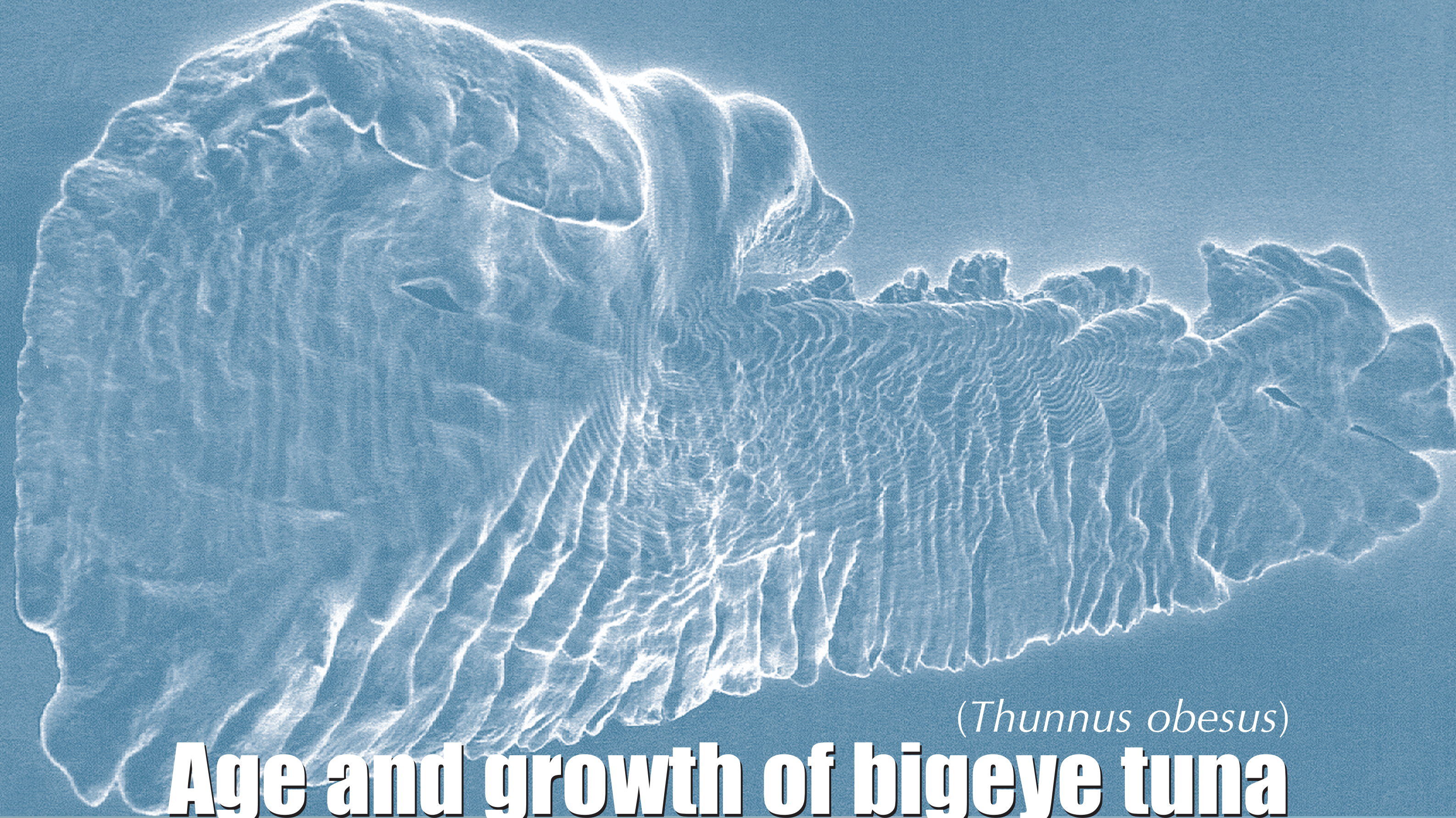

(*Thunnus obesus*)
Age and growth of bigeye tuna
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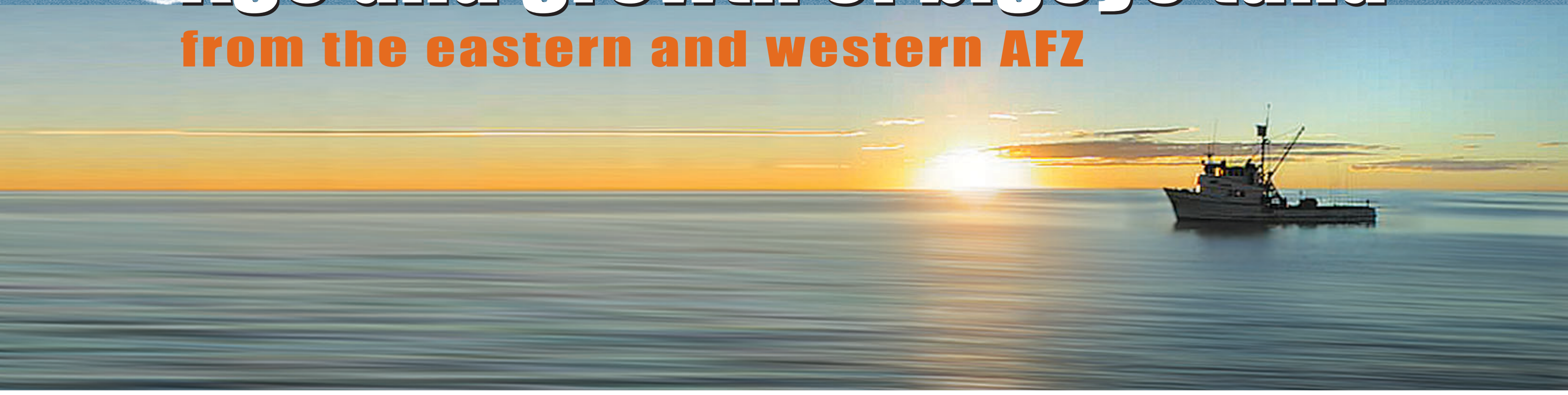

Jessica Farley ■ Naomi Clear ■ Bruno Leroy ■ Tim Davis ■ Geoff McPherson

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Age and growth of bigeye tuna (*Thunnus obesus)* from the eastern and western AFZ
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TABLE OF CONTENTS

1. Non-technical summary 1
2. Acknowledgments 4
3. Background 5
4. Need 7
5. Objectives 7
6. Terminology 8
7. Direct validation of increments in otoliths of bigeye tuna injected with strontium chloride 9
    7.1 Introduction 9
    7.2 Methods 9
    7.2.1 Annual Age Estimates 10
    7.2.2 Daily Age Estimates 14
    7.3 Results 16
    7.3.1 Annual Age Estimates 16
    7.3.2 Daily Age Estimates 18
    7.4 Discussion 19
8. Age, growth and maturity of bigeye tuna 22
    8.1 Introduction 22
    8.2 Methods 23
    8.2.1 Sampling and laboratory processing 23
    8.2.2 Microincrement analysis 25
    8.2.3 Annual age determination 28
    8.2.4 Age corroboration – tag recapture analysis 29
    8.2.5 Growth 30
    8.2.6 Catch-at-age 30
    8.2.7 Maturity 31
    8.2.8 Sex ratio 31
    8.3 Results 32
    8.3.1 Otolith growth 32
    8.3.2 Microincrement analysis 37
    8.3.3 Annual age determination 42
    8.3.4 Age corroboration – tag-recapture analysis 46
    8.3.5 Growth 47
    8.3.6 Catch-at-age 51
    8.3.7 Maturity 51
    8.3.8 Sex ratio 54
    8.4 Discussion 59
    8.4.1 Ageing 59
    8.4.2 Growth and catch-at-age 60
    8.4.3 Implications for stock structure 63
    8.4.4 Maturity 63
    8.4.5 Sex ratio 64
9. Benefits and adoption 64
10. Further Development 65
11. Planned outcomes 65
12. Conclusion 66

13. References 68
14. Intellectual property 74
15. Staff 74
16. List of appendices 75

LIST OF TABLES

Table 7.2.1. Otoliths recovered from Sr-injected bigeye tuna from 3 tagging programs 11
Table 7.2.2. Otoliths analysed for strontium marks in the SEM. 12
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. 17
Table 7.3.2. Results of microincrement counts on strontium-marked otoliths 19
Table 8.2.1. Number of bigeye tuna, *T. obesus*, otoliths selected for age determination by fishery, location and sex. M = male, F = female, U = unknown. 24
Table 8.3.1. Results of regression analysis examining the relationship between fish length (cm) and otolith length (µm). 35
Table 8.3.2. ANCOVA of the effect of Ocean (eastern Indian or western Pacific) on whole and sectioned otolith length. 35
Table 8.3.3. ANCOVA of the effect of sampling location within the western Pacific (Coral Sea and Qld/NSW) and eastern Indian oceans (Western Australia and Indonesia) on sectioned otolith length. The Fork Length*Location interaction was not significant for all locations and was removed from the model. 36
Table 8.3.4. ANCOVA of the effect of sex on sectioned otolith length for four sample locations. The Fork Length*Sex interaction was not significant for all locations and was removed from the model. 36
Table 8.3.5. ANOVA and Bonferroni tests of the effect of sampling location on Y1 and Y2. Y1 and Y2 are the distances from the primordium to the 365th increment (age 1) and 730th increment (age 2) in transverse sectioned otoliths. 39
Table 8.3.6. Summary of microincrement data for bigeye successfully read at the Secretariat of the Pacific Community in Noumea, with corresponding expected and observed counts of annual opaque zones. * indicates low confidence scores (<2.5) associated with the annual age estimates. 40
Table 8.3.6 continued. Summary of microincrement data for bigeye successfully read at the Secretariat of the Pacific Community in Noumea, with corresponding expected and observed counts of annual opaque zones. * indicates low confidence scores (<2.5) associated with the annual age estimates 41
Table 8.3.7. Mean APE (average percent error) by confidence score. 43
Table 8.3.8. Comparison of the expected (from time at liberty) and the observed number of opaque zones in sectioned otoliths from fish tagged and recaptured in the Coral Sea. 47
Table 8.3.9. Proportion of female bigeye sampled by age class. Only age estimates with a confidence score of ≥2.5 are included. 48
Table 8.3.10. Comparison of von Bertalanffy growth models between sexes, sampling locations, and oceans using analysis of the residual sum of squares (ARSS). ns = not significant. 49
Table 8.3.11. Least square estimates and 95% confidence intervals (in brackets) of von Bertalanffy growth parameters by sampling location and sex, and for the combined

western Pacific (Coral Sea and Qld/NSW) and eastern Indian Oceans (Western Australia and Indonesia). For the combined areas, fish with sex not identified are included. 50
Table 8.3.12. Sex ratio of bigeye by sampling area in the western Pacific and eastern Indian Oceans. 57

LIST OF FIGURES

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. 12
Figure 7.2.2. Diagram of a typical left-hand sagittal otolith. Transverse sections were prepared for SEM analysis of strontium marks. 13
Figure 7.2.3. SEM micrograph of a longitudinal section (bigeye #57). Sr-marks were obvious as bright bands across the growth axes. 13
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. 15
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. 16
Figure 7.3.2. An example of a section in which the Sr-mark was weak and was positively identified using EDS. 19
Figure 8.2.1. Location of the fishing ports (*) and areas sampled for otoliths. 24
Figure 8.2.2. Length frequency distribution of bigeye with otoliths selected for age determination by area and sex. Otoliths were selected based on size of fish — stratified sampling rather than random sampling. 26
Figure 8.2.3. Length frequency distribution of bigeye with otoliths selected for microincrement analysis. 27
Figure 8.2.4. Transverse section of the ventral "long" arm of a bigeye otolith. 27
Figure 8.3.1. Comparisons of the internal structures in transverse sections of right and left otoliths of a pair. 33
Figure 8.3.2. Relationship between fork length and (a) whole otolith length (n=1173), (b) sectioned otolith length (n=2295), and (c) whole otolith weight (n=2483) for bigeye sampled in the eastern Indian and western Pacific Oceans. 34
Figure 8.3.3. Relationship between age and otolith weight (n=1670) for bigeye sampled in the eastern Indian and western Pacific Oceans. 37
Figure 8.3.4. Relationship between estimated daily age and otolith length. Only otolith readings with a readability score of good or excellent are included. 38
Figure 8.3.5. Box-plots showing the distribution of measurements of Y1 and Y2 by area. Y1 and Y2 are the distances from the primordium to the 365th increment (n=107) and 730th increment (n=78) in transverse sectioned otoliths. 1 = Coral Sea, 2 = Qld/NSW, 3 = Western Australia, and 4 = Indonesia. Centre line and outside edge of each box indicate the median and 25th/75th percentile around the median respectively. 38
Figure 8.3.6. Estimated birth month by birth year and area of capture for bigeye based on microincrement analysis. Year of capture was 2000 or 2001. 41

Figure 8.3.7. Transverse section of a bigeye otolith showing clear annual increments at the terminal edge (arrows) and the region of unclear increments towards the primordium. 42
Figure 8.3.8. Differences between the first and second reading (or second and third if three reading were conducted) for sectioned bigeye otoliths. 44
Figure 8.3.9. Histograms of otolith measurements from the first inflection point to the first, second, and third opaque growth zones. All otoliths with measurements were included. Grey stripes represent the 25th/75th percentile of the median distance to 365th increment (age 1; Y1) and the 730th increment (age 2; Y2) from microincrement analysis. 44
Figure 8.3.10. Marginal increment measurements by month for bigeye aged 2+ and 3+ for the western Pacific and eastern Indian Oceans. The black circles indicated the mean and standard error for the month. 45
Figure 8.3.11. Percent of otoliths with opaque and translucent growth zones at the edge by month for the western Pacific and eastern Indian Oceans. 46
Figure 8.3.12. Mean length-at-age and standard deviations (circles) for bigeye tuna in the western Pacific and eastern Indian Oceans. von Bertalanffy growth curves fitted to the raw data are shown (lines). 49
Figure 8.3.13. Length distributions of bigeye caught in the Eastern and Billfish Fishery, Southern and Western and Billfish Fishery and the Indonesian longline fishery by six-month period. Data for the ETBF and SWTBF are for Jan 2001 to June 2002, and for the Indonesian fishery from July to December 2002. 52
Figure 8.3.14. Age distribution of bigeye caught in the Eastern and Billfish Fishery, Southern and Western and Billfish Fishery and the Indonesian longline fishery by six-month period. Data for the ETBF and SWTBF are for Jan 2001 to June 2002, and for the Indonesian fishery from July to December 2002. NA = age not assigned as key did not cover the full range of lengths. 53
Figure 8.3.15. Proportion of mature and immature bigeye caught in the western Coral Sea by month and sex. Sample sizes are shown along the top of the graphs. 55
Figure 8.3.16. Proportion of mature females in the north-western Coral Sea by 5 cm length classes. (a) data restricted to the spawning season (Aug-Nov 2000 and Oct-Dec 2001) (n=450) and (b) all data (n=635). Maximum likelihood fit of the logistic regression model is shown for the restricted data. Dotted lines show length at 50% maturity. 56
Figure 8.3.17. Proportion of mature males in the north-western Coral Sea by 5 cm length classes. (a) data restricted to the spawning season (Aug-Nov 2000 and Oct-Dec 2001) (n=500) and (b) all data (n=741). Maximum likelihood fits of the logistic regression model are shown. Dotted lines show length at 50% maturity. 57
Figure 8.3.18. Proportion of males and females by length class and area. 58
Figure 8.4.1 Comparison of growth curves for bigeye caught in the Pacific and Indian Oceans. Symbols marking length-at-age are only shown for ages directly estimated, but the curve has been extended to age 15 years (the maximum obtained in the current study). 62

1. Non-technical summary

2000/100 Age and growth of bigeye (*Thunnus obesus*), from the eastern and western AFZ

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Objectives:

1. Determine the age structure of bigeye caught in both the eastern and western longline fisheries
2. Determine the mean age at first (50%) maturity of bigeye from the Cairns region
3. Investigate sexual differentiation in growth rates of bigeye tuna
4. Investigate regional variation in growth rates
5. Further validation of age estimates based on strontium chloride marking

Outcomes Achieved:

The outputs of this project provide significantly improved understanding of a number of the key biological parameters for one of Australia's most valuable pelagic fish species. Of particular importance is:

- The determination that growth rates of bigeye differ in the eastern Indian and western Pacific Oceans, suggesting the existence of separate stocks within the Australian region,
- The discovery that the longevity of bigeye is at least 15 years, significantly higher than the 8-10 years previously thought, and
- The estimation of the size and age at 50% maturity of bigeye in the north-western Coral Sea.

The timely delivery of these outputs provides for the highly desirable outcome of improved age-based stock assessments of bigeye tuna. The most recent assessments of bigeye at the Standing Committee for Tuna and Billfish and the IOTC highlight uncertainties in input parameters, but suggest that stocks in the Central and Western Pacific Ocean and Indian Ocean, are respectively fully- and over-exploited. The reduction in uncertainty for key stock assessment inputs provided by this project will undoubtedly increase confidence in estimates of stock status. The expectation is that increased confidence in assessment outputs will flow on to increased likelihood of management action to conserve and rebuild stocks.

Non-technical Summary:

Little is known about the age and growth of bigeye tuna, yet it is one of the most valuable components of longline fisheries in the eastern and western Australian Fishing Zone. Accurate age estimates form the basis of calculations of natural mortality, age-at-maturity and longevity, all vital inputs to population stock assessments models. Stock assessments of bigeye in the Pacific Ocean currently use analysis of length frequency and tagging data to determine growth parameters. Estimating the age of large fish through modal progression of length frequency, however, is considered imprecise as size modes merge as fish grow. Similarly, estimates of growth using tag-return data are generally limited to small/young fish as most tagged fish are recaptured within a few years of release.

The project follows a previous FRDC funded study (Clear et al. 2000a) that developed techniques to validate estimates of annual age of bigeye tuna through a strontium chloride mark-recapture experiment in the Coral Sea. Clear et al. (2000a) were able to show that increments 2 to 8 visible in otoliths were deposited annually. The current study has been able to extend the validation of annual increment formation to the 9th increment (Objective 5). Evidence supporting the formation of annual increments was also obtained from analysis of otoliths from fish previously tagged (but not chemically marked) in the Coral Sea. Although fish were at liberty for a short time (0-3 years) the results confirmed that the number of opaque zones observed in otoliths after release from tagging was equal to or within the range expected for all fish. Analysis of strontium marked otoliths were also used to attempt to validate daily increment counts in larger (older) fish; generally above the size usually considered for daily age estimation as the increment are deposited so closely that they cannot be distinguished with confidence. All increment counts underestimated the days at liberty. This underestimate could be explained to some extent by an interruption in growth after tagging. However, the low confidence assigned to the counts from otoliths of the bigger fish analysed indicate using Sr-marked bigeye for validating daily age estimates has limited value for large fish.

To determine the length-at-age of bigeye in the both the western Pacific and eastern Indian Oceans, otoliths were sampled from over 2500 fish caught by Australian longline fisheries (78% from the Pacific Ocean and 22% from the Indian Ocean) between 1999 and 2002. We also estimated ages using otoliths from (1) the CSIRO hardparts archives collected by scientific observers onboard Japanese longliners between 1992 and 1997, and (2) bigeye caught in the Indonesian Indian Ocean longline fishery collected as part of a CSIRO-Indonesian RIMF catch-monitoring program between 2000 and 2002.

Annual age estimation of bigeye using otoliths is not straightforward. The proportion of otoliths that could not be read was high (32%) and only 50% of the otoliths examined were included in the final analyses. The high rejection rate was primarily due to difficulties interpreting the region of the otolith closest to the primordium, where the annual increments are broad and diffuse. Comparisons of assumed daily age estimates with annual increment counts, however, confirmed that the first 1-3 annual increments were being successfully identified in the otolith sections used in the final analysis. Unfortunately, neither marginal increment analysis nor edge type analysis provided

conclusive evidence for the timing of annual increment formation in the otoliths, although winter seems the most likely based on our results. Knowledge of the difficulties encountered in estimating the age of bigeye tuna using sectioned otoliths will benefit further decisions on the practicability of routinely estimating the age of the catch using otolith increment counts.

A significant finding in the study was that the longevity of bigeye is greater than previously thought. The maximum age estimated with high confidence was 15 years for two females sampled in the Coral Sea and Indonesia. The oldest male sampled was 12 years old. A review of the literature revealed that 8-10 years was thought to be the maximum age for bigeye tuna. Given that we analysed no fish over 178 cm FL, and bigeye are known to reach at least 200 cm, it is possible that bigeye may live in excess of 15 years. The recent return of a tag from a bigeye at liberty for 12 years after being tagged as a 2 year old supports our maximum age estimates.

The growth curves fitted to lengths-at-age were significantly different between sexes for fish sampled in the Coral Sea and Western Australia only, with males being slightly larger at age than females (Objective 3). The absence of a statistical difference in mean length-at-age by sex at any of the sampling locations, however, indicates that sexual dimorphism in growth is relatively small in bigeye tuna. Growth rates varied to some extent between areas sampled, but differences were more noticeable between oceans (Objective 4). Estimates of the von Bertalanffy growth parameters indicate faster growth for fish caught in the western Pacific Ocean compared to the eastern Indian Ocean. Estimates of the theoretical maximum fish sizes were close to the observed maximum lengths, but were slightly larger for western Indian Ocean caught fish. Regional differences were also detected in otolith morphology. Otoliths sampled from the western Pacific Ocean were larger on average for the size of fish than those sampled from the eastern Indian Ocean. The differences in fish and otolith growth rates between oceans support the hypothesis of separate bigeye stocks in the Indian and Pacific Oceans.

The ages given in our study represent counts of opaque zones in otoliths, and do not take into account factors such as birth date, timing of opaque zone formation, or date of capture. Since the timing of opaque zone formation could not be precisely identified in our samples, and birth dates may vary substantially, the ages given may be biased by +/- 1 year. A comparison between our age estimates for small fish with those based on counts of daily increments (assumed to represent the “true” age) showed that our technique overestimates length-at-age for age classes 1+ to 3+ years. This type of bias is inherent in annual age estimation techniques as otolith growth after the formation of the last increments is not accounted for in the integer age estimated. This bias does not influence our comparisons of growth between areas or sexes, but must be acknowledged if the growth parameters are to be used for stock assessment purposes.

One of the key objectives of the project was to determine the age structure of bigeye catches by domestic longline fisheries in the eastern and western Australian Fishing Zone (AFZ) (Objective 1) using age-length keys applied to catch-at-length data for the fisheries. The results suggest both regional and inter-annual variation in the catch-at-age. Interpretation of the data, however, is difficult due to the short time series examined and the bias in age estimates in the younger age classes. Overall, however, young fish aged five or less dominated the catch in the AFZ. On the east coast, between 89 and 95% of the catch were age five or less and only 1-6% were age eight or older. On the west coast, the proportion of young fish in the catch was slightly lower than in the east (between 65-90%) and the proportion of old fish was higher (1-17%).

Size at maturity was determined for bigeye caught in the north-western Coral Sea using logistic curves (Objective 2). Length at 50% maturity was 102.4 cm in females and 86.6 cm in males. We estimated fish of these lengths to be 1.8 and 1.1 years old for females and males, respectively. However, using the composite growth curve developed by Hampton et al. (1998) based on daily age estimates and tagging data, fish of these lengths are estimated to be 2.4 and 1.7 years old for females and males respectively. These latter estimates are considered the most appropriate for stock assessment purposes.

The overall sex ratio of bigeye tuna was close to 1:1 in all areas except the Coral Sea where males were more abundant than females. We found that the dominance of males became more prominent as size increased in the Coral Sea, Qld/NSW and WA possibly as a result of sexual dimorphism in growth.

Overall, the knowledge and understanding gained during the project will advance the stock assessments for the species.

Keywords: Bigeye tuna, age validation, growth rate, longevity, age composition, sexual dimorphism, age at maturity.

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There are many people we would like to acknowledge for support during this project. We are especially grateful to those who collected/coordinated otolith sampling around Australia and in Indonesia. These include: Phil Crogan, Geoff Dews, Garry Fry, Mark Green, Daniel Joyce, Sandra Leeds, Toby Patterson, Ian Peel Luke Short, Kiroan Siregar, Retno Andamari, Peter Teale and Kevin Williams. The assistance given to us by Erica Starling, Marie Kruizinga and staff at Indian Ocean Fresh Tuna; Ross McGregor and staff at Lobster Australia; Brett Taylor, Sue Jones and staff at DeBrett Seafoods, are also acknowledged. We also thank the skippers and crew at Great Barrier Reef Tuna Pty Ltd. We are also grateful to the Australian scientific observers aboard Japanese longline vessels who collected otoliths prior to 2000. Otolith sampling in Benoa, Bali, was carried out as part of a catch-monitoring program run by the Research Institute of Marine Fisheries in Indonesia and CSIRO. We thank Tony Lewis and John Hampton at the Secretariat of the Pacific Community (SPC) for permitting Bruno Leroy to conduct the microincrement analysis of otoliths (at no cost to the project). Elemental analysis of otoliths was completed at the scanning electron microscope facility of the Forensic Science Service Tasmania (FSST). We thank FSST staff Frank Halley and Hanh Nguyen for providing access to the SEM facility and for their technical assistance in preparing and analysing the otoliths. We are grateful to Steve Campana who gave some helpful comments, and to Mark Bravington and Toby Patterson for statistical advice. Finally we thank the Central Ageing Facility (Marine and Freshwater Resources Institute) for preparing sections of all our otoliths. The project was funded by FRDC grant 2000/100.

3. Background

Bigeye is one of the most valuable components of the Eastern Tuna and Billfish Fishery (ETBF) with over 1000 tonnes caught in 2001. The catch of bigeye in the Southern and Western Tuna and Billfish Fishery (SWTBF) is much smaller (400 tonnes). However, it is the most valuable component of that fishery and is developing rapidly. Although bigeye is such an important catch species in the eastern and western Australian Fishing Zone (AFZ), little is known about its biology. Information is required on:

- Longevity, which is used to estimate natural mortality rates and productivity.
- Sex/size ratios and sexual dimorphism in growth rates.
- Growth rate estimation and development of an age-length key for future age-based assessment models.
- Age at first (50%) maturity.

To estimate these parameters, validated direct age estimates from appropriate samples are required. While size frequencies now being collected from the fishery identification of newly recruited cohorts, the merging of size modes as the fish grow, means that the age of adult sized bigeye cannot be estimated from size data alone. The limited tagging data from the Coral Sea (Hampton and Gunn 1998) suggest that bigeye may live significantly longer than previously thought. A tag was recently recovered from a bigeye at liberty for 12 years after being tagged as a 2 year old. Direct age estimation is required to develop an age-length key for the species. Determining the age composition and other key biological characteristics of bigeye is ET&BFMAC's priority three in their list of research priority issues in 1999, as it is required in support of stock assessments.

Various hypotheses relating to the movement of fish between the eastern AFZ and the equatorial Pacific can be addressed through estimating the age of fish in these regions in combination with the tagging analysis planned over the next two years by CSIRO and SPC. The results of these studies will greatly assist the parameterisation of any future bigeye population model derived from the generic model currently being developed by CSIRO for broadbill swordfish. Model hypotheses that do not give results consistent with the age structure of the fish caught in the eastern AFZ can be eliminated from further consideration.

Due to the present uncertainties over the catch-at-age, it has been difficult to estimate natural mortality for bigeye tuna. Estimates of natural mortality rates are vital for stock assessment of the species in the Pacific Ocean. Estimating the age of adult bigeye from a representative sample of the population will help address this issue. At the very least, knowing the longevity of the species will indicate the possible boundaries of valid mortality estimates. In general, short-lived species are more resilient to fishing than long-lived species (eg skipjack and southern bluefin tuna), because of higher productivity, higher natural mortality and shorter generation times. Knowledge of the age structure of the population will help define the possible range of mortality estimates.

Present attempts to develop VPA models for bigeye in the Indian and Pacific Oceans are also hindered by the lack of validated catch-at-age data (Fonteneau 1998; Stobberup et al. 1998). The development of an age-length key will overcome these problems. Mean age at 50% maturity is also required to assess the size of the spawning stock. It also indicates the vulnerability of a species to over fishing with early-maturing species usually being more resilient than late- maturing ones.

Bigeye tuna have been aged directly using scales and vertebrae (Tankevich 1982; Yukinawa and Yabuta 1963), but neither technique has been validated. Modal analysis of length frequency data and tagging data have also been used to develop growth curves for bigeye (Iverson 1955; Hampton et al. 1998; Kume and Joseph 1966; Shomura and Keala 1963; Suda and Kume 1967). However, there is considerable uncertainty in growth parameter estimates cased on these methods because of the limited size range of fish examined and the inadequacies inherent in some of the methods. Otoliths have only recently been used to age bigeye tuna. SPC is using microincrements in otoliths to age bigeye from the western and central Pacific Ocean (Lehodey et al. 1999) and IATTC is developing daily age estimation techniques on bigeye in the eastern Pacific Ocean (Anon 2001). Because of the difficulty in resolving microincrements in older fish by light microscopy, the otolith work at SPC is limited to fish less than three years old (approximately 120 cm FCL). The tedious and time-consuming nature of micro increment analysis means that age is estimated for a relatively small number of fish.

CSIRO recently developed techniques to estimate the age of bigeye using annual increments in otoliths in a pilot study "Developing techniques to estimate the age of bigeye and broadbill swordfish off eastern Australia: a pilot project" (FRDC 98/113). The techniques used are similar those used on SBT (Clear et al, 2000b). Nine bigeye injected with strontium chloride and at liberty for 207 to 2,071 days had laid down increments (growth zones) for each year at liberty. These increments appear as alternating opaque and translucent zones, corresponding to changes in the deposition rates of microincrements possibly due to seasonal changes in temperature. Clear et al (2000a), however, noted that there was some difficulty in detecting the first annulus in bigeye otoliths. We propose to confirm the location of these using the microincrement techniques developed at SPC.

Further validation of the ages of older fish will be possible as otolith are recovered from marked fish at liberty for longer intervals. Direct estimation of bigeye age using annual increments will enable us to age adequate numbers to determine the age structure of fished populations, investigate sexual differentiation in growth rates, and determine mean age at 50% maturity. This information can be used to determine stock productivity, growth, mortality and recruitment, and will complement the age estimation programs being carried out in the west, central and east Pacific Ocean.

Because we think the growth rates are similar in the Indian and Pacific Oceans, we propose that this study determine the age structure for bigeye caught in the eastern and western AFZ. Development of an age-length key for bigeye from the western AFZ will greatly improve our capacity to assess bigeye stocks in that fishery, and also the very large bigeye fishery in the north-east Indian Ocean fished by Indonesians. Assessing bigeye stocks in the Indian Ocean is also IOTC's highest priority. The most recent meeting of the Working Party on Tropical Tunas held in September 1999 noted that parameters such as growth and age at 50% maturity are poorly known, and that these are needed to assess the status of bigeye stocks. The results from this proposal will be particularly valuable in any Indian Ocean-based assessment of bigeye stocks. The relationship of Western AFZ bigeye stocks to other populations in the Indian Ocean is currently being investigated through a FRDC Project 97/122 "Determining genetic stock structure of bigeye in the Indian Ocean using mitochondrial DNA and DNA microsatellites".

4. Need

Although bigeye is such an important catch species in the eastern and western AFZ, little is known about its biology. Determining the age composition of bigeye is ET&BFMAC's priority three in 1999, as it is required in support of stock assessments. The limited tagging data from the Coral Sea (Hampton and Gunn 1998) suggest that bigeye may live significantly longer than previously thought. Validated direct age estimates of bigeye are required to estimate natural mortality rates and productivity; investigate sexual dimorphism in growth rates; develop an age-length key for future age-based assessment models and to determine the mean age at 50% maturity.
Due to the present uncertainties over the catch-at-age, it has been difficult to estimate natural mortality for bigeye. Estimates of natural mortality rates are vital for stock assessment of the species in the Pacific Ocean. The direct estimation of adult bigeye ages from a representative sample of the population will help address this issue.

Age-based stock assessments require the breakdown of fish lengths into age classes using a validated length-at-age model. Present attempts to develop VPA models for bigeye in the Pacific are hindered by the lack of catch-at-age data. Mean age at 50% maturity is also required to assess the size of the spawning stock.

Assessing bigeye stocks in the Indian Ocean is also IOTC's higest priority. Parameters such as growth and age at 50% maturity are poorly known, and the information provided by this proposal will be particularly valuable in any Indian Ocean-based assessment of bigeye stocks.

5. Objectives

1. Determine the age structure of bigeye caught in both the eastern and western longline fisheries
2. Determine the mean age at first (50%) maturity of bigeye from the Cairns region

3. Investigate sexual differentiation in growth rates of bigeye tuna
4. Investigate regional variation in growth rates
5. Further validation of age estimates based on strontium chloride marking

The project comprised two main research components that are examined independently in the report. The first component deal with the validation of increment formation in otoliths of fish previously injected with strontium chloride (Chapter 7). The second component encompasses the age, growth and maturity elements of the study (Chapter 8). This second component is based on the direct age estimates of bigeye using sagittae otoliths obtained through a sampling program established for the project, supplemented by existing otoliths held in the CSIRO Hardpart Archives.

6. Terminology

The terminology used in this study is based on Anon (2002). In brief, *growth zones* are the opaque and translucent "bands" visible along sectioned otoliths that form *growth increments*. Under transmitted light, the opaque zone appears dark and the translucent zone appears light. Growth zones can be on a micro (daily) or macro (annual) scale, and the analysis of these is referred to as *microincrement* and *annual increment* analysis, respectively. A *final count* refers to the estimated number of opaque zones present in the otolith after consecutive reading. A *sister* otolith is the second otolith of a pair.

7. Direct validation of increments in otoliths of bigeye tuna injected with strontium chloride

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

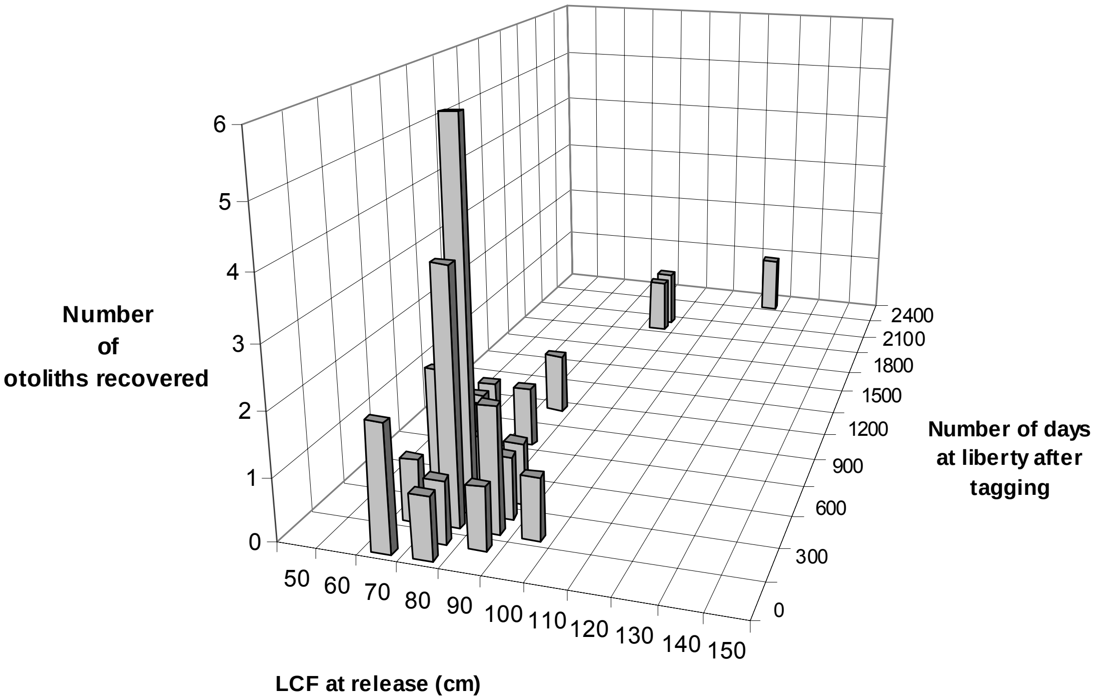

Figure 7.2.1. The size-at-release and period at liberty after tagging for the bigeye tuna from which Sr-marked otoliths were recovered.

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

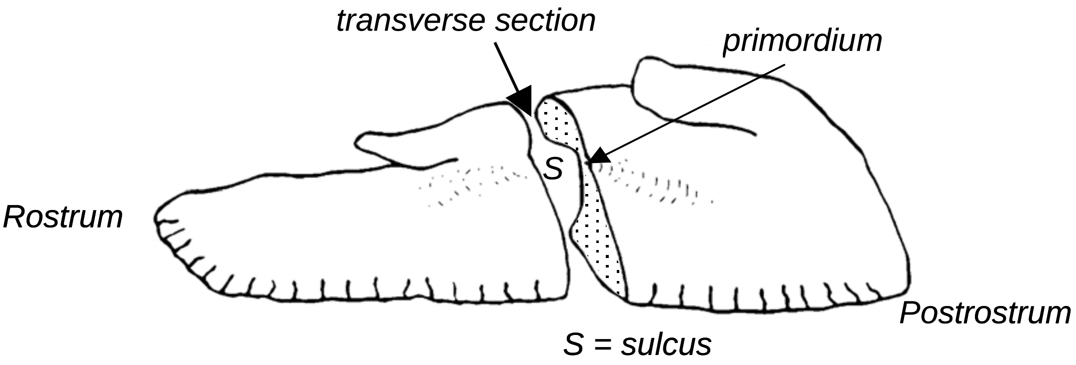

Figure 7.2.2. Diagram of a typical left-hand sagittal otolith. Transverse sections were prepared for SEM analysis of strontium marks.

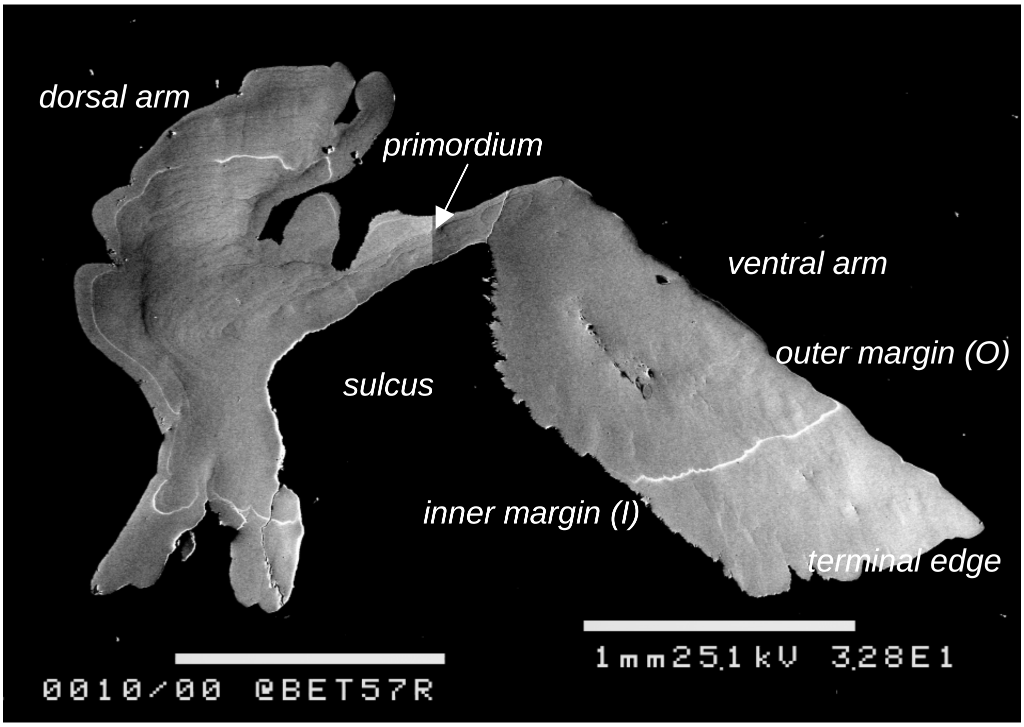

Figure 7.2.3. SEM micrograph of a longitudinal section (bigeye #57). Sr-marks were obvious as bright bands across the growth axes.

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.

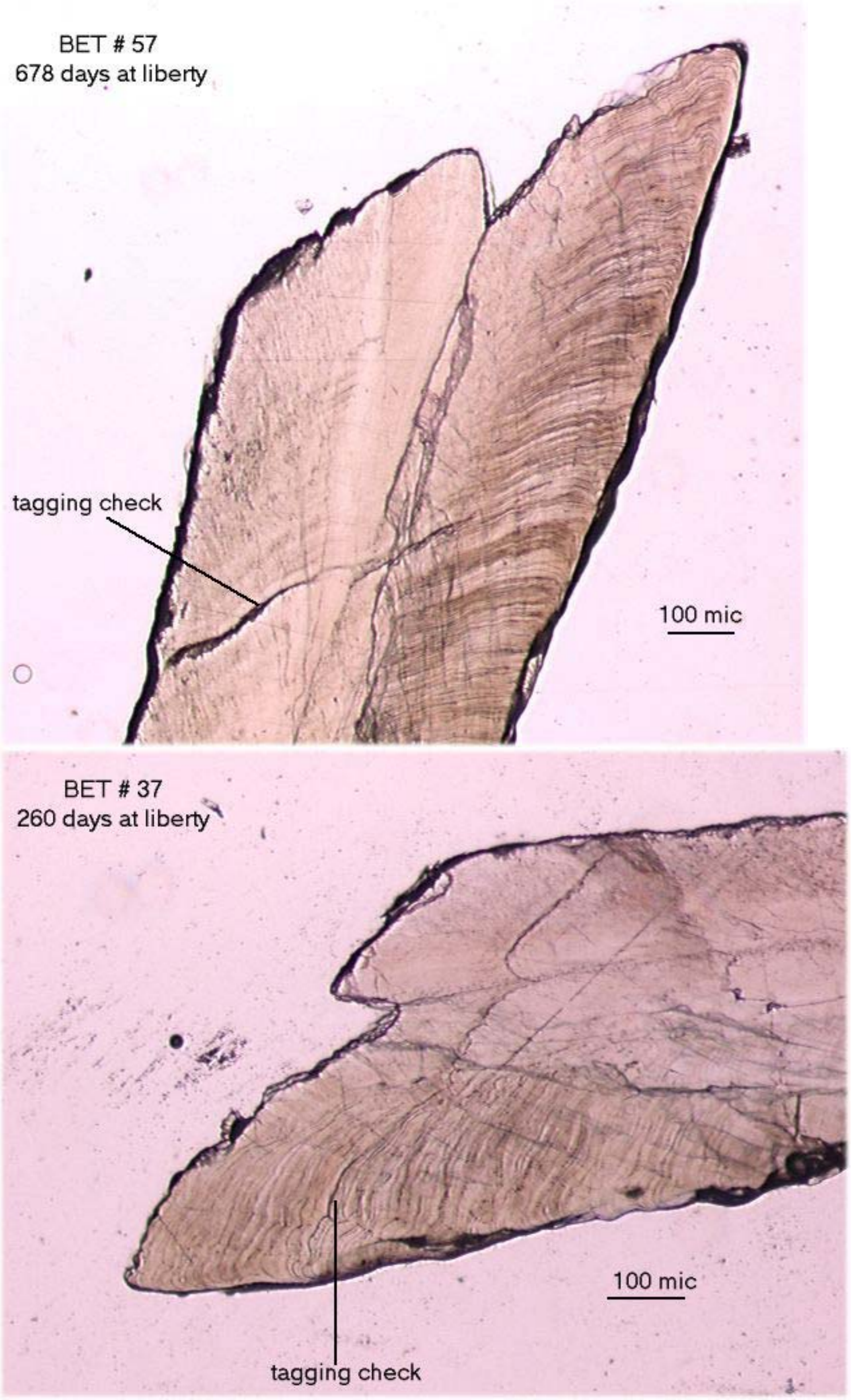

Figure 7.2.4. Images of thin transverse sections used to count microincrements. The reader was told the position of the strontium mark along the section, coinciding with a tagging ‘check’ that was usually visible at the high magnifications used for microincrement counts.

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.

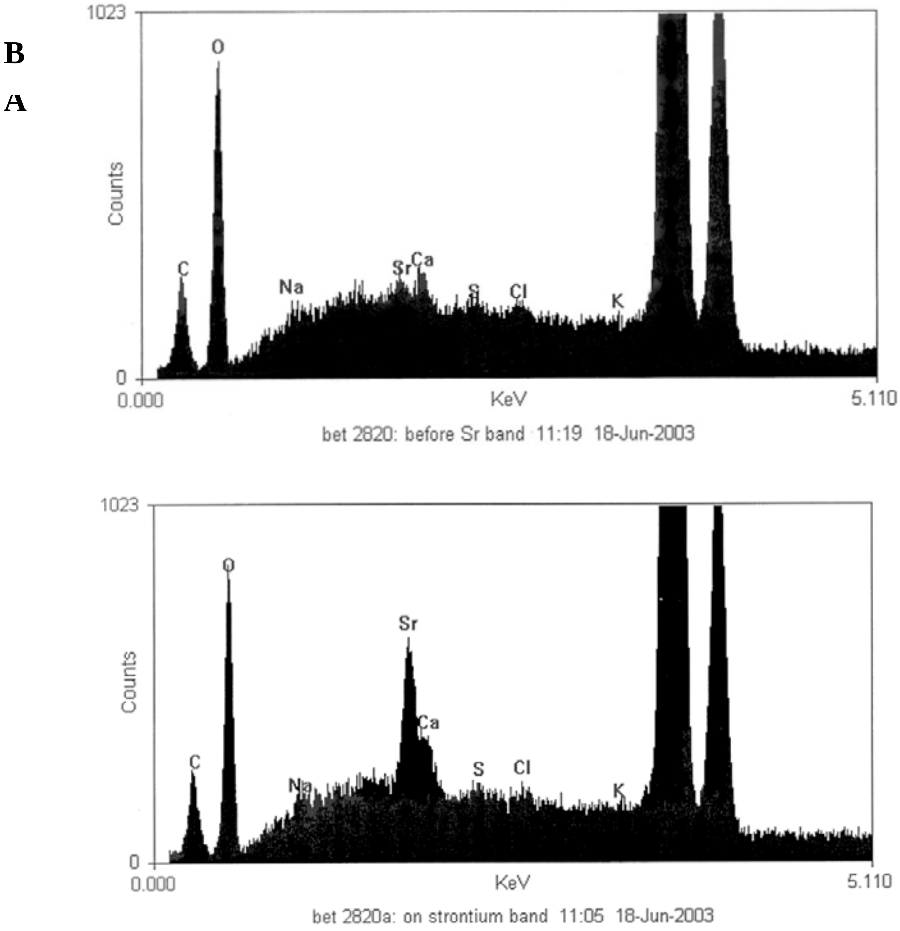

Figure 7.3.1. Examples of EDS spectra from a sectioned bigeye tuna otolith (bigeye #2820) showing peaks due to background levels of strontium (A) and enhanced Sr levels associated with the strontium mark (B). The enhanced peak was used to positively identify the position of the strontium mark.

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.

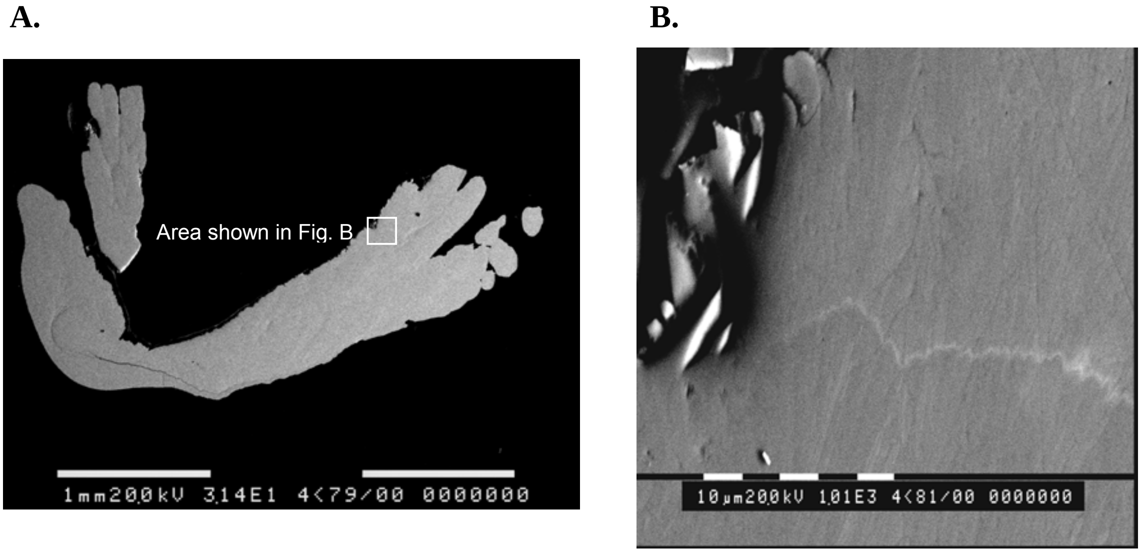

Figure 7.3.2. An example of a section in which the Sr-mark was weak and was positively identified using EDS.

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.

8. Age, growth and maturity of bigeye tuna

8.1 Introduction

Bigeye (*Thunnus obesus*) are a large pelagic species distributed throughout the tropical and subtropical waters of the Pacific, Indian and Atlantic Oceans, spending much of their time below the thermocline (Joseph, 2003). They are the third most common species caught worldwide, after skipjack (*Katsuwonus pelamis*) and yellowfin (*Thunnus albacres*) (Campbell and Miller, 1998).

In Australia, bigeye is one of the most valuable components of an Eastern Tuna and Billfish Fishery (ETBF) with over 1000 tonnes caught in 2001, accounting for less than 1% of the total western and central Pacific Ocean bigeye catch. Although the fishery operates along the eastern seaboard, bigeye catches are predominantly concentrated in northern Queensland (north-western Coral Sea) and off southern Queensland/New South Wales. In the north-western Coral Sea, bigeye can be caught year-round but large concentrations of spawning fish occur during full moon periods from October to January. Bigeye caught in the eastern AFZ are considered to be part of a large stock occurring in the Pacific Ocean (Grewe and Hampton 1998), although the movements of bigeye between eastern Australia and the rest of the Pacific Ocean are unclear, particularly whether fish caught in the south-east AFZ were spawned in the Coral Sea or elsewhere in the tropical Pacific. Spawning of bigeye is known to occur year-round in the tropical Pacific Ocean when sea surface temperatures exceed 26ºC (Hampton et al. 1998). In the western Pacific Ocean, Kikawa (1962) found that mature bigeye are more common between April and September between 10ºN and 10ºS.

The Southern and Western Tuna and Billfish Fishery (SWTBF) is much smaller (400 tonnes caught in 2001) than in the east, but it is the most valuable component of that fishery and is developing rapidly. The catch of bigeye on the west coast is concentrated between 20-35ºS during the winter months, and accounts for about 0.8% of the total caught in the eastern Indian Ocean. Between the north-western coast of Australia and the Indonesian archipelago, an Indonesian longline fishery catches bigeye year-round. This area is a known spawning area for bigeye, yellowfin and southern bluefin tuna. As with the Pacific Ocean, very little is known about the stock structure of bigeye in the Indian Ocean or the movements of fish between the western AFZ and the broader region. Using catch data, Kume et al. (1971) identified two areas of higher bigeye abundance in the Indian Ocean: (1) the equatorial region where spawning occurs year-round when sea surface temperatures exceed 26ºC, and (2) a subtropical region between 25-30ºS where non-spawning fish occur in the Austral winter. Bigeye caught in the western AFZ are thought to form part of this non-spawning group. Grewe et al. (2000) could not reject the hypothesis of a single stock of bigeye in the Pacific and Indian Oceans.

Although it is such an important species in both the ETBF and SWTBF, very little is known about the biology and population dynamics of bigeye tuna in these areas. Accurate age estimates form the basis of calculations of growth, natural mortality, age-at-maturity, and longevity, all vital inputs to population stock assessments models. Much of the early work determining the age and growth of bigeye in the Pacific Ocean was based on modal length (or weight) frequency analysis (Iverson 1955; Kume and Joseph 1966; Shomura and Keala 1963; Suda and Kume 1967). Yukinawa and Yabuta (1963) estimated age from scales although they found it difficult to distinguish the rings in fish greater than 130 cm FL. More recently, Hampton et al. (1998) used assumed daily age estimates and tagging data to produce a composite growth model for bigeye in the western and central Pacific Ocean. Matsumoto (1998) estimated the age of nine bigeye up to approximately 55 cm FL based on the number of assumed daily increments observed on etched whole otoliths. Kato (2001) examined one otolith from a 158 cm FL bigeye and estimated it to be 6.5 years old based also on assumed daily increments. Sun et al. (2001) used dorsal spines to provide maximum age estimates of 10 years old for fish approximately 188 cm FL.

Fewer studies have been conducted on bigeye age and growth in the Indian Ocean. Tankevich (1982) used both scales and vertebrae to estimate annual age but again found that growth marks on scales less distinct in fish over 130 cm FL. Stequert and Conand (2003) compared assumed daily age estimates from otoliths and dorsal spines and found that spines were not suitable for determining the age of fish greater than three years old, while estimates up to 7 years were obtained from otoliths.

Unfortunately, there have been few studies validating the periodicity of the increments counted, either daily or annual, in bigeye tuna. As reported in Chapter 7, Clear et al. (2000a) developed validated techniques to estimate the annual age of bigeye through a strontium chloride mark-recapture experiment in the Coral Sea. The techniques developed were used in the current study for fish sampled from both the eastern and western AFZ and the northeast Indian Ocean. Given that Clear et al. (2000a) had difficulty in detecting the first annulus in sectioned otoliths, we investigated the use of daily age estimation to locate the first few opaque growth zones. We also investigated the use of tag release/recapture data to corroborate our interpretation of otoliths using back-calculation techniques.

8.2 Methods

8.2.1 Sampling and laboratory processing

Bigeye sagittae otoliths were sampled from Australian longline catches in the eastern and western Australian Fishing Zone (AFZ) between 1999 and 2002. Otoliths were collected from three broad regions around Australia: northern Queensland (north-western Coral Sea), southern Queensland/New South Wales (Qld/NSW), and Western Australia (south of 25ºS and west of 130ºE) (Fig. 8.2.1; Table 8.2.1). The otoliths were collected by scientific observers aboard vessels, or by technicians at processing factories and fish markets. Otoliths collected at processing factories were randomly sampled from fish landed on a given day, but not all days were monitored during the year. These otoliths were supplemented by otoliths from the CSIRO hardparts archives collected by scientific observers aboard Japanese longliners between 1992 and 1997. These supplementary otoliths were selected from Qld/NSW and Western Australia only. Additional otoliths were sampled from the Indonesian longline fishery operating south of Bali in 2000-2002 as part of a catch-monitoring program in conjunction with the Research Institute of Marine Fisheries in Indonesia (Davis and Andamari 2002; 2003).

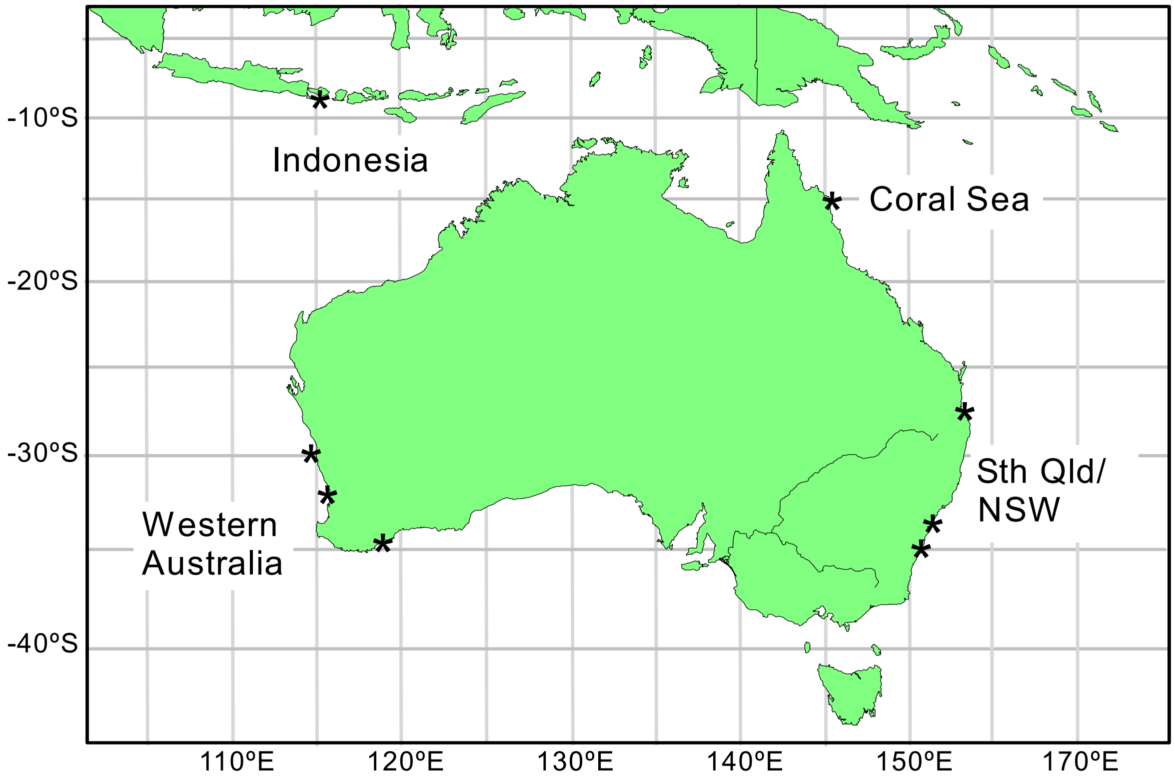

Figure 8.2.1. Location of the fishing ports (*) and areas sampled for otoliths.

Table 8.2.1. Number of bigeye tuna, *T. obesus*, otoliths selected for age determination by fishery, location and sex. M = male, F = female, U = unknown.

Sampling location	Australian			Japanese			Indonesian			Total
	M	F	U	M	F	U	M	F	U	
Coral Sea	468	377	23	-	-	-	-	-	-	868
Qld/NSW	57	56	964	26	27	-	-	-	-	1130
Western Australia	-	-	547	128	134	-	-	-	-	809
Indonesia	-	-	-	-	-	-	192	199	2	393
Grand total	525	433	1534	154	161	-	192	199	2	3200

Fork length (FL) was measured with calipers to the nearest cm for the majority of fish sampled. Where calipers were not available, fish were measured using a measuring board or tape. Any fish measurements made "over the body" were adjusted to standard fork length using the regression:

$y = (0.8948\ x) + 5.4251$

Where Y is the standard fork length and X is the tape measurement over the body of the fish (r^2=0.983). Dressed weight was measured to the nearest 0.1 kg for most fish sampled. Where possible, the sex of fish sampled was recorded. However, as bigeye are usually cleaned and gutted at sea, these data were not available for most fish sampled in Qld/NSW and Western Australia. Sex data were available for the supplementary fish sampled from Japanese longliners. Date of capture or landing was recorded for all fish. Otoliths were removed using a method developed by Clear et al. (2000b) for southern bluefin (*Thunnus maccoyii*) where a core is taken from the underside of the head of the fish with a hole-saw attached to a power-drill. The otoliths were cleaned, dried and archived into the CSIRO hard parts collection.

Of the 3608 otoliths collected from the four regions, 3200 were selected for age estimation. Otoliths were selected based on area of capture and size of fish, with the aim of increasing the number of age estimates to include a representative sample of the size range of SBT caught in each fishery (stratified sampling), although priority was given to fish with sex identified. Figure 8.2.2 shows the size distribution of bigeye with otoliths selected for age estimation.

Whole otoliths were weighed to the nearest 1 mg (n=2483) and the maximum length was measured to the nearest 0.1 mm for a subset (n=1173). The weight of the “sister” otolith was also measured for 528 pairs to assess the similarity in weight of left and right otoliths from the same fish. The relationship between otolith size (length and weight) and fish size (FL) was determined using least-square linear regression. The relationship between fish length and the lengths of left and right otoliths were tested using paired t-tests.

8.2.2 Microincrement analysis

To confirm the location of the first two opaque zones in bigeye otoliths, 113 otoliths were selected for a direct comparison of daily and annual age estimates. One otolith from each pair was prepared for microincrement analysis (daily age estimation), while the other was prepared for annual age estimation. The otoliths were selected from the full size range sampled up to approximately 120 cm fork length (the maximum size for microincrement analysis; Lehodey et al. 1999) from north-western Coral Sea, Qld/NSW, and Western Australia. As very few small fish were sampled, the majority of otoliths were selected from fish between 105 and 120 cm (Fig. 8.2.3).

The preparation for microincrement analysis was conducted at the Secretariat of the Pacific Community (SPC) in Noumea. Scientists at SPC developed methods to estimate the daily age of juvenile bigeye using sectioned otoliths (Lehodey et al. 1999), although there has been no direct validation of the periodicity of growth increments for bigeye in the western Pacific Ocean. The otoliths were embedded in polyester resin and a transverse section containing the primordium (first-formed part of the otolith) was prepared and polished to 50-75 μm thick as described in Chapter 7. The surface of the section was partially decalcified with 5% EDTA (pH 7.4) to emphasize the growth increments. Each otolith was examined under a dissecting microscope and examined along the ventral “long” arm (Fig. 8.2.4).

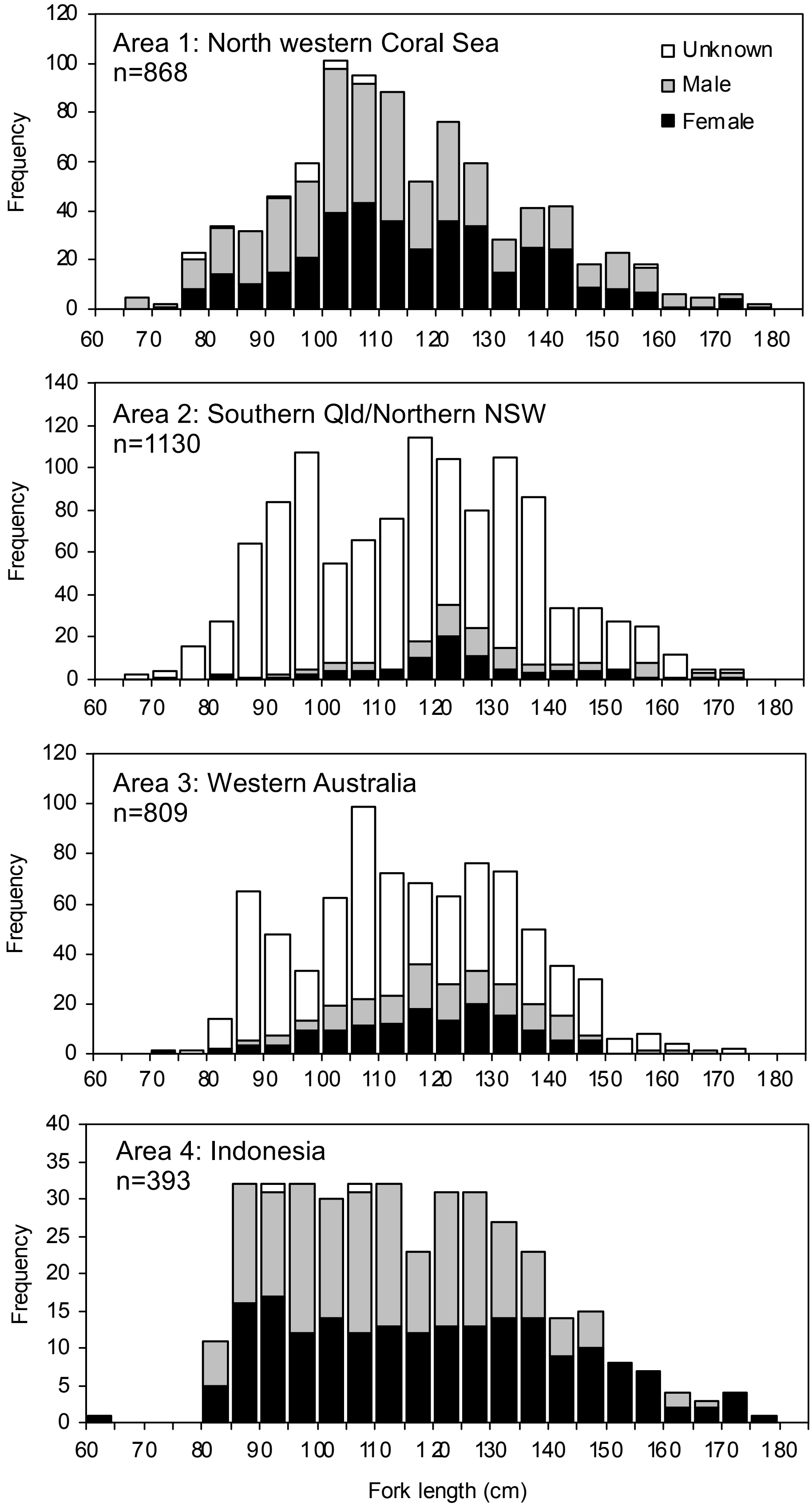

Figure 8.2.2. Length frequency distribution of bigeye with otoliths selected for age determination by area and sex. Otoliths were selected based on size of fish – stratified sampling rather than random sampling.

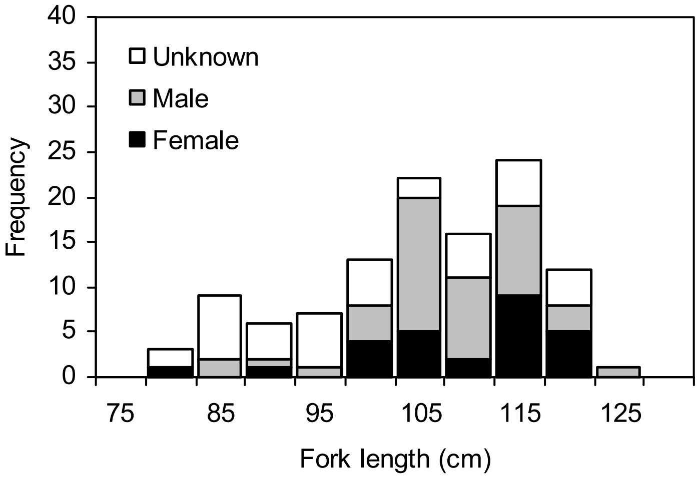

Figure 8.2.3. Length frequency distribution of bigeye with otoliths selected for microincrement analysis.

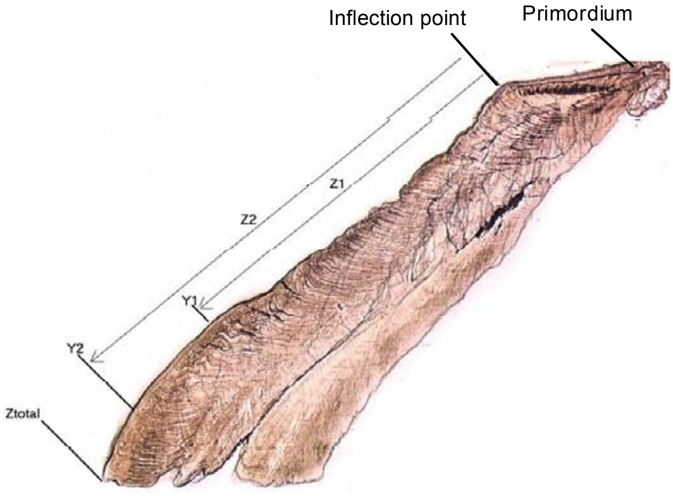

Figure 8.2.4. Transverse section of the ventral “long” arm of a bigeye otolith.

The number of visible microincrements was counted on each otolith. All counts were made from the primordium and were made by the same reader who conducted the microincrement work described in Chapter 7. Assuming daily increment formation, these provided an estimate of age. When present, the distance from the first inflection point to the 365th increment (age 1; Y1), the 730th increment (age 2; Y2) and to the edge of the otolith was measured. All measurements were made along the external side of the ventral edge.

A reliability score based on the entire reading was given to each otolith as either: excellent, good, or doubtful as described in Table 7.3.2 (Chapter 7.3.2). Counting microincrements becomes difficult towards the distal edge of the otolith because the width of the increment decreases. However, even when the reliability score was poor based on the overall reading, it was often possible to locate Y1 and Y2 if they were present. The birth date was back-calculated by subtracting the assumed daily age from the capture date.

The preparation of the second otolith in each pair for annual age estimation was conducted at the Central Ageing Facility in Queenscliff, Victoria (details in Chapter 8.2.3). The otoliths were sectioned along the same plane as for microincrement analysis, and the section passing closest to the primordium of the otolith was used for the comparison. The number of visible opaque growth zones was counted for each otolith and a confidence score was assigned also as described in Chapter 8.2.3.

To compare the age estimates from the two techniques, we calculated the number of annual opaque zones we would expect to see in the sectioned otolith based on the microincrement analysis, and compared this to the observed number of opaque zones. The expected number of annual opaque zones was calculated from the back-calculated birth date, the capture date, and the assumed time that opaque zones form during the year. Since reliable information is not currently available, we assumed that opaque zones form during winter in bigeye tuna (June to August). Therefore, if a fish was born in December 1997 and was captured in August 2000, we would expect two opaque zones to be present (the fish has lived through two winters). However, if a fish was captured during winter, an opaque zone may or may not be visible at the edge of the otolith. We assumed that if a fish were born during winter (June to August), this first slow growth period would not be visible as an opaque zone.

8.2.3 Annual age determination

Transverse sections of the 3200 otoliths were prepared at the Central Ageing Facility (Marine and Freshwater Resources Institute) in Queenscliff, Victoria. Sectiones were prepared in a similar way as for southern bluefin otoliths (Anon 2002). Otoliths were embedded in clear casting polyester resin and four or five serial sections were cut from each otolith (around the primordium) and polished to approximately 350 µm thick. Otolith sections were examined under compound and dissecting light microscopes. To compare the internal structure of otoliths from the same fish, both otoliths from 22 pairs were sectioned and examined.

The “best” section for clarity and interpretability was chosen for reading. The number of visible opaque growth zones was counted along the ventral “long” arm of each otolith, and a confidence score was assigned to each reading as:

1. No pattern obvious
2. Pattern present – no meaning
3. Pattern present – unsure with age estimate
4. Good pattern present – slightly unsure in some areas
5. Good pattern – confident with age estimate
6. No doubt

Each otolith was read two or three times without reference to the previous reading. If the successive readings were in agreement, this estimate was used as the final increment count for the otolith. However, if the readings differed, a further reading was conducted with knowledge of the previous readings to decide on a final count. The final count was assigned an overall confidence based on the mean of the individual confidence scores. If no obvious pattern could be seen in the otolith section, an increment count was not made. All readings were conducted without knowledge of the size of fish or date of capture. The Average Percentage Error method of Beamish and Fournier (1981) was used to examine intra-reader consistency in replicate otolith readings (precision of readings). Comparisons of otoliths that could and could not be interpreted were made using Kolmogorov-Smirnov tests and unpaired t-tests.

Measurements of otoliths were made along the external side of the ventral edge of the section, using a microscope linked via a camera to a computer with image analysis software. For consistency between otoliths, measurements were only made on sections passing through the primordium. Measurements were made from the first inflection point to the first three opaque zones (if present) and to the edge of the otolith. When curvature of the otolith occurred (in older fish) the distance to the edge of the otolith was made along two lines: the first inflection to the second inflection, and then to the edge of the otolith. Measurements were only made if the opaque zones were clearly defined.

Marginal increment analysis (MIA) and edge type analysis (ETA) (Campana 2001) were used to determine if the timing of increment formation during the year could be ascertained. The marginal increment of otoliths was calculated by measuring the distance from the last formed opaque zone to the edge of the otolith (incomplete growth zone) as a proportion of the previous growth zone. This analysis was restricted to otoliths with a final increment count of two or three years. For the ETA, the margin of each otolith section was scored on the presence of an opaque or translucent zone. This analysis was restricted to otoliths with a final increment count of five or more years, and where the margin of otolith section showed distinct alternating opaque and translucent growth zones.

8.2.4 Age corroboration – tag recapture analysis

To corroborate the periodicity of growth increments, we used back-calculation techniques on otoliths collected from fish previously tagged and released in the Coral Sea. In most studies, the aim of back-calculating is to use the measurements of fish length and otolith length at capture to infer fish length at an earlier time (Francis 1995). However, we used fish and otolith length at recapture to infer otolith length at release, and we counted the number of opaque growth zones after this position to compare with the known number of years between tagging and recapture. Similar analysis was used to confirm the periodicity of increments in yellowtail flounder, *Limanda ferruginea* (Dwyer et al. In press).

Of the 3200 otoliths selected for age estimation, 23 were from fish tagged and recaptured in the Coral Sea (Kaltongga 1989). Fish were tagged in October of 1999 and 2001 and ranged in size from 70-101 cm FL. Fish were recaptured between November 2000 and September 2002, after being at liberty for between 15 and 934 days (0.04-2.56 years). Five otoliths were removed from the analysis because of obvious inconsistencies — the recapture fish length was smaller or equal to the release length.

There are several back-calculation techniques available (see reviews in Francis 1990; Francis 1995; Folkvord and Mosegaard 2001) and it is acknowledged that each has advantages and disadvantages depending on the data used and the relationship between fish and otolith size. We estimated otolith size at release using the Fraser-Lee regression method (Carlander 1981):

The Fraser-Lee regression method is based on:

$$O_R = c + (O_r - c)\, L_r^{-1}\, L_R$$

where O_R is the length of the otolith at release, c is the intercept of the regression of fish length at recapture (L_r) to otolith length at recapture (O_r) for fish sampled in the Coral Sea, and L_R is the fish length at release. All measurements of otolith length were taken along the same axis used to estimate the age of the fish. The use of the biological intercept method for back-calculating lengths (Campana 1990) could not be used because the observed initial fish and otolith length (biological intercepts) are unknown.

8.2.5 Growth

Von Bertalanffy growth functions were fitted to these length-at-age data by sex and location using the equation:

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

Where L_t is the fork length (cm) at age t, L_∞ is the theoretical maximum fork length, k is the growth parameter (per year), and t_0 is the theoretical age (year) at zero length. The equation was fitted using the nonlinear regression function. Growth parameters were estimated using the least square method. A modified analysis of the residual sum of squares (ARSS) was used to compare the von Bertalanffy growth functions among locations and sexes (Chen et al. 1992). Unpaired t-tests were also used to compare mean length-at-age among sexes and locations.

8.2.6 Catch-at-age

Age-length keys were developed using our sample of aged fish. Age-length keys give the proportion of fish at age in each 5-cm length class, which enabled the conversion of catch-at-length data to catch-at-age. Neither the Eastern Tuna and Billfish Fishery (ETBF) nor the Southern and Western Tuna and Billfish Fishery (SWTBF) are sampled sufficiently for length, however, weight data is collected for a large proportion of the catch. For example, between mid-1997 and mid-2001, 78% of bigeye retained in the ETBF were sampled (Campbell et al. 2003). Individual weight data collected between January 2001 and June 2002 (n=33,244 in the ETBF and 2,490 in the SWTBF) was converted to lengths using:

$$L = (W / a)^{1/b}$$

where $a = 2.6696 \times 10^{-5}$ and $b = 2.948$ for the eastern AFZ (Campbell and Dowling 2003), and $a = 2.74 \times 10^{-5}$ and $b = 2.908$ for the western AFZ (Stobberup et al. 1989).

The age distribution of bigeye in the Indonesian longline catch was estimated using age-length keys applied to length frequency data obtained as part of the tuna-monitoring program in Bali (Davis and Andamari 2002; 2003) Individual length data was collected for 4,045 fish between July and December 2002.

8.2.7 Maturity

To estimate maturity for bigeye tuna, gonads were removed from 1,376 fish sampled from the north-western Coral Sea. Sampling was conducted in conjunction with otolith sampling (described above) by technicians on board two domestic longliners between August 2000 and September 2002. A visual assessment of the developmental stage was made based on the macroscopic appearance of the gonads using the scheme developed by McPherson (1992) and each fish was classed as either mature or immature based on the presence of vitellogenic oocytes.

An estimate of length at 50% maturity for females and males was obtained for fish sampled in the spawning season from a logistic regression:

$$P(maturity \mid L) = \frac{\exp(a + bL)}{1 + \exp(a + bL)}$$

where P is the estimated proportion of mature individuals at fork length L, and a and b are parameters that define the shape and position of the fitted curve. The predicted length at 50% (L_{50}) maturity was calculated as:

$$L_{50} = -a/b$$

Age at 50% maturity was determined using the age-length data obtained for the north-western Coral Sea (described above). The otoliths used were removed from a subsample of fish used in the maturity study.

8.2.8 Sex ratio

The sex ratio was analysed based on all fish with otoliths sampled and archived into the CSIRO collection (not just those selected for age estimation or given an age estimate). It was assumed that sampling was conducted at random as the sex of bigeye cannot be determined from external features (Schaefer 2001). The data from the Coral Sea and Indonesia were collected during the current project, while data from Qld/NSW and WA were collected from 1994 and 1992 respectively. A comparison was made between areas, and size classes (10 cm) within areas. Statistical differences were analysed using Chi-square tests.

8.3 Results

8.3.1 Otolith growth

No significant difference was detected in otolith weight between left and right otoliths (t-test comparison); 88% of otolith pairs were less than 5% different in weight. Comparisons of transverse sections of otolith pairs show remarkable similarity (examples in Fig. 8.3.1) indicating that either can be used for age estimation, and different age estimation techniques can be used on separate otoliths.

The initial analysis of fish length and otolith length (either whole or sectioned) indicated a linear relationship (Fig. 8.3.2a, b; Table 8.3.1) with a significant difference between the eastern Indian and western Pacific Oceans (ANCOVA; Table 8.3.2). The *p* values for both Ocean and the Fork Length*Ocean interaction terms were significant for whole otoliths. The *p* values for the Fork Length*Ocean interaction term was not significant for sectioned otoliths, and when removed from the model, the *p* values for Ocean was significant. The *p* value for Fork Length (<0.001) shows that it is clearly useful for predicting otolith length.

By restricting the analysis of otolith growth data to sectioned otoliths (as there were higher numbers of measurements), the fish length to otolith length relationship was significantly different between sampling locations within the western Pacific Ocean (Coral Sea and Qld/NSW), which may indicate some level of geographic separation of bigeye populations within the region (Table 8.3.3). A significant difference was not found in the fish length to otolith length relationship within the eastern Indian Ocean (Western Australia and Indonesia). At each location, the fish length to otolith length relationship was not significant between sexes (Table 8.3.4.). The linear relationship of fish length and sectioned otolith length for fish sampled in the Coral Sea was used for back-calculation using the Fraser-Lee technique (Carlander 1981) in Chapter 8.3.5.

Analysis of otolith weight and fish length (Fig. 8.3.2c) showed a second order polynomial relationship (r^2=0.835 for the Pacific Ocean and r^2=0.823 for the Indian Ocean), and showed differences between oceans. Fish age was linearly related to otolith weight (r^2=0.886 for the Pacific Ocean and r^2=0.846 for the Indian Ocean), although age varied substantially for a given weight making it an imprecise predictor of age in bigeye tuna (Fig. 8.3.3).

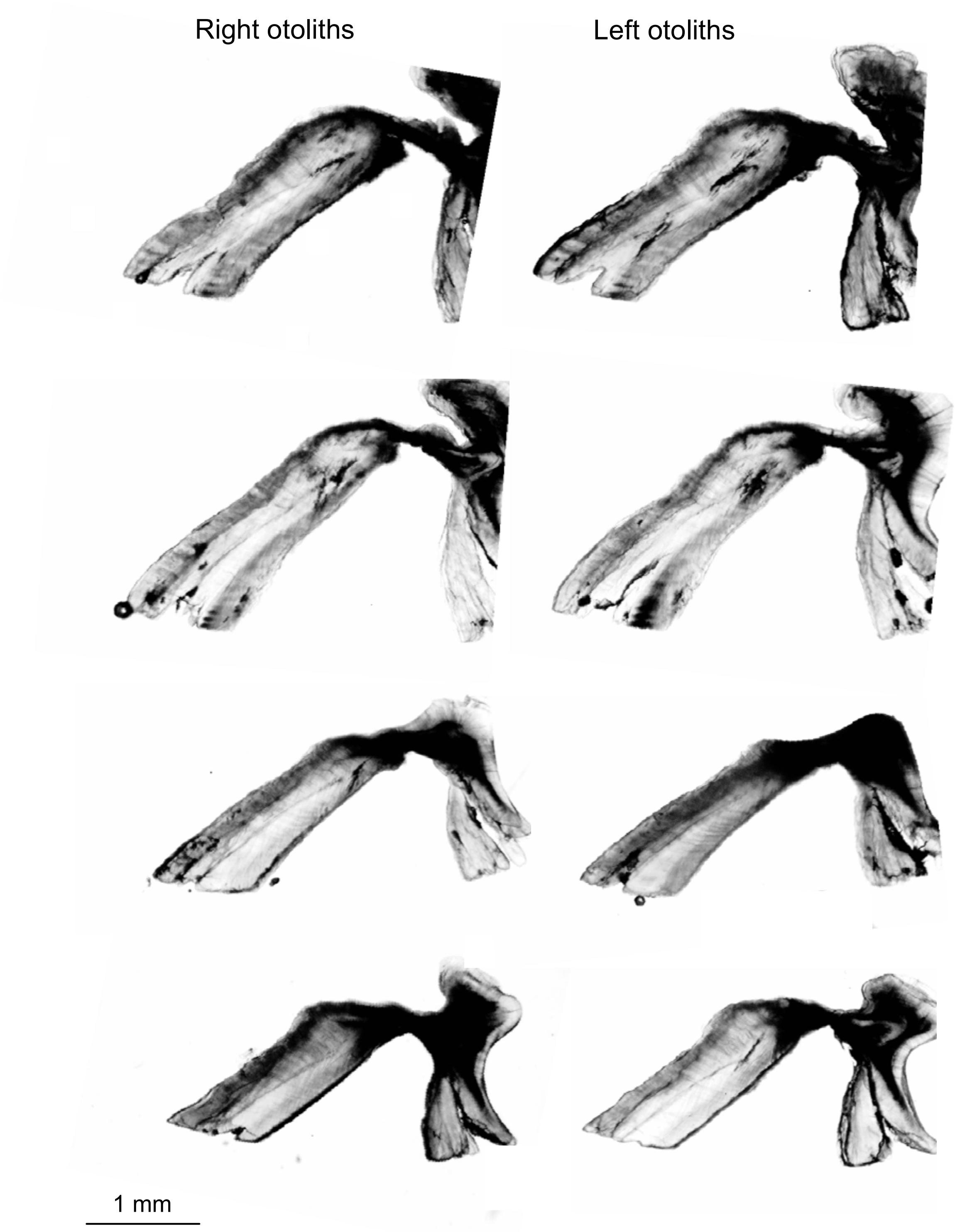

Figure 8.3.1. Comparisons of the internal structures in transverse sections of right and left otoliths of a pair.

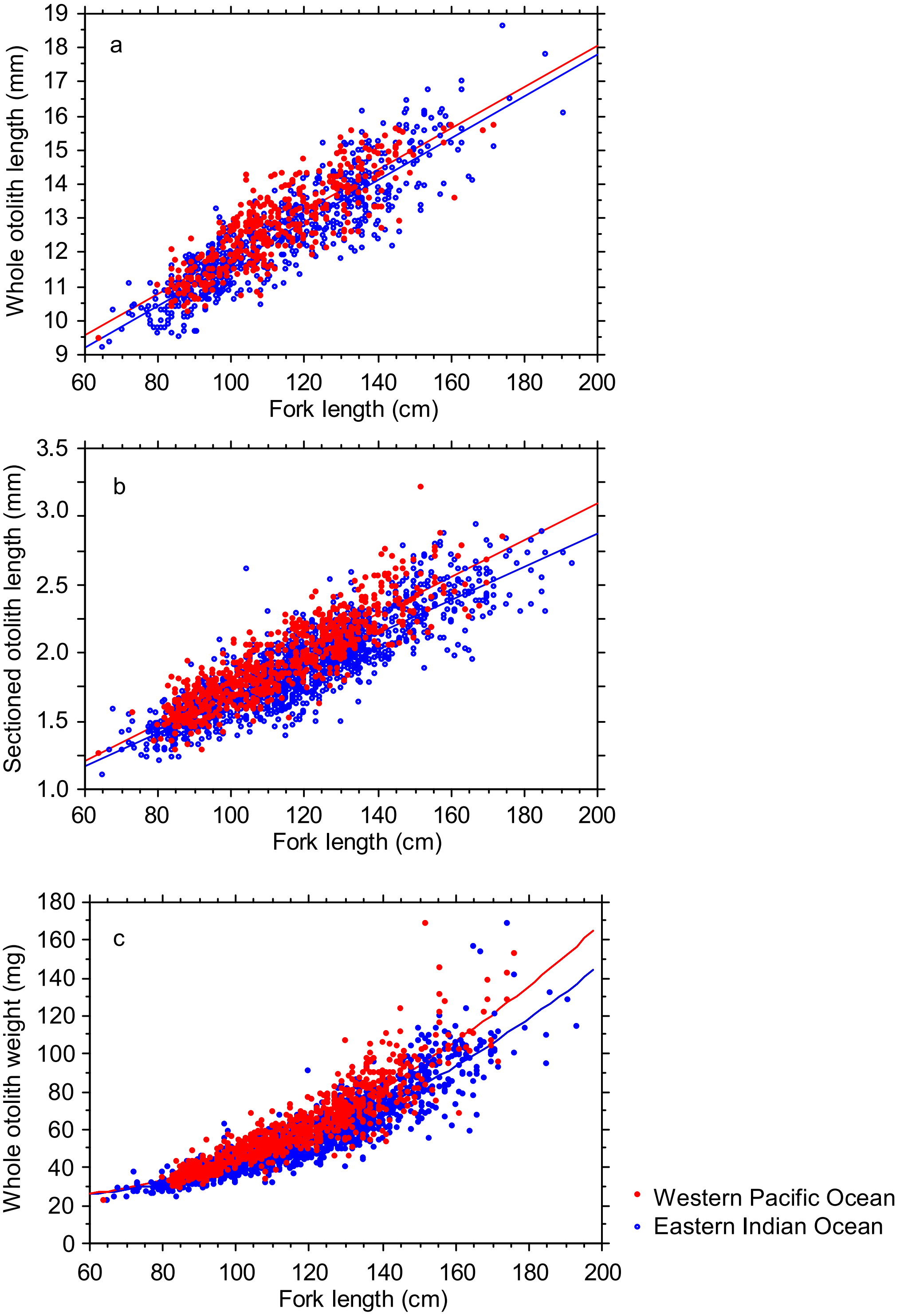

Figure 8.3.2. Relationship between fork length and (a) whole otolith length (n=1173), (b) sectioned otolith length (n=2295), and (c) whole otolith weight (n=2483) for bigeye sampled in the eastern Indian and western Pacific Oceans.

Table 8.3.1. Results of regression analysis examining the relationship between fish length (cm) and otolith length (μm).

	Whole otolith		Sectioned otolith	
	Indian Ocean	Pacific Ocean	Indian Ocean	Pacific Ocean
Slope	0.061	0.061	0.014	0.012
Intercept	5.942	5.535	0.392	0.442
R-squared	0.717	0.799	0.778	0.771

Table 8.3.2. ANCOVA of the effect of Ocean (eastern Indian or western Pacific) on whole and sectioned otolith length.

ANCOVA table for whole otolith

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	1416.994	1416.994	3130.482	<.0001	3130.482	1.000
Ocean	1	1.216	1.216	2.686	.1015	2.686	.356
Fork length * Ocean	1	.071	.071	.158	.6913	.158	.068
Residual	1169	529.141	.453				

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	1830.720	1830.720	4047.415	<.0001	4047.415	1.000
Ocean	1	26.073	26.073	57.643	<.0001	57.643	1.000
Residual	1170	529.212	.452				

ANCOVA table for sectioned otolith

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	141.633	141.633	6489.747	<.0001	6489.747	1.000
Ocean	1	.039	.039	1.785	.1816	1.785	.251
Fork length * Ocean	1	.396	.396	18.150	<.0001	18.150	.996
Residual	2288	49.934	.022				

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	170.080	170.080	7735.244	<.0001	7735.244	1.000
Ocean	1	5.651	5.651	257.022	<.0001	257.022	1.000
Residual	2289	50.330	.022				

Table 8.3.3. ANCOVA of the effect of sampling location within the western Pacific (Coral Sea and Qld/NSW) and eastern Indian oceans (Western Australia and Indonesia) on sectioned otolith length. The Fork Length*Location interaction was not significant for all locations and was removed from the model.

ANCOVA table for the western Pacific Ocean

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	117.518	117.518	5493.689	<.0001	5493.689	1.000
Location	1	1.288	1.288	60.206	<.0001	60.206	1.000
Residual	1554	33.242	.021				

ANCOVA table for the eastern Indian Ocean

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	52.988	52.988	2518.059	<.0001	2518.059	1.000
Location	1	2.047E-9	2.047E-9	9.725E-8	.9998	9.725E-8	.050
Residual	732	15.403	.021				

Table 8.3.4. ANCOVA of the effect of sex on sectioned otolith length for four sample locations. The Fork Length*Sex interaction was not significant for all locations and was removed from the model.

ANCOVA table for the Coral Sea

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	64.670	64.670	2407.693	<.0001	2407.693	1.000
Sex	1	.019	.019	.705	.4015	.705	.128
Residual	754	20.252	.027				

ANCOVA table for the Tasman Sea

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	2.806	2.806	118.371	<.0001	118.371	1.000
Sex	1	.001	.001	.045	.8329	.045	.055
Residual	77	1.825	.024				

ANCOVA table for Western Australia

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	.652	.652	26.139	<.0001	26.139	1.000
Sex	1	.063	.063	2.509	.1225	2.509	.321
Residual	34	.848	.025				

ANCOVA table for Indonesia

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Fork length	1	19.848	19.848	947.941	<.0001	947.941	1.000
Sex	1	1.951E-12	1.951E-12	9.319E-11	>.9999	9.319E-11	.050
Residual	251	5.255	.021				

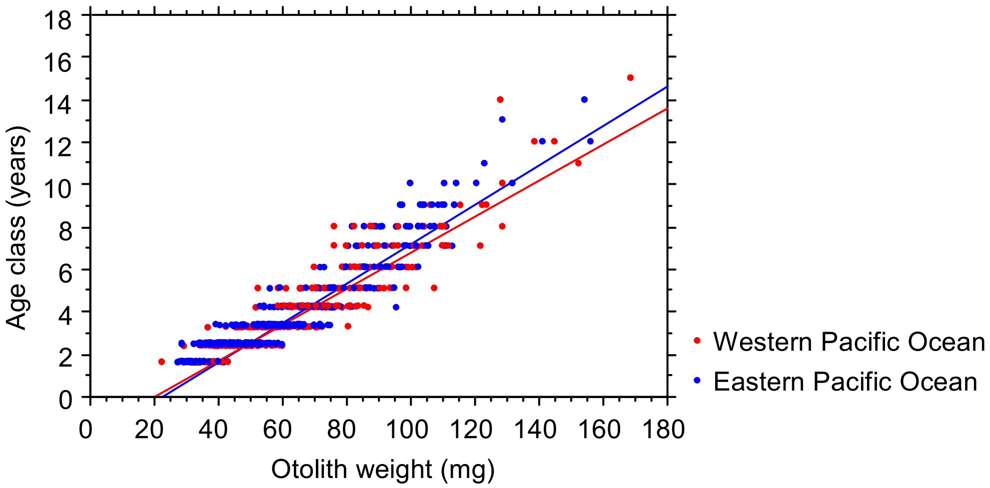

Figure 8.3.3. Relationship between age and otolith weight (n=1670) for bigeye sampled in the eastern Indian and western Pacific Oceans.

8.3.2 Microincrement analysis

Seventy three otoliths were read in the analysis of microincrement counts. Increment counts ranged from 544 (1.5 years) to 1300 (3.6 years). A linear relationship (r^2=0.731) was found between otolith length and estimated daily age (Fig. 8.3.4).

The mean distance was 1124 µm to the 365th increment (age 1; Y1), 1639 µm to the 730th increment (age 2; Y2) and 2015µm to the 1095th increment (age 3; Y3) for all areas combined. Otoliths from Indonesia showed the smallest variability in Y1 and Y2 (Fig. 8.3.5), but the mean of these were not significantly different between the four regions (Table 8.3.5). This is confirmed by the multiple comparisons analysis using the Bonferroni test (Table 8.3.5). As only four measurements of Y3 were obtained, regional comparisons were not made.

Of the 73 otoliths read successfully in the microincrement analysis, an annual age was estimated for 53 based on the sister otolith. Table 8.3.6 shows the comparison of daily age and annual age estimates for each fish. The results indicate that estimated age based on counts of observed opaque growth zones was generally correct. For 89% of fish, the number of opaque zones observed equalled the number expected or was within the expected range. Of the incorrect estimates (n=6), only two annual age estimates had a confidence score ≥2.5 and would have been included in the final analysis (see Chapter 8.3.4 below). The number of opaque zones counted in these two was one less than expected. Appendix 2 gives examples of otoliths with the location of Y1, Y2 and observed annual opaque zones marked.

Although the sample size was small, the back-calculated birth dates of bigeye based on the microincrement analysis varied considerably in both the Indian and Pacific Oceans (Fig. 8.3.6). The majority of bigeye analysed from the north-western Coral Sea (n=36) had birth dates between February and July, while birth dates of fish caught in Western Australia (WA) show a year-round distribution.

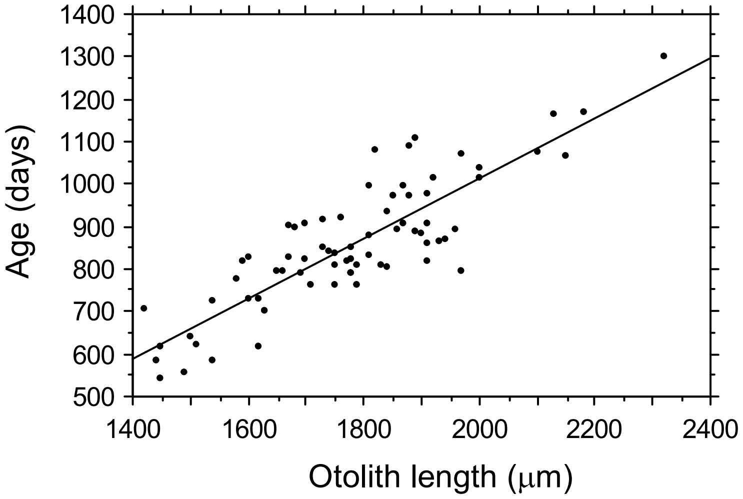

Figure 8.3.4. Relationship between estimated daily age and otolith length. Only otolith readings with a readability score of good or excellent are included.

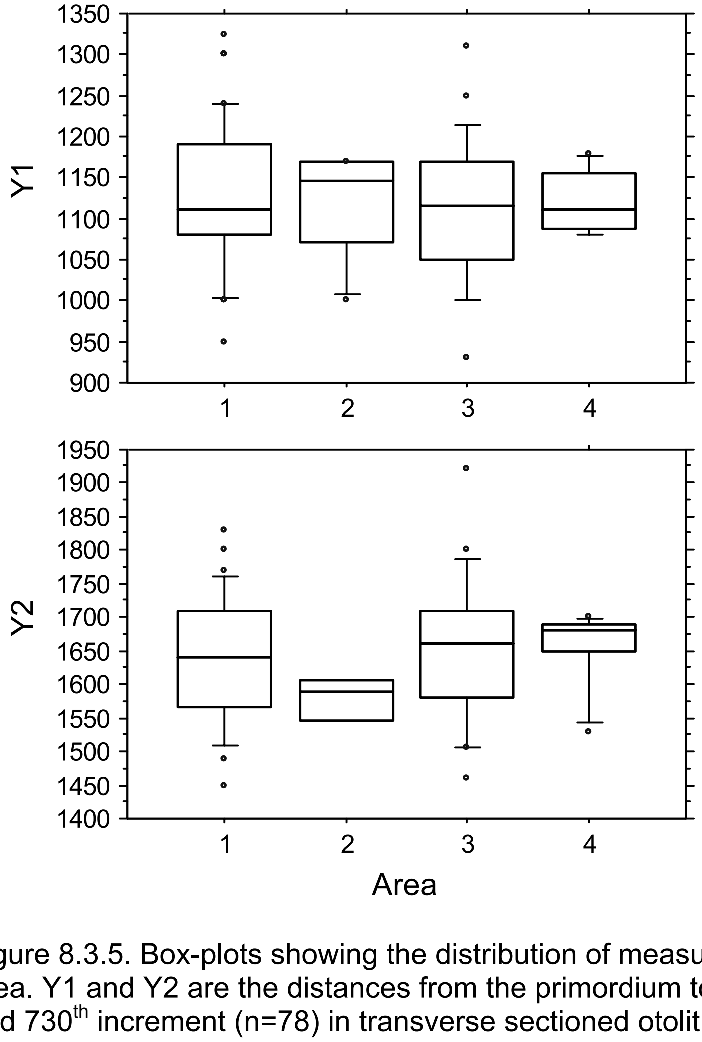

Figure 8.3.5. Box-plots showing the distribution of measurements of Y1 and Y2 by area. Y1 and Y2 are the distances from the primordium to the 365th increment (n=107) and 730th increment (n=78) in transverse sectioned otoliths. 1 = Coral Sea, 2 = Qld/NSW, 3 = Western Australia, and 4 = Indonesia. Centre line and outside edge of each box indicate the median and 25th/75th percentile around the median respectively. Dots indicate all observations <10th and >90th percentile.

Table 8.3.5. ANOVA and Bonferroni tests of the effect of sampling location on Y1 and Y2. Y1 and Y2 are the distances from the primordium to the 365th increment (age 1) and 730th increment (age 2) in transverse sectioned otoliths.

ANOVA Table for Y1

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Area #.2	3	6175.689	2058.563	.295	.8292	.884	.103
Residual	71	496090.977	6987.197				

Bonferroni/Dunn for Y1
Effect: Area
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value
Area 1, Area 2	1.216	93.512	.9719
Area 1, Area 3	20.307	61.081	.3699
Area 1, Area 4	12.327	84.323	.6927
Area 2, Area 3	19.091	98.453	.6003
Area 2, Area 4	11.111	114.336	.7927
Area 3, Area 4	-7.980	89.772	.8100

Comparisons in this table are not significant unless the corresponding p-value is less than .0083.

ANOVA Table for Y2

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Area #.2	3	11586.350	3862.117	.420	.7391	1.261	.127
Residual	59	542205.365	9189.921				

Bonferroni/Dunn for Y2
Effect: Area
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value
Area 1, Area 2	42.800	125.127	.3542
Area 1, Area 3	-7.082	77.372	.8035
Area 1, Area 4	-16.200	115.644	.7035
Area 2, Area 3	-49.882	133.150	.3106
Area 2, Area 4	-59.000	158.480	.3136
Area 3, Area 4	-9.118	124.281	.8419

Comparisons in this table are not significant unless the corresponding p-value is less than .0083.

Table 8.3.6. Summary of microincrement data for bigeye successfully read at the Secretariat of the Pacific Community in Noumea, with corresponding expected and observed counts of annual opaque zones. * indicates low confidence scores (<2.5) associated with the annual age estimates.

Fish number.	Fork length (cm)	Estimated age from daily increment counts (yrs)	Expected number of opaque zones based on birth and capture dates	Estimated annual age from observed opaque zone counts (yrs)	Confidence score of estimated annual age
1516	81	1.49	1	1	3.50
1727	82	1.60	1 or 2	1	2.00*
1559	88	1.61	1	1	3.50
1033	88	1.69	2	2	2.00*
1287	86	1.70	2	2	3.50
1371	84	1.76	2	1	2.50
1798	87	1.93	1 or 2	1	3.00
1762	89	1.99	1 or 2	1	3.00
1742	90	1.99	1 or 2	2	2.00*
1744	89	2.00	1 or 2	2	2.50
1791	89	2.08	2 or 3	1	3.00
1683	99	2.08	2	2	3.00
1106	99	2.09	2	2	2.50
2011	110	2.13	1 or 2	2	2.50
1640	95	2.16	2	2	3.50
1753	97	2.18	2 or 3	2	2.50
840	104	2.18	1 or 2	2	3.00
1782	100	2.21	2 or 3	2	3.00
867	106	2.21	1 or 2	2	3.00
1035	90	2.24	2	2	1.00*
977	101	2.24	2	2	3.00
875	96	2.25	2 or 3	2	1.33*
1643	97	2.26	2	2	4.00
831	104	2.26	2 or 3	2	2.67
1051	99	2.27	2	2	2.00*
996	101	2.27	2	2	2.33*
1266	103	2.29	2	2	3.50
945	109	2.30	2 or 3	2	2.33*
942	113	2.30	2 or 3	2	2.67
1123	113	2.36	2	3	2.00*
1110	104	2.37	3	2	1.50*
1139	104	2.38	2	2	2.00*
839	99	2.41	2 or 3	2	2.33*
922	111	2.42	2 or 3	2	2.67
1308	112	2.44	3	3	2.50
1695	92	2.45	2 or 3	2	3.00

Table 8.3.6 continued. Summary of microincrement data for bigeye successfully read at the Secretariat of the Pacific Community in Noumea, with corresponding expected and observed counts of annual opaque zones. * indicates low confidence scores (<2.5) associated with the annual age estimates.

Fish number	Fork length (cm)	Estimated age from daily increment counts (yrs)	Expected number of opaque zones based on birth and capture dates	Estimated annual age from observed opaque zone counts (yrs)	Confidence score of estimated annual age
816	99	2.46	2 or 3	2	2.33*
913	106	2.48	2 or 3	2	2.00*
835	102	2.49	2 or 3	2	2.00*
1131	110	2.51	3	2	1.67*
834	102	2.52	2 or 3	3	1.50*
884	109	2.67	2 or 3	3	2.67
1133	107	2.68	3	3	2.00*
847	107	2.73	2 or 3	2	2.33*
1501	114	2.73	2	2	3.50
1474	103	2.78	3	2	3.00
1068	120	2.78	3	3	2.00*
1331	116	2.85	3	3	2.50
1029	118	2.95	3	3	2.33*
1277	98	2.96	3	3	2.50
1093	117	2.99	3	3	2.33*
1063	115	3.04	3	3	1.67*
1466	120	3.20	3	4	1.50*

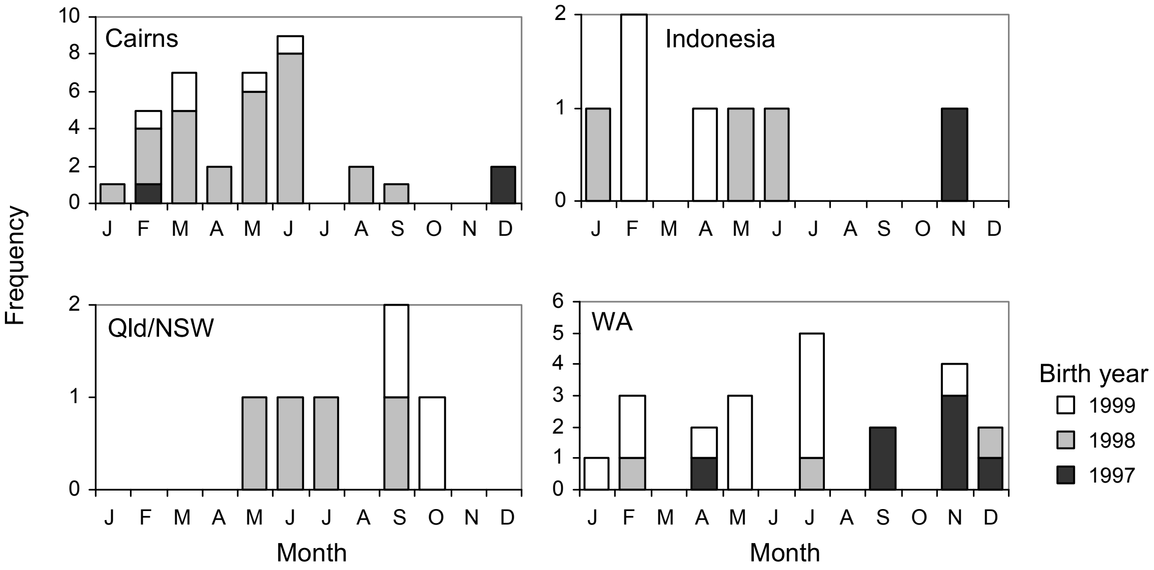

Figure 8.3.6. Estimated birth month by birth year and area of capture for bigeye based on microincrement analysis. Year of capture was 2000 or 2001.

8.3.3 Annual age determination

The clarity and interpretability of growth increments varies substantially between otoliths. Some show clear and well-defined opaque zones while others are less well defined, making it difficult to distinguish between these and the translucent zones. In general, the first two or three annual opaque zones out from the primordium appeared broad, diffuse, and often contained multiple translucent and opaque sub-annual bands. Subsequent opaque zones are usually clearer and in large otoliths, the distance between the opaque zones becomes regular in width and appearance towards the terminal edge of the otolith (Fig. 8.3.7). These outer zones are generally darker and clearer than inner or middle zones. In large otoliths, the opaque zones towards the terminal edge occasionally appear as double increments, similar to that observed in southern bluefin (Gun et al. In press). These pairs of increments are counted as one opaque growth zone.

The mean average percentage error (APE) between blind readings was 5.98. Not surprisingly, the precision of readings was related to the confidence score from the readings: lower precision being correlated with lower confidence scores of readings (Table 8.3.7). If only those otoliths with a mean confidence of ≥2.5 are included, mean APE decreased to 4.34% — a precision level above that recommended by Morison et al. (1998). When readings differed, most were by only +/-1 year (Fig. 8.3.8), indicating a high level of precision.

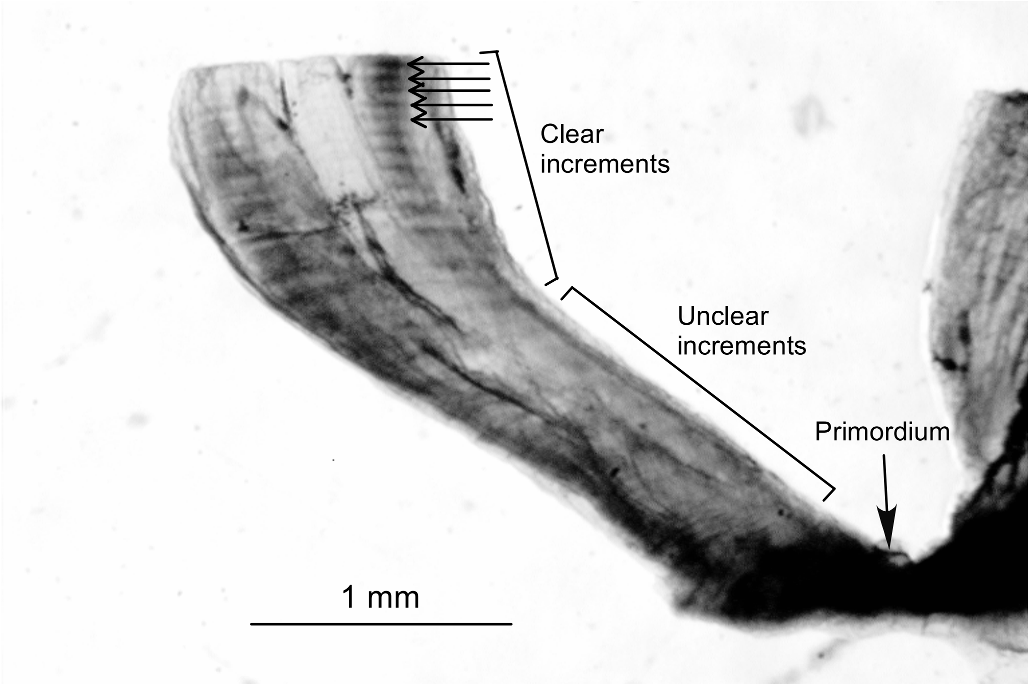

Figure 8.3.7. Transverse section of a bigeye otolith showing clear annual increments at the terminal edge (arrows) and the region of unclear increments towards the primordium.

A final increment count was assigned to 2,185 (68%) of the otoliths read. Of these, only 1,612 were included in the final data analysis as age estimates with mean confidence scores below 2.5 were not included due to low reading precision. Kolmogorov-Smirnov tests and unpaired t-tests showed no significant difference in all of the comparisons of otolith size (within 5 cm fork length classes) for otoliths included and excluded from the final analysis. A comparison of otolith growth rates from microincrement analysis also failed to show a significant difference between otoliths included and excluded from the analysis (Kolmogorov-Smirnov p=0.06). This suggests that otolith size in relation to fish size does not appear to influence the readability of otoliths in bigeye tuna.

The estimated positions of the first three opaque zones were consistent with measurements of Y1 and Y2 from the microincrement analysis (Fig. 8.3.9) confirming that the first few growth increments are being successfully identified in sectioned otoliths. The variability among otoliths in the distance from the first inflection point to the first opaque zone is probably due to a combination of factors such as different growth rates between fish, variability in the time of year of opaque zone formation or spawning, and measurement error associated with the precision of locating each opaque zone.

The timing of increment formation was investigated using marginal increment analysis (MIA) and edge type analysis (ETA). The total number of otoliths examined for MIA and ETA was 825 and 263, respectively. For both oceans the marginal increment was highly variable even within a single month (Fig. 8.3.10), and a consistent cyclic annual pattern was not apparent in either ocean. However, in the Pacific Ocean in 2001, the mean MI declined consistently from January to July before increasing again, suggesting a winter formation time for opaque zones in that year. The proportion of otoliths with an opaque growth zone at the edge showed a clearer pattern of formation during the year but only when the data was pooled across all years (sample sizes were too low to compare consecutive months within a 12 month period). Figure 8.3.11 indicates that opaque zones at the otolith edge were more common in early winter in both the western Pacific and eastern Indian oceans, although the pattern appears clearer for Indian Ocean bigeye tuna.

Table 8.3.7. Mean APE (average percent error) by confidence score.

Mean confidence score	Number of otoliths	Mean APE
1	150	8.02
2	999	7.16
3	904	3.70
4	134	1.00
5	1	000
Total	2279	5.98

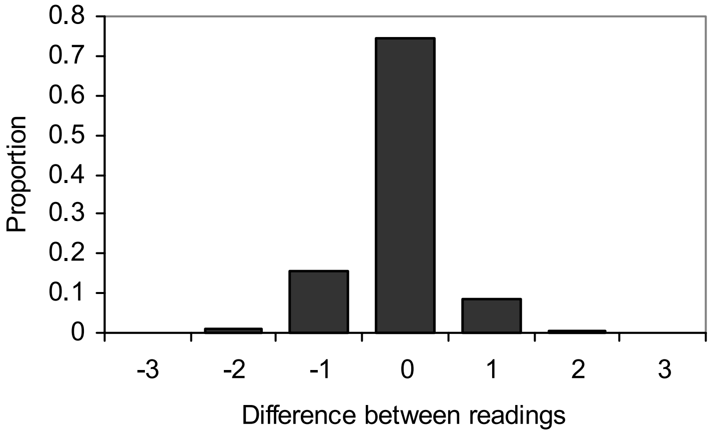

Figure 8.3.8. Differences between the first and second reading (or second and third if three reading were conducted) for sectioned bigeye otoliths.

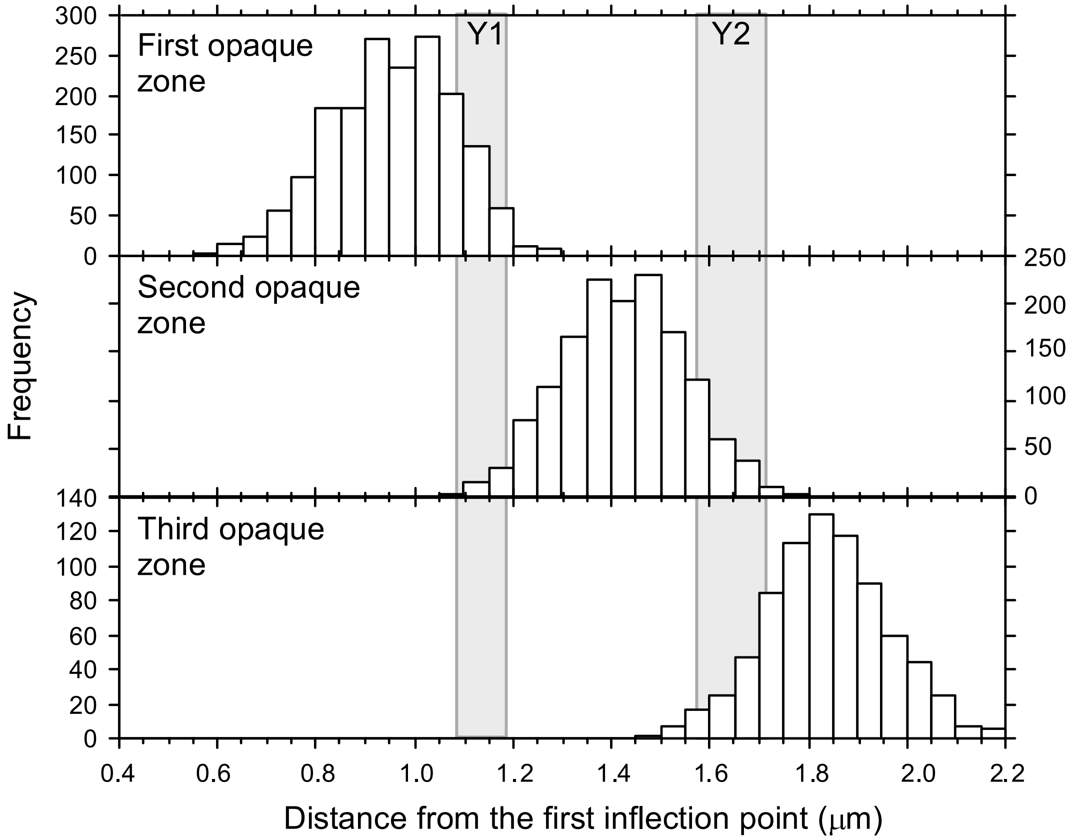

Figure 8.3.9. Histograms of otolith measurements from the first inflection point to the first, second, and third opaque growth zones. All otoliths with measurements were included. Grey stripes represent the 25th/75th percentile of the median distance to 365th increment (age 1; Y1) and the 730th increment (age 2; Y2) from microincrement analysis.

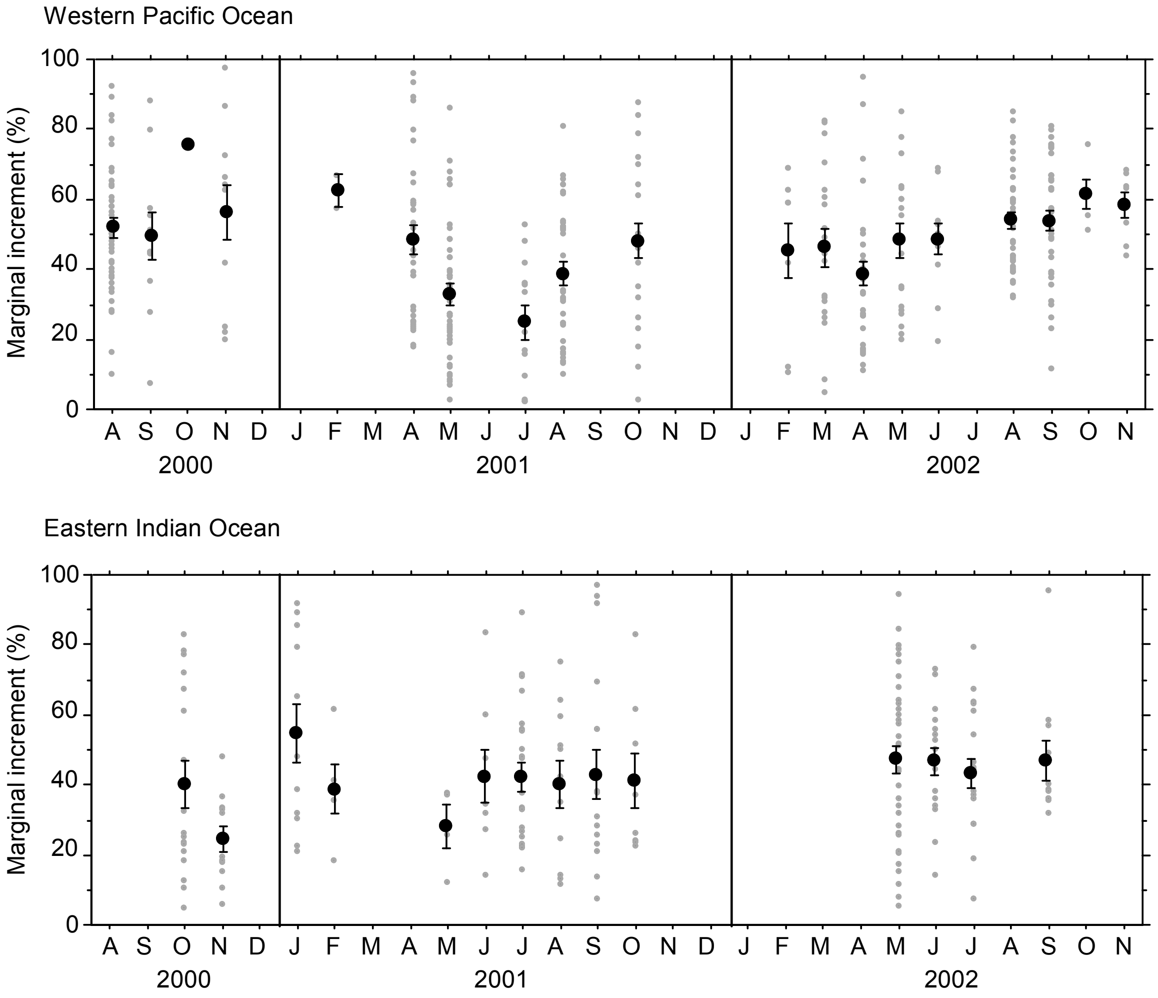

Figure 8.3.10. Marginal increment (% of the previous growth zone) by month for bigeye aged 2+ and 3+ for the western Pacific and eastern Indian Oceans. The black circles indicated the mean and standard error for the month.

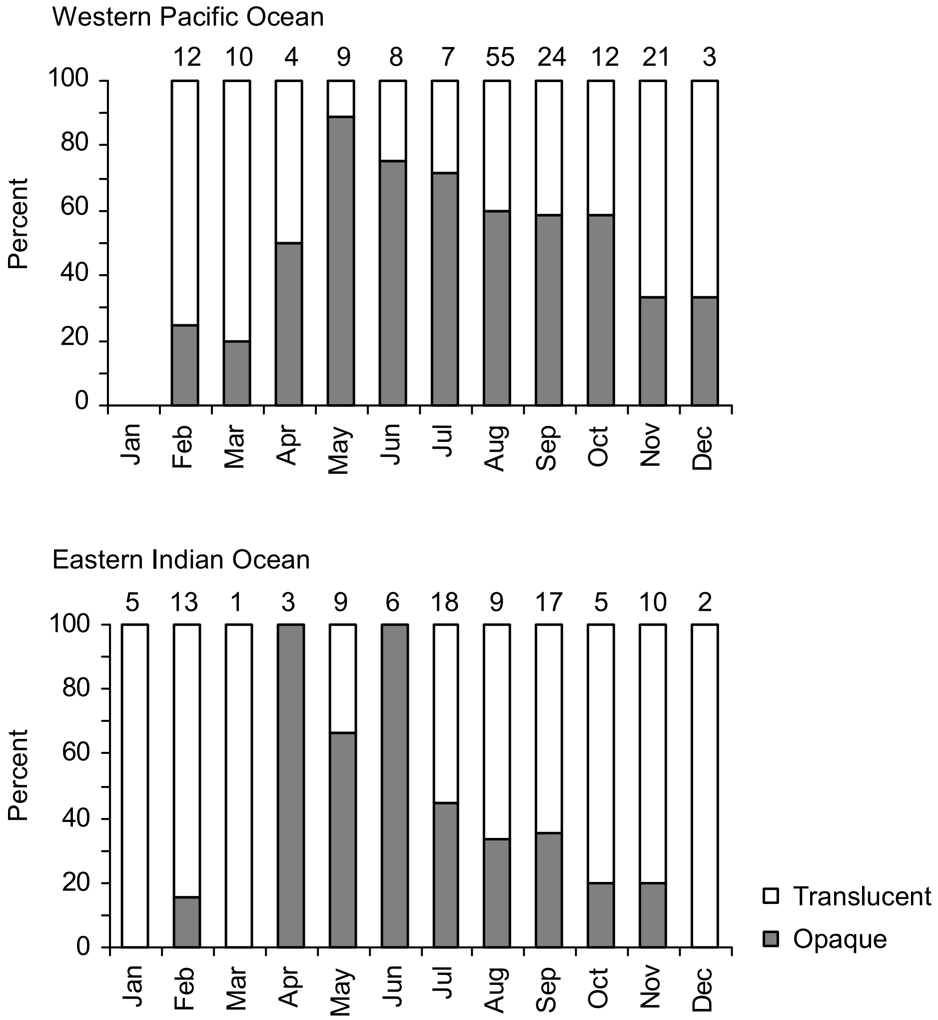

Figure 8.3.11. Percent of otoliths with opaque and translucent growth zones at the edge by month for the western Pacific and eastern Indian Oceans.

8.3.4 Age corroboration – tag-recapture analysis

Back-calculated lengths of otolith at release were obtained for 16 of the 18 fish tagged in the Coral Sea. The number of opaque zones observed after release from tagging was equal to or within the range expected for all fish (Table 8.3.8) corroborating the results of the direct validation of annual increments work (Chapter 7). Although most fish were at liberty for less than one year, the results confirm that one growth ring is deposited annually in bigeye tuna. For example, the fish at liberty the longest (2.5 years) had two opaque zones present after the back-calculated length of the otolith at release from tagging.

Table 8.3.8. Comparison of the expected (from time at liberty) and the observed number of opaque zones in sectioned otoliths from fish tagged and recaptured in the Coral Sea.

Fish number	Fork length at release (cm)	Fork length at recapture (cm)	Otolith length at recapture (mm)	Back-calculated otolith length at release (mm)	Time at liberty (years)	Expected number of opaque zones	Observed number of opaque zones
2125	77	83	1.48	1.40	0.04	0	0
2132	77	80	1.43	1.39	0.07	0	0
2126	78	80	1.48	1.45	0.07	0	0
2327	81	88	1.47	1.38	0.53	0-1	0
2612	91	101	1.79	1.65	0.61	0-1	0
2611	83	101	1.63	1.41	0.71	0-1	0
3387	101	113	1.82	1.67	0.93	1	1
3388	82	107	-	-	0.93	1	-
3386	98	116	1.82	1.60	0.94	1	1
3397	81	102	1.62	1.37	0.95	1	1
3394	81	103	1.81	1.51	0.95	1	1
3395	86	106	1.62	1.39	0.95	1	1
3389	80	107	1.74	1.40	0.95	1	1
3393	82	104	-	-	0.96	1	-
3396	93	116	1.8	1.52	0.96	1	1
1047	75	102	1.77	1.41	1.07	1	1
2037	83	130	2.06	1.46	1.92	2	2
2328	82	141	2.19	1.44	2.56	2-3	2

8.3.5 Growth

Age estimates were combined with those of Clear et al. (2000a) giving a total number of age estimates with high confidence scores of 1657; 783 with known sex. Large variations in age were detected within all length classes. The majority of fish were estimated to be less than five years old (84%) and only 1.7% were >10 years old. The oldest fish sampled was 16+ years old, however, the confidence level of this estimate was low and was not included in the analysis. The two oldest fish included in the analysis were 15+ year-old females sampled from the Coral Sea (170 cm FL) and Indonesia (152 cm FL). The oldest males were two 12 year-old sampled in Western Australia (150 and 162 cm FL). The ratio of females to males with otolith sampled was biased slightly towards females (0.9:1). However, males were more dominant in the younger (1+ to 4+) age classes (Table 8.3.9).

The von Bertalanffy growth curve was fitted to lengths-at-age by sex for each of the four sampling locations. The growth curves were significantly different between sexes for fish sampled in the Coral Sea and Western Australia only, with males being slightly larger at age than females (Table 8.3.10). However, no significant difference was detected in mean length-at-age between sexes at any of the sampling locations (unpaired t-tests) suggesting that the sexual dimorphism in growth in bigeye is relatively small. In all locations, however, the asymptotic length was greater for males than females (Table 8.3.11).

Estimates of the von Bertalanffy growth-curve parameters by sampling location and ocean (sexes combined) are given in Table 8.3.11. The growth curves were significantly different between all locations compared (Table 8.3.10) with the smallest differences occurring between sampling locations in the same ocean, and the largest differences occurring between sampling locations in different oceans. Using a single set of parameters for the eastern Indian (Western Australia and Indonesia) and western Pacific Oceans (Coral Sea and Qld/NSW), a significant difference between the von Bertalanffy parameters was found between growth rates in the two oceans. The growth parameter *k* was greater for bigeye sampled in the western Pacific Ocean compared to those sampled in the eastern Indian Ocean. Estimates of L_∞ were close to the observed maximum length, and were slightly larger for the Indian Ocean fish. The fitted growth curves are shown in Figure 8.3.12. Mean length-at-age with 95% confidence intervals are given for each sampling location in Appendix 3.

Table 8.3.9. Proportion of female bigeye sampled by age class. Only age estimates with a confidence score of ≥2.5 are included.

Age class (years)	Total no.	% Female
1	70	37.3
2	214	49.1
3	200	51.5
4	98	47.9
5	52	57.7
6	39	76.9
7	47	63.8
8	30	60.0
9	14	50.0
10	11	9.1
11	2	100.0
12	6	66.7
13	1	100.0
15	2	100.0

Table 8.3.10. Comparison of von Bertalanffy growth models between sexes, sampling locations, and oceans using analysis of the residual sum of squares (ARSS). ns = not significant.

Group	Comparison between		F	d.f	p
Cairns	Females	Males	3.23	3, 328	<0.05
Qld/NSW	Females	Males	1.96	3, 193	ns
Western Australia	Females	Males	2.85	3, 150	<0.05
Indonesia	Females	Males	2.27	3, 88	ns
All sexes	Coral Sea	Qld/NSW	5.63	3, 993	<0.01
All sexes	Western Australia	Indonesia	3.24	3, 652	<0.05
All sexes	Coral Sea	Indonesia	12.78	3, 545	<0.001
All sexes	Coral Sea	Western Australia	10.35	3, 799	<0.001
All sexes	Qld/NSW	Indonesia	17.86	3, 846	<0.001
All sexes	Qld/NSW	Western Australia	26.03	3, 1100	<0.001
All sexes	Pacific Ocean	Indian Ocean	31.64	3, 1651	<0.001

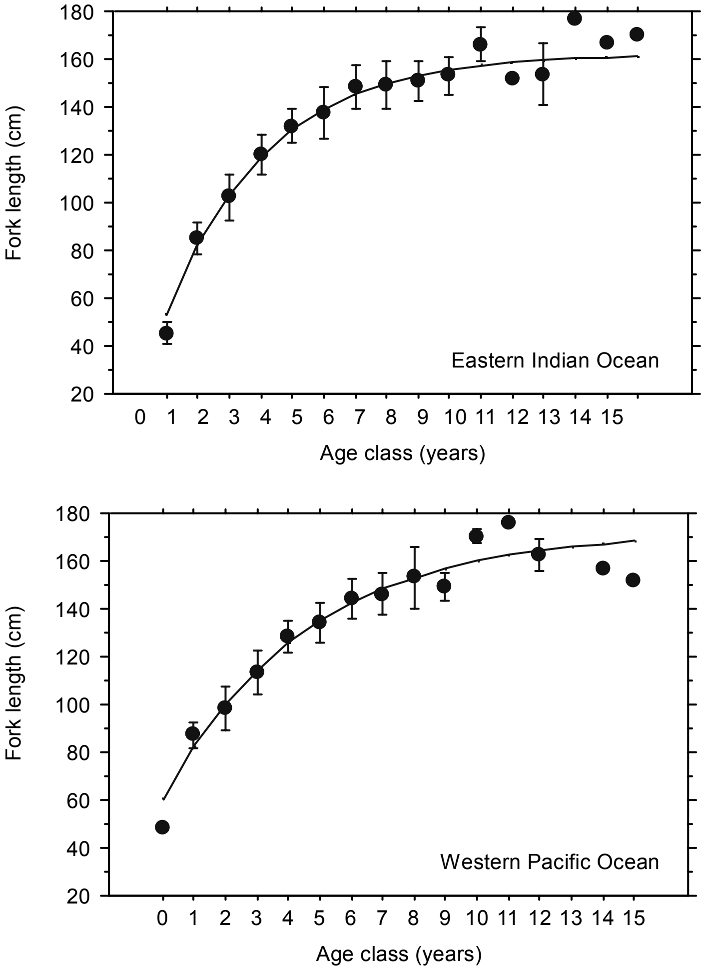

Figure 8.3.12. Mean length-at-age and standard deviations (circles) for bigeye tuna in the western Pacific and eastern Indian Oceans. von Bertalanffy growth curves fitted to the raw data are shown (lines).

Table 8.3.11. Least square estimates and 95% confidence intervals (in brackets) of von Bertalanffy growth parameters by sampling location and sex, and for the combined western Pacific (Coral Sea and Qld/NSW) and eastern Indian Oceans (Western Australia and Indonesia). For the combined areas, fish with sex not identified are included.

Location	Sex	Length range	N	L_∞	K	t_0
Coral Sea	F	78-176	162	157.78 (151.5, 164.1)	0.299 (0.23, 0.37)	-1.712 (-2.34, -1.09)
	M	69-178	172	168.629 (159.7, 177.6)	0.242 (0.19, 0.30)	-1.879 (-2.45, -1.31)
	All	39-178	349	158.37 (154.7, 162.0)	0.327 (0.29, 0.36)	-1.26 (-1.49, -1.03)
Qld NSW	F	73-165	47	156.193 (145.3, 166.7)	0.382 (0.25, 0.51)	-0.811 (-1.51, -0.11)
	M	93-171	47	174.214 (154.3, 194.2)	0.276 (0.13, 0.43)	-1.309 (-2.74, 0.13)
	All	67-171	650	168.57 (163.6, 173.6)	0.279 (0.25, 0.31)	-1.41 (-1.66,- 1.17)
Western Australia	F	73-158	84	163.928 (137.6, 190.3)	0.228 (0.08, 0.37)	-2.484 (-4.34, -0.73)
	M	83-169	72	172.941 (158.56, 187.33)	0.248 (0.16, 0.33)	-1.587 -2.52, -0.66)
	All	73-174	202	169.58 (161.2, 177.9)	0.222 (0.18, 0.26)	-2.05 (-2.51, -1.59)
Indonesia	F	83-176	112	172.656 (160.1, 185.2)	0.243 (0.17, 0.32)	-1.463 (-2.21, -0.71)
	M	80-168	87	215.947 (114.8, 317.1)	0.117 (0.00, 0.24)	-3.202 (-5.22, -1.19)
	All	48-174	456	176.80 (164.9, 155.8)	0.214 (0.16, 0.26)	-1.78 (-2.33, -1.24)
Western Pacific Ocean		39-178	999	162.59 (159.72, 165.45)	0.308 (0.29, 0.33)	-1.30 (-1.45, -1.14)
Eastern Indian Ocean		48-174	658	172.24 (165.39, 179.09)	0.219 (0.19, 0.25)	-1.96 (-2.31, -1.61)

8.3.6 Catch-at-age

Separate age-length keys were developed for 2001 and 2002 for both the western Pacific and eastern Indian Oceans using our sample of aged fish. Appendix 4 gives the four keys developed. Due to the small differences in growth between sexes, and the overall sex ratios being close to 1:1, we did not develop separate keys for males and females. The age-length keys were applied to length frequency data (shown in Figure 8.3.13) to estimate the age distribution of the catch for the eastern and western AFZs for 2001 and the first half of 2002. Similarly, the catch-at-age of the Indonesian longline fishery was estimated for the second half of 2002.

It is clear that fish aged five or less dominated the catch of bigeye in the AFZ (Fig. 8.3.14), although the age distribution varied between area and seasons. On the east coast, between 89 and 95% of the catch were age five or less (depending on the area and period examined) and only 1-6% were age eight or older. In 2001, 2+ and 3+ age classes comprised the largest component of the catch, while 1+ fish were generally rare. In the first half of 2002, fewer 2+ fish were caught compared to the same time in the previous year, while a greater proportion of 4+ fish were caught.

On the west coast, young fish again dominated the catch but the age distribution was more evenly spread across all age classes than on the east coast. The proportion of fish age five or less was between 65-90% and the proportion of fish aged eight or older was 1-17%. The older fish (>8+) were in lower abundance in the first half of 2002 than in the previous year. For comparison, the age distribution of the Indonesian catch in the second half of 2002 was again dominated by 2+ and 3+ age classes, and 89% were five years or less. Less than 1% were age eight or older.

8.3.7 Maturity

Females classed as mature were predominantly caught in the north-western Coral Sea from August to November in 2000 and from October to December in 2001 (Fig. 8.3.15) indicating the main spawning period for bigeye in the area sampled. The relatively low proportion of mature females in October and to some extent November 2001 was due to a higher proportion of small (immature) fish sampled rather than it being outside the main spawning period. Mature males were caught in high proportions in nearly all months sampled.

The smallest mature female and male sampled were 80 cm and 65 cm FL respectively, although most mature fish were >100 cm (females) and >80 cm (males). Using the logistic curve fitted to the maturity data collected during the spawning seasons, length at 50% maturity (L_{50}) was estimated to be 102.4 cm in females and 86.6 cm in males (Fig 8.3.16a; 8.3.17a). Limiting the data to the period of highest reproductive activity for females remove the bias associated with misidentifying mature but post-spawning or resting females as immature. Figure 8.3.16b indicates the effect of such bias as a greater proportion of large and presumably mature fish are classed as immature. Since males classed as mature were present in high proportions in all months sampled, we estimated L_{50} for all males sampled as 88.2 cm (Fig 8.3.17b), slightly larger than if restricted to the spawning season.

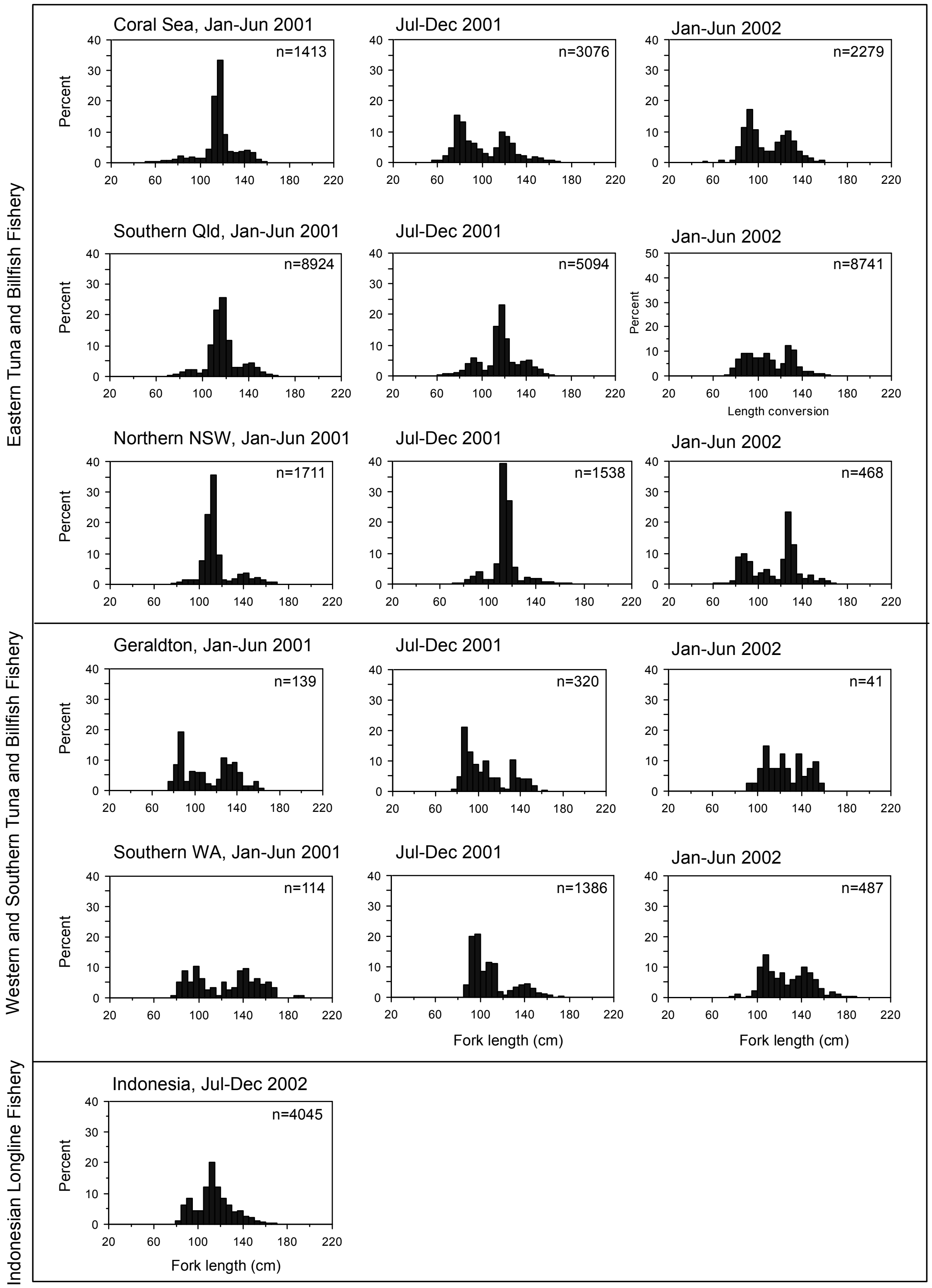

Figure 8.3.13. Length distributions of bigeye caught in the Eastern Tuna and Billfish Fishery, Southern and Western Tuna and Billfish Fishery and the Indonesian longline fishery by six-month period. Data for the ETBF and SWTBF are for Jan 2001 to June 2002, and for the Indonesian fishery from July to December 2002.

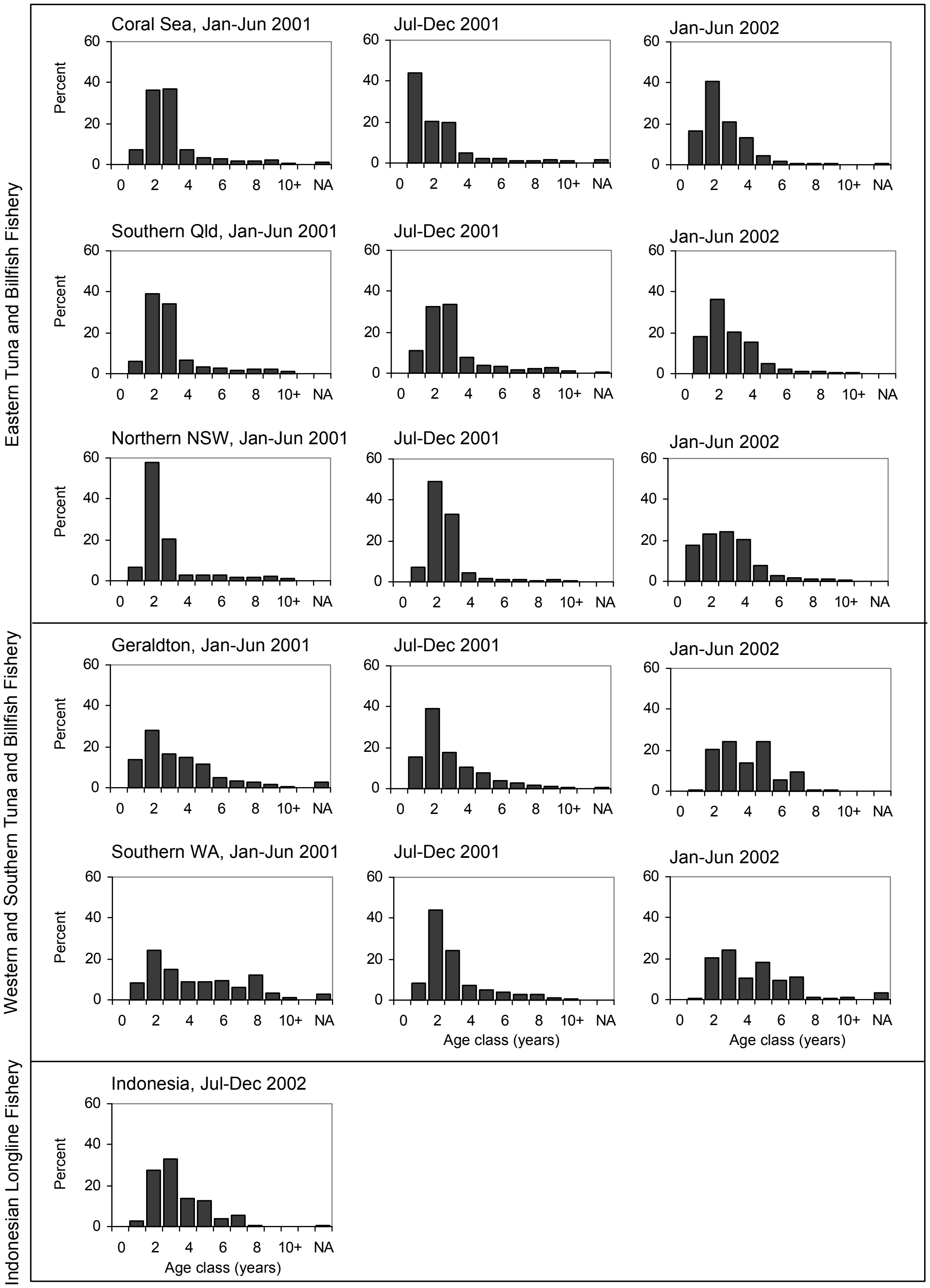

Figure 8.3.14. Age distribution of bigeye caught in the Eastern Tuna and Billfish Fishery, Southern and Western tuna and Billfish Fishery and the Indonesian longline fishery by six-month period. Data for the ETBF and SWTBF are for Jan 2001 to June 2002, and for the Indonesian fishery from July to December 2002. NA = age not assigned as key did not cover the full range of lengths.

Parameters estimates from the three logistic curves were:

Females (spawning season): $P(maturity\,|\,L) = \dfrac{\exp(-12.1141 + 0.1183L)}{1 + \exp(12.1141 + 0.1183L)}$

Males (spawning season): $P(maturity\,|\,L) = \dfrac{\exp(-5.6605 + 0.0654L)}{1 + \exp(-5.6605 + 0.0.654L)}$

Males (all): $P(maturity\,|\,L) = \dfrac{\exp(-6.7852 + 0.0769L)}{1 + \exp(-6.7852 + 0.0769L)}$

Estimating age at 50% maturity using a logistic regression was not possible due to insufficient estimates of age for fish with gonads sampled. However, we assigned ages to L_{50} based on all the ages estimated for females and males sampled in the Coral Sea. Of the females in the 100-104.9 cm length class (the class encompassing female L_{50}), 80% were estimated to be 2+ and 20% to be 3+ fish. The minimum age for mature female sampled was 2 years old. Using the von Bertalanffy growth parameters for females (Table 8.3.11), a fish 102.4 cm FL would be 1.8 years old. If the von Bertalanffy growth parameters of Hampton et al. (1998) based on assumed daily ageing were used, a fish 102.4 cm FL would be 2.4 years old. Using a similar approach for males, of the fish in the 85-89.9 cm length class, 86% were estimated to be 1+ fish and 14% to be 2+ fish. From our growth models, an 86.6 (or 88.6) cm male would be 1.1 (or 1.2) years old. Using growth models reported by Hampton et al. (1998), the estimated age would be 1.7 (or 1.8) years old.

8.3.8 Sex ratio

There was a significant difference from 1:1 in the overall sex ratio in the Coral Sea (biased towards males) but not in Qld/NSW, Western Australia or Indonesia (Table 8.3.12). When the size classes were examined separately, males were generally dominant in size classes over 140-150 cm in the Coral Sea, Qld/NSW and WA (Fig 8.3.18). In the Coral Sea, however, there were more females than males in the largest size class sampled, although the sample size was small. In Indonesia, a reversal of the pattern was observed as females dominated in all size classes over 130 cm.

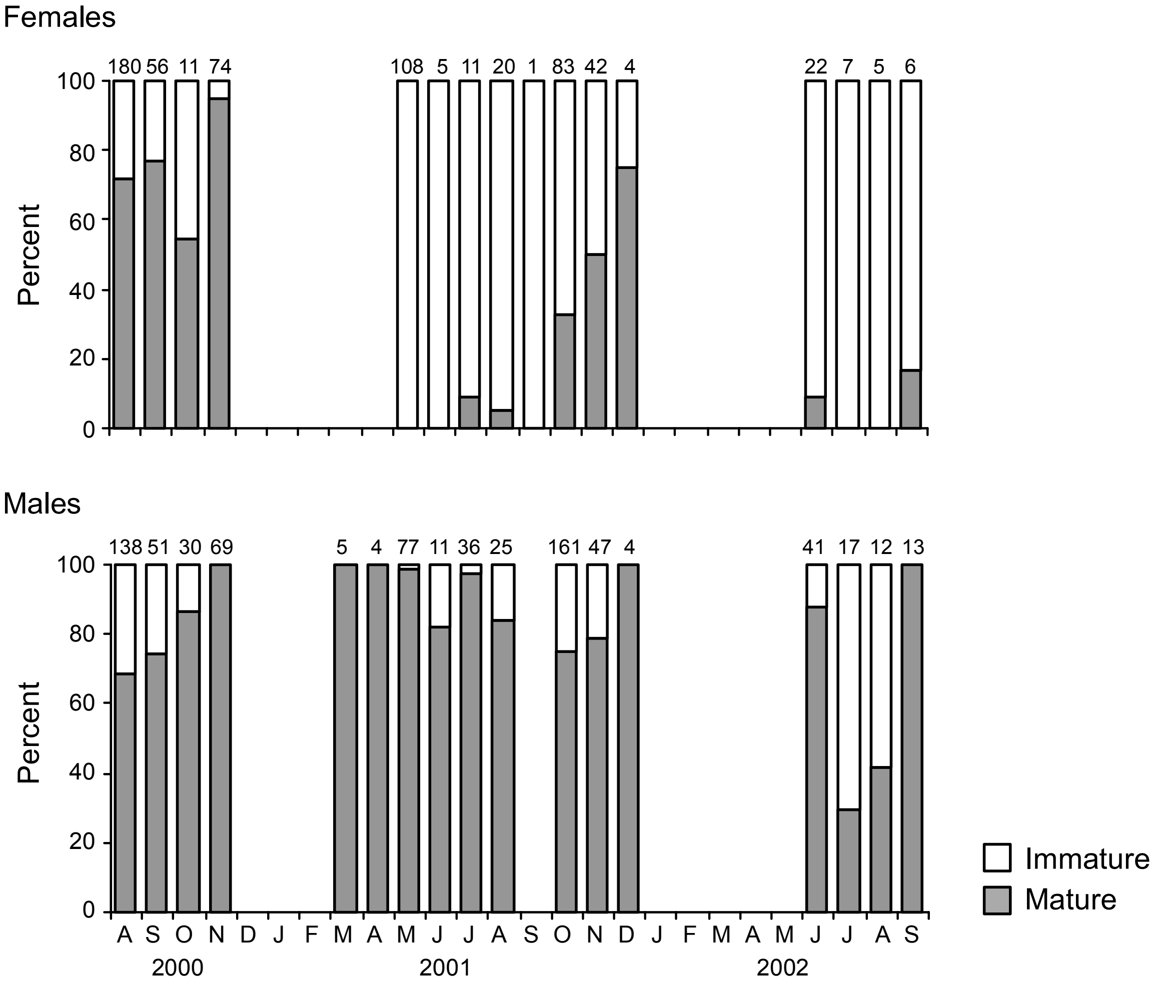

Figure 8.3.15. Proportion of mature and immature bigeye caught in the western Coral Sea by month and sex. Sample sizes are shown along the top of the graphs.

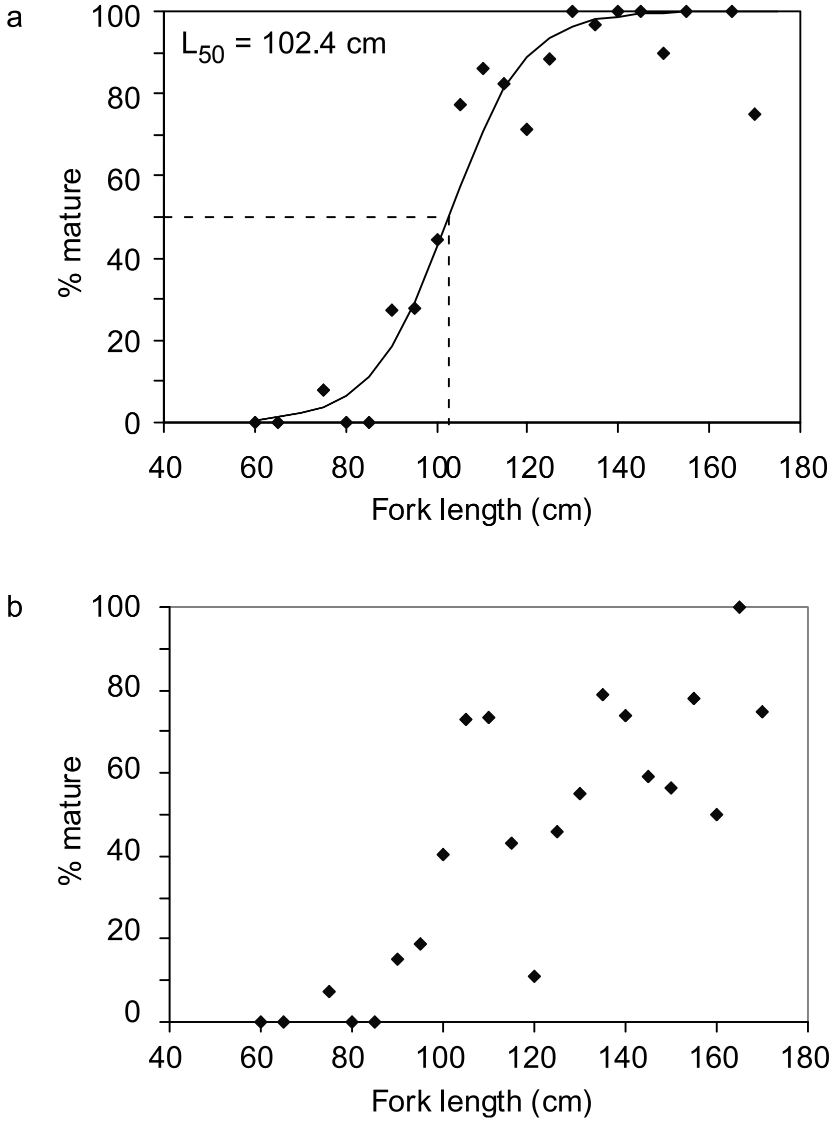

Figure 8.3.16. Proportion of mature females in the north-western Coral Sea by 5 cm length classes. (a) data restricted to the spawning season (Aug-Nov 2000 and Oct-Dec 2001) (n=450) and (b) all data (n=635). Maximum likelihood fit of the logistic regression model is shown for the restricted data. Dotted lines show length at 50% maturity.

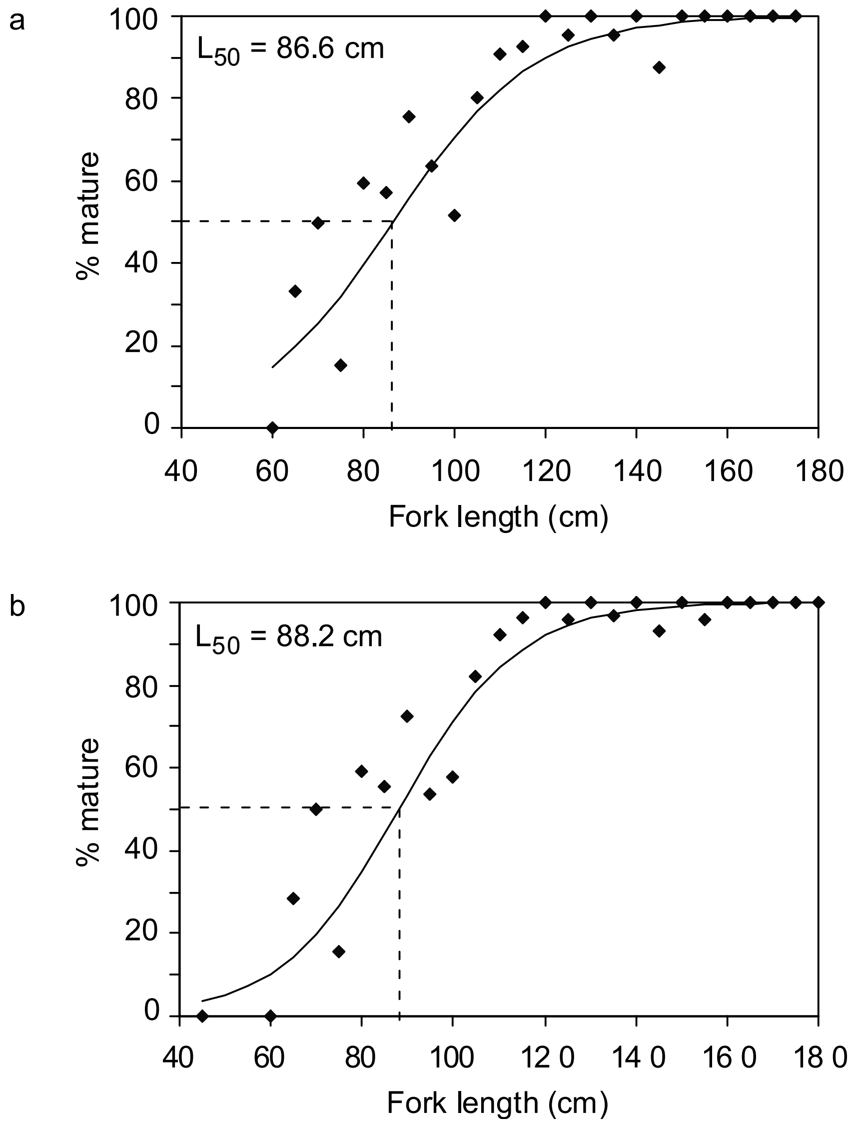

Figure 8.3.17. Proportion of mature males in the north-western Coral Sea by 5 cm length classes. (a) data restricted to the spawning season (Aug-Nov 2000 and Oct-Dec 2001) (n=500) and (b) all data (n=741). Maximum likelihood fits of the logistic regression model are shown. Dotted lines show length at 50% maturity.

Table 8.3.12. Sex ratio of bigeye by sampling area in the western Pacific and eastern Indian Oceans.

Ocean	Area	No. Males	No. Females	Sex ratio	χ^2	p
Pacific	Coral Sea	485	391	1.24:1	10.09	0.001
	Qld/NSW	92	103	0.89:1	0.62	0.431
Indian	Indonesia	218	240	0.91:1	1.057	0.304
	WA	165	170	0.97:1	0.08	0.785

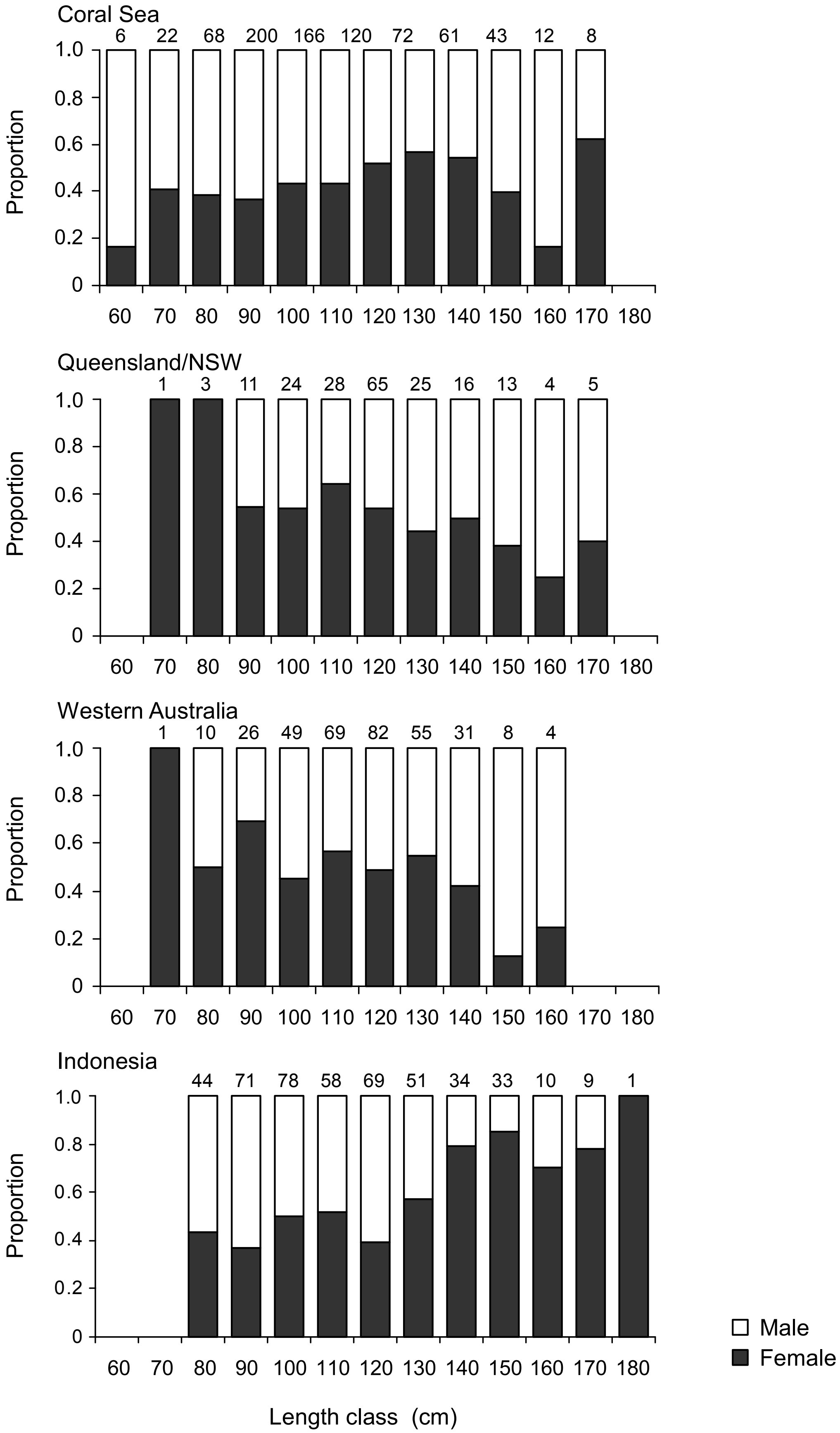

Figure 8.3.18. Proportion of males and females by length class and area.

8.4 Discussion

8.4.1 Ageing

The use of sagittae otoliths for annual age estimation of bigeye is not straightforward. The proportion of otoliths that could not be read was high (32%) and only 50% of the otoliths examined were included in the final analyses. The high rejection rate was primarily due to difficulties in interpreting the region of the otolith closest to the primordium where the annual increments appear broad and diffuse. In some otoliths, the first few opaque zones were especially indistinct and these otoliths were not assigned a final increment count.

Microincrement analysis of a subsample of sister otoliths confirmed the position of the first few opaque growth zones in bigeye otoliths, and showed that for otoliths that could be read, the first three opaque zones were being successfully identified. Unfortunately, no direct validation of the daily periodicity of growth increments in bigeye has been undertaken in either the western Pacific or eastern Indian Oceans, although Lehodey et al. (1999) used tagging data to indirectly validate the daily nature of microincrements. The validation work described in Chapter 7 indicated that for larger fish, counts of microincrements after Sr-marked on otoliths underestimated the time at liberty after tagging by up to 30%. However, given that we selected otoliths from fish smaller than the maximum size considered for microincrement analysis (120 cm FL) and reading with poor confidence scores were excluded, we believe our estimates of daily age are reasonably accurate but may underestimate age to some extent. Given this, our back-calculated birth dates may also be misleading. Nevertheless, the large range of birth months calculated from only a small number of samples, suggests that bigeye tuna caught in the eastern and western AFZ may have been spawned over a considerable proportion of the year. Further analysis of otoliths sampled throughout the year may provide a better understanding of the spawning period of bigeye in the region.

Clear et al. (2000a) directly validated the annual formation of growth increments during a mark-recapture study using strontium chloride in the Coral Sea. Their work confirmed that the 2nd to 9th opaque zones are formed annually for fish caught off the east coast of Australia. Our otolith interpretation was further corroborated by the analysis of 18 otoliths from fish tagged but not chemically marked in the Coral Sea. The data supports the annual periodicity of the first two increments in bigeye tuna. Unfortunately, direct validation of annual increments in otoliths sampled from the Indian Ocean, or for fish older than nine years has not been undertaken and results should be considered as preliminary. Although it may be possible to validate the age of older bigeye using bomb radiocarbon chronometry (Kalish 1993; 1996), neither CSIRO nor SPC have otoliths collected prior to 1990 in their collections.

The precise time of opaque zone formation is still unclear in bigeye tuna. Marginal increment analysis was conducted on otoliths from young fish (2+ and 3+ years) but the values varied considerably each month in both the Pacific and Indian Oceans. Mean monthly marginal increment showed a cyclic pattern only for otoliths sampled in the Pacific Ocean in 2001, with low values occurring in winter. The absence of a consistent annual pattern in MI may be due in part to the poor measurement precision associated with the first few increments, problems associated with resolving increments at the otolith edge (Campana 2001), and/or variability in the timing of increment formation between individuals. Edge type analysis (ETA) was conducted on otoliths from older fish (≥5+ years) and although opaque edges were found year-round, a higher proportion was found from May to October in the western Pacific Ocean, and from April to June in the eastern Indian Ocean. These patterns were visible only when samples were pooled across years and year classes, and should be considered preliminary. Sun et al. (2001) used ETA on dorsal spines to show that February to September was a period of slow growth for bigeye caught in the north-west Pacific Ocean, which coincided with the spawning period of fish in the region. In general, seasonal changes in the rate of growth and structure of otoliths are thought to be either a physiological response to environmental variation or cyclical physiological changes such as the increased somatic growth that often occurs in fish during spring (Wright *et al.* 2002).

The ages estimated made in our study do not take into account factors such as birth date, timing of opaque zone formation, and date of capture. Since the timing of opaque zone formation could not be precisely identified in our samples, and birth dates may vary substantially, the integer ages given may be biased by +/- 1 year. For example, if we assume that opaque growth zones are formed on 1 August, a fish that was born on 1 September 2001 and captured on 1 July 2003 would have one visible opaque zone (age 1+) but would actually be 1.8 years old. Conversely, if the fish was actually born on 1 June 2002 and captured on 1 September 2002, it would still have one visible opaque zone but would only be three months old.

8.4.2 Growth and catch-at-age

Bigeye tuna are a relatively slow growing and long-lived species. Their growth rate is most rapid in the first few years of life and asymptotic length is reached at about age 8 to 10 years. The maximum age of bigeye obtained in this study (15 years for both the Pacific and Indian Oceans) is higher than obtained in any previous study. Since the number of large fish sampled was small (of the 3200 sampled, only 20 were ≥ 170 cm FL) and the maximum length is over 200 cm, it is possible that fish could live longer than 15 years. However, of the 136 fish ≥150 cm FL in the final data analysis, only 12 were estimated to be ≥10 years old (9%) suggesting that old fish are rare in the populations sampled. Lehodey et al. (1999) suggested that bigeye live to at least age 8 years, after several fish tagged in the western Pacific Ocean aged between two and three years were recaptured over six years later. Very recently, a tag was returned from a bigeye 12 years after release as a two year old in the western Pacific Ocean suggesting a maximum age of at least 14 years. Sun et al. (2001) estimated a maximum of 10 years for a 189 cm fish caught in the western Pacific based on sectioned dorsal spines. In the Indian Ocean, Stequert and Conand (2003) used reading of daily increments in otoliths in preference to the first dorsal spine to obtain a maximum age of 7 years for a ~160 cm fish, while Tankevich (1982) obtained a maximum age of 8 years for a 183 cm fish based on counts of increments in vertebrae. All of these age estimates may be possible given the large range of ages we obtained for each given length class, although the maximum fish size we sampled was only 178 cm FL. However, given the direct validation of the annual periodicity of opaque growth zones in bigeye (Clear et al. 2000a) and the clear alternating growth zones visible in the otoliths of large bigeye (eg

Fig 8.3.7), we believe our estimates of longevity for bigeye are accurate for fish from the Pacific and Indian Oceans.

From the results presented, differences in growth between sexes appear to be relatively small in bigeye tuna, with males being slightly larger at age than females. Kume and Joseph (1966), Shomura and Keala (1963), and Suda and Kume (1967) all reported sexual dimorphism in growth in Pacific caught bigeye, with faster growth in males after approximately 130-150 cm FL. In the Indian Ocean, Tankevich (1982) gave growth parameters by sex that had very different L_∞ estimates, although Stobberup et al. (1998) suggested that these differences might be due to the different size range of fish examined.

The von Bertalanffy growth curves obtained in the current study are compared to previous studies in Figure 8.4.1. There is significant variance in the L_∞ and k terms among these studies, especially between early and more recent work. In the Pacific Ocean, growth parameters obtained by Kume and Joseph (1966), Shomura and Keala (1963), and Suda and Kume (1967) were all based on length-frequency analysis. The variation in age found in the current study as bigeye reach their asymptotic length (up to 10 years) indicates that growth parameters estimated from length-frequency analysis are likely to overestimate length-at-age. Yukinawa and Yabuta (1963) determined growth parameters based on counts of rings on scales, but assumed that rings formed twice a year giving a higher growth rate compared to most other studies. Sun et al. (2001) based their growth estimates on counts of growth rings on dorsal spines, however Stequert and Conand (2003) suggest this method is only suitable for fish up to age 3 due to vascularisation of the core. Tankevich (1982) provided the only growth parameters for the Indian Ocean until recent work by Stequert and Conand (2003).

The von Bertalanffy growth parameters obtained by Hampton et al.'s (1998) composite model for the western and central Pacific Ocean and Stequert and Conand (2003) for the western Indian Ocean are very similar to each other. Both authors used assumed daily microincrement counts from otoliths to estimate the age of fish up to age three. To estimate the age of larger fish, Hampton et al. (1998) used tag-recapture data while Stequert and Conand (2003) continued to use daily increment counts but used a scanning electron microscope to resolve the microincrements. Both authors obtained a maximum age of approximately 7-8 years. The L_∞ estimates from both of these studies, however, are similar to our estimates for the Pacific and Indian Oceans. Given that we directly estimated the age of bigeye up to 15+ years, our study supports the results of these previous studies.

Our estimates of the *k* term, however, are lower than Hampton et al. (1998) and Stequert and Conand (2003). This is most likely due to the bias inherent with annual age estimation compared to daily age estimation. As already discussed, our integer estimate of age does not take into account birth date, capture date and time of opaque zone resulting in a bias of +/-1 year. Our age estimates overestimate length-at-age for age classes 1+ to 3+ years, but this bias is less obvious in the older age classes as somatic growth slows.

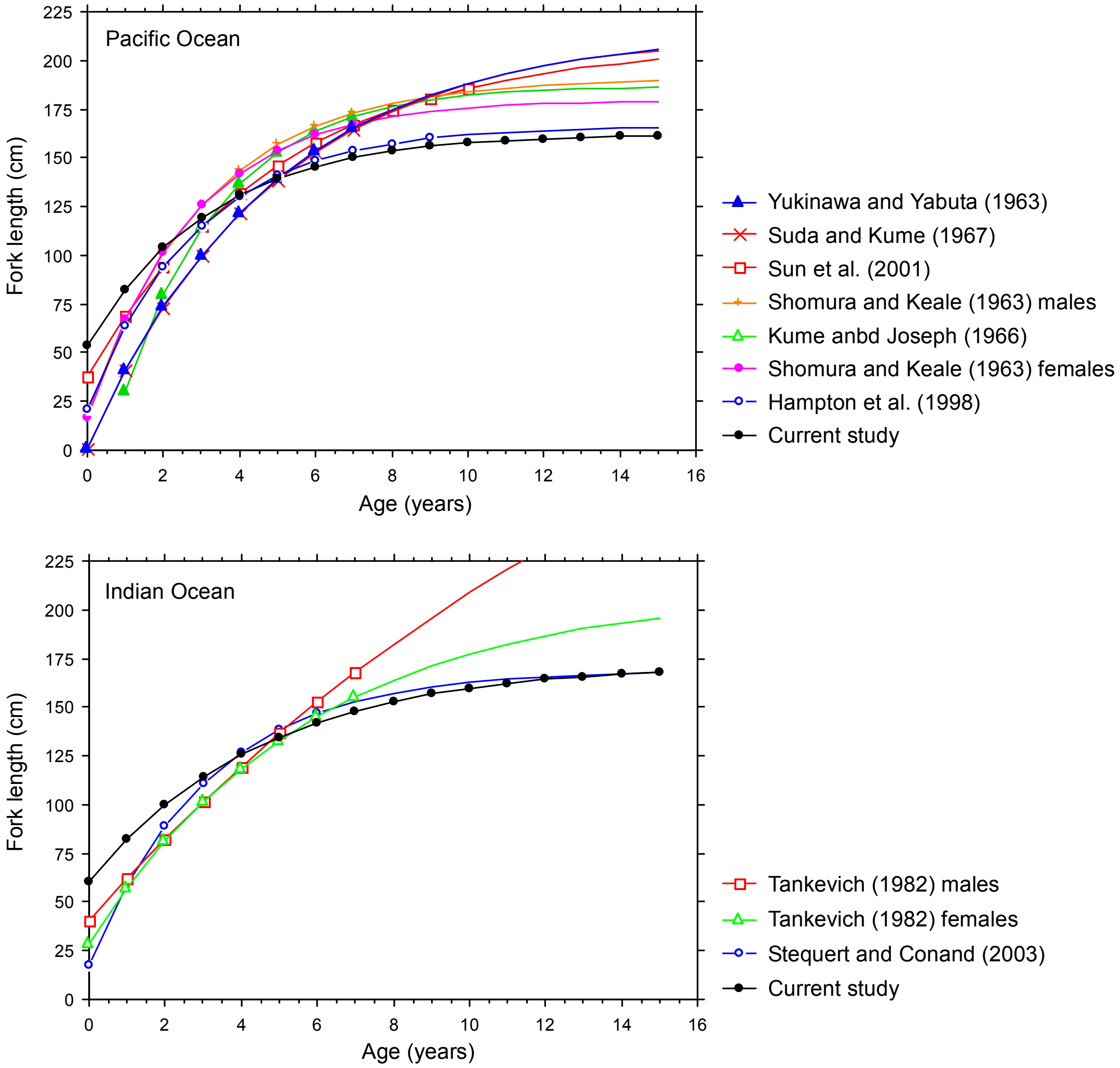

Figure 8.4.1 Comparison of growth curves for bigeye caught in the Pacific and Indian Oceans. Symbols marking length-at-age are only shown for ages directly estimated, but the curve has been extended to age 15 years (the maximum obtained in the current study).

Given the bias in age estimates for young fish, the von Bertalanffy growth model may not be the most appropriate model to examine growth in bigeye tuna. It may be possible through more rigorous statistical investigation to fit a growth curve that accounts for the uncertainty in age.

Our study provides the first validated age-length keys and estimated catch-at-age for bigeye in Australian and Indonesian waters. The results suggest regional and inter-annual variation in the catch-at-age in the eastern and western AFZs. However, interpretation of the data is difficult as the time series is short and there may have been changes in fishing operations, fishing areas and target species that cannot be accounted for. The bias in age estimates in younger age classes produces an imprecise (underestimate) estimate of catch-at-age. This may have the effect of reducing the ability to detect and follow strong year classes in the catch. From inspection of the age structure, however, it does not appear that fish move out of either the eastern or western AFZs at a particular age – becoming unavailable to the fisheries. The most dramatic decline in the relative abundance of fish occurred between age 3+ and 4+ in the eastern AFZ. The decline, however, was within that expected from natural mortality if we assume rates of between 0.4-0.8 (Anon 1998).

8.4.3 Implications for stock structure

For management purposes, bigeye tuna in the Indian and Pacific Oceans are considered separate stocks. Grewe et al. (2000), however, found no evidence to suggest that bigeye in the Indian Ocean were genetically different to Pacific Ocean bigeye studied earlier by Grewe and Hampton (1998). Our study showed that the growth rates of bigeye tuna varied to some extent between areas, but more noticeably between oceans with faster growth but smaller maximum size in the western Pacific Ocean compared to the eastern Indian Ocean. Regional differences were also detected in otolith morphology between oceans; otoliths sampled from the eastern Pacific Ocean were larger on average for the size of fish than those sampled from the eastern Indian Ocean. Although these results may not reflect genetic differences, they are consistent with separate stocks of bigeye in the Indian and Pacific Oceans.

8.4.4 Maturity

Macroscopic staging of ovaries is not recommended as the most appropriate method of determining maturity (Schaefer 2001) as post-spawning or resting ovaries may be classed as immature rather than mature, resulting in an overestimation of length at maturity. However, McPherson (1992) compared the reliability of macroscopic assessment of bigeye ovaries sampled in the Coral Sea with histological classification and demonstrated that if fish were mature the macroscopic scheme would classify them correctly. If a fish were immature, however, the macroscopic scheme would incorrectly classify ovaries as mature in 6% of cases (1 out of 16 were incorrectly classified). If this is the case (although the number of ovaries analysed was small), our estimate of length at 50% maturity for females of 102.4 cm may be slightly underestimated.

An estimate of size and/or age at 50% maturity is considered the most important parameter describing attainment of sexual maturity within a population. Schaefer (1998) considered minimum size at first maturity as having “no practical value except perhaps in establishing the minimum size at which fish should be sampled for investigations of reproductive biology”. Unfortunately, previous studies of bigeye maturity have focused on the minimum size at first maturity and have concentrated predominantly on females. Hisada (1973), using Kiwawa’s (1966) report that females with a gonad index >3.1 were fully mature, showed that the minimum size at first maturity was 64 cm for fish caught in the handline fishery in the north-western Coral Sea (Figure 14 in Hisada 1973). Hisada’s figure showed that most females, however, were not mature until over 100 cm in the handline fishery and 130 cm in the longline fishery. McPherson (1992) reported the minimum size at first maturity for the same region to be 100 cm and 125 cm in areas fished by handlines and longlines respectively.

Other studies in the Pacific Ocean have recorded the minimum size of mature females as 81-90 cm (Kikawa 1962), 81-100 cm (Kikawa 1966), 14-20 kg (~90-100 cm; Yuen 1955), 115 cm (Kume and Joseph 1966) and 119 cm (Yasutake et al. 1973) although the last two were based on very small samples sizes. Alverson and Peterson (1963), Calkins (1980) and Stobberup et al. (1998) report the minimum age at first maturity as 3 years. Stequert and Conand (2003) suggest that estimates of size at first maturity of round 100 cm equate to the end of the second year of life. In the Indian Ocean, Kume (1962) found that the smallest spawning female caught by longline was 92 cm, and Tankevich (1982) reported the minimum length at first spawning for males and females to be 106 cm (25 kg), which corresponds to an age of 3-4 years. Size and/or age at maturity have not been determined for Atlantic bigeye tuna.

8.4.5 Sex ratio

The overall sex ratio was close to 1:1 in all areas except the Coral Sea where males were more abundant than females. An overall dominance of males has been observed for bigeye in both the Pacific (Iversen 1955; Kikawa 1966; Kume and Joseph 1966; Kume 1969; Hampton et al. 1998) and the Indian Oceans (Mimura et al. 1963). These studies also found that the dominance of males became more prominent as size increased. We found a similar pattern of male dominance in larger size classes in the Coral Sea, Qld/NSW and WA possible due to sexual dimorphism in growth, but not in Indonesia. Our finding of an increase in the dominance of females in larger size classes in Indonesia has not been recorded before, but may be related to sexual differences in catchability or increased mortality in larger males. Further investigation is required to remove the possibility of a sampling bias towards females in the larger size classes.

9. Benefits and adoption

The project established an understanding of a number of the key biological parameters required for age-based stock assessments of bigeye tuna including estimates of longevity, growth rates (particularly for large fish), age-length keys, catch-at-age and size/age at maturity. The information gained will not only improve the capacity to assess stocks within the eastern and western Australian Fishing Zones, but will be valuable for stock assessments in the broader eastern Indian and western Pacific Oceans. Although it was not an objective of the study to estimate the age of bigeye tuna caught in the Indonesian longline fishery, we considered that the knowledge gained would be important for regional stock assessments and would provide useful information on stock structure. Assessing bigeye stocks in the Indian Ocean is IOTC’s highest priority. Knowledge of some of the difficulties encountered in estimating the age of bigeye tuna using sectioned otoliths will benefit further decisions on the ability to undertake routine age estimation.

10. Further Development

This project provides the first validated age estimates for bigeye tuna within Australian waters. It is essential, however, that as strontium-injected fish are recaptured in the future, analysis of otoliths for validation purposes continues especially for fish that have been at liberty for long periods or fish assumed to be aged 0+ when released. If a further tagging program takes place, it is suggested that higher doses of strontium chloride be injected into large fish to ensure that Sr-marks are detected in the otoliths.

The estimates of longevity reported in this study are a significant result for bigeye tuna. As very few large fish were sampled, and otoliths from no fish over 180 cm FL were analysed, it is recommended that where possible, otoliths from large fish continue to be collected and analysed to provide a better estimate of growth which is a vital input within the stock assessment context. Unfortunately, fish over 180 cm are rare in Australian catches.

The project identified a bias inherent with integer annual age estimates compared to daily age estimates, which are assumed to represent the "true" age. Our age estimates did not take into account birth date or time of opaque zone formation as neither could be precisely identified in our samples, resulting in a bias of +/-1 year that is most distinct in young fish. Given this bias, the von Bertalanffy growth model may not be the most appropriate model to examine growth in bigeye tuna. It may be possible through more rigorous statistical investigation to fit a growth curve that accounts for the uncertainty in age. This would allow for further statistical analysis comparing growth between sexes, areas and oceans. It would also allow for comparison of growth over time as some age estimates were for fish sampled as early as 1992. Any change in length-at-age over time must be provided for in stock assessment models.

It is also recommended that detailed statistical analysis on how to appropriately estimate the age distribution of the catch of bigeye from length-at-age and length frequency data. Morton and Bravington (2003) reported on similar work for southern bluefin tuna where two "hybrid estimators" provided more precise estimates of proportion-at-age than age-length keys especially in fisheries catching high proportions of small fish where length more closely reflects age. If appropriate methods could be developed, it may be possible to determine the catch-at-age annually for years otoliths were sampled (1992+) even though sample sizes are small in the initial years.

Finally, estimates of birth dates from microincrement analysis proved to be useful for investigating spawning period in the Indian and Pacific Oceans. Further analysis of otoliths sampled throughout the year may provide a better understanding of temporal variability in bigeye spawning in the region.

11. Planned outcomes

The planned outcomes of the project were to improve biological parameters, primarily growth rate estimates, required for age-based stock assessments of bigeye tuna in the

Australian region. The results of this project have contributed markedly to the body of knowledge of bigeye tuna, and it is anticipated that the results from this project will be used in future stock assessments of bigeye tuna in the eastern and western AFZs.

12. Conclusion

All of the objectives of the study were met. The project established an understanding of a number of the key biological parameters required for age-based stock assessments of bigeye tuna. The results of this project will contribute markedly to the body of knowledge of the biology, life-history traits and population dynamics of bigeye tuna.

A significant finding in the study was that the longevity of bigeye is greater than previously thought. The maximum estimated age is at least 15 years. Since bigeye are known to reach over 200 cm FL and the largest fish we analysed was only 178 cm, it is possible that the maximum age may be higher than 15 years. The theoretical maximum fork length (L_∞) estimates obtained from von Bertalanffy growth analysis are similar to those of Hampton et al. (1998) and Stequert and Conand (2003) for the Pacific and Indian Oceans respectively, but are lower than in other studies. Both Hampton et al. (1998) and Stequert and Conand (2003) used counts of assumed daily increments in otoliths to estimate the age of fish up to three years, and used either tag-recapture data (Hampton) or further daily age estimates (Stequert and Conand) for fish up to age 7-8 years. The similarity of our L_∞ estimates with both these studies provides support for their age estimation techniques, but it is considered that counts of annual growth increments in otoliths will provide a more accurate estimate of age in larger fish.

Our estimates of the von Bertalanffy growth parameter *k* are lower than both Hampton et al. (1998) and Stequert and Conand (2003). This is most likely due to the bias inherent with integer annual age estimates compared to daily age estimates that are assumed to represent the “true” age. Our age estimates do not take into account birth date, capture date or time of opaque zone formation, resulting in a bias of +/-1 year. Our age estimates overestimate length-at-age for age classes 1+ to 3+ years, but this bias is less distinct in the older age classes as somatic growth slows. This bias does not influence our comparisons of growth between areas or sexes, but must be acknowledged if the growth parameters are to be used for stock assessment purposes.

The age structure of the longline catch of bigeye in the eastern and western AFZ (Objective 1) was determined using age-length keys based on our direct age data, applied to catch-at-length data for the fisheries. Overall, young fish aged 5 years or less dominated the catch in eastern AFZ (89-95% of the catch) and to a slightly lesser extent in the western AFZ (65-90%). The proportion of the catch aged eight or older ranged between 1-6% on the east coast and 1-17% in the west. The results suggest both regional and inter-annual variation in the catch-at-age.

Collection of data for maturity estimates (Objective 2) was made in the north-western Coral Sea, a known spawning area for bigeye tuna in the AFZ. The project established the length at 50% maturity at 102.4 cm in females and 86.6 cm in males. We estimated fish of these lengths to be 1.8 and 1.1 years old for females and males respectively. However, using growth curves determined by Hampton et al. (1998) based on assumed daily age estimates and tagging data, fish of these sizes are estimated to be 2.4 and 1.7 years old for females and males respectively. These latter estimates are considered the most appropriate for stock assessment purposes.

The growth curves fitted to lengths-at-age were significantly different between sexes for fish sampled in the Coral Sea and Western Australia only, with males being slightly larger at age than females (Objective 3). The absence of a statistical difference in mean length-at-age by sex at any of the sampling locations, however, indicates that sexual dimorphism in growth is relatively small in bigeye tuna. The overall sex ratio of bigeye tuna was close to 1:1 in all areas except the Coral Sea where males were more abundant than females. We found that the dominance of males became more prominent as size increased in the Coral Sea, Qld/NSW and WA possibly as a result of the slight sexual dimorphism in growth.

Significant differences in growth rates were apparent between the Pacific and Indian Oceans (Objective 4) with larger theoretical maximum fork length (L_∞) estimates in both Western Australia and Indonesia compared to the Coral Sea and Qld/NSW. Regional differences were also detected in otolith morphology between oceans; otoliths sampled from the eastern Pacific Ocean were larger on average for the size of fish than those sampled from the eastern Pacific Ocean. Although these results may not reflect genetic differences, they are consistent with separate stocks of bigeye in the Indian and Pacific Oceans.

The final objective of the study (Objective 5) was to continue the validation of age estimates based on a strontium chloride mark-recapture experiment in the Coral Sea. Results of the analysis of a marked otolith that had been at liberty after tagging for over six years, support the annual formation of growth increments in older bigeye otoliths, and confirm that our technique of counting increments provides reliable estimates of age. The annual nature of growth increments in bigeye tuna otoliths has now been validated in fish with 2 to 9 increments on their otoliths. Supporting evidence for annual increment formation was also obtained from analysis of otoliths from fish previously tagged (but not chemically marked) in the Coral Sea. Although fish were at liberty for a short time (0-3 years) the results confirmed that the number of opaque zones observed in otoliths after release from tagging was equal to or within the range expected for all fish.

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14. Intellectual property

No commercial intellectual property arose from this work

15. Staff

Tim Davis*	Principal Investigator
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Naomi Clear*	Fisheries biologist
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Bruno Leroy[+]	Technician
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16. List of appendices

Appendix 1:

Images of strontium-marked otolith from bigeye tuna used for validation of annual increments. Two types of images of each otolith are shown: scanning electron microscope (SEM) micrographs and light microscope photographs. In the SEM micrographs (A and B) the strontium mark is obvious as a bright band on the transverse otolith sections. In the light microscope images of the same otoliths (C) the positions of increments observed after the strontium mark are indicated (broken arrows).

For some specimens the images from the SEM and light microscope don't appear to match. This is due mainly to the different imaging techniques: the SEM visualises only the surface of the otolith section whereas the light microscope image has "depth", i.e. we see not only the surface but the part of the otolith that is below the surface of the surrounding embedding resin.

Appendix 2:

Examples of transverse sections of bigeye tuna otolith showing the location of the 365th (Y1) and 730th daily increment (Y2) signifying the length of the otolith at age 1 and 2 years from microincrement analysis. The locations of the opaque zones (inc 1-3) are also indicated.

Appendix 3:

Mean length-at-age (with standard deviations) estimated from bigeye tuna otoliths sampled in Cairns, southern Queensland/New South Wales, Western Australia and Indonesia.

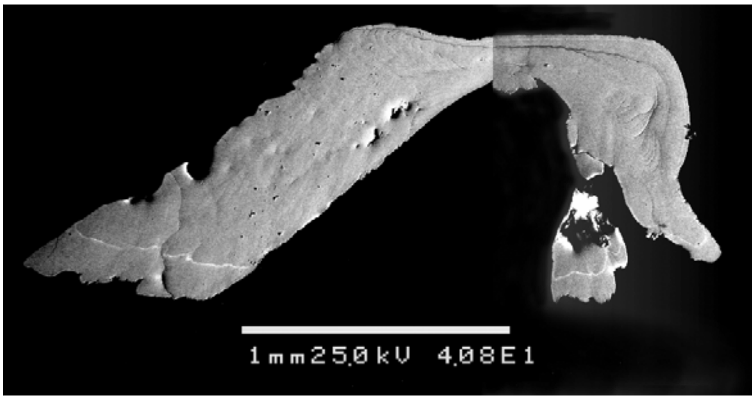
Appendix 4:

Age-length keys developed using our sample of aged bigeye tuna. Only age estimates with a confidence score of ≥2.5 are included. Mean length-at-age (LatA) and standard deviation (SD) are shown.

Images of strontium-marked otolith from bigeye tuna used for validation of annual increments

BET#37

A.



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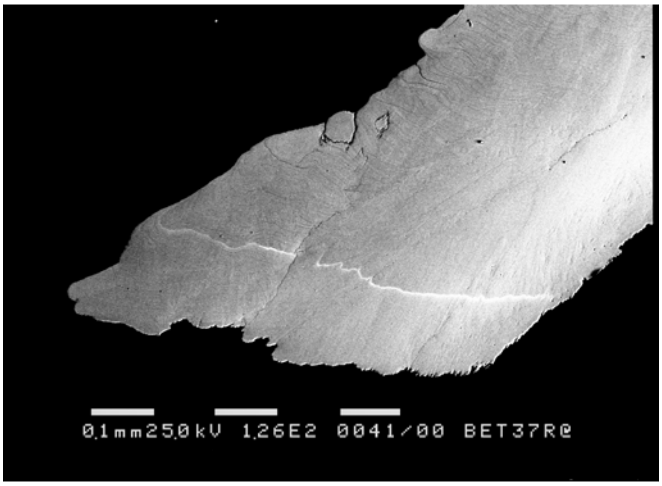

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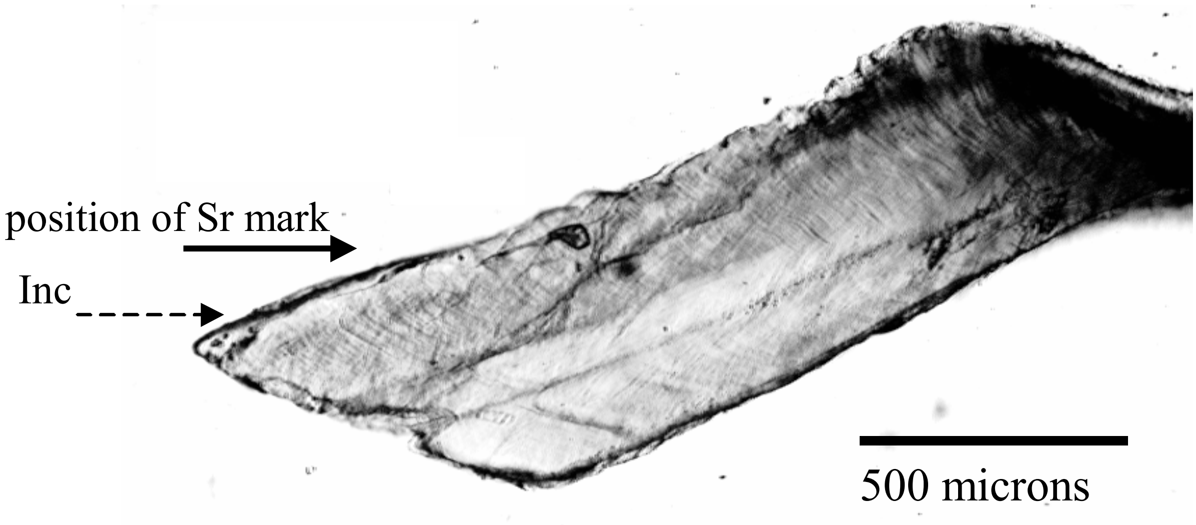

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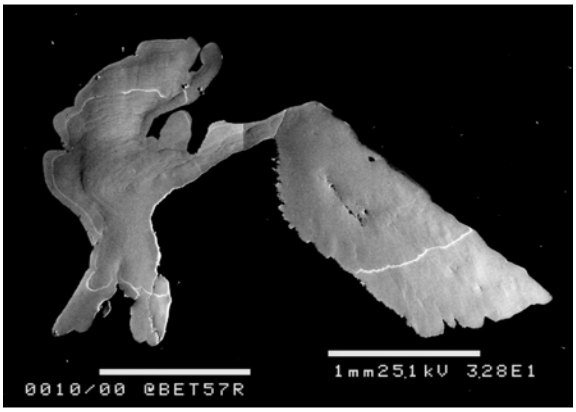

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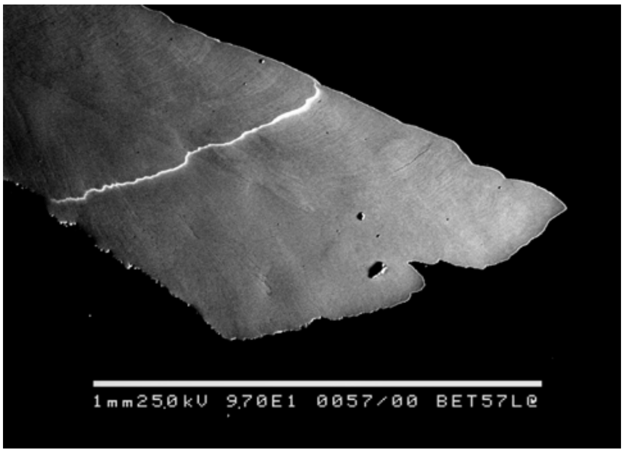

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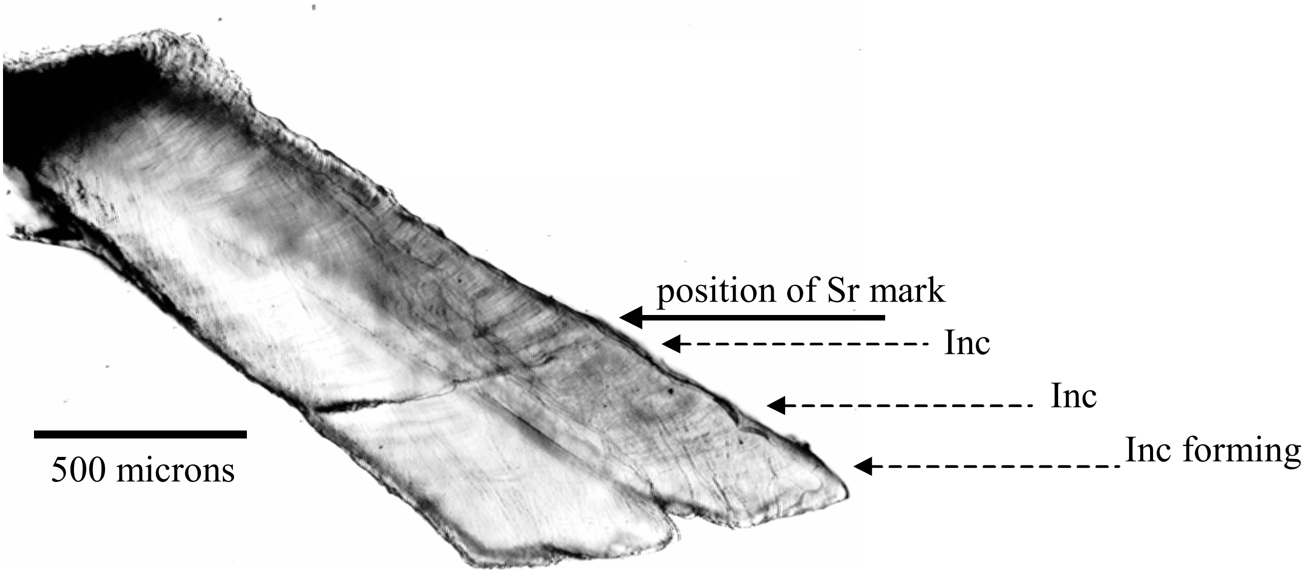

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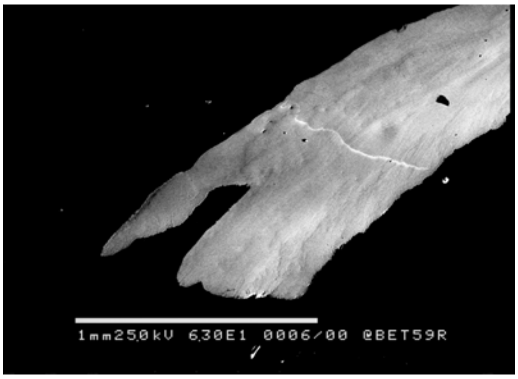
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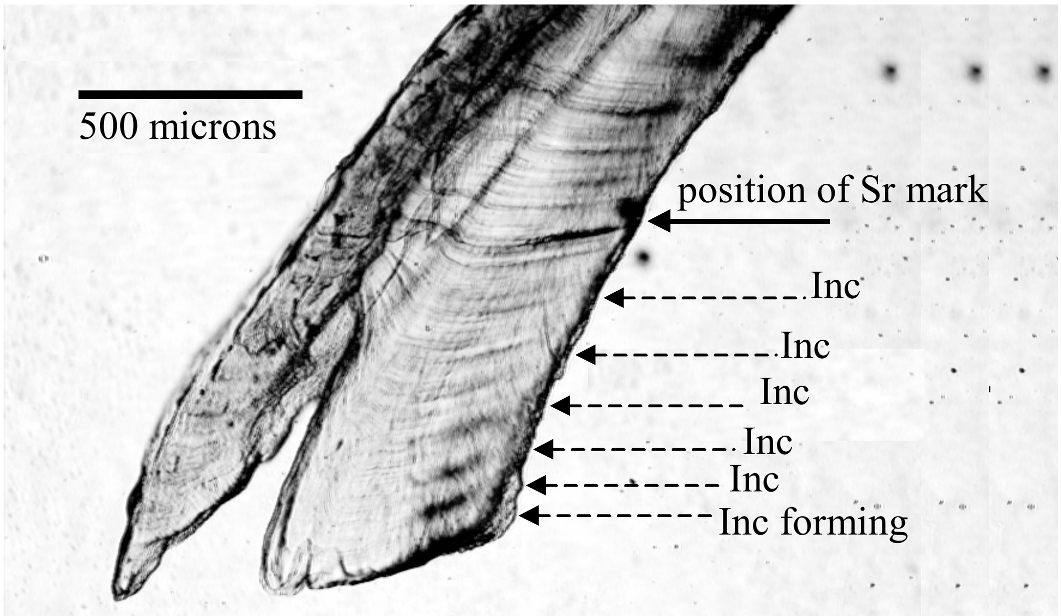
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position of Sr mark
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Inc
Inc
Inc
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Inc forming

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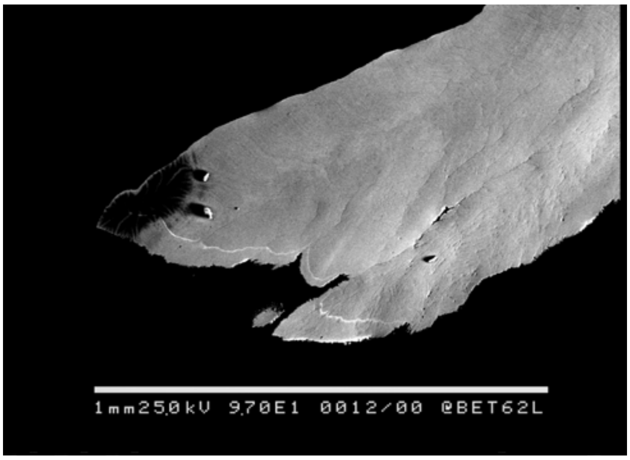
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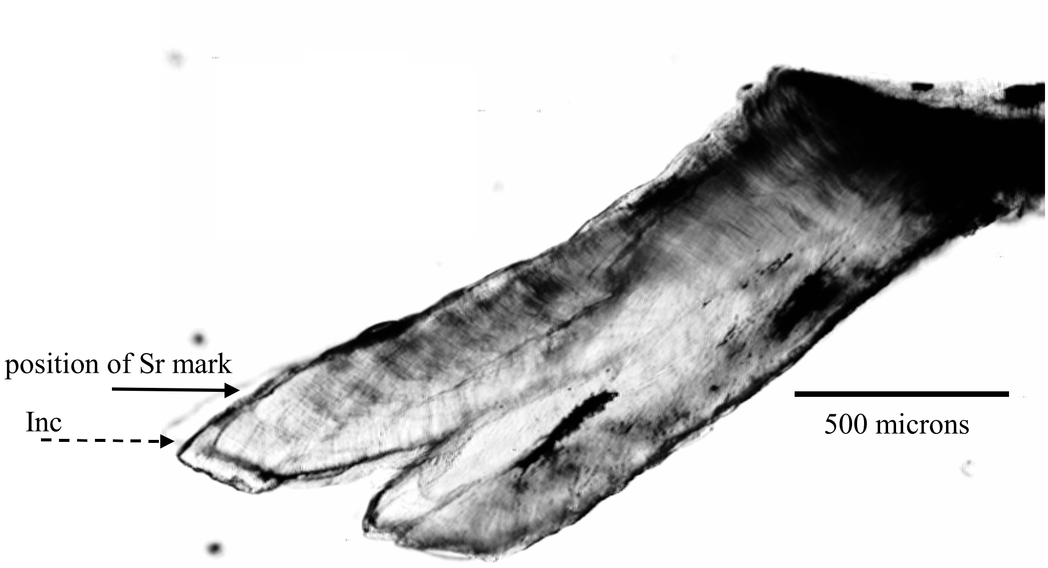
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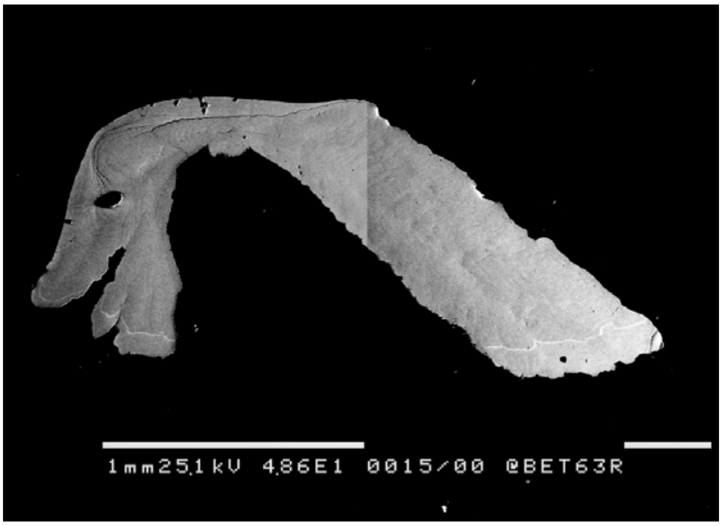
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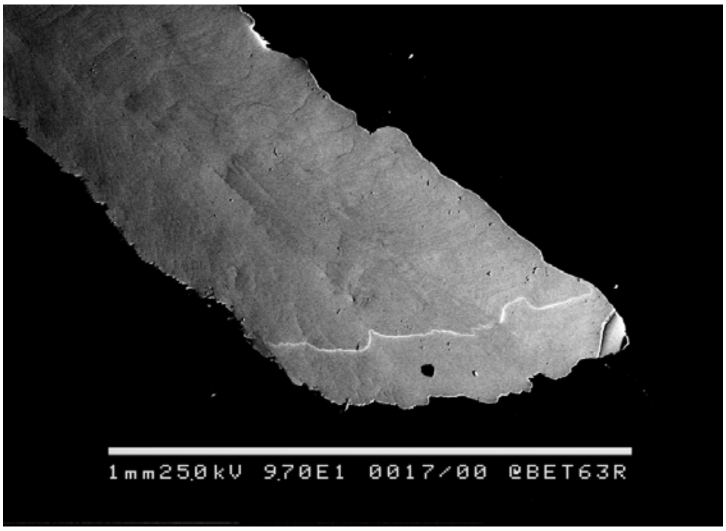
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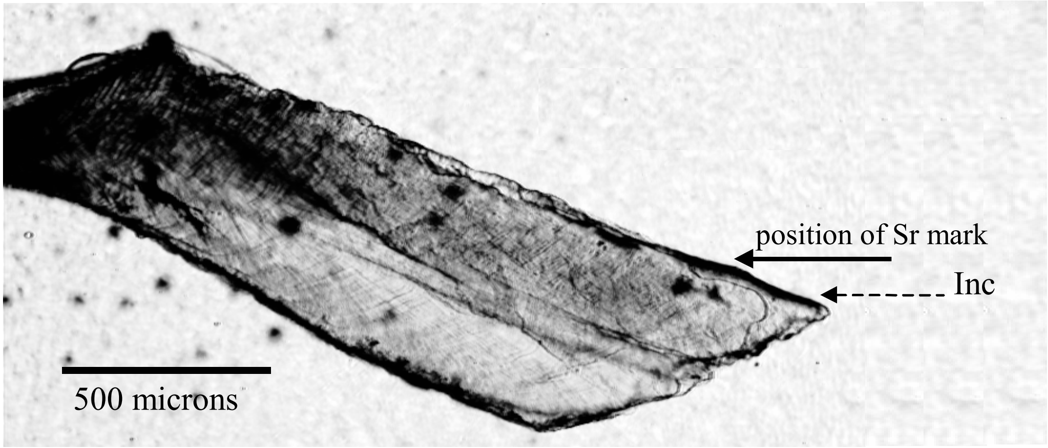
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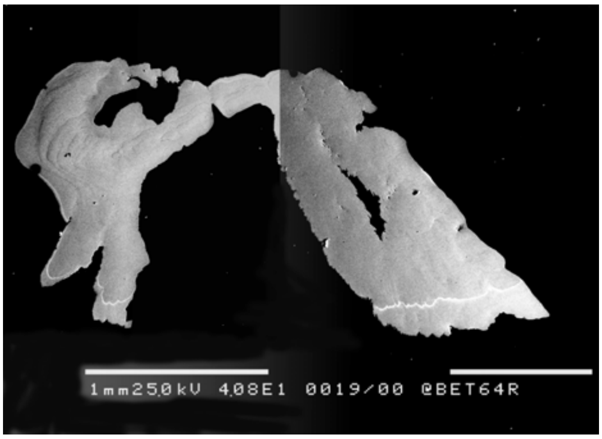
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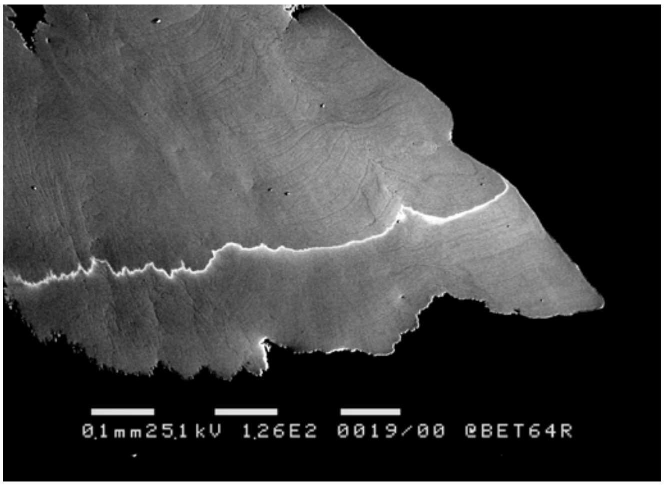
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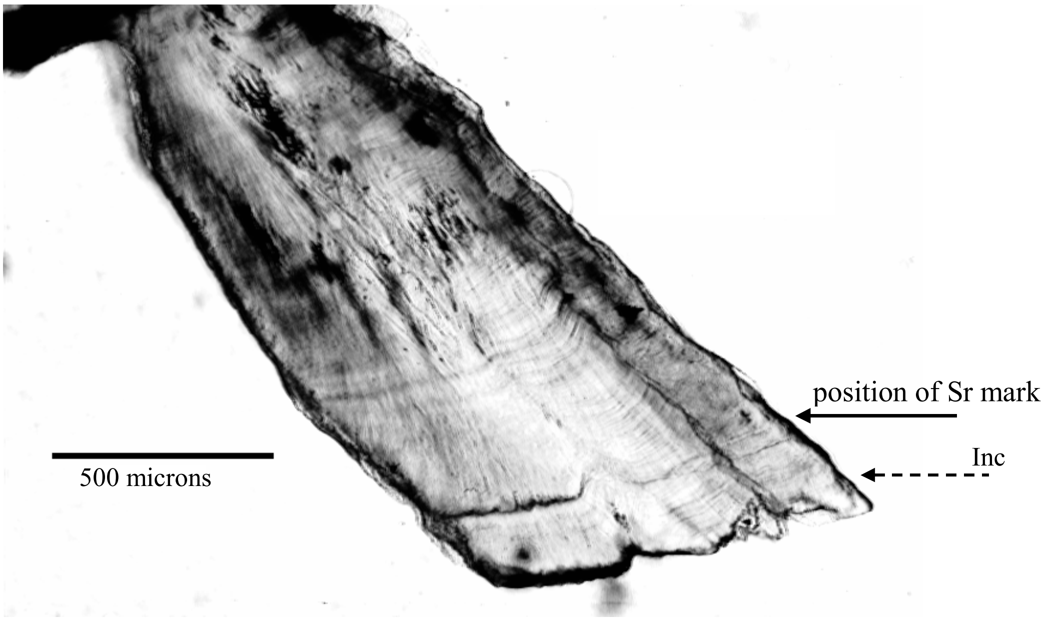
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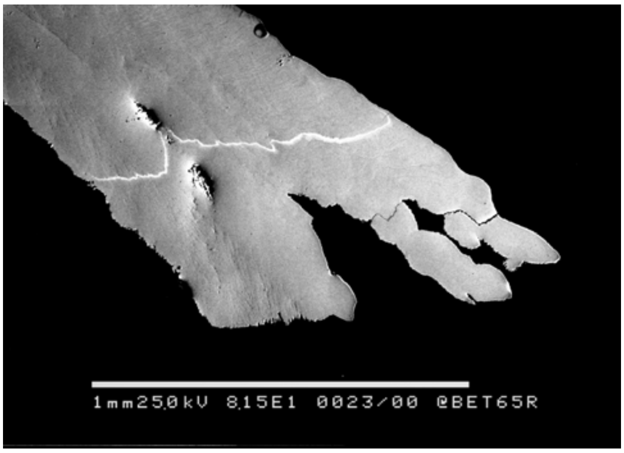
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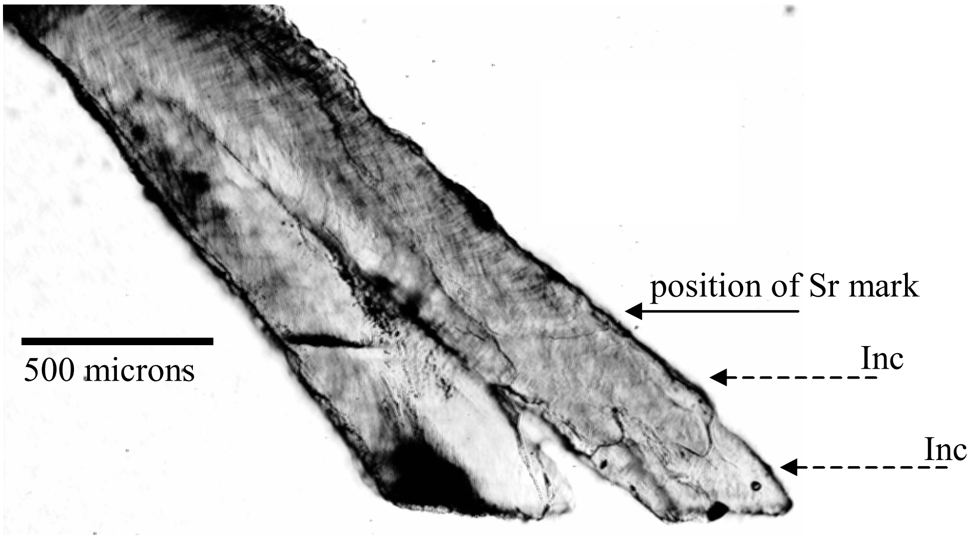
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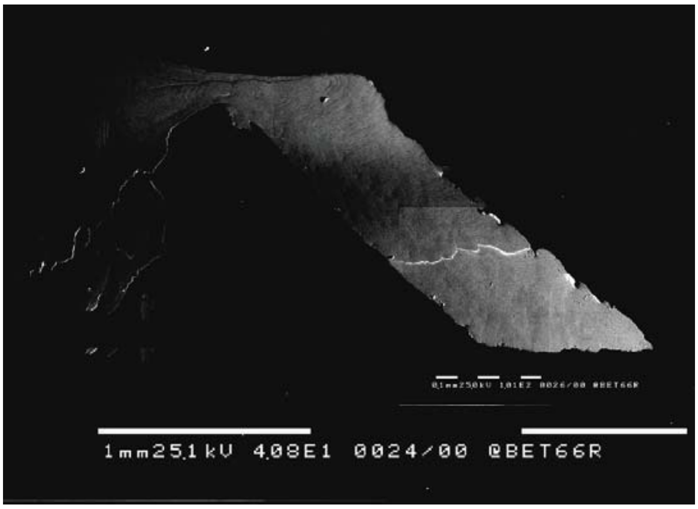
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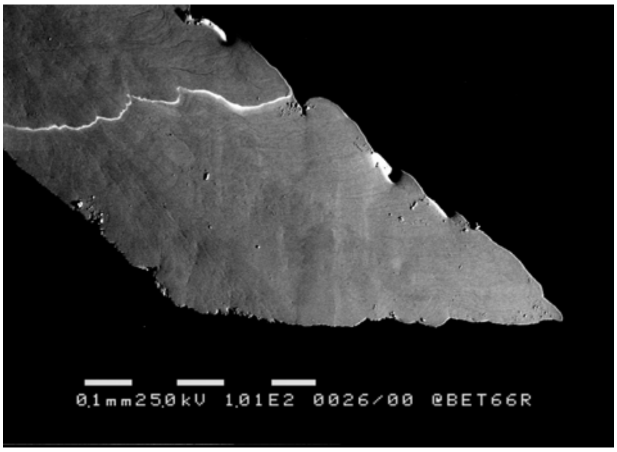
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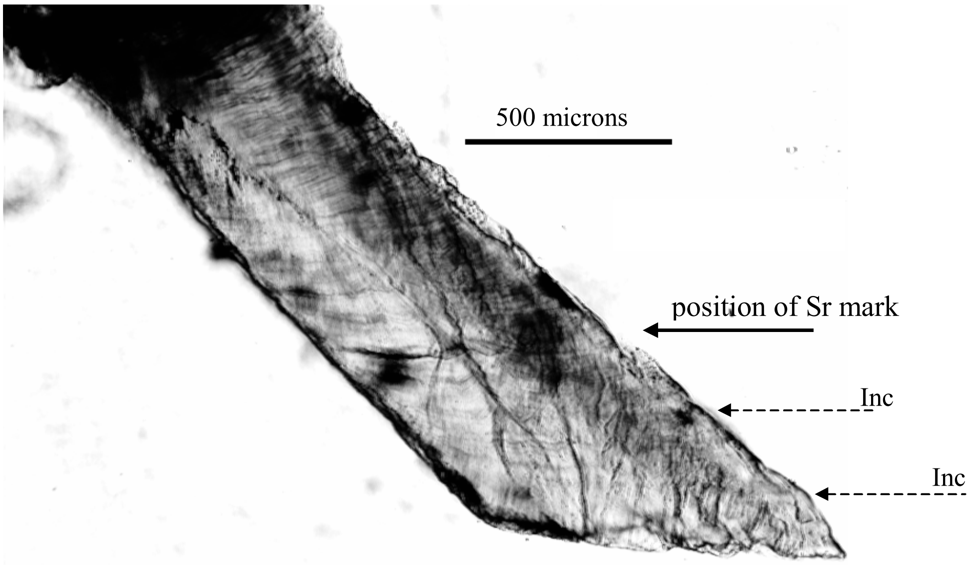
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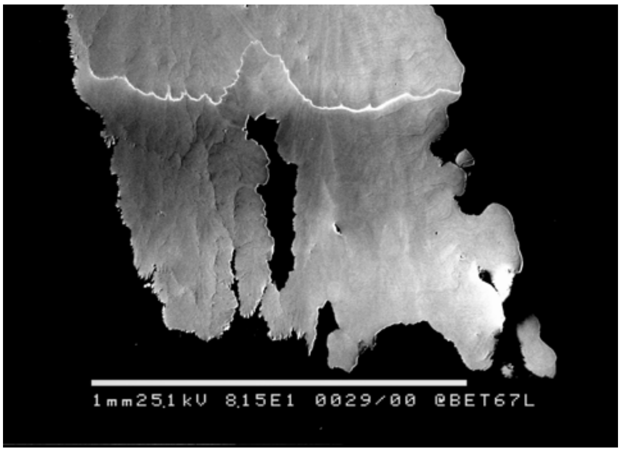
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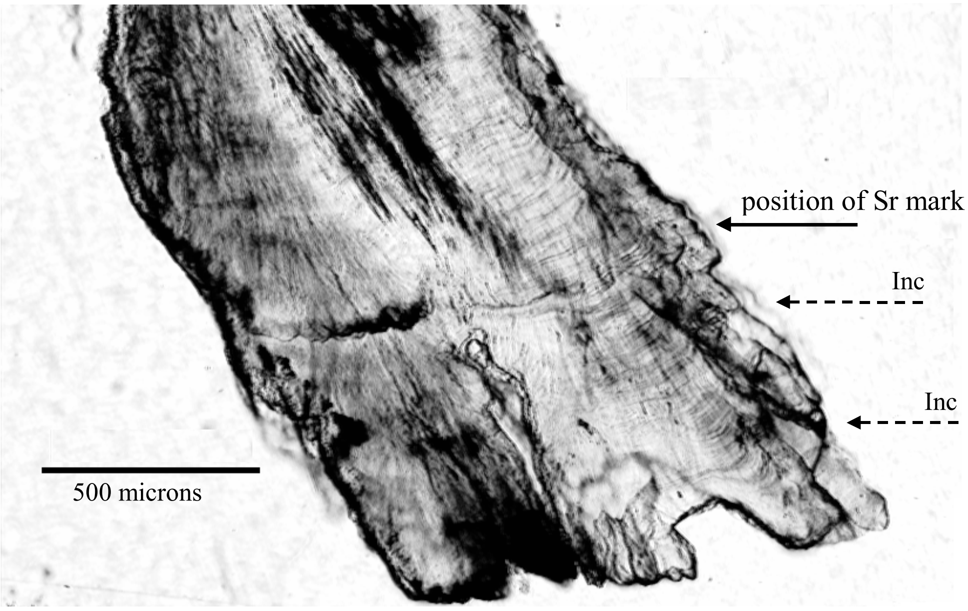
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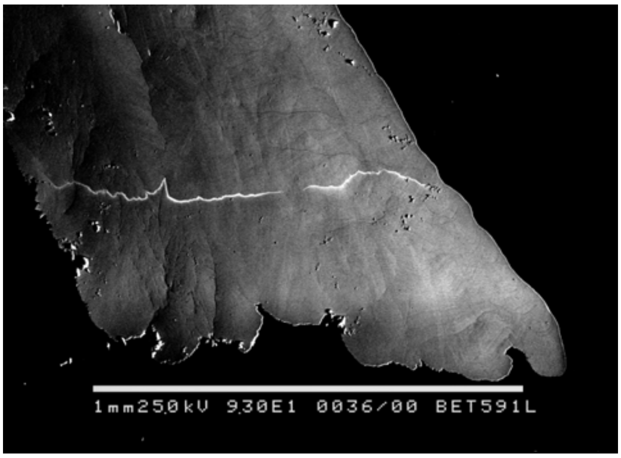
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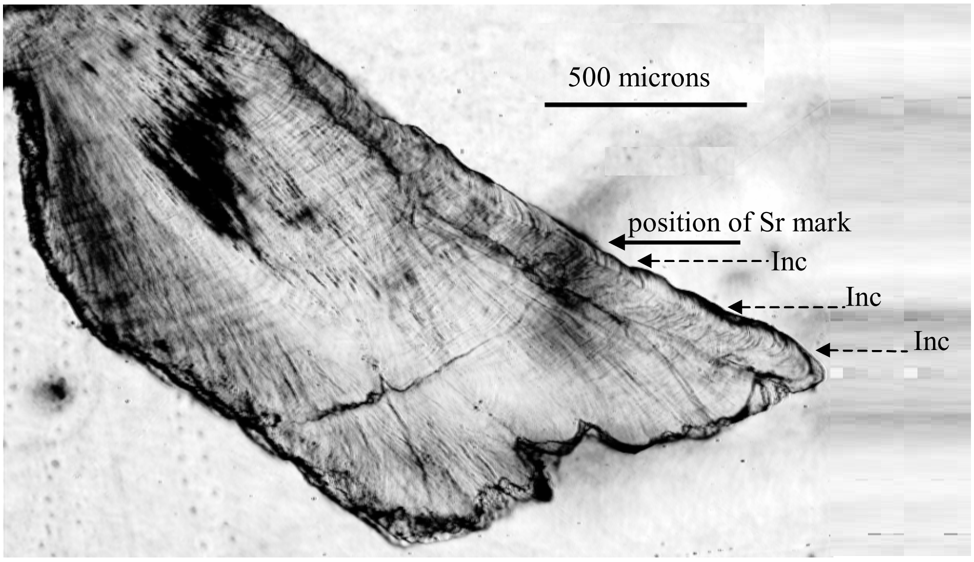
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position of Sr mark
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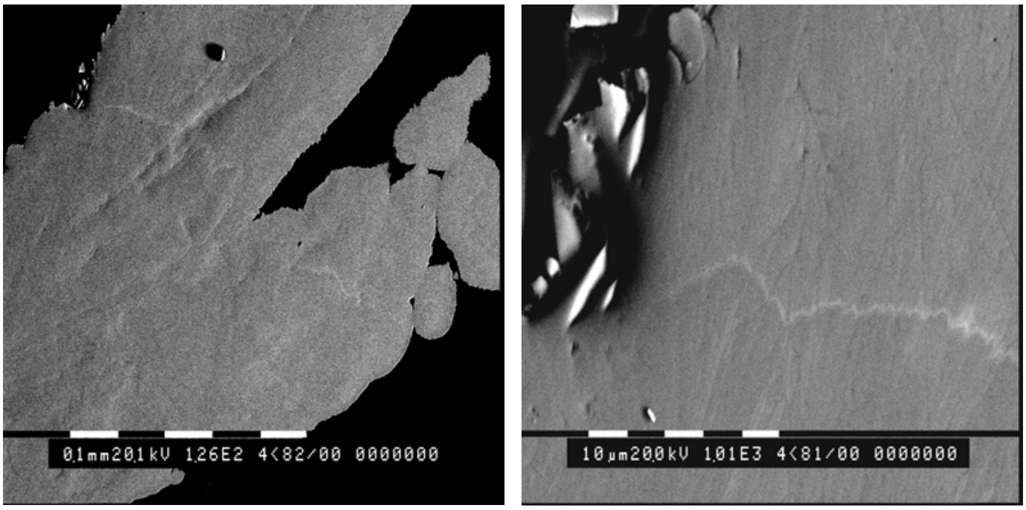
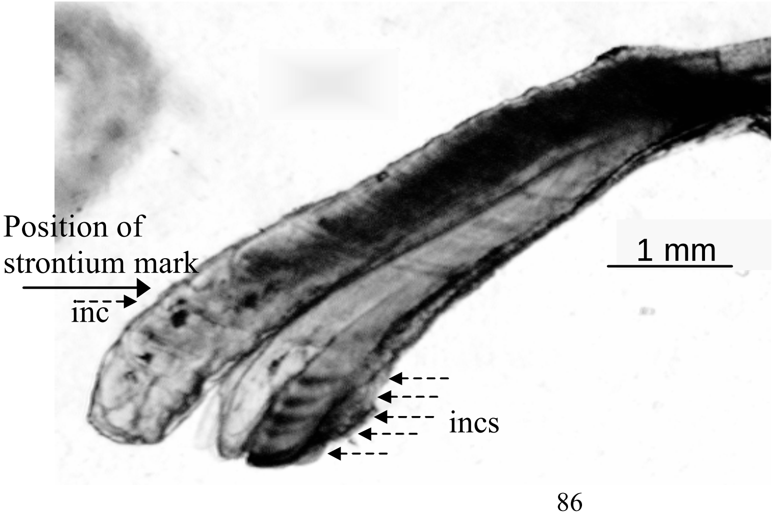
A.


1mm20.0kV 3.14E1 4<79/00 0000000

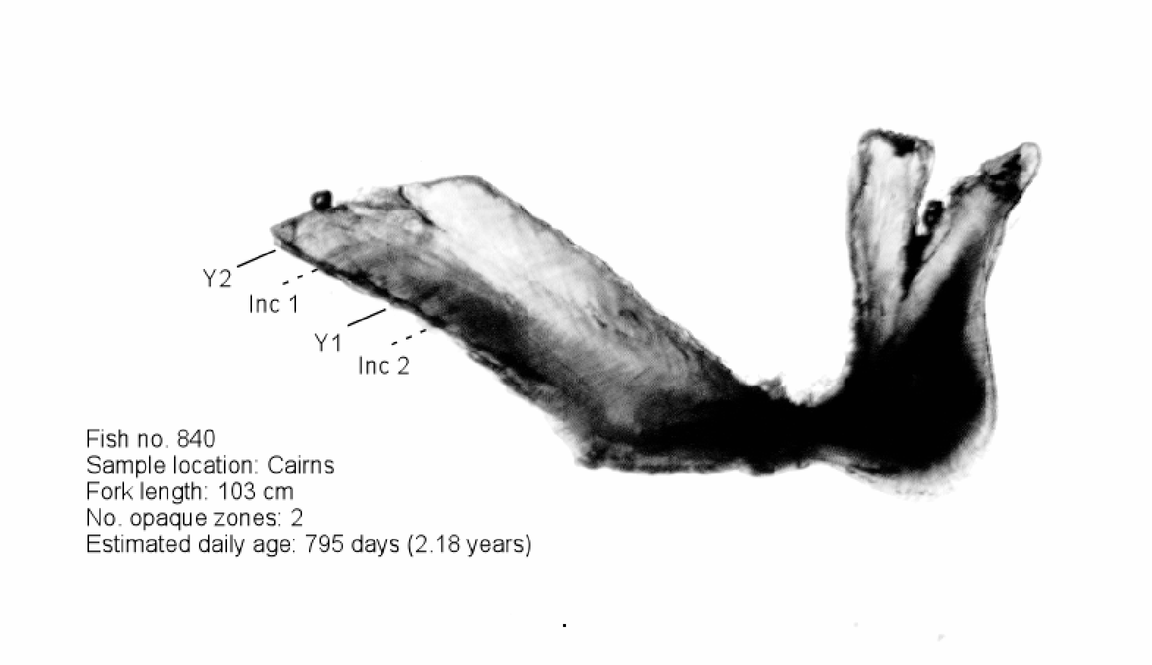
B.


0.1mm20.1kV 1.26E2 4<82/00 0000000
10µm20.0kV 1.01E3 4<81/00 0000000

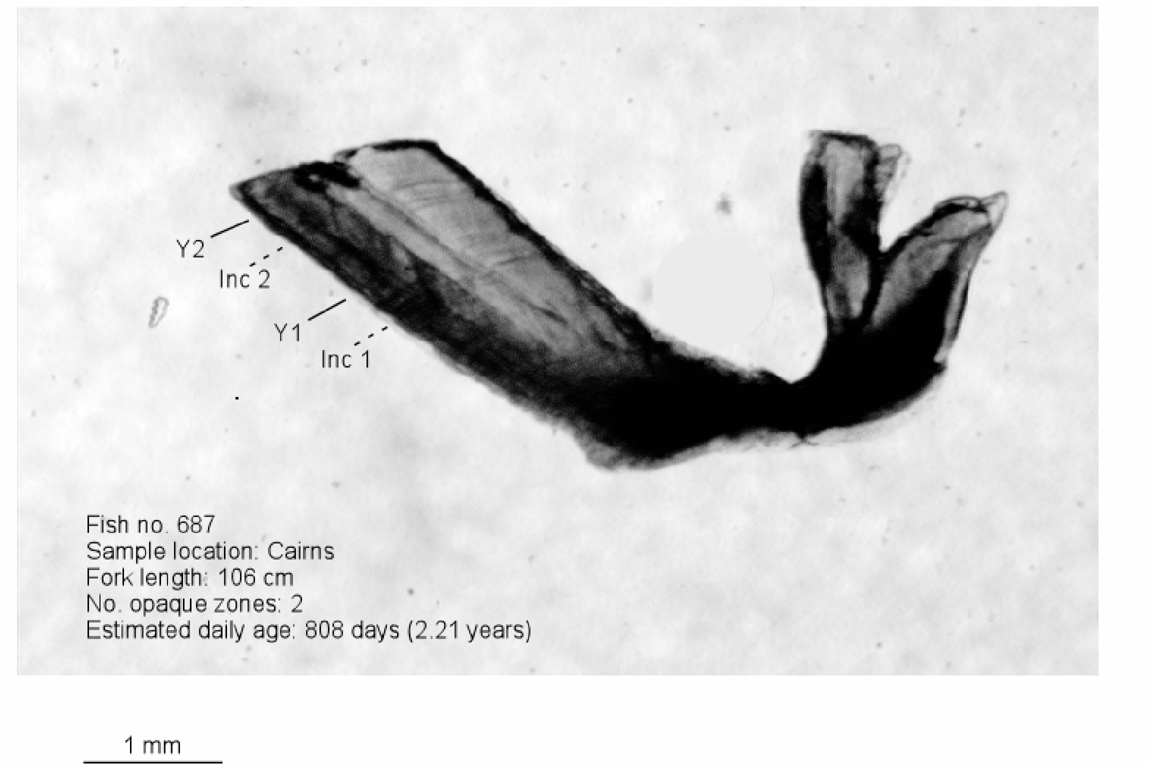
C.


Position of strontium mark
inc
1 mm
incs

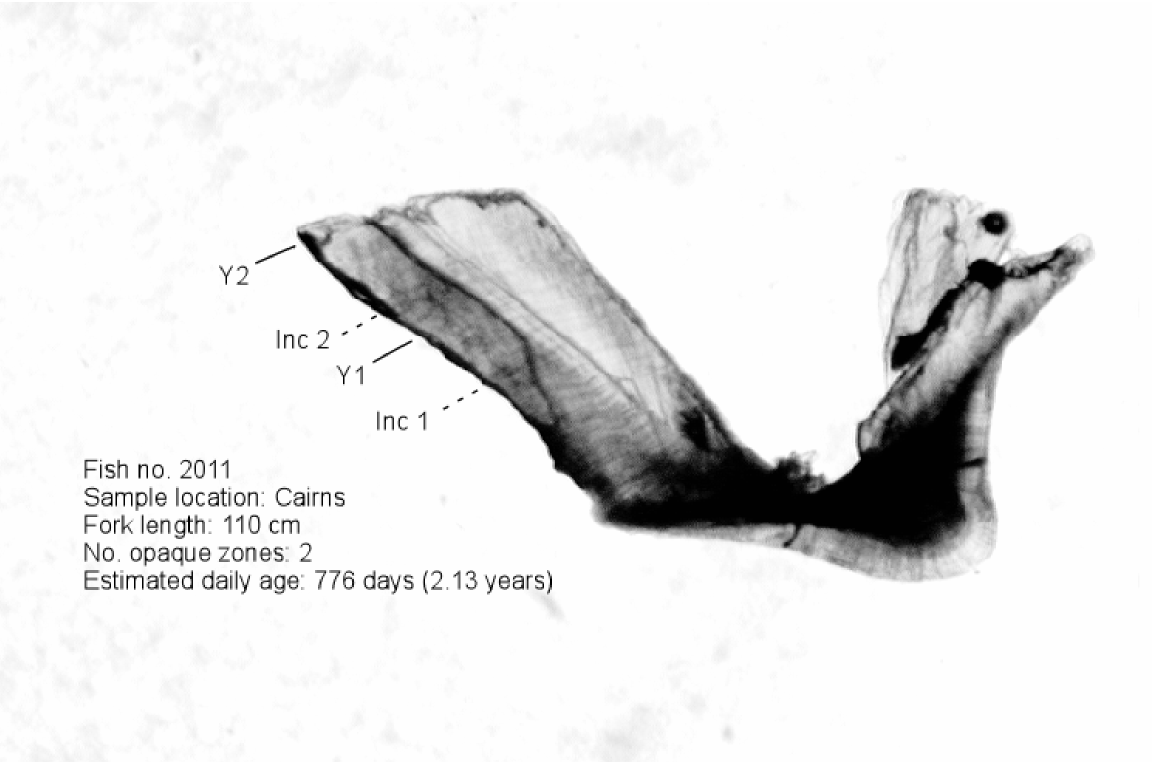
Examples of transverse sections of bigeye tuna otolith showing the location of the 365th (Y1) and 730th daily increment (Y2) signifying the length of the otolith at age 1 and 2 years from microincrement analysis. The locations of the opaque zones (inc 1-3) are also indicated.



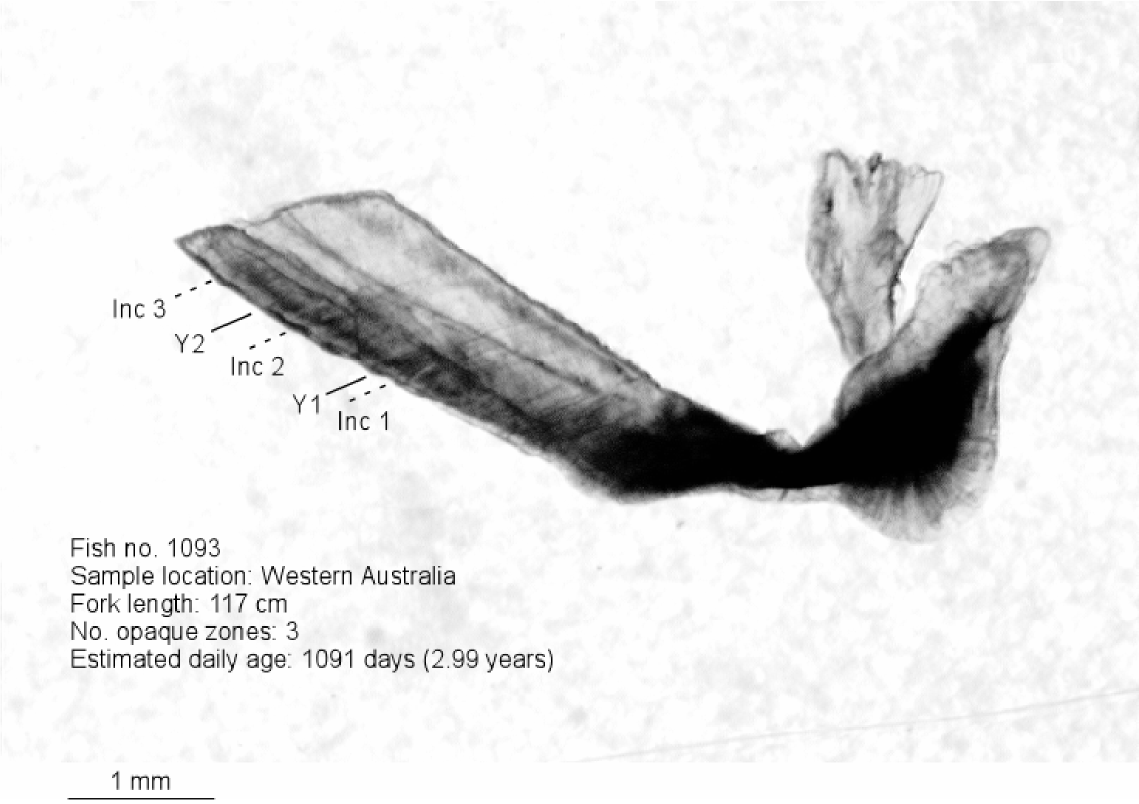
Fish no. 840
Sample location: Cairns
Fork length: 103 cm
No. opaque zones: 2
Estimated daily age: 795 days (2.18 years)



Fish no. 687
Sample location: Cairns
Fork length: 106 cm
No. opaque zones: 2
Estimated daily age: 808 days (2.21 years)



Fish no. 2011
Sample location: Cairns
Fork length: 110 cm
No. opaque zones: 2
Estimated daily age: 776 days (2.13 years)



Fish no. 1093
Sample location: Western Australia
Fork length: 117 cm
No. opaque zones: 3
Estimated daily age: 1091 days (2.99 years)

Mean length-at-age (with standard deviations) estimated from bigeye tuna otoliths sampled from four regions

Age class (years)	Cairns			Qld/NSW		
	Mean FL (cm)	SD	n	Mean FL (cm)	SD	n
0	45.4	4.44	8			
1	84.6	6.71	47	85.2	6.57	66
2	104.4	8.60	105	101.5	9.93	209
3	119.7	9.68	62	119.9	7.91	156
4	127.5	6.99	20	132.6	6.74	107
5	131.7	10.95	20	140.4	9.47	33
6	144.6	5.32	15	149.8	10.09	30
7	147.7	10.86	28	151.5	8.14	22
8	149.3	8.42	21	153.1	8.15	10
9	150.0	7.93	10	155.8	7.02	11
10	169.3	5.84	7	160.5	4.80	4
11	151.3	-	1			
12	149.8	12.95	3	164.9	-	1
13	176.3	-	1			
14				167.0	-	1
15	170.1	-	1	85.2	6.57	66
Total			349			650

Age class (years)	Western Australia			Indonesia		
	Mean FL (cm)	SD	n	Mean FL (cm)	SD	n
0				48.0	-	1
1	87.7	3.99	26	86.8	7.06	16
2	99.1	9.28	122	96.0	8.43	66
3	114.0	8.98	125	111.5	9.70	53
4	128.1	6.42	73	129.4	8.23	27
5	134.4	8.13	50	133.9	9.37	7
6	142.2	6.65	23	148.8	10.04	10
7	143.8	8.33	16	149.8	7.83	10
8	149.8	9.14	11	158.7	17.75	6
9	149.2	4.21	5	148.0	11.31	2
10	170.3	3.21	3			
11				176.0	-	1
12	162.0	-	1	162.5	9.19	2
13						
14	157.0	-	1			
15				152.0		1
Total			456			202

Age-length key developed for the eastern AFZ for 2001.

	Age (years)										
Fork length (cm)	1	2	3	4	5	6	7	8	9	10+	Total
65	2										2
70	1										1
75	10										10
80	12	1									13
85	9	3									12
90	11	9	2		1						23
95	2	9	1								12
100		7	1								8
105	1	7									8
110		10	4								14
115		16	24	3			1				44
120		6	41	8	1						56
125		1	21	7	2						31
130			6	7	1			1			15
135			2	1	3	1	1		1	1	10
140			1	1	1	1		1	1		6
145					2	4	2	3	2	1	14
150						3	1	1	3		8
155					2	1	1	2		1	7
160						1			1	1	3
165						1	1			1	3
170							1			1	2
175										2	2
Total	48	69	103	27	13	12	8	8	8	8	304
Mean LatA	84.7	107.0	121.7	126.9	136.0	151.1	149.6	148.1	149.3	169.1	
SD	7.44	11.02	7.38	5.64	16.97	7.78	16.48	7.87	8.92	-	

Age-length key developed for the eastern AFZ for 2002.

	Age (years)										
Fork length (cm)	1	2	3	4	5	6	7	8	9	10+	Total
65	1										1
70	1										1
75	9										9
80	14	1									15
85	26	15									41
90	6	43									49
95	2	45	1								48
100		22	5								27
105		21	5								26
110		14	20	1							35
115		5	24								29
120		4	10	4	1						19
125			11	4	3						18
130			3	30	4	2					39
135			2	26	6	2		2			38
140				9	4	2	2	3			20
145				1	4	7	2				14
150					1	3	3	2	2	1	12
155				1		1	5	3	3	1	14
160					1			1	2		4
165										1	1
170									1		1
175											1
Total	59	170	81	76	24	17	12	11	8	3	461
Mean LatA	85.0	99.2	117.4	134.3	138.7	145.3	152.0	149.3	158.3	158.1	
SD	5.66	8.45	8.13	5.97	9.17	7.20	6.25	8.79	6.23	8.07	

Age-length key developed for the western AFZ for 2001.

	Age (years)										
Fork length (cm)	1	2	3	4	5	6	7	8	9	10+	Total
80	4	3									7
85	12	18									30
90	5	10									15
95		7	1								8
100		4	5								9
105		11	13								24
110		1	8	2							11
115			3	2							5
120			4	2							6
125			6	13	3	1	1				24
130			1	10	6	1					18
135			1	2	9			1			13
140			1	1	1	5	4	1	1		14
145				1	2	3	3	3	1		13
150						2	1		1	1	5
155						2	1	1	1		5
160								2			2
165								1			1
170								2			2
175										1	1
Total	21	54	43	33	21	14	10	11	4	2	213
Mean LatA	85.2	92.6	112.1	126.4	133.3	142.9	142.5	153.6	147.5	164.0	
SD	3.35	8.51	10.48	7.32	5.55	8.48	7.91	12.27	6.45	17.68	

Age-length key developed for the western AFZ for 2002.

	Age (years)										
Fork length (cm)	1	2	3	4	5	6	7	8	9	10+	Total
60	1										1
65											0
70											0
75											0
80	2	4	1								7
85	8	15									23
90	3	14	1								18
95		13	3								16
100		11	15								26
105		28	12								40
110		5	17								22
115		1	4	2	1						8
120		1	7	5	2						15
125				3	1		1				5
130				1	2	1					4
135				1	5		1				7
140						3	3				6
145				2	1	3	5		1		12
150					2						2
155						1	1	1			3
160					1						1
165											0
170										1	1
Total	14	92	60	14	15	8	11	1	1	1	217
Mean LatA	86.2	98.9	108.5	127.7	135.9	144.1	143.7	155.0	145.0	174.0	
SD	7.13	9.03	8.06	10.03	12.32	7.20	7.68	7.13	-	-	