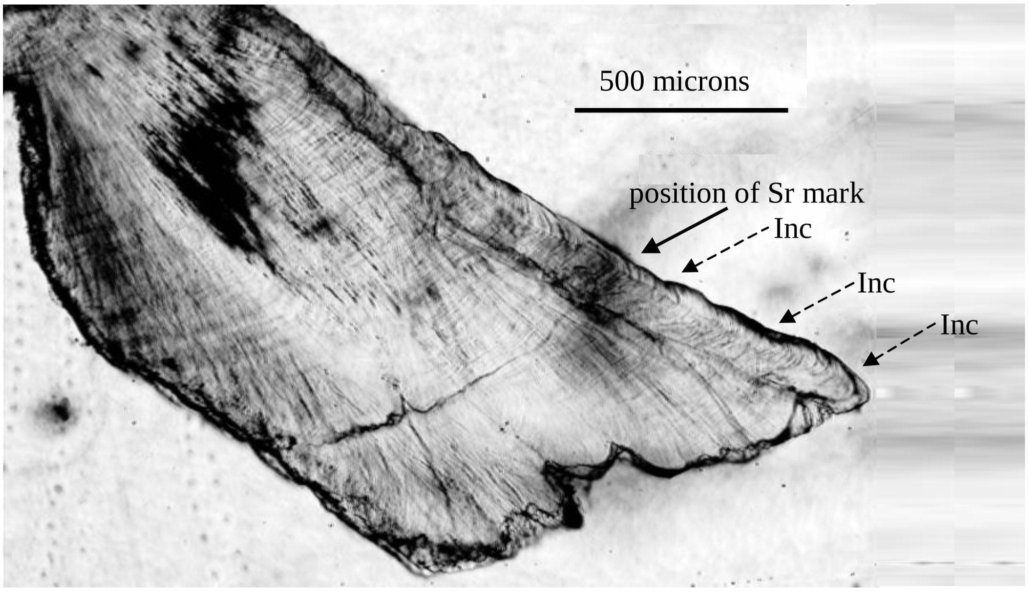
A.


1mm25.0kV 3.14E1
BET591L

B.


1mm25.0kV 9.30E1 0036/00 BET591L

C.


500 microns
position of Sr mark
Inc
Inc
Inc