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The direct estimation of age and growth of Southern Bluefin Tuna

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CSIRO
AUSTRALIA

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RESEARCH &
DEVELOPMENT
CORPORATION

Project 92/42

NON TECHNICAL SUMMARY

92/42 The Direct Estimation of Age and Growth of Southern Bluefin Tuna.

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Objectives:

1. Develop techniques appropriate for the direct ageing (i.e. interpretation of banding patterns in calcified tissues such as otoliths, scales and vertebrae) of juvenile and adult SBT. In the future, the techniques developed would allow the Central Ageing Facility at the Victorian MSL to undertake routine assessment of the age composition of SBT catches.
2. Validate estimates of age for as many year classes as possible using calcified tissues from fish injected with oxytetracycline or strontium chloride during CSIRO's 1983/4, 1990/1 and 1991/2 tagging projects and through the integration of data from direct ageing, tagging and length frequency analyses.
3. Produce an age-length key for SBT populations exploited by domestic and high seas fisheries using data from direct ageing, length frequency and tagging analyses.
4. Compare length-at-age and growth rates of fish caught during the 1960's, 1980's and 1990's to examine the hypothesis that these parameters have changed in response to changes in population levels.
5. Determine age-at-recruitment to the southern Western Australian fishery and the distribution of spawning dates for fish recruiting into the fishery each year.
6. In conjunction with studies of the reproductive biology of SBT currently under way at CSIRO, estimate age at maturity of SBT and the distribution of ages of spawners.

Non Technical Summary

This project was developed in response to calls from the SBT Trilateral (now the CCSBT) Scientific Committee for the development and validation of techniques for the direct estimation of age and growth in the species. Since the early 1980’s, the stock assessment methods used by Australian, Japanese and New Zealand

scientists to examine the status of SBT stocks have depended heavily on accurate data on the age structure of the catch, and population. However, there was significant concern that the methods being used to estimate the age composition of the catch - which were based principally on the conversion of lengths or weights to age using growth curves derived from tagging data - were not accurate, particularly for large fish. As the conversions produced indirect estimates of age they could not be validated. The resulting uncertainty surrounding their accuracy introduced significant uncertainty into the assessments. Given the serious concerns over the status of the SBT stock, which is considered to be at historically low levels, a new approach that allowed direct and validated estimates of age was urgently required.

In 1992, with funding from the FRDC, CSIRO and the Japanese Marine Research Agency, JAMARC, we began a large and integrated project designed at developing and validating new techniques for directly estimating the age and growth of SBT. A large mark and recapture experiment was initiated within a JAMARC-CSIRO tagging program and over the four years 20,204 were injected with strontium chloride ($SrCl_2$); otoliths, scales and vertebrae collected by CSIRO, Australian Fishing Zone and RTMP Observers, and scientists from the Japanese National Research Institute for Far Seas Fisheries were prepared using adaptations of techniques reported in the literature; a relational data base was developed allowing all of the age and growth data produced by the project to be integrated into the central CSIRO SBT data base, the key repository for data used in the annual CCSBT assessments; a collaborative project was developed with Dr John Kalish and his team at the Australian National University using a new technique based on bomb radiocarbon chronometry to provide independent validation of our age estimates for large fish; and samples from our study were sent overseas for review by international experts.

The project met all of its objectives and has undoubtedly been a major success. Our findings revealed serious errors in the historical data used within the Tri-lateral/ CCSBT assessments (see Table 1) and the new data produced by the project have had an immediate and significant impact on both the assessment process and our understanding of the SBT population. The new techniques and data have been accepted by the CCSBT Scientific Committee, prompting an agreement at the 1996 CCSBT meeting to introduce a fishery-wide program of sampling and routine age estimation over the next few years.

In addition to the achievements made within the project, the new techniques we have developed and validated have the potential over the next few years to resolve many of the outstanding uncertainties surrounding the population biology and demographics of the SBT populations.

The major outcomes and achievements of the project are listed below and Table 1 summarises the changes to our understanding of key population parameters resulting from the study.

Major outcomes and achievements

1. A new technique for revealing and reading daily growth increments in the otoliths (or ear bones) was developed and used to confirm that the age at which SBT recruit to the WA coast is one year rather than two, as previously accepted.
2. A large otolith marking experiment using a benign salt (strontium chloride) rather than commonly-used fluorochrome dyes that can be harmful to humans was successful in providing validation of the annual formation of bands in otoliths of 1-6 year-old fish.
3. After considerable efforts, the Japanese SBT fishing industry accepted our case that extraction of SBT otoliths using a high-powered drill and hole saw did not affect product quality, or decrease market value. This facilitated the collection, for the first time, of large numbers of SBT otoliths, from throughout the size range of the catch and the geographic range of the fishery. Without these samples the project could not have succeeded.
4. Through strontium marking and bomb radiocarbon chronometry we validated that slow growth bands form once a year, throughout life in SBT otoliths. This represented a major breakthrough as it meant that we could be confident that our otolith band counts could be used to directly estimate the age of individual fish of all sizes.
5. Rings on vertebrae do not form annually after fish reach sexual maturity, meaning that vertebrae cannot be used for estimation of age in anything but juvenile fish.
6. Using the new otolith techniques we found that SBT mature approximately 10-12 years of age, 3-5 years later in life than previously accepted.
7. It was also discovered that SBT can live for more than 40 years, rather than 20 years as accepted previously, suggesting strongly that current assumptions about natural mortality rates in the species are invalid and that these are lower than previously accepted, at least at larger sizes.
8. A large fraction of the SBT population was found to be older than 20 years of age, i.e. older than the previously accepted maximum age for the species.

Table 1. Key population parameters resolved by the study, compared with the assumptions and estimates previously used within the assessment process.

Population parameter	Prior to this study	New Estimate
Age at recruitment to surface fisheries in WA	2 years	1 year
Age at recruitment to high seas longline fisheries	5-6 years	4 years
Age at maturity	7 years	10-12 years
Maximum Age	20 years	40+ years
Natural Mortality	Constant	Non-linear, likely to be very low post-maturity

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Project Background and Justification

The project was developed in 1992 following repeated calls, over a number of years, from the SBT Trilateral Scientific Committee (now the CCSBT Scientific Committee) for an improvement in the accuracy of data on the age composition of the SBT catch. The background to these calls and the history of previous attempts to directly estimate the age of SBT using hard parts such as otoliths, scales and vertebrae, were outlined as follows in the original proposal:

i. The requirement for accurate and validated age data on SBT

"It is essential to get accurate age composition estimates for use in SBT Virtual Population Analyses (VPA's)" (Summary Document, 9th Trilateral Scientific Meeting, 1990). Uncertainties in the age structure of SBT populations, resulting from the lack of a validated direct method for ageing the species, severely limit our ability to manage exploited stocks. In lieu of validated length-at-age data, estimates of the age distribution of the catch are based on the conversion of lengths and weights to ages based on estimated growth curves. These conversions introduce a large degree of unmeasurable uncertainty into the catch-at-age distribution and tend to dampen and smooth out differences over time in the relative proportion of different year classes within the catch (Hearn et al.,WP5 SBT Recruitment Monitoring and Tagging Program Workshop, August 1991). Since the primary method of assessing SBT stock condition has been VPA, and the primary input into these analyses is the catch-at-age distribution, large and unmeasurable uncertainties in the catch-at-age distribution translate into uncertainties in the assessment results. The development of methods appropriate for ageing SBT and the determination of a validated length-at-age key for fished populations would thus improve the ability of fishery scientists to assess the status of stocks and in so doing would benefit the long-term viability and profitability of the Australian (and Japanese) tuna fishing industry.

At the same time, validated data on the age of fish from throughout the exploited population would improve our understanding of a number of aspects of the species' biology and ecology - all of which are of importance in the development of optimal management strategies. Specifically:

1. The age of recruitment of SBT to fisheries in southern Western Australia and South Africa is not known. The growth curves of Murphy (1976) and Kirkwood (1983) that form the basis of age conversions used in VPA are based on tagging and length frequency data and assume that fish recruit when two years old (i.e. at approx. 60 cm LCF). However, the accuracy of these curves has been questioned recently following the daily ageing of a small sample of 25 cm SBT. These fish were estimated to be 70-100 days old (Thresher, unpubl. data) rather than the predicted 1 year of Kirkwood (1983). If Thresher's estimates are correct, and similar growth rates have been validated for northern bluefin tuna, SBT may recruit at age 1, a factor that will have implications for VPA's where incorrect assignment of cohorts have significant effects on the accuracy of assessments. Daily ageing of large samples of pre-recruit and newly-recruited SBT will provide a means of estimating accurately the age of recruitment.
2. The effect of changes in population size on the growth rates of juvenile SBT has been examined by Hearn (ms. in prep.), using tagging data, and Leigh (1991), using length frequency data. Their analyses suggest that during the 1970's and 80's growth rates of 3-5 year olds changed significantly. However, the nature of these data, and in particular those from tagging, result in uncertainties regarding the generality of

the findings. The CSIRO scale and otolith archives contain material collected during the 1960's, 80's and 90's. Using the techniques developed during the proposed study, this material should provide direct and validated estimates of age and growth from periods in which the SBT fishery was developing, declining and possibly recovering and thus provide independent data with which to test the hypothesis of Hearn and Hampton (1990).

3. Hynd (1965) described the recruitment each year into the Western Australian SBT fishery of two (or more) size classes within the 2+ cohort. He concluded that each size class or group of fish was the result of a separate spawning event and that this in turn indicated the presence of more than one stock of SBT. Hynd's hypothesis has received little attention among SBT fisheries researchers. However, if more than one stock of SBT are present in the fished population, it could have enormous ramifications on the strategies used in managing the fishery.
   Recent detailed analysis of length frequency data collected over twenty-five years from the SBT fishery off southern Western Australia (Leigh, 1991) point to the recurrent presence of two or three sizes modes in recruiting 2+ fish. These data, Hynd's observations, and anecdotal reports from Japanese longline fishing masters of two pulses of fish migrating north towards the spawning grounds each year, all suggest that there may be two (or more) separate pulses of spawning by SBT each year during their protracted spawning season. Confirmation of temporal partitioning of spawning effort can be obtained using the daily ages of fish recruiting to the Western Australian fishery. Knowing a fish's age in days it is possible to back-calculate its birth date and with adequate samples one can reconstruct a spawning frequency distribution for a recruiting cohort. For SBT, these data would provide a baseline for investigations into adult spawning behaviour, larval distribution/ecology and genetic heterogeneity within a cohort.

4. Despite their importance in assessing the health of a fished population, no data are available on the age at which SBT mature or the age distribution of spawning fish. As the likely impact of high fishing mortality on juvenile cohorts during the early 1980's has been a reduction in the numbers of fish spawning during the 1990's, an accurate estimate of the current age distribution of spawners would be a valuable indicator of parental stock condition.

ii. Background to related research undertaken in Australia and overseas.
The first attempts to age SBT were made in the early 1960's when CSIRO and the Japanese Far Sea Fisheries Research Laboratory assessed the utility for ageing of a number of different hardparts. Despite references to successful pilot studies (Hynd, 1965), CSIRO's efforts with scales appear to have come to nothing, although samples from collections made during the 60's remain in the Division's archives. The Japanese studies resulted in a single publication (Yukinawa, 1970), in which age and growth of fish up to the age of eight years were estimated using scales.

Until the early 1980's, when the condition of SBT stocks began to cause concern, there was little call for accurate data on the age composition of exploited populations. Given the 1960's experiences, it was also generally accepted that SBT were very difficult or impossible to age directly using conventional techniques. Thus, with the introduction of Virtual Population Analysis (VPA) as the primary method of stock assessment for SBT, data from tagging studies and length frequency analyses were used to produce growth curves from which age-length conversions were calculated. However, following the first attempts at VPA for SBT, the inadequacy of length-at-age data derived from these sources was evident, particularly those for older cohorts.

In 1982, in response to then "critical requirement for validated age estimates", J.Thorogood (CSIRO/University of Sydney) began a pilot project evaluating the use of the otoliths and bones for ageing of SBT. For a variety of reasons, support from CSIRO and FIRTA for Thorogood's work was curtailed after two years. The pilot project (Thorogood, 1987), suffered from inadequate sample sizes and poor coverage of older year classes (post 7 years old). Independent attempts to verify Thorogood's readings have also resulted in questions regarding his techniques and interpretation of structure (D. Smith, MSL, National Ageing Facility, pers. comm.).

In 1987, CSIRO began a pilot study examining the potential for electron microprobe analysis in estimating the age of SBT. This work was reported in a Final Report to FRDC (Grant 83/30), but has not been published. In brief, the results suggest that concentrations of elements such as strontium and sulphur vary cyclically throughout the life of fish. However, as a result of problems with obtaining samples from one study area throughout a twelve month period, it was not possible to demonstrate unambiguously that these cycles are deposited annually. As well as data on the chemistry of otoliths, the study provided invaluable experience in the specialised sectioning techniques that are required for ageing of tunas (A.Wild, IATTC, pers. comm.).

Research into the age and growth of other *Thunnus* species is of particular relevance to the proposed study. Studies of the northern or Giant bluefin tuna, *Thunnus thynnus thynnus*, using fin spines, vertebrae and otoliths, either independently or in combination, have provided reliable and validated estimates of age for fish up to 12 years old and have recorded ages to 30+ years (Hurley and Isles, 1983; Lee et al., 1983). Various preparation techniques have been used in these studies, many of which have potential for use in SBT ageing. Similarly, in ageing studies of yellowfin tuna, *Thunnus albacares*, Wild and associates at the IATTC have used sectioning techniques specifically adapted for their species combined with detailed analysis of otolith microstructure. Combined with oxytetracycline marking/ validation experiments these techniques have allowed daily ageing of yellowfin up to two years and annual ageing of adults (Wild and Foreman, 1980).

The lesson from overseas experiences with age estimation in tunas is that, contrary to the prevailing Australian dogma, it is possible through improvement of techniques and use of the latest technology (scanning electron microscopes, digitising soft and hardware etc.) to age long-lived species such as SBT.

In summary, validated data on age and growth are urgently required for assessment of Southern Bluefin Tuna stocks. In addition, there are important questions regarding the reproductive biology and stock structure of SBT for which accurate age data are essential baselines for future research. Given the value of the SBT fishery today and over the past thirty years it is somewhat surprising that accurate age and growth data are not available. Explanations for this shortcoming seem to revolve around a dogma that the species is difficult to age. However, experiences overseas and over the past four years at CSIRO suggest that with careful technique development, SBT can be aged through interpretation of the banding patterns on hardparts such as scales, otoliths and vertebrae.

Objectives

1. Develop techniques appropriate for the direct ageing (i.e. interpretation of banding patterns in calcified tissues such as otoliths, scales and vertebrae) of juvenile and adult SBT. In the future, the techniques developed would allow the Central Ageing Facility at the Victorian MSL to undertake routine assessment of the age composition of SBT catches.
2. Validate estimates of age for as many year classes as possible using calcified tissues from fish injected with oxytetracycline or strontium chloride during CSIRO's 1983/4, 1990/1 and 1991/2 tagging projects and

through the integration of data from direct ageing, tagging and length frequency analyses.

3. Produce an age-length key for SBT populations exploited by domestic and high seas fisheries using data from direct ageing, length frequency and tagging analyses.
4. Compare length-at-age and growth rates of fish caught during the 1960's, 1980's and 1990's to examine the hypothesis that these parameters have changed in response to changes in population levels.
5. Determine age-at-recruitment to the southern Western Australian fishery and the distribution of spawning dates for fish recruiting into the fishery each year.
6. In conjunction with studies of the reproductive biology of SBT currently under way at CSIRO, estimate age at maturity of SBT and the distribution of ages of spawners.

Methods

Technical papers for submission to international fisheries science journals have been written on each of the major work modules within the project. These are included as appendices to this FRDC final report. Rather than duplicate the considerable detail provided in these technical papers, we refer you to them for descriptions of the following methods and approaches:

- Validation techniques and strontium chloride marking methodology are described in Appendix 1 - “Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, through a large-scale mark-and-recapture experiment using strontium chloride” - Clear, N.P., Gunn, J.S. and Rees, A.J.
- Daily ageing techniques are described in Appendix 2 - “Age determination of juvenile southern bluefin tuna, *Thunnus maccoyii*, based on scanning electron microscopy of otolith microincrements” - Rees, A.J., Gunn, J.S. and Clear, N.P.
- Bomb radiocarbon chronometry methods are described in Appendix 3 - “Use of the bomb radiocarbon chronometer to determine age of southern bluefin tuna

(*Thunnus maccoyii*). Kalish, J.M., Johnston, J.M., Gunn, J.S. and Clear, N.P. *Mar. Ecol. Prog. Ser.* (in press).

- Methods used to estimate age from slow growth bands on otoliths, rings on vertebrae and annuli on scales are described in Appendix 4 - “The direct estimation of age and growth in Southern Bluefin Tuna, *Thunnus maccoyii*, using otoliths, scales and vertebrae”. Gunn,J.S, Clear, N.P., Carter, T.I., Rees, A.J., Stanley, C.J. and J. Farley.
- Methods used to estimate the age at first maturity are described in Appendix 5 - “Preliminary Estimates of the Age Structure of the SBT Spawning Stock”. Gunn, J.S., Davis, T.L.O., Farley, J.H., Clear, N.P. and K.A. Haskard. CCSBT/SC/96/10.

Results

The results of each of the project’s key work modules, and a discussion of their importance, are provided in the five technical papers included as Appendices to this report. The major outcomes of the project, and the Appendix in which they are covered, are listed below.

1. Strontium chloride marking (Appendix 1) and bomb radiocarbon chronometry (Appendix 3) were used to validate that slow growth bands in SBT otoliths form once a year, throughout life. Thus, counts of slow growth bands on otoliths can be used to directly estimate the age of individual fish of all sizes (Appendix 4).
2. Counts of vertebral rings and scale annuli were reliable estimators of age for ages up to 8-10 years and 4 years, respectively. Above these limits, counts from both structures resulted in significant underestimates of age (Appendix 4). It appears that vertebral rings either form at a rate of less than one per year after fish mature, or that changes after maturity in the growth plane of vertebrae make counting difficult and estimates of age unreliable.
3. The age at which SBT first recruit to Western Australian surface fisheries was estimated to be at approximately one year old using

otolith microincrements (Appendix 2). Prior to this project, recruitment was thought to be at 2 years of age, an estimate based on un-validated scale annuli counts. The new microincrement data resulted in adjustments to the value of t_o in von Bertalanffy growth curves used within the CCSBT assessment process.

4. Age estimates from otoliths revealed that SBT can live for as long as 40+ years, more than twice the estimates of longevity accepted previously within the assessment process (Appendix 4). This suggested strongly that natural mortality rates in SBT are much lower than previously assumed, especially at larger sizes.
5. Age estimates of representative samples of the Japanese longline catch off Tasmania from 1992-94 (Appendix 4) and Indonesian catches on the spawning grounds from 1992-95 (Appendix 5) indicate that a significant fraction of the SBT population is over 20 years of age. Data from the Indonesian catches also suggest that the age at maturity in SBT may be much higher than the 7 years previously accepted, with fish first appearing on the spawning grounds as 10-12 year olds (Appendix 5).
6. Otolith-based length-at-age data from fish throughout the age and size range of the species were used to derive a von Bertalanffy growth curve. The parameters of the growth function were significantly different to those produced during the 1960's-80's from tagging and length frequency data. The reasons for this are discussed in Appendix 4. Reassuringly, the growth parameters used most recently by the CCSBT in its assessments, which were developed using tagging data, but with significant tuning using data from our project, are very similar to our parameter estimates from directly-estimated ages.

In summary, the project developed and validated methods that will allow the direct estimation of age in all sizes of SBT, using otoliths. These methods were used to estimate the age at recruitment, age at first spawning, maximum age and age composition of catches. The estimates were significantly different from those used previously within the CCSBT assessment process. The new data have been fully accepted by the CCSBT Scientific Committee and the resulting new perspectives on the life history of SBT have been used in the most recent assessments, with significant impact.

In addition, the CCSBT has recently agreed to develop a program for the routine sampling of otoliths and estimation of age of the SBT catch. The CCSBT Scientific Committee agreed at its 1996 meeting that the validated age estimation methods developed by this project provide the basis for improved catch-at-age matrices for input into the VPA process. This is the outcome we were looking for when this project was first proposed. We believe the study represents a major step forward in our understanding of SBT biology and population dynamics.

Benefits

The project was designed to provide methods for improving the accuracy of data used in SBT assessments, and in so doing reduce uncertainties within the assessment process. The successful completion of all objectives has paid immediate dividends in the refinement of basic population biology parameters used in the assessments. It has also provided the potential for routine collection of validated catch-at-age data for the complete size and age range exploited by the diverse range of SBT fisheries.

The benefits of accurate assessments will hopefully come in the short term, through the reduction of the risk of further declines in the SBT stock. In the medium to longer term, it is to be hoped that accurate assessments will assist in the development of management strategies facilitating stock rebuilding.

It would be naive to suggest that these ends will be met simply through improvements in the quality and accuracy of data such as those made by this project; political and economic factors obviously play an important role in the management of international stocks. However, to ensure that uncertainties in the stock assessments are not used as a vehicle for ignoring ESD and Precautionary Principle guidelines, it is essential that, wherever possible, data and analytical methods are of the highest standards. The project represents a significant step forwards in reducing uncertainty surrounding critical data and it is to be hoped this progress will benefit the SBT stock and the industries that exploit it.

Intellectual Property and Valuable information

No Intellectual Property of commercial importance resulted from this project. However the techniques developed and validated for estimation of age from SBT otoliths will undoubtedly be useful for other species of tunas and pelagic fishes. The results from the project have produced four technical papers for submission to international fisheries journals, a result that should ensure the work funded by FRDC will be exposed to the international fisheries science community.

If and when the CCSBT begin routine estimation of age of the SBT catch, we are committed to training Central Ageing Facility staff in the techniques we have used.

Further Developments

Having developed and validated methods for direct estimation of age in SBT, there is now the potential for further investigations into key life history and population parameters. Improved data on population demographics, age at maturity, catch-at-age distributions of the various fishing fleets that exploit the SBT stock and direct estimates of natural mortality would all further reduce uncertainties within the assessment process. As a follow up to FRDC 92/42, CSIRO have developed a proposal to address the following objectives:

- Using methods recently developed and validated by CSIRO within an FRDC project (92/42), and otoliths collected from SBT fisheries throughout the species' range, determine the age distribution of catches in each of the major SBT fishing grounds.
- Using these catch-at-age matrices, examine the hypothesis that there are significant differences among fishing grounds in the age distribution of catches.
- Determine whether observed changes in the size distribution of Indonesian catches on the spawning grounds during the 1990's reflect a fish-down of older age classes.
- Use catch-at-age distributions to examine patterns and rates of natural mortality in SBT, in particular in "plus group" or mature age classes.
- Using measurements of the radius of the first and second winter growth bands of otoliths from throughout the age distribution of the catch, examine historical changes in the growth rate over the first two years of life.

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Final Cost

Appendix 6 is a Final Audited Statement of Receipts and Expenditure for the project.

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 1

Project 92/42

Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, through a large-scale mark-and-recapture experiment using strontium chloride

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Key Words: validation, strontium, otolith, chemical marker, southern bluefin tuna, age determination, energy dispersive spectroscopy

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Abstract

From 1990-95, 20,204 southern bluefin tuna (SBT), *Thunnus maccoyii*, were injected with strontium chloride ($SrCl_2$) as part of a large-scale tag-and-release program in the Great Australian Bight. The objectives of the marking experiment were to examine the efficacy of $SrCl_2$ as an otolith marker and to determine the periodicity of increment formation in SBT otoliths.

Otoliths were sampled from 623 of the 961 marked fish recaptured; the high level of sampling success being attributed to a major liaison effort throughout the multinational SBT fishery. The same return rates for tags in marked and unmarked fish indicated that the injection of strontium had no effect on the survival of tagged fish. Strontium marks were detected on 95% of the otolith sections of marked fish examined, using a Robinson detector and/or an Energy Dispersive Spectrometer (EDS) linked to a scanning electron microscope. A relationship was demonstrated between the intensity of the strontium mark and the dosage rates, and a dosage of 100 mg/kg fish weight is recommended to sure easy identification of the strontium mark. The advantages of using strontium chloride included the high success rate in detecting marks, the benign nature of the marking compound to both fish and humans, the ability to unambiguously validate the presence of a strontium mark using EDS, the insensitivity of $SrCl_2$ to light and the longevity of the strontium mark. Although detection of strontium marks may be more expensive than for the more commonly-used fluorescent markers, the advantages of using $SrCl_2$ suggest it is an excellent option for large-scale marking experiments on commercial fishes where fluorescent markers such as tetracycline may present a health risk.

Using the strontium marks, we established that in SBT with 1-6 increments in their otoliths, one increment is laid down per year at liberty. In the 59 marked fish examined, there was 100% agreement between the expected and observed number of increments after marking. These results and the data from two supplementary tag returns from unmarked fish recaptured after extended times at liberty, provide unambiguous evidence for the annual formation of increments on the otoliths of SBT, to at least the age of 13 years. In addition, the findings from a recent study using bomb-radiocarbon levels to estimate ages of older SBT provide strong evidence that annual increments are deposited in the sagittal otoliths of SBT throughout life.

Introduction

Southern bluefin tuna (SBT), *Thunnus maccoyii,* is a large, long-lived, highly migratory pelagic fish with a circumglobal distribution between 30°S and 50°S (Caton, 1991). Its only known spawning ground lies in the Indian Ocean south of Java, between 7°S and 20°S (Caton, 1991). Since it was first exploited in the 1950s the stock has declined dramatically; to between 16% and 25% of its initial level (Sainsbury[1]). The fishery is currently managed by individual transferable quotas (ITQ) and annual assessments are conducted to determine the annual total allowable catch (TAC).

Virtual population analysis (VPA) has been the principal method used to assess SBT stock condition since 1980 (Murphy and Majkowski, 1981). However, unmeasurable uncertainties in the VPA assessments have been introduced because of the absence of a validated direct method for estimating age in the species which produces uncertainties in determining the age structure of SBT populations. Without a validated age key, estimation of the age distribution of the catch has been made by the conversion of lengths and weights to ages, using growth curves derived from tagging data (Hampton, 1991, Polacheck et al.[2]).

In 1992 we began a study to develop reliable techniques for determining ages of SBT. At the same time, a large-scale mark and recapture experiment was initiated to provide the basis for validation of age estimates from otoliths. The overall objective of these two studies was to develop a validated length-at-age key for the entire size range of the SBT population. This paper gives details of the mark and recapture experiment and our evidence for annual formation of increments in otoliths.

The requirement to validate assigned ages is critical in age estimation studies (Beamish and McFarlane, 1983; Smith, 1992; Secor, 1995). In validating the method that was used to determine ages of SBT, we aimed to confirm the temporal meaning of the zones being counted on the otoliths.

Previous attempts to estimate SBT ages directly, included either no attempt at validation, or validation was restricted to a small number of age classes. Hynd (1965) used scales to estimate ages of fish up to 80 cm fork length (LCF) but did not attempt to validate his age estimates. Yukinawa (1970) counted up to 8 rings on scales using marginal increment analysis to show that the rings form at the same time each year. Thorogood (1987) used otoliths to estimate age in fish from 42 to 167 cm LCF and, using marginal increment analysis, was able to show seasonal band formation in what he called ages 2 to 4. Jenkins and Davis (1990) examined microincrements in the otoliths of SBT larvae of 3.5 to 12 mm standard length (SL) collected from the same cohort over consecutive days. Using marginal increment analysis they were able to validate daily increment formation and assigned ages of approximately 7 to 18 days to their samples.

Few of the many age determination studies of other tunas have validated annual increments over a wide range of age classes. Tetracycline has been most widely used in marking experiments to validate daily increment formation in wild and captive tunas: e.g. yellowfin tuna, *Thunnus albacares* (YFT), in the wild (Wild and Foreman, 1980, Wild et al., 1995) and in captivity (Yamanaka[3]); skipjack in the wild (Wild et al., 1995); black skipjack in captivity (Wexler, 1993); Atlantic bluefin in the wild (anon.[4]).

Despite the success of tetracycline as a marker in other *Thunnus* species, experiments on SBT in the 1980s using oxytetracycline produced mixed results with a high proportion of injected fish having unmarked otoliths (CSIRO unpublished data[5]). Given this previous failure, and public health concerns over the use of tetracyline (in the USA, the Federal Drug Agency prohibits its use in wild fisheries), we selected strontium chloride ($SrCl_2$) as an alternative marker.

Strontium chloride is a non-toxic salt that occurs naturally in sea water. It is readily incorporated into the bloodstream of fish and although it has not been used previously on scombrids, Sr has been used successfully to mark fish vertebrae, by introduction into the diet or water (Behrens Yamada and Mulligan,1990) and otoliths, by immersing and injecting fish (Brothers, 1990). Strontium is chemically and biologically similar to calcium. As calcium and strontium ions have the same valency (2+) and a similar ionic radius (Ca, 0.99Å; Sr, 1.13Å) strontium readily substitutes for calcium during deposition of the calcium carbonate/protein otolith matrix.

Public health concerns were thought unlikely as strontium chloride is a component of some foods and is considered to be benign at the concentrations used in the marking agent (Sax and Lewis, 1987). Having chosen strontium chloride without the opportunity to test its efficacy in tuna, the first two objectives of this study were to:

1. evaluate whether intramuscular injection of strontium chloride results in effective and reliable marking of otoliths , and
2. examine the effect of strontium chloride injections on recapture rates.

If successful and benign marking was demonstrated, we planned to use the strontium chloride marks to validate the techniques used to estimate ages for as many year classes of SBT as possible.

Materials and Methods

Tagging and Marking

The marking experiment that formed the basis of this study was run as a component of a six year tag-and-release program conducted in the Great Australian Bight and was designed to examine movement patterns, growth and mortality rates in juvenile SBT. From 1990-96, 64,497 SBT were tagged and released and 20,204 of these fish were injected with $SrCl_2$ (Table 1). All fish were double tagged (Williams, 1992); strontium injected fish with orange tags, un-injected fish with yellow tags. To examine quantitatively whether the injection of $SrCl_2$ affected the tag return rate, equal numbers of fish from each school, chosen at random, were tagged with yellow and orange tags. The lengths of orange tagged fish ranged from 41-120 cm LCF.

The strontium chloride solution for injection was prepared in the laboratory before going into the field. A stock solution of 1 g $SrCl_2.6H_2O$ /ml was made up by dissolving 1 kg of analytical grade $SrCl_2.6H_2O$ crystals in 1 litre of distilled water, resulting in a 0.21 g/ml solution of Sr^{2+}. The solution was buffered to pH 7.0 using KOH, and stored at between 0°C and 4°C.

To allow rapid injection into large numbers of fish, a 5 ml automatic vaccinator fitted with a 0.2 mm needle was used. Flexible tubing connected the vaccinator to a plastic storage container that was either worn as a back pack or attached to the tagging cradle (Williams, 1992).

Fish were injected approximately 2 cm below the dorsal midline, in line with the centre of the first dorsal fin. Particular care was taken to expel all the air from the applicator as the pressure caused by the injection of an air bubble into the muscle of the SBT can result in the loss of the injected solution. In these cases we noted the loss and the fish was injected a second time.

The dose of injected $SrCl_2$ was varied according to the length of the fish (Table 2). To determine a suitable dosage rate for SBT, initial trials with strontium were made on three non-tuna species, silver trevally, *Pseudocaranx dentex*, sand flathead, *Platycephalus bassensis*, and purple wrasse, *Pseudolabrus fucicola*. The dosages we used followed the results of immersion and injection trials on trout by Brothers (1990) who injected 100 mg/kg, and tetracycline experiments on tuna (Wild and Foreman, 1980) where the dosage of approximately 0.3 ml/kg was used. Investigation of various dosages (maximum of 200 mg Sr/kg) resulted in clear Sr marks on otoliths sections and no mortalities were recorded from any dosages (CSIRO unpublished data[6]). These dosages were increased for fish longer than 90 cm after examination of the first recovered otoliths from larger fish showed faint marks, indicating low strontium uptake. The adjusted dosages followed the relationship of at least 80 mg of Sr per kilogram of fish.

Tag Liaison and Otolith Sampling

A critical factor in the experiment was the sustained liaison effort over many years, to recover otoliths from recaptured fish. An extensive campaign was conducted to educate the SBT fishing industry on the objectives of the marking experiment and to develop a protocol for the collection of samples. Posters and information notes, in Japanese and English, were distributed throughout the fishery. Rewards were offered for the return of tags with a substantial bonus for the recovery of otoliths from orange tagged fish. To clearly identify fish that had orange tags when they came on board, and to aid in otolith recovery, kits containing large orange discs and a means of attachment to the fish were distributed to Japanese vessels.

Given the high value of SBT on the Japanese sashimi market, it was essential that otoliths could be sampled without affecting the external appearance of the fish. To achieve this, we used a hole-saw attached to a drill to remove a core of 35 to 44 mm diameter, anterior and dorsal to the basi-occipital plate that contained sections of the cranium, the semi-circular canals and sagittal otoliths. Sagittal otoliths were removed from the cores, cleaned in distilled water and dried at 28°C.

Validation Procedures

An age estimate was made from the otoliths before attempting to identify a Sr mark. Sagittae were prepared and read using the whole otolith method described in Gunn et al.[7] (Figure 1). A digital image of each otolith was taken (using the public domain "NIH Image" program and a video camera mounted on a Wild M5A dissecting microscope) and measurements made of the otolith length, the length of the postrostral (PR) and transverse axes and the distance between the primordium and the inside of the translucent zones along and postrostral (PR) and transverse axes (for terminology see Rees et al.[8]). For each specimen, 3 independent age estimates were made without reference to the length of the fish nor the time that the fish was at liberty after tagging and injection.

Detection of strontium marks

Two methods were used to detect a strontium-rich band on the otoliths which we refer to as the strontium mark:

1. Scanning electron microscope (SEM) with a Robinson backscatter detector

In the early stages of the project we used a Robinson backscatter detector coupled to a Philips 515 SEM for detection of the strontium mark in the calcium carbonate matrix. The Robinson detector allows visualisation of differences in the total atomic weight (Z) across a specimen. As a strontium mark within the otolith matrix contains a higher concentration of Sr and hence a higher Z than surrounding uncontaminated $CaCO_3$ matrix, it appears as a weak to intense bright band across the growth axis of the section (Figures 2 and 3). The intensity of the band depends on the magnitude of the difference in Z between the two portions of the matrix.

This kind of analysis requires a flat, polished surface so we sectioned the sagittal otoliths either along the postrostral axis, producing an oblique longitudinal section, or along a transverse axis (for terminology see Rees et al.[8]). The rostral axis often shows clear increments but we did not find distinct Sr marks in this part of the otolith. The sections were ground and polished using the methods described by Gunn et al. (1992). An evaporated carbon coat (25-30 nm) was applied to the sections to minimise charging in the SEM. The position of the Sr mark along the axis was measured using the vernier attached to the SEM stage drives.

2. Energy-Dispersive Spectroscopy (EDS)

In the later stages we used an EDS x-ray microanalysis system that allowed confirmation of the presence of Sr in the bright bands, and also detection of Sr marks in unsectioned otoliths. The system comprised a Link 133 eV Si(Li) detector with light element capability and a Thomson Scientific "WinEDS" PC-based analyser attached to a Philips 515 SEM. Before x-ray analysis whole (unsectioned) otoliths were acid-etched along the postrostral axis from the surface with 1 N and 3.5 N HCl, to expose the growth plane, then rinsed in bleach and distilled water and dried. Immediately prior to analysis we dipped the otoliths in a dilute carbon solution, to minimise charging in the SEM. Strontium marks were detected by operating the SEM in "spot" mode and searching for a point or zone where a significant Sr signal was detected on the x-ray microanalyser (typically a strong peak at 1.81 keV in the spectrum, corresponding to emission of Sr La x-rays). If a strontium mark was detected during x-ray analysis its position along the PR axis was measured and the mark photographed either with conventional SEM photography (Figure 4) or a rapid, low-grade video print which still showed the features of interest. To confirm that the suspected mark was strontium-rich two plots of the x-ray spectra from the otolith were taken: one on the strontium mark and one just before the mark. Acceptable evidence of the correct identification of a strontium mark was considered to be presence of an enhanced Sr level in the area analysed together with an absence of Sr (except for background levels) immediately prior to the area (Figure 5).

The measurements of increments on the whole otoliths and the strontium mark in sections were made along the same axes without reference to the other. This allowed a direct comparison between the number of increments observed after the strontium mark and the number expected, calculated using the known time at liberty after tagging.

Quantitative EDS analyses for linescans were carried out on carbon coated polished sections in the Philips 515 SEM operated at an accelerating voltage of 20 kV, using a focused electron beam of approximately 0.15 mm diameter, and analysis times of 60-200 seconds. The effective area analysed by the beam is larger than the diameter of the beam itself, on account of spreading of the beam within the specimen after entry; examination of superficial beam damage to specimens following analyses suggests that this area is in the order of 2 mm diameter. Elemental concentrations were calculated by reference to appropriate standards (calcite and celestite for Ca and Sr, respectively) using the "WinEDS" software.

Results

Of the 20, 204 fish injected with $SrCl_2$ between 1990 and 1995, 943 had been reported recaptured and 616 sets of sagittal otoliths recovered to 1 January 1996. Seventy sets of otoliths were chosen from these to use during the validation study, collected from fish of 45 to 102 cm LCF at release and 57 to 133 cm LCF at recapture. The smallest fish caught during the tagging program was 40 cm LCF. We were unable to tag and inject fish smaller than this as they are generally considered pre-recruits, ie. are not available to the fishery. Otoliths took from a half a day to a day to prepare and analyse so, as this study was only part of a larger study to determine ages of SBT, the numbers of otoliths analysed was restricted to 70. This number allowed samples to be selected from the range of size classes in the recaptures and the range of times at liberty.

Recapture rates of orange tagged fish and recovery of otoliths

There were no statistically significant differences between the return rates of yellow and orange tags for any of the release years (Table 3). The number of otoliths recovered as a percentage of orange tags recaptured, varied between 20%, in the first year of the tagging program, and 88%, in the final year (Table 4); overall, otoliths were recovered from 65% of the orange tagged fish recaptured.

Detection of Sr marks in the otoliths of orange tagged fish

Otoliths removed from fish injected with strontium chloride typically showed a bright band in backscattered electron images of polished sections through appropriate growth planes, e.g. oblique LS following the PR axis. This bright band was frequently associated with a local growth interruption immediately prior to the band, presumed to be a tagging check (Figure 4). This check was not discernible from the other surface features on the whole otolith when increments were counted. The visibility of the Sr mark (brightness in the backscattered electron image) was highest in fish which had been relatively small at time of injection (e.g. 50-55 cm LCF) e.g. specimen OB102 (Figures 2 and 3) which measured 49 cm at time of release and was injected with 2 ml $SrCl_2$ solution. Vertebral sections from fish injected with $SrCl_2$ were examined in the SEM but no Sr marks were found.

The presence of strontium in the bright bands was demonstrated by EDS spectra which showed a strong peak of strontium La x-rays when the electron beam was directed to the Sr mark, compared with very low (background) levels in the regions of the otolith preceding the mark (Figure 5). Subtraction of a pre-bright band spectrum from a bright band spectrum confirmed that only strontium is present in the bright band in elevated amounts, while there is a reduction in calcium level (Figure 5).

In particular, there is no evidence of increased chlorine incorporation associated with the injection of $SrCl_2$ into the fish. A quantitative EDS linescan across a bright band in one specimen, SBT OB96, sectioned in oblique LS and analysed along the direction of maximum observed growth, revealed "background" levels of 0.1 to 0.25% Sr by weight up to 5 microns before the band and a measured peak of 7.1% Sr on the band, falling to 3.5 % (50% of peak level) 6 microns after the start of the band and 0.7% (10% of peak level) approximately 15 microns after the start of the band. There is some indication of continuing slightly elevated Sr levels out to around 50 microns after the band, although this is at the limits of the EDS technique to resolve (Figure 6).

Accompanying the measured 7.1% increase in Sr level in the bright band is a fall in measured Ca concentration from 39-40% before the band to a minimum of 35.5% on the band, a decrease of 3.5-4.5% in absolute value or 10% relative. Within the limits of accuracy of the EDS technique, this supports the theoretical scenario where Ca atoms are being 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. Calculation of the increase in mean atomic number of the specimen resulting from a 7% increase in Sr and a 3.5% decrease in Ca gives a value of approximately 104 for the Sr enriched zone as compared with 100 for the unaltered $CaCO_3$ matrix - a difference well resolvable with backscattered electron imaging on the SEM on a suitably polished and coated specimen. The extent of the bright band in this specimen (OB96) coincided with measured Sr levels in the range 5-7%, thus it is possible that Sr concentrations in the range 0.5-5% may not be detectable by backscattered imaging although they should still be detectable by EDS. The EDS system is also essential for testing the identity of weak bright bands in sectioned specimens when it is not clear from the backscattered imaging which, if any, is a strontium mark.

Reliability of marking through injection of strontium

Three sets of otoliths were excluded from the experiment because we considered the increments on the whole otoliths either ambiguous or uninterpretable and could not give an age estimate with confidence. Of the remaining 67 otoliths, strontium marks were detected in 19 of the 20 sectioned otoliths using the Robinson detector (95% detection rate) and 40 out of 47 whole otoliths using EDS (85% detection rate). Strontium marks were as visible in the oblique longitudinal section (postrotral axis) as in the transverse section. However, as increments are more widely spaced along the postrostral axis and hence easier to measure, we generally used the postrostral axis on the whole otolith for age estimates and to locate the strontium mark from a section, using the Robinson detector, or from the whole, etched otolith, using EDS.

Early in the experiment we found that the Sr marks in fish tagged and injected at lengths of 90 cm or larger were consistently fainter than those of smaller fish. The concentration of Sr in the Sr marks of large fish was also significantly lower than in smaller fish. To overcome this, dosage rates for large fish were increased in 1993 (Table 2). Following the increase in dosage rates the bands in large fish were markedly more intense and easier to detect.

There was no apparent correlation between the time at liberty (i.e. time between Sr injection and recapture) and the intensity of the Sr marks, with the maximum time at liberty for a fish from which otoliths were analysed for Sr was 1638 days. There was also no correlation between the intensity of the Sr marks and the delay between otolith recovery and analysis. Unlike tetracycline, which is known to be photosensitive, the strontium mark did not fade after exposure to light.

Validation of annual increment formation from Sr marks

The 59 fish in which Sr marks were located ranged from 45 to 102 cm LCF at the time of release, which corresponds to estimated ages of 1 to 4 (Gunn et al.[7]). Times at liberty varied from 8 to 1638 days. The oldest recaptured fish was estimated to be 6+ years old and had been at liberty for 1242 days (over 3 years).

There was 100% agreement between the number of increments observed after the strontium mark and the number of increments expected, calculated from the time at liberty. Thus, the annual periodicity in formation of increments 2-6 has been validated for the otoliths analysed. As we were unable to tag young-of-the-year fish, in which the first increment on the sagitta had yet to form, the strontium experiment could not determine when this increment is laid down. However, Rees et al.[8] have determined through daily increments that the approximate size at age 1 is 50 cm. We have found otoliths of 50 cm fish to have one increment.

Of the otolith increments counted, this first increment was typically the most difficult to measure. The beginning of the first translucent zone occurred between 2.4 and 3.2 mm from the primordium along the postrostral axis, the most commonly used axis for analysis. Rees et al.[8] have found microincrements in this region to be narrower than those deposited earlier, indicating a period of slow growth of the fish.

Additional validation of annual increment formation from tagged fish at liberty for extended periods

During the course of our experiment, two fish tagged by CSIRO in the 1980's were recaptured and their otoliths sampled. From lengths at first recapture of 45 cm and 82 cm LCF, the fish had grown to 163 cm and 162 cm after being at liberty for 9 years, 7 months and 10 years, 8 months, respectively. Based on the age-length key developed by Gunn et al.[7] the 45 cm fish tagged in 1983 was one year old at release, while the 82 cm fish tagged in 1984 was a 2 year old. The ages at recapture of these two fish were estimated from transverse sections through the primordium of the sagittal otoliths. Eleven increments were present in the fish released as a one year old and caught 9.58 years later; 13 increments in the fish released as a two year old and recaptured 10.75 years later.

Discussion

Validation

This study has demonstrated that the second to sixth increments inclusive, in the sagittae of SBT are deposited annually. This validation is independent of when the marked fish were tagged or recaptured. As daily age estimates have been used to demonstrate that the first major increment in the sagitta forms in the first year of life (Rees et al.[8]), the annual formation of translucent zones appears to hold for the first six increments in SBT sagittae - incorporating fish up to lengths of approximately 133 cm fork length.

The close agreement between increment counts on otoliths and the sum of age at tagging and their time at liberty for two fish tagged during the 1980's and recaptured in the 1990's, indicated that increment formation continues to be annual in fish up to 13 years old. Although these increment counts were made with the knowledge that the times at liberty were in excess of 10 years, i.e. they were not "blind" counts, we are confident that the readings were objective because of the agreement between replicate counts.

Further evidence for annual formation of increments in SBT sagittae throughout life has been provided by a recent comparison of increment counts with age estimates derived from levels of bomb-radiocarbon in the early growth zones of sagittae (Kalish et al. in press). In this study there was close agreement between the two methods of estimating age for fish up to 34 years old.

Three sources of data, those from our marking experiment, the increment counts for two fish at liberty for over a decade, and the bomb radiocarbon data, provide very strong evidence that seasonal changes in growth are expressed as clearly identifiable annual increments in the sagittal otoliths of SBT. These increments can be used to estimate the age of individual fish at any point in their lifespan.

Prior to our studies, Yukiniawa (1970 using scales) and Thorogood (1984 using otoliths) had used marginal increment analyses to demonstrate the annual check or translucent band deposition in what they considered 2-4 year-old fish. Their results differ from our data only in the identity of year classes; their two to four year-olds correspond to the year classes one to three in our study. The difference in scale readings derives from Hynd's (1965) observation of two “checks” on the scales of new recruits (approximately 50 cm LCF) to the Western Australian fishery. Interpretation of otolith microincrements (Rees et al.[8]) indicates that these fish are only one year old. Unequivocal validation of the competing estimates is not possible at this stage as samples from pre-recruits were not available to either Hynd or Yukinawa and we were not able to tag and mark pre-recruit fish. In a number of other *Thunnus* species 50-60 cm fish are around one year old and our otolith microincrements and the data based on their interpretation are consistent with this. Therefore, we believe it is most likely that the interpretation of Rees et al.[8], that 50 cm FL fish are one year old, is correct .

An explanation for the discrepancy between our results and those of Thorogood (1987), who also worked with otoliths, is less obvious. Throughout our samples we found no evidence for two increments in the otoliths of new recruits. The early zones on all axes of otolith growth are difficult to read on some otoliths, and the increments in these areas are less distinct than those deposited later. In some fish a poorly defined "band" is also present very close to the primordium, i.e. within 2 mm along the postrostral axis. Although these two factors may cause confusion to an inexperienced reader in some fish, Thorogood makes no mention of difficulty in reading his first increment. An alternative explanation for Thorogood's interpretations may be that his readings were influenced by the findings of Hynd (1965) and Yukinawa (1974) based on scales, as their estimates of age at recruitment were entrenched within the SBT population dynamics dogma of the 1970's and 1980's.

Berry et al. (1977) and Lee et al. (1983) hypothesised that two increments are formed per year in otoliths from mature females of Atlantic bluefin tuna, *Thunnus thynnus.* One translucent zone may correspond to a winter slow growth period, the other to a spawning period (Lee et al., 1983). In the two large tagged fish examined in our study, only one increment per year was deposited throughout life. The outer increments (i.e. those deposited after sexual maturation) were consistent in both their width and optical density and were visually equivalent to the increments described by Lee et al. (1983), comprising a wide opaque region and a narrow translucent area which, on a black background, appears dark under reflected light. The close agreement between otolith increment counts and bomb-radiocarbon age estimates for mature SBT up to 34 years old (Kalish et al., in press) support our hypothesis that one increment continues to form per year as does the consistency of the width and optical density of increments deposited after sexual maturation in the otoliths aged by Kalish et al. (in press). In summary, there is no significant evidence to suggest that mature female SBT deposit two increments per year. In this regard our findings are similar to those of Hurley and Iles (1983) and Hurlbut and Clay (1988) for *T. thynnus* who found, albeit in the absence of direct validation, that a single increment is laid down per year in medium and giant size classes.

The use of strontium chloride to mark otoliths of large fish

This study has shown that intramuscular injection of strontium chloride leaves a distinct mark on the otoliths of SBT that is clearly visible as a SEM backscatter image in the Robinson detector. 95% of otolith sections from marked fish examined had detectable marks and, on this basis, we conclude that the compound is an efficient marker. Success rates of this level using OTC require dosages that lead to high mortalities (McFarlane and Beamish, 1987). The high detection rates are not surprising as $SrCl_2$ occurs naturally in sea water, the mean concentration being 3.8-8.2 ppm (Carriker et al., 1991) or 0.09 mM/kg (Bruland, 1983), and both Sr and Cl are major constituents of the otoliths of SBT (Gunn[9]). When $SrCl_2$ is injected into the muscle it is taken up into the blood stream and is subsequently incorporated into the endolymph and then the otolith as a substitute for Ca within the $CaCO_3$ portion of the aragonite matrix. The combined weight fraction of Ca and Sr (approximately 42%) within the matrix does not change as a result of the injection. However, the Ca:Sr ratio changes from 250:1 before injection to as low as 5:1 during the period over which the Sr spike induced by the injection is metabolised. At 6 mm and 15 mm after the injection mark the Sr levels have dropped to approximately 50% and 10% of peak values (Figure 6), corresponding to time periods in the order of 2 and 5 days respectively, based on median growth rates of approximately 3.0 mm/day estimated in this axis (Rees et al.[8]).

Detecting Sr marks on whole otoliths using EDS was successful, although less so, (85%), however there were two advantages in using this method. Firstly, the preparation time was approximately half that required to prepare sections suitable for the Robinson detector. Secondly, the age estimate and measurements of increments was made along the postrostral axis on whole otoliths from these smaller fish (up to six years old) and the method of locating the strontium mark using EDS meant that the measurement of the position of the strontium mark along the axis was made in the same plane. Using a section of the axis with the Robinson detector gave a measurement of the same axis but in a cross section of the axis. In fish older than about 6 years increments can be unclear on whole otoliths and can only be counted in sections. Transverse sections were used to determine ages of older fish (Gunn et al.[7]) and, in the future as strontium marked otoliths are returned from older fish, we will use transverse sections to estimate an age, make measurements of the increments and locate the strontium mark in the SEM.

The recapture rates of orange tagged, injected SBT were not significantly different from the recapture rates of yellow tagged SBT, indicating that Sr injections had no effect on the survival rate of tagged fish. The dosages of Sr varied between 65 and 250 mg/kg of fish. There is nothing to suggest that there were dosage related mortalities, as SBT injected with the highest doses were among those recaptured and sampled for otoliths. The CSIRO trials with 3 other species in 1990/91 (CSIRO unpublished data [6]) produced a 100% detection rate and showed that there was a direct relationship between the dosage and the intensity of the mark. Early problems with indistinct marks on otoliths of large SBT prompted an increase in the dose, which proved very successful. These less intense marks may be explained by changes in the growth rate of otoliths, as deposition of narrower daily microincrements occurs over the increased surface area of the otoliths in large fish, resulting in less strontium in the areas analysed. Another factor may have contributed to the less distinct marks. Sometimes after injection into large fish, there was a loss of strontium solution from the muscle. Although more solution was injected if a loss was noticed, there could have been some subsequent loss after the fish were returned to the water, resulting in a lower effective dose. Thus, as a general guideline, we recommend a dose of 100 mg Sr/kg for marking of otoliths in SBT but with a caution to ensure that injection is effective.

The problems with low dosage rates and indistinct marks demonstrated one of the most appealing aspects of using $SrCl_2$ as a marker. Unlike the situation with fluorescent markers, where it is very difficult to objectively evaluate faint or ambiguous marks, particularly if these are close to the outside edge of the otolith, it is possible to objectively evaluate Sr marks using X-ray analysis. As the concentrations of the Ca and Sr within the matrix are high, this can be conducted using very simple Energy Dispersive Spectroscopy systems, which are available in many SEM facilities. Although not a trivial procedure, x-ray analysis requires similar preparation methods to those used for examination of fluorescent markers and can usually be contracted to facilities at low cost. Given the often substantial investment in tagging programs, and the common combination of low recapture rates and even lower otolith sampling rates, every sample is extremely valuable in a marking experiment. The safety net of chemical analysis is thus very advantageous.

Comparison of strontium and fluorescent markers

At the beginning of this project we chose strontium chloride over the more commonly used fluorescent markers because previous work on SBT using oxytetracycline had been unsuccessful. Although immersion in high concentrations of strontium and/or feeding with strontium-laced food had been used successfully for marking otoliths of larval and juvenile stages of hatchery-reared salmon (Behrens Yamada and Mulligan, 1982;1990), sea trout and a range of postlarval tropical fish species (Brothers, 1990) and squid (Hurley et al., 1985), strontium had not previously been injected, or used to mark otoliths of large fish. Brothers' (1990) had concluded on the basis of his experiments that detection of strontium marks was expensive and involved relatively difficult preparation, at least compared with fluorescent markers and other marking techniques such as thermal inducement (Volk, 1990).

As noted, in the case of large otoliths where time at liberty is long and so the marks are covered by a large amount of matrix, sectioning is necessary for either marker and thus, preparation times are not significantly different. For smaller fish and those at liberty for short periods, fluorescent markers can be detected in the whole otolith, while strontium detection without an EDS system would require sectioning and thus involve significantly longer preparation time.

Brothers' (1990) comment regarding expense is certainly pertinent in evaluating and comparing marking methods and compounds, although it must be emphasised that the expense of analysing marked otoliths is often a small fraction of the cost of a marking experiment, particularly where large numbers of fish have been marked and released. This being the case, perhaps the most important factor in the cost equation is the rate of success of detecting marks in the otoliths of marked fish rather than the comparative cost of analysis. However, there are significant differences in the cost of equipment required for detection of fluorescent and strontium marks. For fluorescent markers, a light microscope equipped with an ultraviolet illumination source is required, along with filters matching the wave length of the fluorescence emitted from the marker when excited by this light source (e.g. see Wild and Foreman, 1980). For strontium, an SEM equipped with a Robinson detector is the minimum requirement; an EDS system a useful extra. Although a SEM is a common facility in large research laboratories, there is usually an hourly cost to the user that can be high. The consideration then comes down to efficiency; we have found that with well prepared specimens 4 otoliths can be examined and analysed per hour using an SEM.

Preparation time and costs are factors in which strontium marking is at a disadvantage to fluorescent marking. There are a number of factors in which it has a significant advantage however. One benefit of requiring both a light microscope and an SEM for this method of validation is that measurements of increments and strontium marks are independent: the strontium cannot be detected in the light microscope and the annual increments cannot be observed in the SEM.

Allergic reactions to compounds such as oxytetracycline in a significant proportion of the human population have led to bans by the USFDA on the use of these compounds in commercial fisheries. Strontium chloride on the other hand is recognised as a safe compound for human consumption, being a salt with a low order of toxicity (Sax and Lewis, 1987) and is used in toothpaste by some manufacturers (e.g. “Sensodyne”).

Unlike fluorescent markers such as oxytetracycline, strontium chloride is not photosensitive. Hence, there is no need to store the marking solution or the marked otoliths in the dark, and the mark does not fade with exposure to light, or with time. There is no indication in our samples that the strontium mark fades while the fish is at liberty; strontium marks were as evident in fish that had been at liberty for long periods as those that were recaptured soon after release. We aim to increase the number of annual increments validated by this method as fish are recaptured in the future that have been at liberty for longer periods.

In summary, strontium chloride injection has proven a very successful way to mark the otoliths of SBT; 95% of SBT marked and recaptured had detectable Sr marks present in sectioned otoliths. Although detection of strontium marks may be more expensive than for fluorescent marks, this high “success rate”, the benign nature of the marking compound to both fish and humans, the ability to unambiguously validate the presence of the mark using EDS, the insensitivity of $SrCl_2$ to light and the longevity of the strontium mark suggest it should be seriously considered by those interested in large-scale marking experiments on commercial fishes.

Acknowledgments

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Table 1. Numbers of SBT released with yellow tags and numbers of SBT injected with $SrCl_2$ and released with orange tags. (Data to 16 February 1996. Release period = 4th quarter of one year, first, second and third quarter of the next)

Release Year	Yellow Tags	Orange Tags + $SrCl_2$
90-91	6909	835
91-92	4543	3595
92-93	5907	5304
93-94	8253	8251
94-95	15683	2219
95-96	2998	0
All Years	44293	20204

Table 2. Dosage of injected $SrCl_2$ used for each size class. After examination of the first recovered otoliths from large fish showed faint strontium marks the dosages for fish larger than 90 cm LCF were increased.

LCF	Approx. Wt	Dose 1990-92			Dose 1993-96		
(cm)	(kg)	$SrCl_2$ (ml)	Sr (mg)	mg Sr/kg fish	$SrCl_2$ (ml)	Sr (mg)	mg Sr/kg fish
40-50	1.5-3.0	2	400	130-270	2	400	130-270
50-70	3.0-7.0	3	600	86-200	3	600	86-200
71-80	7.0-10	4	800	80-114	4	800	80-114
81-90	10-15	5	1000	67-100	5	1000	67-100
91-95	15-18	6	1200	67-80	7	1400	78-93
96-100	18-21	7	1400	67-78	9	1800	86-100
>100	>21	8	1600	76	12	2400	114

Table 3. A summary of yellow and orange tag recaptures from 1990 to 1996. The number of recaptured yellow and orange tags, released in each year of the tagging program, and the number of recaptured tags as a percentage of the total number released in the year are shown. There were no statistically significant differences between the return rates of yellow and orange tags (from Sr injected fish) released in all years of the program (χ^2 = 2.10, p = 0.56) nor between the return rates of yellow tags and orange tags for any of the release years (χ^2, p shown).
(Release and recapture periods = 4th quarter of one year, first, second and third quarter of the next)

Number of Yellow and Orange Tags Recaptured
(Number of Tags Recaptured as a Percentage of the Number of Releases)

	Fish Tagged 1990-91		Fish Tagged 1991-92		Fish Tagged 1992-93		Fish Tagged 1993-94		Fish Tagged 1994-95	
	yellow tags	orange tags	yellow tags	orange tags	yellow tags	orange tags	yellow tags	orange tags	yellow tags	orange tags
n	6909	835	4543	3595	5907	5304	8253	8251	15683	2219
Fish Recaptured 1990-91	183 (2.65)	25 (2.99)	-	-	-	-	-	-	-	-
Fish Recaptured 1991-92	180 (2.61)	19 (2.28)	84 (1.8)	55 (1.53)	-	-	-	-	-	-
Fish Recaptured 1992-93	111 (1.61)	11 (1.32)	116 (2.6)	86 (2.39)	56 (0.95)	63 (1.19)	-	-	-	-
Fish Recaptured 1993-94	62 (0.90)	6 (0.72)	102 (2.2)	76 (2.11)	130 (2.20)	90 (1.70)	50 (0.61)	47 (0.57)	-	-
Fish Recaptured 1994-95	29 (0.42)	6 (0.72)	43 (0.95)	36 (1.00)	177 (3.00)	116 (2.19)	171 (2.07)	168 (2.04)	67 (0.43)	20 (0.90)
Fish Recaptured 1995-96	2 (0.03)	0	6 (0.13)	6 (0.17)	29 (0.49)	17 (0.32)	86 (1.04)	89 (1.08)	94 (0.60)	25 (1.13)
All Recapture Years	567 (8.22)	67 (8.03)	351 (7.68)	259 (7.20)	392 (6.64)	286 (5.40)	307 (3.72)	304 (3.69)	161 (1.03)	45 (2.03)
Difference Between Yellow and Orange Tag Recaptures χ^2 (p)	5.02 (0.83)		3.17 (0.94)		1.44 (0.61)		3.22 (0.40)		1.68 (0.39)	

Table 4. The number of tags released and recaptured, and the number of otoliths recovered from orange tagged fish during each year of the tagging program. (Data to 16 February 1996. Recapture period = 4th quarter of one year, first, second and third quarter of the next)

Recapture Year	Yellow Tags Recaptured	Orange Tags Recaptured	Otoliths Recovered (% of orange tags recaptured)
1990-91	183	25	5 (20)
1991-92	264	74	49 (66)
1992-93	282	160	94 (59)
1993-94	344	219	107 (49)
1994-95	488	346	248 (72)
1995-96	238	137	120 (88)
Total	1799	961	623 (65)

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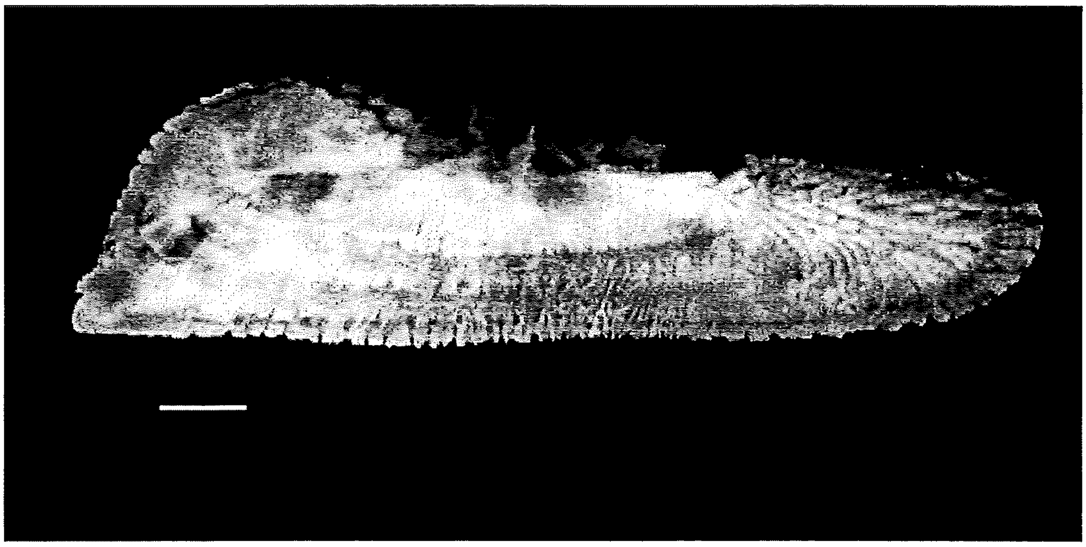

Figure 1. A whole, burnt otolith of specimen OB 33, showing the increments used to estimate the age of small SBT. Three increments were counted on this right hand sagitta: the darker translucent zones can be followed from the rostral axis to the postrostral axis along the otolith surface parallel to the ventral margin.
Scale Bar: 1mm

Figure 2

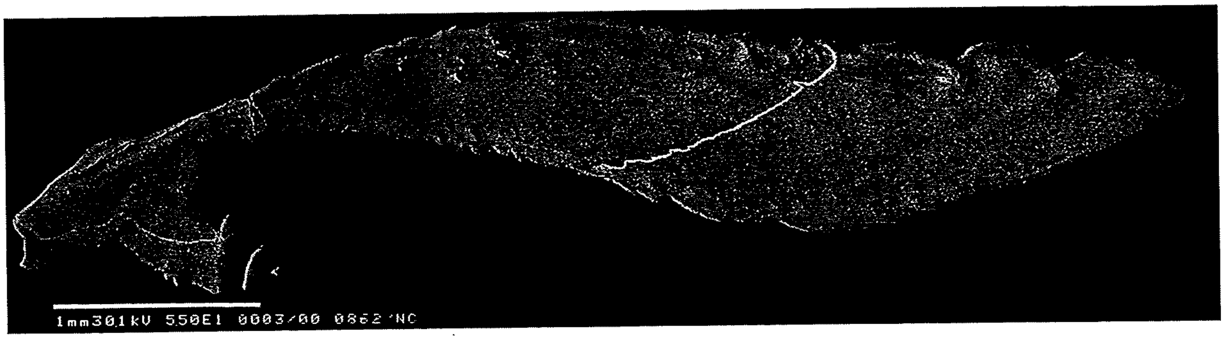

Figure 3

Figures 2 and 3. SEM micrographs showing strontium marks apparent as bright bands on sections taken from sister otoliths of specimen OB 102. Figure 2 is an oblique longitudinal section through the post rostral axis and figure 3 is a transverse section.

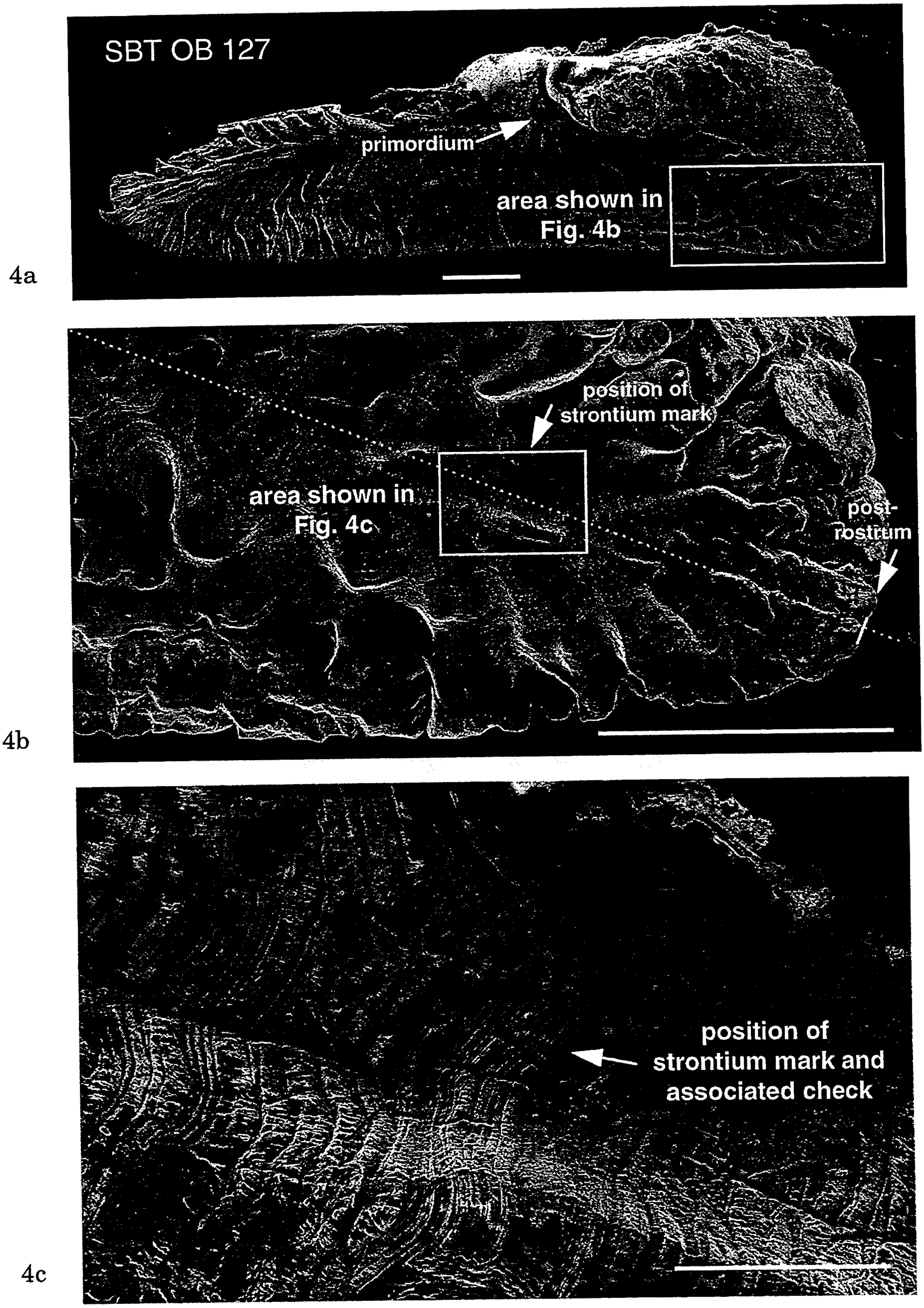

Figure 4. SEM micrographs of a carbon coated whole etched otolith of specimen OB 127, showing the position of the strontium mark and the check associated with tagging and injection. The dotted line (Fig. 4b) indicates the axis along which the position of the strontium mark was measured in relation to the primordium and the postrostrum.

Scale bars: 1 mm (Fig. 4a, 4b), 0.1 mm (Fig. 4c).

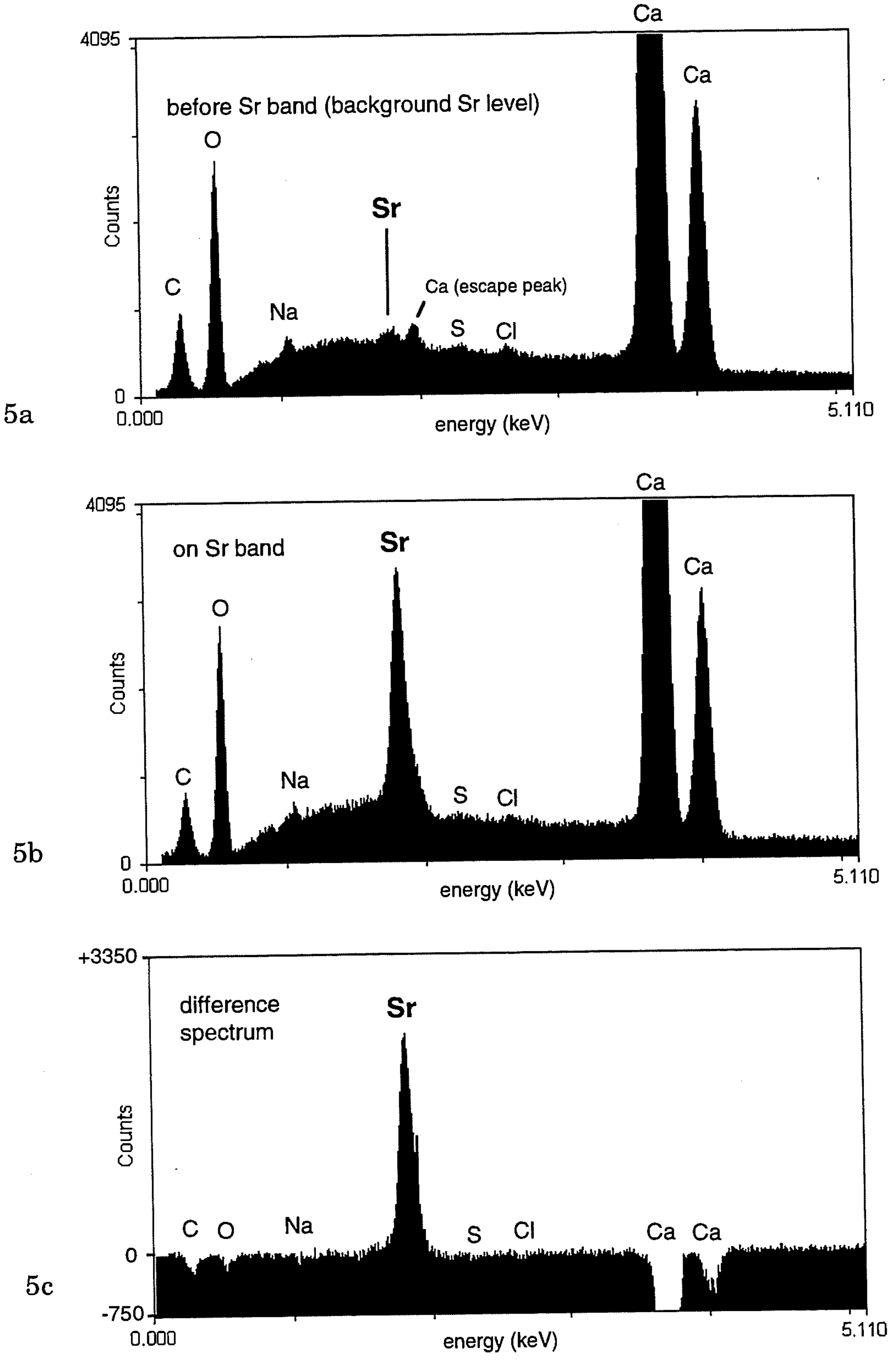

Figure 5. Examples of EDS spectra from SBT otoliths showing peaks due to background levels of Sr (Fig. 5a) and enhanced Sr levels associated with the strontium mark (Fig. 5b). The difference spectrum between the two (Fig. 5c) reveals that strontium is the only element present in elevated levels as a result of the strontium chloride injection, while levels of calcium are decreased.

Spectra similar to that shown in Figure 5b were used to positively identify the location of strontium marks in sections and etched whole otoliths (see text for further details).

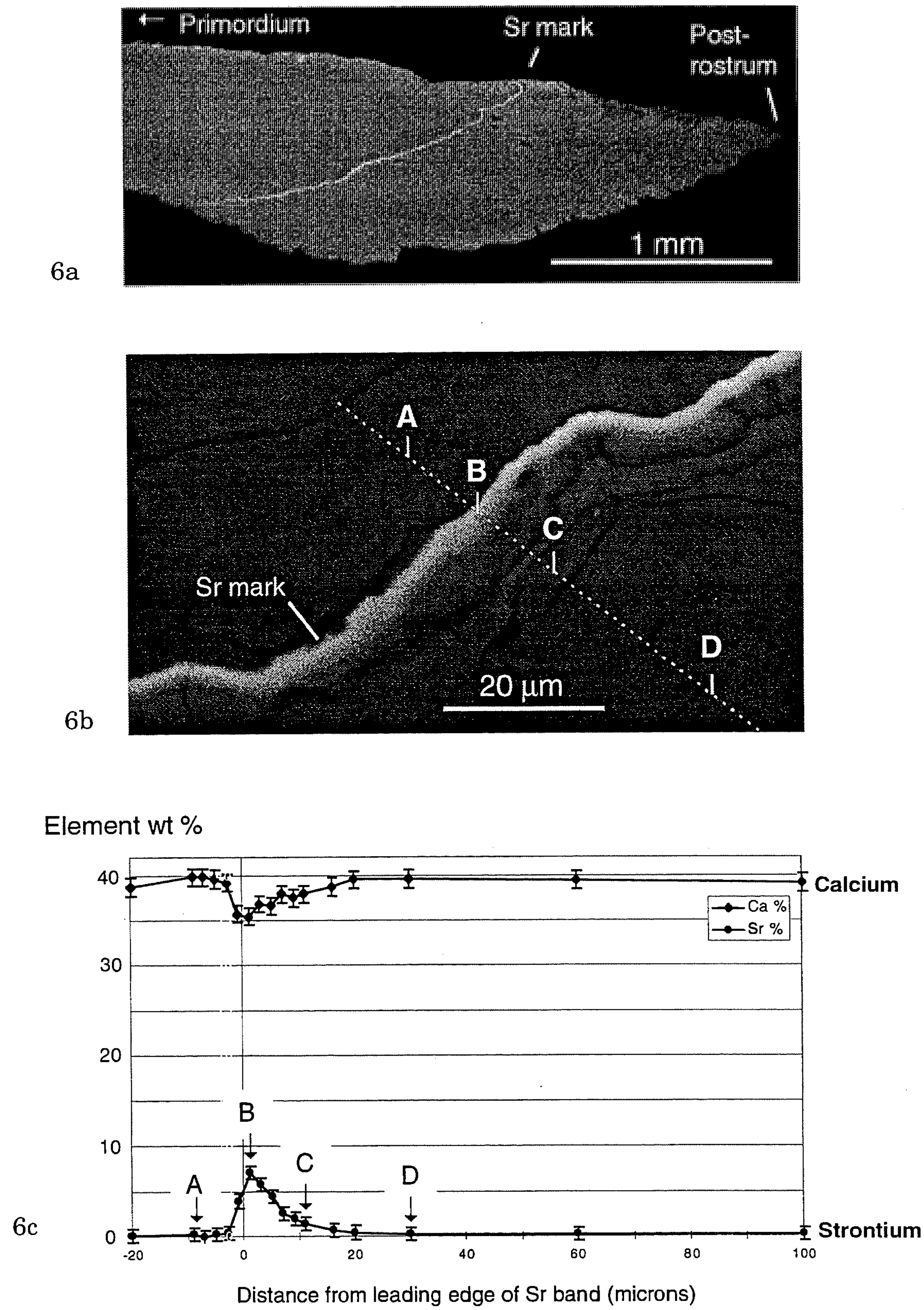

Figure 6. Details of the strontium mark on polished LS of specimen OB 96.

Figs. 6a, 6b: Backscattered SEM images showing overall location of mark (Fig. 6a) and detail of region analysed for Sr and Ca levels (Fig. 6b). Dashed line indicates transect followed for x-ray microanalysis.

Fig. 6c: Measured variation in Sr and Ca levels along transect shown in Fig. 6b; points A-D indicate representative positions along the transect to enable comparison of figures 6b and 6c.

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 2

Project 92/42

Age determination of juvenile Southern Bluefin Tuna, *Thunnus maccoyii*, based on scanning electron microscopy of otolith microincrements

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draft manuscript as at December 1996 - not to be cited without permission

Abstract

The sequential growth of sagittal otoliths of juvenile southern bluefin tuna (SBT), *Thunnus maccoyii* is reconstructed based on examination of otoliths from larvae (<12 mm long), juveniles (25-78 cm FL) and some larger specimens. Microincrements, ranging in width from >50 to <1 microns, were visualised by a number of methods; light microscopy of thin sections or acid etched surfaces proved effective only for fish up to approximately 35 cm fork length (FL), while scanning electron microscope examination of transverse section fracture surfaces in some cases provided a means of recognising a continuous record of microincrements in fish up to approximately 80 cm FL. A partial validation of the hypothesised daily formation of microincrements was obtained by observing post-strontium mark otolith growth and visible microincrements in recaptured wild fish which had previously been caught, tagged, and injected with a solution of strontium chloride before release.

Results from age determination of 50 SBT (FL 25-78 cm) from otolith microincrements suggest that SBT in the samples studied measure approximately 25 cm at 3 months, 32 cm at 4 months, 50 cm at one year and 70-80 cm at two years old. These data indicate that previous estimates of SBT ages, based on the assumption of age 2 at 50-55 cm FL, have overestimated fish age by approximately one year for any given length. The revised ages do however fit well with reported growth rates for other, northern hemisphere *Thunnus* species.

Introduction

Accurate age determination of southern bluefin tuna (SBT), *Thunnus maccoyii* has been identified as a critical need in underpinning the models and stock assessments used for managing the fishery based on this commercially important species (Gunn, 1993). While apparent daily otolith microincrements have been used as a tool for age determination of other tunas, e.g. yellowfin tuna *Thunnus albacares* (Wild & Foreman 1980, Wild 1986, Yamanaka 1990, Stecquert et al. 1996), skipjack tuna *Katsuwonus pelamis* (Wild & Foreman 1980, Uchiyama & Struhsaker 1981), albacore tuna *Thunnus alalunga* (Laurs et al. 1985) and northern bluefin tuna *Thunnus thynnus* (Brothers et al. 1983; Radtke 1984; Foreman 1996), this approach has not been applied to date to southern bluefin, with the exception of larvae <12 mm in length, caught close to their spawning ground in the Indian Ocean, studied by Jenkins & Davis (1990). Macro-increments (presumed annual bands) have been reported to occur in otoliths of adult SBT by Thorogood (1987), but his age assesments of SBT based on the recognition of annual banding in otoliths appear to be questionable, since they were unable to be reproduced when the specimens were subsequently re-examined by an independent laboratory, and were also at variance with age data suggested by other methods in a number of cases (see discussion in Proctor et al., 1993).

We have further investigated the nature and occurrence of both macro-increments (annual bands) and microincrements in otoliths of southern bluefin tuna. This paper describes the microincrements which are present within SBT otoliths, reconstructs patterns of otolith growth from examination of individuals of a range of sizes, and provides preliminary evidence that observed microincrements are likely to be daily structures as reported in many other fish species. The ages of a range of SBT (FL 25-78 cm) are then estimated based on microincrement counts from otoliths, and compared with previous published data on length-at-age of SBT and other tunas based on a variety of sources. The nature and patterns of annual banding in SBT, together with age determinations based on these and other data, are described in two companion papers (Clear et al., in press; Gunn et al., in press).

Material and methods

Material studied

Specimens of southern bluefin tuna from which otoliths used in this study were retrieved came from the following sources:

(i) research cruise by Japanese vessel "Shoyo Maru", December 1990-January 1991 (SBT juveniles, FL 25-30 cm and 44-47 cm)

(ii) "Shoyo Maru" research cruise December 1992-January 1993, (SBT juveniles, FL 30-35 cm)

(iii) specimens caught in West Australian fishery between 1985 and 1993 (40-78 cm fish)

(iv) specimens returned as part of a tag-recapture experiment involving strontium chloride marking of fish (OB number sequence, fish 49-89 cm); for full details see Clear et al., in press.

(v) otoliths of tuna larvae previously studied by Jenkins & Davis (1990), collected January-February 1987, location approx. 16° S, 116° E (east Indian Ocean, off NW Australia) (SBT larvae, standard length 3-12 mm approx.).

All specimens numbered SBT-- or OB-- form part of the SBT otolith collection held at CSIRO Division of Fisheries, Hobart.

Otoliths were extracted from fish, cleaned, and stored in vials as described in Clear et al. (in press).

Terminology

We have used standard otolith morphological terms in this paper as far as possible, following Panella (1980), Wild (1986) and Kalish et al. (1995). Our terminology is illustrated in Fig. 1. The path from the primordium to the postrostrum parallel to the distal surface of the otolith we term the PR path; this is the counting path adopted by A. Wild of the Inter-American Tropical Tuna Commission for light microscope studies of microincrements in yellowfin tuna and other species (e.g. Wild, 1986). We have also studied otoliths sectioned in a plane passing through the same portion of the otolith but at right angles to the distal surface (our "oblique longitudinal section" or oblique LS), and in transverse section (TS), as illustrated in Fig. 1.

Otolith preparation - method 1

Initially, otoliths were prepared for light microscope (LM) observation of microincrements using the acid etching-acetate replica technique of Wild (1986). Otoliths were acid-etched along the PR path and replicas taken using “Bioden” cellulose acetate film (0.034 mm thickness) for study in the light microscope. These replicas were examined using x10 to x40 objectives under brightfield and/or Nomarski illumination, and photographed where appropriate onto 35mm black-and-white film for examination as photographic enlargements.

Otolith preparation - method 2

Some SBT otoliths were available which had previously been embedded in resin and sectioned and polished to expose either an oblique longitudinal or transverse section, following the procedure for microprobe analysis described in Gunn et al. (1992); others were embedded and sectioned for the study reported by Clear et al. (in press.), and studied here for the possible occurrence of daily microincrements and to examine otolith growth in the plane of transverse sections. Thin (c. 300 micron) polished sections were examined either in the LM using transmitted light, or in the scanning electron microscope (SEM) after mounting on standard SEM stubs. Specimens intended for normal (topographic) imaging using secondary electrons were lightly etched using 1N HCl for 1-3 seconds, rinsed in distilled water, dried and then sputter coated with platinum for SEM observation; specimens intended for backscattered electron imaging (for atomic number contrast) and/or X-ray microanalysis in the SEM were coated with approximately 20 nm of evaporated carbon in a vacuum coating unit and examined without etching. SE images were taken at 15-20 kV accelerating voltage using a conventional secondary electron detector, while BS images were taken using 30 kV accelerating voltage and a Robinson backscattered electron detector, set to maximum contrast, on a Philips 515 SEM.

Otolith preparation - method 3

The majority of specimens in this study were prepared by fracturing the selected otolith under pressure from a scalpel blade along the TS axis, dipping briefly (1-2 seconds) in 1N HCl, rinsing with distilled water, and then dipping in bleach before a second distilled water rinse. In most cases, a series of fractures parallel to the first TS fracture were obtained by repeatedly breaking the portion of the otolith containing the rostrum along fracture paths displaced from the primordium (e.g. F2, F3, F4 in Fig. 1); for each case, the displacement of the fracture from the primordium was noted (to the nearest 0.1 mm) at time of fracturing, under a calibrated binocular microscope. The resulting portions were mounted on SEM stubs to reveal a number of fracture faces from the same specimen, and were sputter coated with platinum and examined in the SEM to determine which, if any, displayed a clear uninterrupted sequence of microincrements. Selected fracture faces were photographed in the SEM to produce an overlapping sequence of micrographs on large format (6.5 x 9 cm) film, taken at magnifications between x500 and x2000 on the SEM.

Microincrement recognition and counting procedure

The term “increment” is used to describe the material laid down in an otolith between recognisable structural features, usually considered to represent a specific interval of time. Microincrements are distinguished from macroincrements (annual bands, etc.) on the basis of their small size (typically <50 microns); in many fish species they represent or are believed to represent daily (or sometimes subdaily) units of otolith growth. For ease of enumeration, we use the boundaries between increments (D-zones) as the basic unit for counting; these appear dark in acetate replicas studied in the light microscope (e.g. Figs. 12, 13), or as incised or depressed regions in SEM study of etched material, particularly in the region immediately surrounding the primordium (Figs. 2-4). They also appear as the darkest regions in backscattered electron images (e.g. Figs 5, 10). Further out from the primordium in etched SEM sections or fractures, microincrements appear as more diffuse patterns of alternating dark and light regions, the dark bands again representing small depressions but without the deep incision seen in the earliest formed microincrements. Again, these dark bands (light bands on SEM negatives) were used as the unit of counting. In both oblique LS and TS sections, maximum visibility of microincrements was encountered along a sloping or sometimes curved counting path (see Figs 1, 5, 16) which was not parallel to either the distal or proximal surface. This differed from the PR path, chosen to represent the plane of maximum extension, which is the target of surface etching with the acetate replica method.

Between approximately 20 microns and 1.2 mm out from the primordium along the long axes of the otolith (i.e. the PR path and the path from primordium to rostrum), subsidiary structures to the “main” microincrements appear (Figs. 3-5) which we interpret as representing subdaily growth bands. Such bands appear as incomplete arcs both when sectioned parallel to the distal surface (Fig. 3) and through a plane at right angles to this (Fig. 4). Close in to the primordium (e.g. for the first 50-100 microns along the PR path), such presumed subdaily structures are comparatively easy to recognise, but beyond this region, in an apparent “rapid extension zone” persisting until approximately 1.2 mm along the PR path, interpretation is more difficult since there may be arcs present at approximately 20-25 micron spacing (Fig. 5) which appear at first sight to be possibly analogous with “daily” microincrements elsewhere in the otolith. However, tracing these structures to repeating features both towards the distal surface and deeper into the otolith reveals that there are in fact two “arcs” on the PR path for every unit of structure above (represented by concentric ridges) and below the PR path (represented by normal D-zones), and so it appears that each true microincrement is represented by a double arc along the PR path in this region of the otolith only.

One further source of potential difficulty when counting microincrements along any counting path is the presence of “checks” which appear to represent periods of temporary cessation of growth along any one axis. In places, especially when counting TS fracture faces, such checks may form a quasi-repeating pattern of their own, which can only be differentiated from true microincrements by the more regularly spaced and slightly diffuse boundaries of the latter (Fig. 6). Checks may also be present separating microincrement zones along the chosen counting path. In most cases, it was possible to follow these laterally until a new area of otolith growth was encountered which displayed the “missing” microincrements. Minor “checks” which could not be traced in this way were counted as representing a minimum of two increments, although it was appreciated that the presence of too many of these could lead to underestimating the true increment count for that specimen. The absence of such checks in the counting path was the key reason for selecting specific fracture faces for counting, even if these involved a degree of displacement from the primordium. “Normal” patterns of microincrement deposition are illustrated for “winter” and “summer” growth (wider and narrower increments) in Fig. 7.

Small areas where increments were unclear, possibly through incomplete etching or unfavourable crystal orientation, were counted by interpolation using increment widths visible in immediately adjacent regions, although if more than a few such regions occurred the otolith fracture would be rejected as unreadable.

After some time spent developing criteria for counting and recognising microincrements as above, specimens were counted by reader 1 (A. Rees) on two successive occasions or as many times as necessary until estimates converged to a value reproducible to +/- 5%. A subset of 17 otoliths representing all size classes in the study were then re-counted without knowledge of fish size or previous count results by reader 2 (Gary Critchley), and any sources of discrepancy (differences of interpretation) discussed. These were used to refine criteria for recognition of microincrements until counts could be made which were reproducible and consistent between readers to within +/- 5%.

In cases where the fracture face showing the clearest continuous microincrement path was from a TS displaced from the primordium by a known distance towards the rostrum (see Fig. 1), a correction was applied to the total number of microincrements visible to include those increments presumed to be unrepresented in the fracture face. An estimate of elapsed increments versus distance along the long axis of the otolith was obtained from study of acetate replicas and sectioned otoliths (Table 1) and the value corresponding to the known displacement of the fracture added to the observed increments, to produce an estimate of total increments present in the otolith.

Table 1. Approximate increment number represented by a known distance from primordium towards rostrum or postrostrum (estimated from acetate replicas), used for calculation of "missing" increments from displaced TS fractures

Displacement from primordium (mm)	Estimated increment number to be added
0.1	12
0.2	14
0.3	16
0.4	18
0.5	20
0.6	22
0.7	24
0.8	26
0.9	28
1.0	30
1.1	32
1.2	35
1.3	38
1.4	40
1.5	43

Validation experiment methodology

Otoliths which had been previously marked with strontium chloride as part of the mark-recapture experiment described by Clear et al. (in press.) were also prepared by the fracture method, to study otolith growth and any increments visible between the strontium marking point and the otolith margin. Strontium marks (typically associated with a prominent check in etched specimens or with a bright band in backscattered images of polished sections) were identified by using an EDS (energy dispersive spectroscopy) x-ray microanalysis system, attached to the Philips 515 SEM, operated in "spot" mode, and searching for a region of the otolith showing a peak of strontium Lα x-rays at 1.81 keV in the spectrum (full details and example spectra are given in Clear et al., in press). Specimens for location of strontium marks were coated with carbon in a vacuum evaporator (for polished sections), or, in the case of fracture surfaces, either dipped in a weak solution of carbon paint in dichloroethane and allowed to air-dry, or sputter-coated with platinum. The latter method was not used for sections, since it destroyed the capability for recognition of strontium marks through backscattered electron imaging, but was employed to improve secondary electron imaging of etched specimens for which the backscatter imaging method was less usable. The EDS system retained the capability to detect Sr peaks through the platinum coating.

Specimens in which a strontium mark was identified were photographed in the SEM for the study of post-marking otolith growth and the presence of any visible microincrements.

Results

Patterns of growth in the SBT otolith

Otoliths from larval and juvenile-adult SBT examined in the present study were measured in various axes using either a calibrated light microscope or the SEM, prior to subsequent preparation by sectioning, fracturing, etc. Selected morphometric features are plotted against fish length (SL for larvae or FL for juveniles/adults) in Fig. 8; additional data on larval otoliths are available in Fig. 9 of Jenkins & Davis (1990). These data show that overall otolith lengths are between 30 and 280 microns (0.03-0.28 mm) for larvae of SL 3.5-11.5 mm, around 4.5 mm and 7 mm for 25 cm and 45 cm FL juveniles, respectively, and increase from approximately 7 to 12 mm for SBT between 50 and 100 cm FL. In general, for otoliths of fish up to at least 100 cm FL, the length of the PR path (maximum observed growth plane towards the postrostrum) is a little under half (45-48%) of the overall otolith length, and the length transverse section counting path (estimated as the distance from primordium to ventral margin x 0.9) approximately 15% of the overall otolith length.

As prepared by all three methods employed in this study, a clear pattern of microincrements surrounds the primordium of the otolith, with the first D-zone (counted as increment 1 for present purposes) located approximately 6 microns out from the primordium (e.g. Fig. 2). Successive D-zones occur at narrow intervals (2-3 microns) until approximately increment 10, where spacing starts to increase rapidly in the plane parallel to the distal surface of the otolith, and formation of subdaily increments becomes noticeable (Figs. 3, 4). Over the period between increments 1 and 10, a small initial amount of growth proceeds towards the proximal surface but rapidly ceases, leaving a pronounced check below the first 10-12 increments in this direction, clearly visible in Figs 4 and 10. At the same time, growth occurs in an "upward" direction (i.e., towards the distal surface), leading to the formation of a dome-shaped structure around the primordium, which in turn ceases to grow for a period following increment 10 or thereabouts (Fig. 10). This "dome" and the prominent check above it remain visible in the central portions of otoliths of larger fish indefinitely, despite the deposition of later layers of material over the top, which can reach 40 microns or more in thickness (e.g. in Fig. 5). The later-deposited "overburden" (which is required to be removed by acid etching for the preparation of acetate replicas in older fish) covers but also preserves intact original the distal surface of the post-larval or early juvenile fish (compare Fig. 5 with Figs 10 and 11a).

From approximately increment 10, when the "dome' is complete and growth temporarily ceases in the distal direction, new increments are laid down "beneath" the primordium coinciding with a change the in nature of the lateral development of the increments. These have hitherto been more or less circular in plan view, but begin to extend only in anterior and posterior directions and start to become wider (>10 microns) and display partial (presumed subdaily) arcs (eg. Fig. 3, 5, 10). At the same time as growth is proceeding rapidly in the anterior and posterior directions (beginning of the "rapid extension zone"), a series of prominent ridges forms on the distal surface of the otolith (Fig. 11a), also visible in transmitted light in suitably small specimens where little overgrowth has occurred (Fig. 11b). Comparison with Fig. 5 reveals that each of these ridges corresponds with one increment along the clearest LS counting path, and with a pair of arcs along the PR path which are accordingly interpreted by us as a daily-plus-subdaily pair. The spacing of these ridges and corresponding microincrements counted is of the order of 40-50 microns at their maximum development, gradually reducing in size towards the end of the "rapid extension zone" which typically occurs around 1.0-1.2 mm along the PR path.

These extremely large microincrements are not well defined in acetate replicas of the PR path, and the presence of numerous subdaily arcs almost equal in definition to the rings interpreted as daily increments produces some difficulties in interpretation of these replicas in the light microscope. However, examination of a slightly out of focus image appears to aid correct interpretation of the microincrement structure and throw only the 40-50 micron periodicity structures into prominence, whereby they can be counted through this region (Figs 12, 13).

Following the end of the "rapid extension zone", which encompasses approximately increments 15-40 according to our analysis, deposition of subdaily rings ceases and microincrements become better defined and closer together (e.g. 15-20 micron spacing along the PR path). SBT in the 25 cm size-class caught in January (mid-summer in the southern hemisphere) maintain this pattern out to the margin of the otolith, which occurs at approximately 2.0 mm along the PR path (Fig. 12); in other specimens, e.g. 45 cm fish caught at the same point in the year, increment width declines rapidly at around 1.8 mm along the PR path and a sequence of very narrow microincrements persists which scarcely increase in width again before reaching the margin at approximately 3.0 mm from the primordium (Fig. 13). These increments, typically 2-3 microns in width, are consistent with slowing of otolith growth through a winter period, with little sign of resumption of significant otolith growth before the next mid-summer.

Difficulties encountered in recognising the finest "winter" increments in acetate replicas, together with problems of interpretation of increment structure in the rapid extension zone, led us to consider alternative preparation techniques and counting paths. One possibility appeared to be recognition of clear increments in oblique longitudinal sections, along a sloping counting path which extended progressively deeper into the otolith than the PR path favoured for the acetate replica technique (Fig. 5). However, increment visibility tended to decline with increasing distance from the primordium, and at the same time would appear to go through phases of improved or reduced visibility, possibly owing to minor variation in crystal orientation relative to the plane of section. In addition, such sections were time-consuming to prepare (approximately 1 day per specimen for embedding, sectioning and polishing, with frequent inspections required to locate the preferred plane intersecting the primordium), and were not pursued in favour of the more rapid fracture-etch technique described earlier. This technique proved to be possible to apply only in the TS rather than the oblique LS direction, but provided a relatively rapid result with a reasonable degree of success, estimated at 80-90% for small (25 cm FL) SBT, decreasing to, for example, 20-30% in the largest fish studied fish (50-70 cm). Microincrements visible in the TS counting path were smaller than those revealed by acetate replicas or longitudinal sections along the PR path, but visibility was not a problem except in larger fish (60 cm +) during presumed "winter" growth. As implied by the morphometric data presented in Fig. 8, microincrements along the TS counting path were around 15% of the width of those visible along the PR path, i.e. 6-7 microns in the rapid extension zone, declining to 2-3 microns thereafter (first "summer" growth) and less than 0.5 microns in the first "winter", before increasing again to around 1.5 microns width in the following second "summer" (Figs 7a-c). In general, specimens displaying more than one "winter" growth period proved difficult to count (for exceptions see below), but summer increment deposition in older fish was observable in some cases (e.g. Figs 14, 15) with a microincrement spacing of around 0.7-0.8 microns in this axis; from a comparison of growth rates in the two axes studied (Fig. 8), this would be expected to be equivalent to approximately 4-5 micron increments along the PR path.

Results of validation experiment using otoliths of strontium marked fish

This experiment was only partially successful, owing chiefly to the non-availability of SBT which had been tagged and strontium marked when small (e.g. <60 cm FL) and recaptured the same season (the only fishes tagged in this size range from which otoliths had been recovered had been at liberty for times in excess of one year). However, otoliths were examined from 22 fish with short times-at-liberty which had been tagged as larger individuals (75-89 cm), 20 from summer-tagged fish and 2 from winter-tagged fish, to see if the strontium marking point could be recognised and any microincrement patterns discerned between this and the margin. In addition, three fish with longer times-at-liberty were examined, one an 82 cm individual at liberty for approximately 14 months, and the other two the fish tagged at <60 cm FL described above. In all cases, the maximum observable extension to the TS counting path external to the strontium mark was measured using the SEM, and acid-etched specimens were photographed in the SEM for a study of possible microincrements present.

The results are tabulated in Table 2, with example otolith portions illustrated in Figs. 14-15. Sr marks were successfully located in a high proportion (80%) of otoliths examined, "failures" including two specimens with very short times-at-liberty (13 and 18 days), which may in fact contain a mark too close to the margin to detect. In all cases, the position of the band with enhanced Sr was associated with a major check or growth interruption (e.g. Figs 14, 15) and cessation of "normal" microincrement deposition for some distance thereafter (5-50 microns in different specimens). In a proportion of specimens examined, regular "summer" increment deposition could be observed prior to the Sr mark (e.g. the 0.7-0.8 micron spacing observable in Figs 14 and 15a), but in only one specimen (SBT OB 29, illustrated in Fig. 15) was a semi-clear pattern of microincrements visible after the Sr mark. In this specimen we estimate approximately 34 microincrements are visible in a specimen at liberty for 39 days after tagging, suggestive of a daily rhythm of deposition with perhaps a 4-5 day growth interruption represented by the "check" associated with the Sr marking event.

In the absence of more specimens showing clear microincrements post-marking, an analysis was made of mean otolith growth rates along the TS counting path in strontium marked fish, visible in the otolith between the strontium mark and the otolith margin. These data are tabulated in Table 2, and show that summer-caught fish, tagged in the size range 80 +/-7 cm and at liberty from 0.5 to 5 months, showed otolith growth in the range 0.22-0.69 microns/day with a mean value of 0.46 microns/day. These values suggest that observed pre-mark summer growth increments of the order of 0.7 to 0.8 microns represent growth periods of 1-2 days at the most, with a daily rhythm being the most likely explanation.

The single winter-tagged fish recovered within a short time in which a strontium mark was recognised displayed a low otolith growth rate in this axis (0.23 microns/day following tagging) (table 2b), while of the summer-tagged fish with times-at-liberty in excess of one year (Table 2c and 2d), the fish 82 cm at time of tagging showed a mean otolith extension rate of 0.34 microns/day through one summer, one winter and part of the next summer, while the two smaller individuals at tagging (49 cm and 57 cm) showed higher mean otolith growth rates through the next 1.5-2 years (0.93 and 0.80 microns/day, respectively). The significance of these values is explored further in the Discussion.Table 2. Summary of observed otolith growth along the transverse section counting path following strontium marking, in marked and recaptured SBT.

Table 2. Release and recapture data on strontium marked fish used in the validation experiment for daily growth increments in otoliths, together with observed otolith growth post-strontium marking.

(a) summer tagged fish, recaptured within 5 months (150 days) of release (all fish are in size range 75-87 cm FL at tagging) n=20

SBT#	length at tagging	days at liberty	length at recapture	months during which growth occurred	Sr mark located?	observed max. otolith growth (TS counting path)	mean growth rate (microns per day)
OB 62	79 cm	13	79 cm	Mar 93	no	--	--
OB 163	n.d.	15	82 cm	Jan-Feb 94	yes	6 microns	0.40
OB 162	87 cm	18	89 cm	Jan-Feb 94	yes	4 microns	0.22
OB 158	79 cm	18	81 cm	Jan-Feb 94	no	--	--
OB 161	75 cm	22	78 cm	Jan-Feb 94	yes	7 microns	0.32
OB 29	78 cm	39	80 cm	Feb-Mar 92	yes	23 microns	0.59
OB 28	73 cm	41	76 cm	Feb-Mar 92	yes	19 microns	0.46
OB 30	80 cm	43	81 cm	Jan-Mar 92	no	--	--
OB 22	86 cm	52	88 cm	Jan-Mar 92	yes	14 microns	0.27
OB 21	n.d.	54	87 cm	Jan-Mar 92	yes	27 microns	0.50
OB 8	85 cm	65	88 cm	Jan-Mar 92	yes	30 microns	0.46
OB 18	78 cm	70	84 cm	Feb-Apr 92	yes	40 microns	0.57
OB 63	83 cm	79	86 cm	Jan-Apr 93	no	--	--
OB 36	85 cm	82	90 cm	Jan-Mar 92	yes	35 microns	0.43
OB 20	85 cm	82	89 cm	Jan-Apr 92	yes	37 microns	0.45
OB 114	81 cm	85	88 cm	Jan-Apr 93	yes	35 microns	0.41
OB 43	81 cm	109	90 cm	Feb-May 92	yes	75 microns	0.69
OB 41	82 cm	110	97 cm	Feb-May 92	yes	50 microns	0.45

OB 167	77 cm	118	88 cm	Jan-May 94	yes	70 microns	0.59
OB 44	81 cm	133	86 cm	Jan-May 92	yes	60 microns	0.45

(b) winter tagged fish, recaptured within 5 months (150 days) of release (size range 86-89 cm FL at tagging) n=2

SBT#	length at tagging	days at liberty	length at recapture	months during which growth occurred	Sr mark located?	observed otolith growth (TS counting path)	mean growth rate (microns per day)
OB 32	86 cm	8	89 cm	Jun 91	no	--	--
OB 31	89 cm	26	89 cm	Jun 91	yes	6 microns	0.23

(c) summer tagged fish, recaptured more than 12 months after release (Fl >80 cm at tagging) - example

SBT#	length at tagging	days at liberty	length at recapture	months during which growth occurred	Sr mark located?	observed otolith growth (TS counting path)	mean growth rate (microns per day)
OB 83	82 cm	416	99 cm	Jan 92-Mar 93 (S+W+part S)	yes	140 microns	0.34

(d) summer tagged fish, recaptured more than 12 months after release (Fl <60 cm at tagging) n=2

SBT#	length at tagging	days at liberty	length at recapture	months during which growth occurred	Sr mark located?	observed otolith growth (TS counting path)	mean growth rate (microns per day)
OB 102	49 cm	546	92 cm	Jan 92-Jun 93 (S+W+S)	yes	410 microns	0.93
OB 81	57 cm	704	94 cm	Jan 91-Dec 92 (S+W+S+W)	yes	560 microns	0.80

Results of application of age determination techniques to individual SBT

Microincrement counts from 50 SBT ranging in size from 25 to 78 cm FL are presented in Table 3, and plotted as assessed age in days vs. fork length in Fig. 17, based on the assumption that these counts represent the age of the fish in days. As a comparison, the most frequently used von Bertalanffy curve for SBT for the past 10 years, the Kirkwood (1983) curve, is also shown on this plot, with its assumed age at FL 55 cm adjusted backwards from 2 years to 1 (see further discussion below). Back-calculated birth dates for representative individuals are plotted in Fig. 18. Taking the boundary between successive spawning seasons as 1 July, it is apparent from these data that year class 0+ is represented in this study by 25-35 cm fish caught between November and February (assessed age between 3 and 4 months); year class 1+ fish are represented by specimens between 38 and 62 cm captured between November and March; and year class 2+ by 3 fish measuring 69-78 cm in November-January and 1 fish measuring 60 cm in September. Spawning appears to occur between September and April, with the 0+ fish (captured in the 1991/2 and 1992/3 field seasons) spawned relatively early in the season (August-October), the “smaller” (40-55 cm) 1+ fish spawned in mid season or later, and the few “larger” 1+ fish (55-62 cm) spawned early in the season. It is apparent that fish of certain “borderline” lengths, e.g. FL 60 cm, may either represent year class 1+ if caught late in the season and spawned early the previous season, or year 2+ if caught early in the season and spawned late in the spawning season two years previously, although their chronological ages are very similar (500 days approx.)

Table 3. Microincrement counts of otoliths from SBT used in this study

SBT reference	Fork Length (cm)	Date of capture	Assesed age (days) from microincrement counts
SBT 1521	24.7	9/12/90	85
SBT 1514	24.7	7/12/90	94
SBT 1516	24.7	7/12/90	86
SBT 1518	25.8	9/12/90	101
SBT 1520	26.6	9/12/90	94
SBT 1520	26.6	9/12/90	94
SBT 1512	27	23/11/90	92
SBT 3079	30	4/2/93	124
SBT 3080	31.2	4/2/93	122
SBT 3097	32	5/2/93	120
SBT 3007	33.2	30/1/93	112
SBT 3003	33.6	30/1/93	123
SBT 285	39	6/12/88	316
SBT 272	40	6/12/88	245
SBT 304	40	15/12/88	331
SBT 305	41	15/12/88	298
SBT 309	42	15/12/88	286
SBT 2021	45	20/1/92	262
SBT 268	45	25/11/88	266
SBT 325	45	10/12/88	275
SBT 319	45	15/12/88	298
SBT 1533	45.5	15/12/90	286
SBT 1536	45.5	16/12/90	308
SBT 1533	45.5	15/12/90	286
SBT 1538	47	16/12/90	299
SBT 3020	48	3/2/93	332
SBT 3022	48	3/2/93	341
SBT 3012	48.3	3/2/93	328
SBT 2012	49	14/1/92	382
SBT 2120	49	13/1/93	383
SBT 2108	49	15/1/93	380
SBT 2454	50	28/1/93	402
SBT 2483	50	8/2/93	351
SBT 2484	50	8/2/93	444
SBT 308	50	10/12/88	300
SBT 2121	50	18/1/93	387
SBT 3025	50	3/2/93	342
SBT 2132	52	7/1/92	336
SBT 418	53	28/2/89	388

SBT 2282	53	5/3/93	385
SBT 2284	54	5/3/93	411
SBT 2263	55	24/2/93	378
SBT 2015	57	22/1/92	444
SBT 2135	58	12/1/92	426
SBT 1485	60	26/1/90	506
SBT 2379	60	11/9/85	497
SBT 2257	62	9/1/93	393
SBT 248	69	20/11/88	604
SBT 2209	72	27/11/92	604
SBT 2019	78	7/1/92	793

Discussion

Evidence for the daily nature of observed microincrements

It was initially hoped that information from the strontium marking experiment would provide a direct validation of the daily formation of microincrements in the transverse axis, at least during summer growth for SBT in the size range 75-85 cm FL. Although in this respect the experiment was generally unsuccessful, most likely on account of the observable disruption to patterns of matrix deposition immediately following strontium marking, four fish (OB 29, OB 78 and OB 43 and OB 167) showed mean otolith growth rates in the region of 0-6-0.8 microns per day in the transverse axis which are close to the observed microincrement spacing of 0.7-0.8 microns for pre-mark summer growth in these fish (e.g., Figs 14, 15). This is consistent with the hypothesis that the regular (pre-mark) microincrements represent consecutive days of growth. In addition, there is a variety of evidence which provides indirect support for the hypothesis that the observed microincrements represent a record of days. Firstly, at least for fish greater than 40 cm FL, "summer" and "winter" microincrement widths in the transverse axis are similar to calculations of the daily growth increments which would be expected based on knowledge of fish growth rates (from tagging data), a generalised model for seasonality, and observed relationships between fish length and otolith morphometry (see appendix). Second, observed patterns of "winter" and "summer" growth in the counting paths correspond with the expected timing of these seasons in the life of the fish, back-calculated from the dates of capture and assuming that microincrements represent days. Third, the early microincrements in larval SBT (e.g. between days 5 and 15) have been shown to be formed daily, on the basis of marginal increment analysis plus repeated sampling of a single cohort in the field (Jenkins & Davis, 1990), and in other tuna species, mark-recapture experiments using oxytetracyclene have indicated daily formation of microincrements for larger juveniles and adults, e.g. yellowfin tuna over the size range 25 cm - 148 cm (Wild & Foreman 1980; Wild, 1986; Yamanaka, 1990) and northern bluefin between 19 cm and 68 cm (Foreman 1996). Lastly, the microincrement-derived ages for southern bluefin in this study appear to be reasonable, e.g. they are consistent with data from annual banding in both otoliths and vertebrae presented in Gunn et al. (in press), and the lengths-at-age of small juvenile SBT (e.g. 25-30 cm fish) are consistent with data for northern bluefin juveniles presented by Brothers et al. (1983), in which species, spawning typically occurs off Florida in early May, and juveniles measuring around 30 cm can be found in mid-August (assessed age around 100 days old).

The above evidence suggests that the microincrement-derived ages are likely to be essentially correct, although practical difficulties remain in respect of counting "problem" zones such as areas where increments are unclear (for which a simple interpolation is applied), checks or apparent hiatuses in matrix deposition (for which the "check" is traced laterally wherever possible until an uninterrupted pattern of increments is encountered), and areas where features interpreted as subdaily structures are present in addition to the main microincrements. The main region where this latter problem occurs is between about increments 15 and 45 along the PR axis, where the microincrement spacing is largest (up to 50 microns) and features we interpret as subdaily bands are of almost equal prominence to the daily ones (Fig. 5). We resolve this problem by counting along an oblique path displaced from the normal PR path, or by use of the transverse fracture, where the "subdaily" features are not developed to the extent which they occur on the PR path. Nevertheless, this remains as a possible area of difference of interpretation between readers if only the PR path is analysed, and it may be a possible explanation, for example, for some of the discrepancies apparent between published growth curves based on microincrement counts for the same species where consistent differences are apparent (e.g. Fig. 1.19a of Yamanaka, 1990). This point is explored further later in this section.

Comparison of lengths-at-age from present data with previous growth curves for SBT

The most significant feature of the present data is that SBT in the size class 50-55 cm can now confidently be assigned to the age class 1+ (i.e., spawned one season previously, and close to their first birthday) rather than 2+ as assumed by previous authors (e.g. Kirkwood 1983; Hampton 1991), and that previously published ages of SBT from all year classes should be revised downwards by one year. These findings have been taken into account in the latest year partitions used for stock assessment (Anon, 1994), but not yet published elsewhere. Thus, for example, the two-year-old fish discussed by Leigh & Hearn (1993), with modes centred on 52 cm and 65 cm in February 1986, we now consider to be 1+ fish, their three-year-olds become 2+, etc. The most widespread growth curve for SBT used for age partitioning of the catch through the 1980s, the Kirkwood (1983) von Bertalanffy growth curve, predicted a length-at-age 1 of 33.9 cm; our data suggest that fish of this size are instead approximately 4 months old.

The origin of the interpretation that the 50-55 cm mode in the catch frequency data corresponded to two-year-old fish may be traced back to scale readings by Hynd (1965) and Yukinawa (1970), both of whom reported the presence of one scale ring in the smallest mode taken late in the season (35-50 cm in May-July) and 2 rings in the next larger mode (60-70 cm in May-July); these latter are the fish which would be expected to have been around 50-55 cm in January, around their assumed mean "birthday". Since these scale data are incompatible with the present data from otolith microincrements, and also with other data from annuli in otoliths and vertebrae of SBT (Gunn et al., in press) and in addition seem unlikely from consideration of growth rates of other tunas where these are known (see below), either the fish studied by Hynd and Yukinawa fish were laying down their "winter" scale ring earlier in the season than expected, which seems unlikely on the basis of marginal increment data shown by the same authors, or the scale readings may have been mis-interpreted, particularly in the identification of the position of the first annual band. The phenomenon of a variable and possibly sub-annual “partial” ring before the first true “winter” band is not unknown in otoliths (see Gunn et al., in press) and may well account for a consistent error of one year in ages assigned from the historical scale reading data. This interpretation seems plausible on the evidence of some of the published scale photographs (e.g. Yukinawa 1970) and is supported by investigation of scales from fish of similar size classes in our laboratory (C. Stanley, pers. comm.).

Figure 19 demonstrates the mis-match between the regression fitted to present microincrement-derived age data and the Kirkwood (1983) growth curve, which passes through 55 cm at age 2. A closer agreement is found if the Kirkwood ages are revised downwards by one year (Fig. 17), but the slope of the latter curve appears too shallow to match the bulk of the present data.

In 1994, the Commission for Conservation of Southern Bluefin Tuna (CCSBT) took into account indications from the present data that SBT in the size range 50-55 cm FL were likely to represent year-class 1+ and not 2+ as previously assumed, and also indications from tagging data that juvenile (<86 cm) SBT caught in the 1980s and 1990s appeared to be growing faster than equivalent fish in the 1960s and 1970s (Hearn & Polacheck, 1993). The current CCSBT growth curve (Anon, 1994) takes into account both of these factors, and is shown in Fig. 18 for comparison with the present data. This curve is fitted to length 55 cm at age 1 by adjustment of the parameter T_0 in the von Bertalanffy growth equation (see Anon, 1994 for details), based on apparent evidence from modal progression data from West Australia in the 1960s and South Australia in the 1980s; for example, Kirkwood (1983) illustrates “age 2” modes (which we would now consider to represent 1+ fish) around 52-55 cm on 1 January in SA and NSW fish between 1964 and 1979. For comparative purposes, we also show the effect of incorporating a suggested seasonal component to this growth curve, adjusting the value of T_0 so that the value of FL 55 cm at age 1 is unaffected (see Appendix). Although this suggested seasonal element does not affect the interpretation of the majority of present data (since in the main, samples in the present dataset are from fish collected between November and February, i.e. the austral summer), it would become increasingly relevant for fish taken in autumn/early winter, which would be expected to be significantly longer for their age than the present growth curve would predict.

The slope of the present regression is effectively identical to that of the present, steeper CCSBT curve, but it is also noteworthy that, with only one exception, all of our present data points fall below it by 3-5 cm on average for any given age. This discrepancy remains whether or not an additional seasonal growth component is added (see Fig. 19). While this discrepancy is relatively small, it may point to a more significant error in assigned ages of larger fish if continued to subsequent year-classes.

It is difficult at this point to resolve the reason for this discrepancy, since it could be due to one or the interaction of several factors. Possibilities include a systematic overestimation of microincrement-derived ages, of the order of 2-3 months (considered unlikely in the first instance); variation between the two curves on the basis that SBT on average may be a little older than 1 year on 1 January (this may be the case, but no trend to this effect is evident from Fig. 18); an error due to the present data forming an unrepresentative subset of the complete picture based on a variety of years, localities and fish size classes; or an error in the fitted CCSBT curve to tag-and-recapture data, possibly in the designation of 55 cm for size-at-age 1, for which the term T_0 is selected in the von Bertalanffy growth equation. A test of the latter possibility is provided in the first instance by evidence from age data based on other hard parts (e.g. annuli in otoliths and vertebrae: Gunn it al., in press), which do suggest mean lengths-at-age lower than the current CCSBT curve, and cohort (length-frequency) data, which are ambiguous: for example, the majority of the 1980s cohorts illustrated by Leigh (1991: graph 18) fall below the present CCSBT curve on 1 January at assigned ages 2 and 3, but not at age 1. On the other hand, the "age 2+" (now considered 1+) SBT tagged by CSIRO in South Australia in January-February 1984 and recaptured in May-June the same year grew almost exactly as predicted by the present CCSBT curve with added seasonal component as described in the Appendix (Fig. 21), i.e. from approximately 58 cm to 74 cm over this period (data of Hearn, 1986 unpublished, quoted in Burgess et al., 1991). Clearly, more work is required before the various factors affecting sizes of 1+ fish around their presumed 1 January "birthday" can be resolved.

From Fig. 17 it is also apparent that growth of SBT between 0 and approximately 30 cm FL does not follow a von Bertalanffy type function. Indeed, this must be true for any species for which the von Bertalanffy function includes a negative value for T_0, since in reality a length of zero centimetres must correspond with age zero and not some negative value. Interpolation indicates that the true growth of SBT between FL 0 and 30 cm must involve a rapid stanza of growth, of the order of perhaps 15 cm/month (5 mm/day) or higher, at some point between FL 1 and 24 cm. A similar scenario has been discussed by Brothers et al. (1983) in relation to northern bluefin over the size range 0-30 cm; these authors concluded that growth rates of the order of 6 mm/day were likely for post-larvae/early juveniles between approximately 30 and 50 days old (their Fig. 8). In our samples, this interval would correspond to the microincrements formed through the "rapid extension zone" of the otolith, which may well correlate with similarly rapid growth of the fish following an early phase of exponential growth, as illustrated in Jenkins & Davis (1990). Further microstructural aspects of the otolith through the rapid extension zone are discussed in more detail below.

Comparison of present age-length data with growth curves for other tuna species

Growth curves in the literature for other tuna species (e.g., northern bluefin and yellowfin), largely derived from otolith microincrement counts but checked against tagging and/or modal data where possible, are compared with the present regression for SBT over the size interval 30-80 cm in Fig. 20. These comparisons indicate that in general, yellowfin tuna grow more rapidly than bluefin between age 1 and 2, but at age 1 both species are likely to be in the range 45-60 cm FL. One additional feature apparent from these growth curves is that there is a discrepancy between ages based on otolith microincrement data for yellowfin from the western Pacific (Yamanaka) and eastern Pacific (Wild), i.e., curves A and D in Fig. 19; this is in the order of 30-60 days for equivalent sized fish. One possible explanation for this could be different authors' interpretations of daily and/or subdaily increments through the "rapid extension zone"; in particular, features we consider to represent alternate daily and subdaily structures could, if counted as both daily by other authors, lead to a divergence of assigned ages of the order of 30 days. While there is no tagging data on the smallest fish (e.g. FL 5-15 cm) to prove or disprove our hypothesis that the daily features in this region of the otolith are the relatively large (50 micron) structures along the PR path, preliminary otolith microchemical data from SBT and yellowfin tunas do support this interpretation by showing cyclic changes in otolith constituents (e.g. calcium and strontium levels) with an exactly equivalent periodicity (J. Gunn, unpublished data). In addition, from the relationship between otolith morphometry and fish length presented in Fig. 8, implied growth rates for SBT corresponding to 50-micron "daily" increments in the PR path during the "rapid extension zone" would be around 6 mm/day, similar to values postulated by Brothers et al. (1983) for for late larval/early juvenile northern bluefin from both field cohort data and otolith microincrement counts. From these lines of evidence, as well as otolith microstructure in areas other than the PR path, we believe that our interpretation of daily and subdaily microincrements through the "rapid extension zone" is sustainable, together with the resulting age data on juvenile SBT.

In conclusion, our study demonstrates the following key points regarding the growth of juvenile SBT as recorded in their otoliths:

(1) Southern bluefin tuna entering the Australian fishery in the size range 50-55 cm belong to the year class 1+ and not 2+ as believed prior to the commencement of this work. Young-of-the year (class 0+) examined in this study ranged from FL <1 cm to 34 cm (age range <1 to 4 months). These data are based on counts of microincrements which, although not completely validated as being daily in formation, are consistent with calculated values for "expected"

daily otolith growth rates adjusted for seasonality, show the anticipated patterns of summer and winter zones for the assessed age and known time of recapture of the fish, and are in agreement with independent data from other hard parts of SBT and from reported growth rates of tunas studied elsewhere.

(2) The growth curve for small (<86 cm) 1980s and 1990s SBT adopted by the CCSBT in 1994, incorporating the above information regarding approximate size-at-age 1, agrees in slope with a regression fitted to the present data from FL 32 cm - 78 cm, but differs in average length-at-age by 4-5 cm over this size range. Present data do not permit the cause of this discrepancy to be studied in detail, but it is a suggested area for further investigation (e.g. by comparison with cohort data and additional direct ageing information).

(3) The growth of SBT below approximately 30 cm is not adequately represented by a von Bertalanffy-type function, and probably involves an early logarithmic growth phase leading to a brief rapid growth stanza of at least 5 mm/day before declining to values predicted from an appropriate von Bertalanffy growth equation. The postulated rapid growth period most likely corresponds to a "rapid extension zone" in the otolith featuring relatively large microincrements (<50 microns width in the primordium to post-rostrum counting path) with prominent subdaily banding. Counting of these presumed subdaily bands as additional "days" represents a possible source of discrepancy in published age data based on microincrement counts between different authors.

(4) The study of etched transverse fractures simplifies the recognition of microincrements in the "rapid extension zone" and allows essentially continuous records of microincrements to be studied in some fish up to approximately 80 cm (2 years old). Larger fish are probably precluded from successful study by this method on account of the increasingly narrow spacing of "winter" microincrements.

(5) The use of strontium chloride as a marking agent, injected into tagged fish before release, appears to cause temporary interruptions to the daily growth record and the organisation of the otolith aragonite matrix over a period of possibly one to a few weeks, limiting its usefulness as a method for validating the daily formation of microincrements. Its true value is for creating a permanent, unambiguous mark in the otolith for fish where time-at-liberty is in the order of years rather than weeks or months (cf. Clear et al, in press).

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Appendix

Estimation of "expected" daily growth in SBT otoliths from first principles

For this procedure, the following assumptions are made:

(1) SBT growth increments between successive "birthdays" (i.e. 1 Jan) are reasonably represented by the present CCSBT von Bertalanffy curve for 1980s/1990s fish (note: a possible systematic error of the order of 3-5 cm in assigned lengths-at-age, as postulated in the Discussion, would not affect the calculated annual growth increments significantly for present purposes)

(2) An additional seasonal component is allowed for, in its simplest form considered to be a sinusoidal function centred on mid-February (austral summer), with a seasonal minimum growth rate approaching zero in mid-winter (Fig. 20). This component has been calculated using the seasonal growth model of Somers (1988), and then adjusting the T_0 value of the resulting function until size-at-age 1 agrees with that from the unmodified von Bertalanffy curve. This model for seasonal growth is in general agreement with field data on the timing of modal progressions (e.g. as illustrated by Leigh, 1991) as well as data from short time-at-liberty tag and recapture data on summer growth rates (e.g. the fish recaptured as part of the strontium marking experiment, Table 2a) and winter growth of SBT in sea cages (C. Proctor, unpublished data). It predicts January-April as the season for most rapid fish growth and July-September as the "winter minimum" growth period.

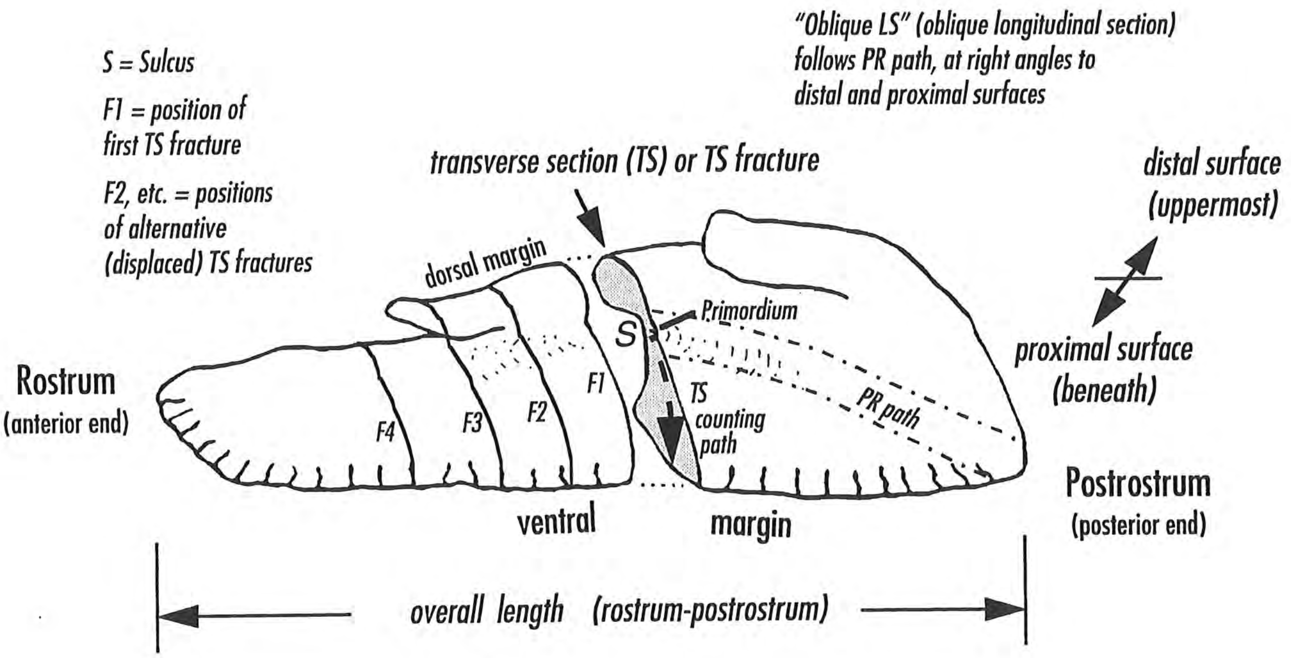
According to this model, "summer" growth over the 3 months January-March is estimated to be 45% of the total annual increment rather than 26% without seasonality, while "winter" growth (July-September) would be 6% of the annual increment rather than 25% if no seasonal allowance is made.

(3) Over the size range FL 45-90 cm (+), the mean otolith extension is 50 microns along the PR path and 9 microns along the TS counting path for every 1 cm increase in FL (see Fig. 8), and this relationship is maintained throughout the year, as a first approximation.

From the presently used CCSBT growth curve for SBT (Anon, 1994), the following mean growth rates are predicted for the fish and by extrapolation, the otolith in the two counting paths of relevance:

Assigned year class plus predicted FL on 1 January	Predicted annual growth increment (from CCSBT curve)	Expected "summer" growth (45% of annual total) in FL (3 months)	Expected "winter" growth (6% of annual total) in FL (3 months)	*Calculated equivalent otolith growth rate in PR path*	*Calculated equivalent otolith growth rate in TS counting path*
Age 1 (55 cm) to age 2 (81.5 cm)	26.5 cm	11.9 cm (4.0 cm/month)	1.6 cm (0.53 cm/month)	*summer: 6.6 microns/day* *winter: 0.9 microns/day*	*summer: 1.2 microns/day* *winter: 0.2 microns/day*
Age 2 (81.5 cm) to age 3 (99.4 cm)	17.9 cm	8.1 cm (2.7 cm/month)	1.1 cm (0.37 cm/month)	*summer: 4.5 microns/day* *winter: 0.6 microns/day*	*summer: 0.8 microns/day* *winter: 0.1 microns/day*
Age 3 (99.4 cm) to age 4 (113.4 cm)	14.0 cm	6.3 cm (2.1 cm/month)	0.8 cm (0.27 cm/month)	*summer: 3.5 microns/day* *winter: 0.4 microns/day*	*summer: 0.6 microns/day* *winter: 0.08 microns/day*

Note: no prediction is made for rates of fish or otolith growth for fish younger than age 1, since growth over the interval FL 0-30 cm (+) cannot be adequately represented by a von Bertalanffy type function (see Fig. 18).



Generalised representation of a left hand sagittal otolith of a southern bluefin tuna

Fig. 1. Generalised SBT otolith, showing terminology used and locations of regions examined for microincrements.

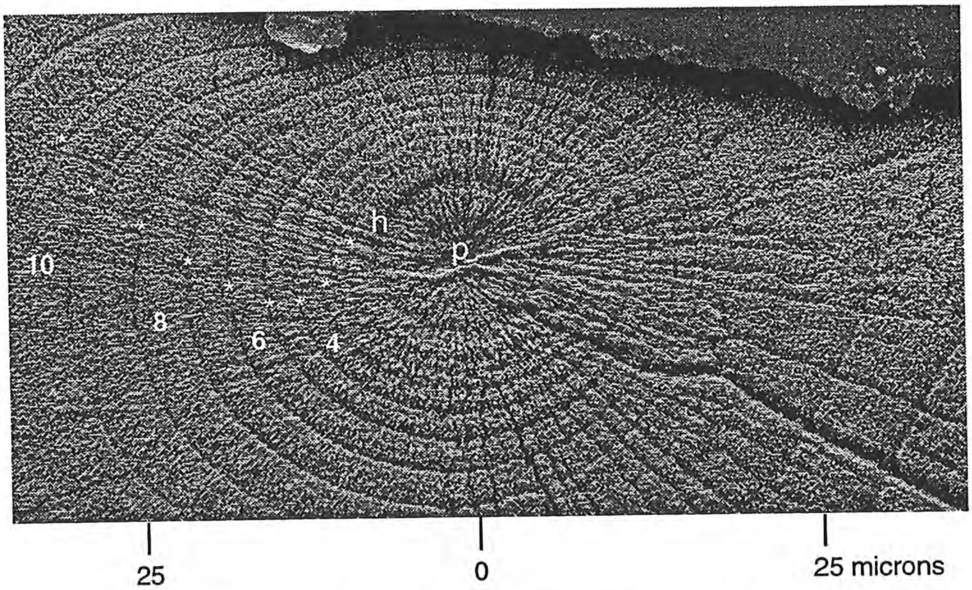


Fig. 2. Central portion of a larval (9 mm SL) SBT otolith, sectioned parallel to the distal face, showing location and nature of primordium (p), presumed hatching mark (h) and first 10-12 micrincrements separated by D-zones (asterisks). SEM image of specimen etched with 0.02N HCl, photographed at x1,300 on SEM. Distances shown are from primordium in anterior/posterior directions.

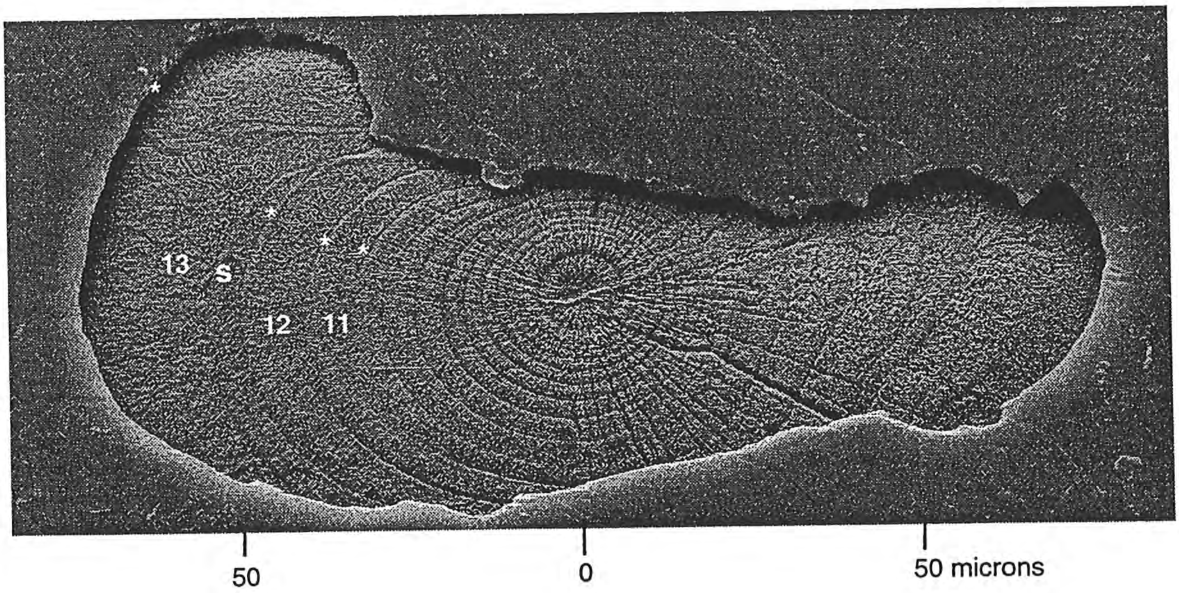


Fig. 3. Image showing outer portions of the larval SBT otolith in Fig. 2, showing onset of significant anterior and posterior development, with progressively widening growth increments (11-13) the last containing an apparent subdaily ring ("s"). SEM image, photographed at x650.

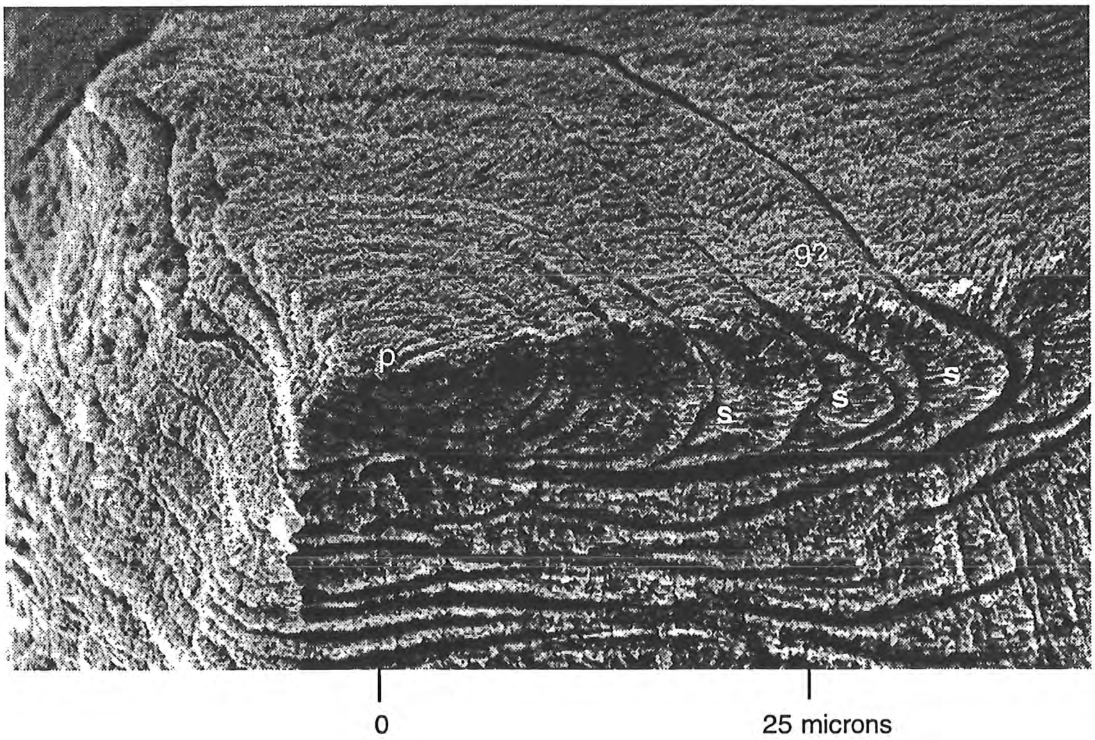


Fig. 4. Image of the central region of a fractured otolith from a juvenile (27 cm FL) SBT, with superficial calcium carbonate removed by acid etching to reveal primordial plane. This image shows the region corresponding to larval otolith growth, to approximately microincrement 9 (the first 4-5 microincrements are unclear in theis specimen). Note the "partial" development of presumed subdaily rings ("s") in the vertical (i.e., dorso-vental) plane in the region of microincrements 7-9. SEM image, photographed at x1,600.

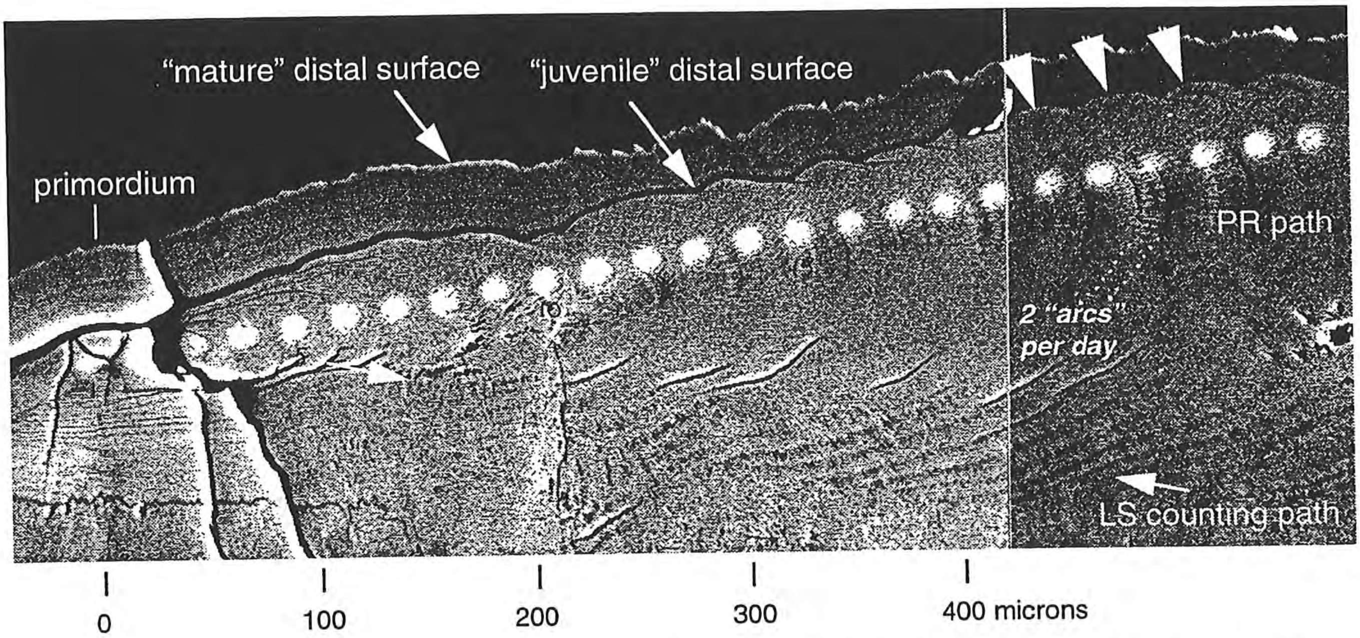


Fig. 5. Polished oblique longitudinal section showing the primordium and successive 0.6 mm along PR path, through an otolith from a 134 cm FL SBT. This image shows part of the "rapid extension zone" (increments 15-25 approx.) with our interpretaion of the nature of daily and subdaily features. Note the presence of a series of "daily" ridges in the preserved juvenile ventral surface (broad arrowheads), the presence of two "arcs" (daily and subdaily D-zones) per increment along the PR path, and the occurrence of well defined single D-zones (dark bands) along a sloping LS counting path (narrow arrows). Backscatter SEM image of unetched, carbon-coated specimen, photographed at x200 on SEM.

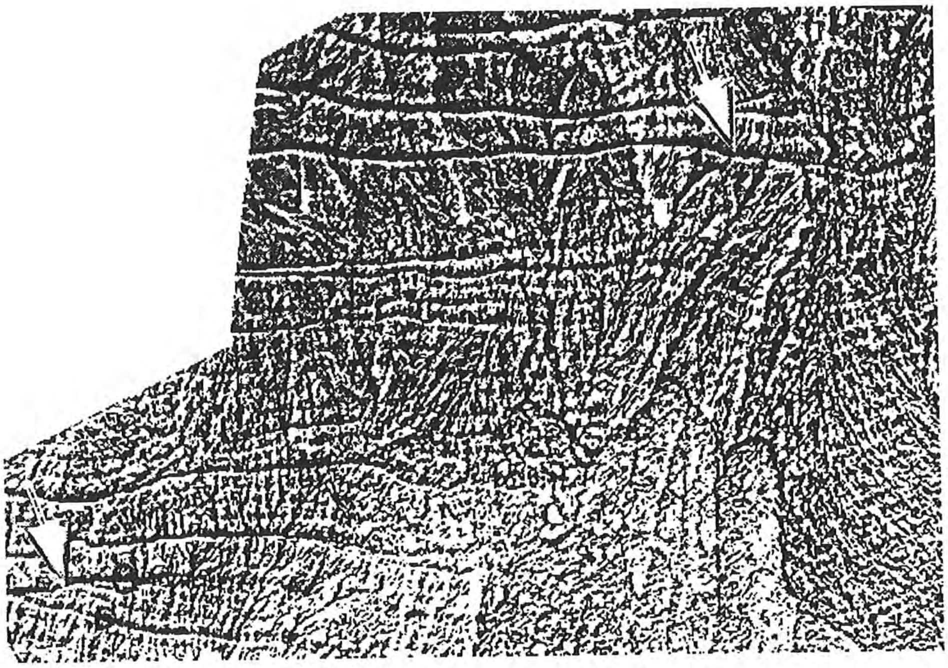


Fig. 6. Portion of an acid-etched TS fracture of an SBT otolith illustrating the presence of "good" presumed daily microincrements (lower right) along the TS counting path, with area to left containing numerous "checks" and missing growth increments. Arrows indicate areas where "checks" can be seen to represent periods of temporary growth cessation in this region of the otolith. SEM image of acid-etched specimen, photographed at x500 on SEM.

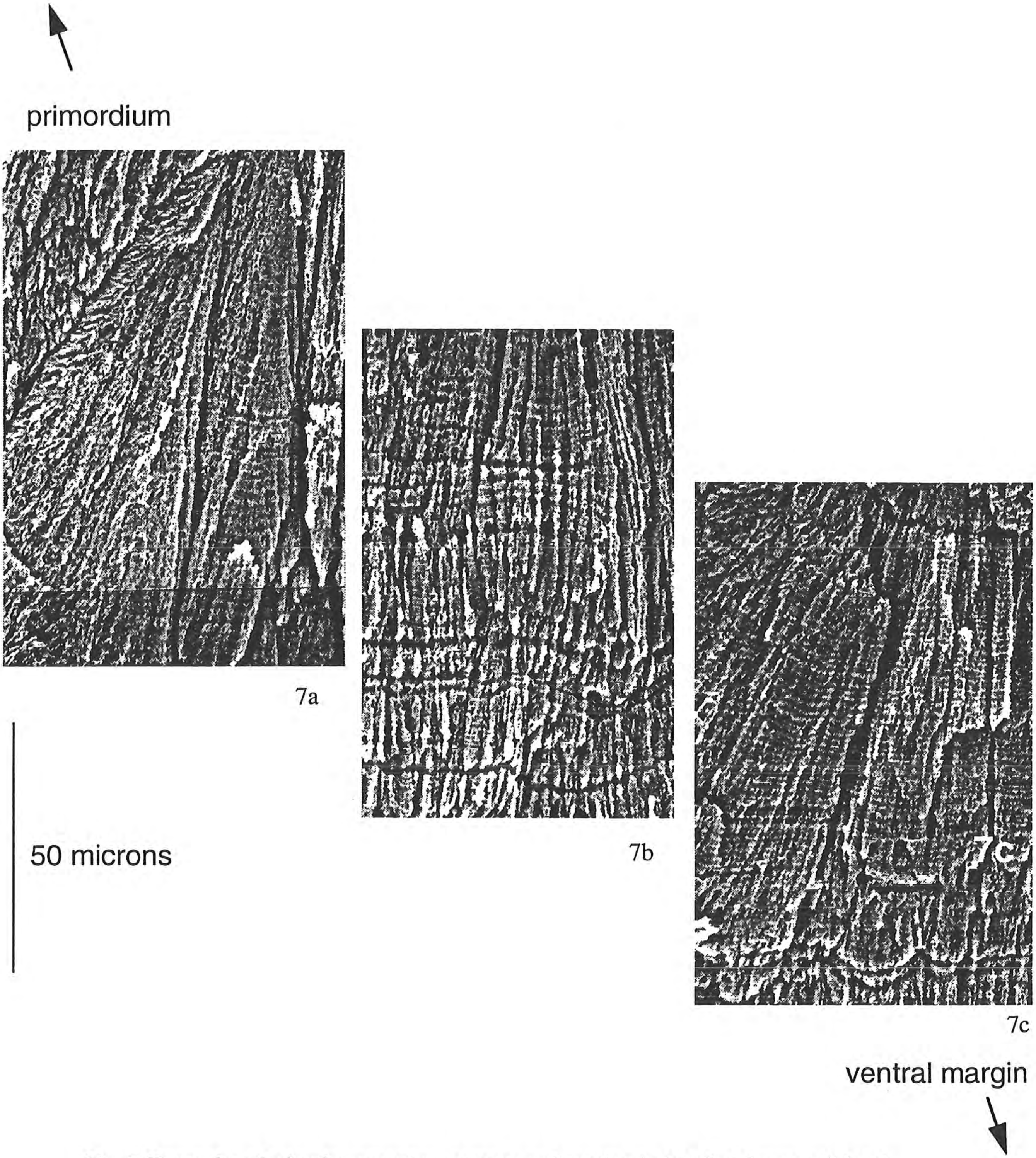


Fig. 7. Examples of microincrement appearance and spacing in late first "summer" (post rapid extension zone) (Fig. 7a) , first "winter (Fig. 7b), and second "summer" (Fig. 7c) in a TS fracture of the otolith from a 60 cm SBT. SEM image of acid-etched specimen, photographed at x500 on SEM.

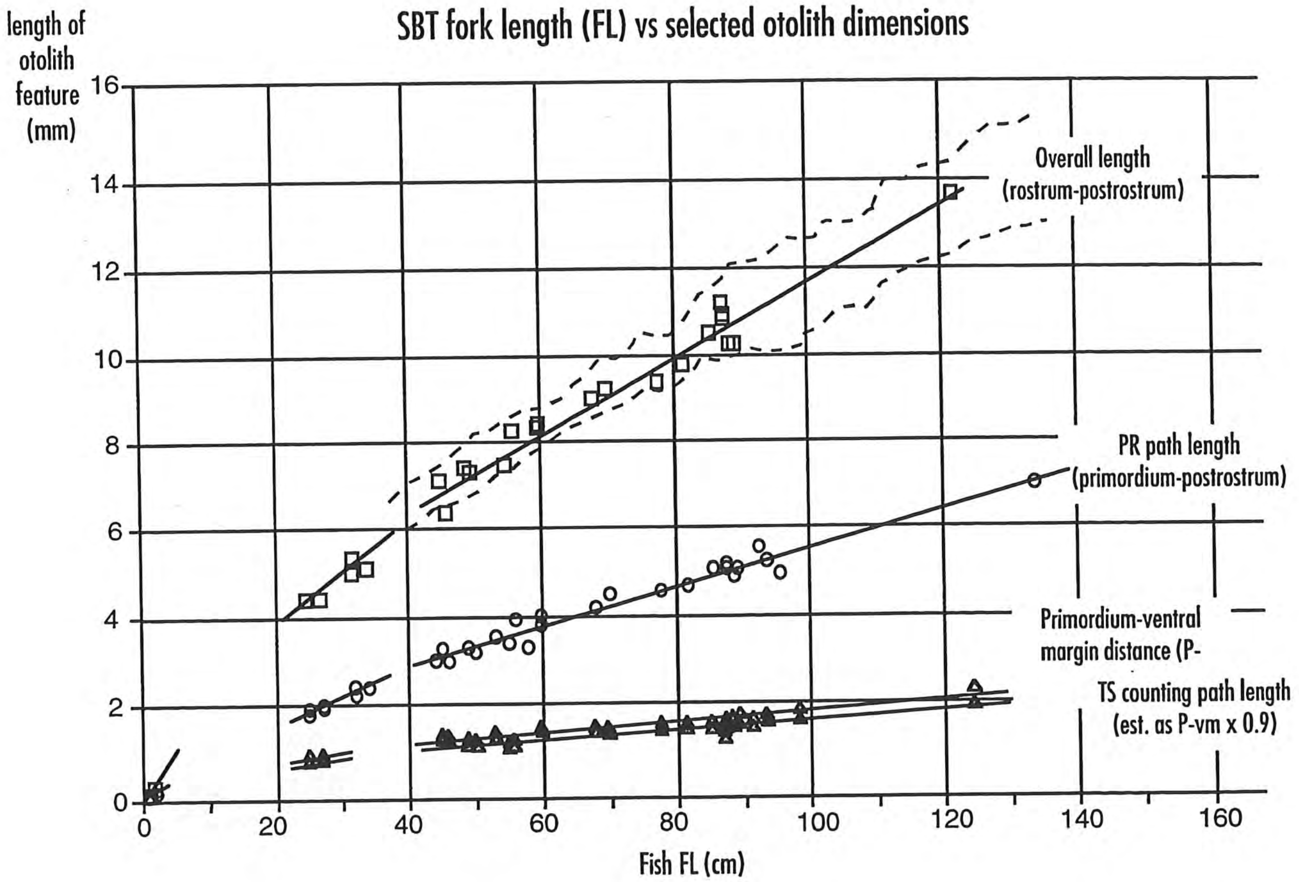


Fig. 8. Morphometric data from selected otoliths used in this study. PR path length is the counting path used for acetate replicas; TS counting path length is the path used for SEM study of microincrements in TS fracture faces. Dashed lines indicate the approximate limit of data obtained by Clear et al (in press) for the relationship between otolith length and fork length for SBT from additional specimens over a larger size range than the present dataset.

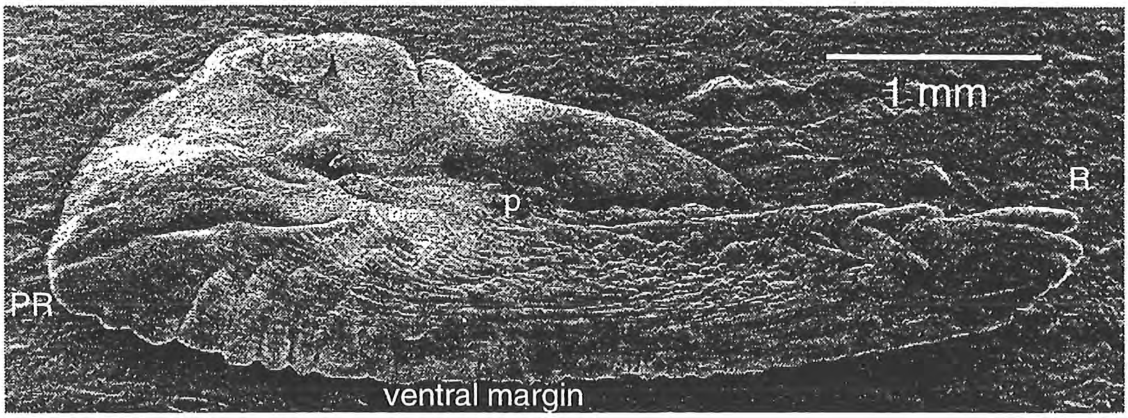


Fig. 9. SEM image of a whole otolith from a small (25 cm) juvenile SBT, showing principal features and the presence of concentric ridges on the distal surface in the "rapid extension zone" surrounding the primordium. Low kV image of uncoated specimen, photographed at x20 on SEM. p=primordium, R=rostrum, PR=post-rostrum.

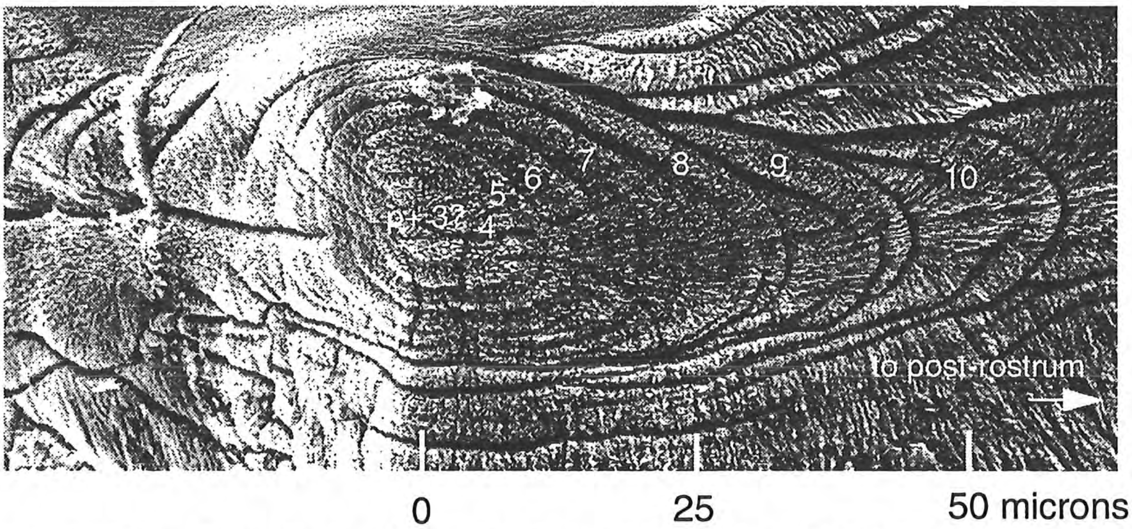


Fig. 10. Primordial region in a fractured, etched otolith of a 25 cm SBT. This region shows the primordium covered by a "dome" formed by approximately the first c. 8 microincrements, and the subsequent posterior development towards the "rapid extension zone". Innermost c.3 microincrements are presumed not visible. SEM image, photographed at x1,300.

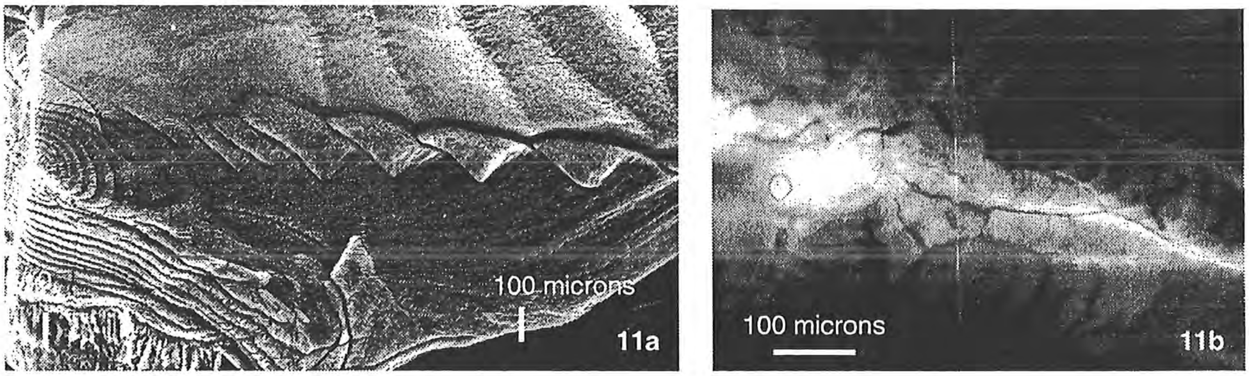


Fig. 11a, 11b. Images showing the presence of prominent surface ridges on the distal surface of otoliths of SBT in the "rapid extension zone" surrounding the primordium. Fig. 11a: lightly etched, fractured specimen viewed in SEM, photographed at x400; Fig. 11b, otolith from small (25cm) SBT, photographed in light microscope with transmitted light at x30.

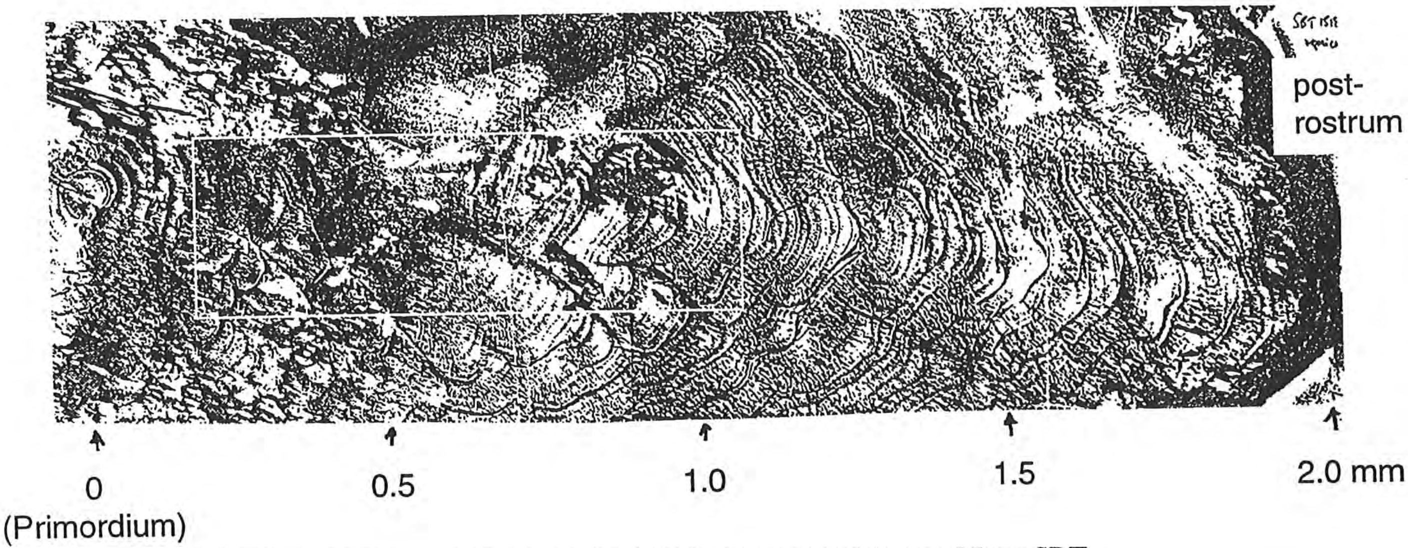


Fig. 12. Acetate replica along PR path of acid-etched otolith from a 25 cm SBT, photographed in the light microscope at x200. Note presence of primordium and surrounding increments, "rapid extension zone" (white rectangle) with 30 to 50-micron microincrements, and 15-20 micron increments persisting to otolith margin.

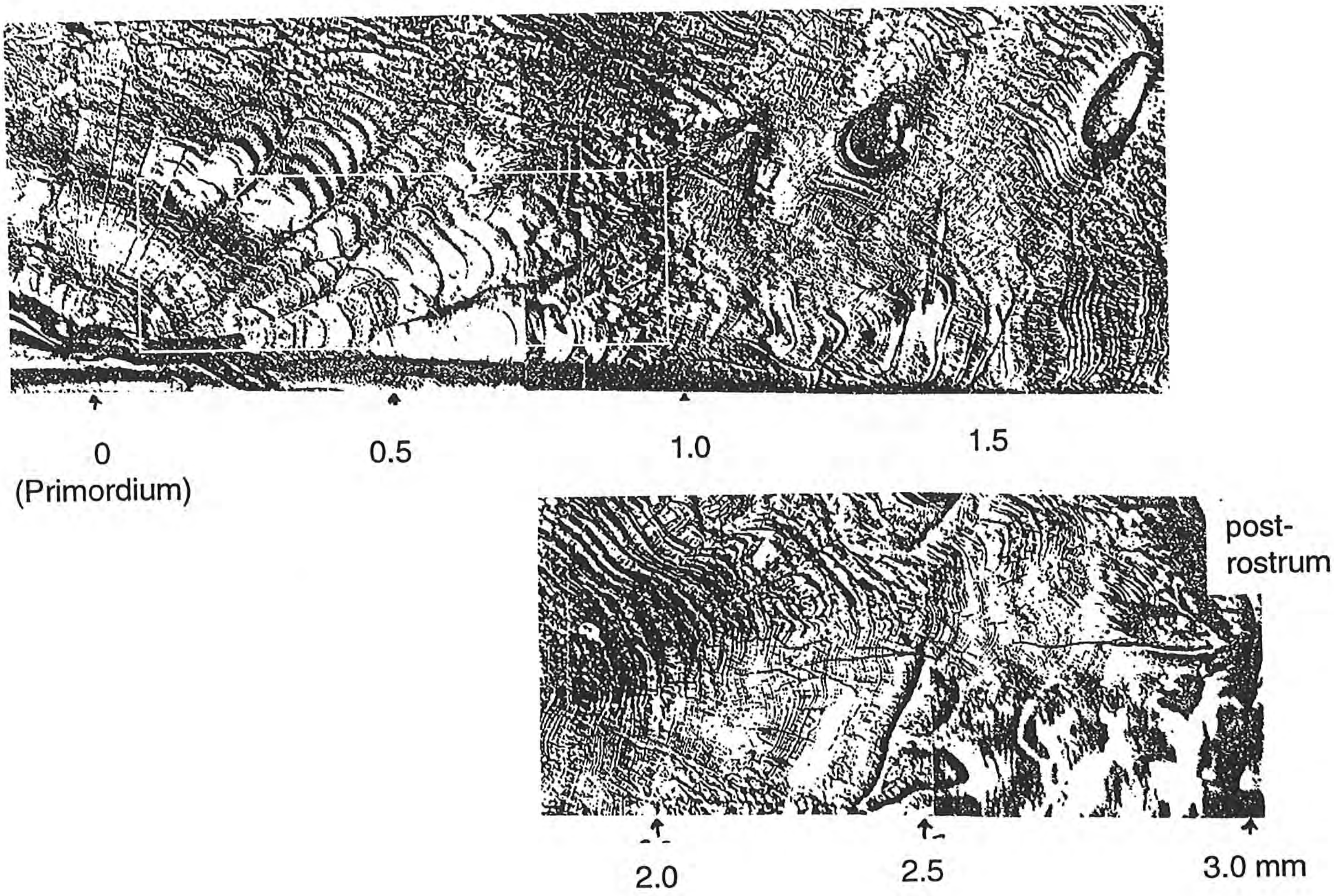


Fig. 13. Acetate replica along PR path of an etched otolith from a 46 cm SBT, photographed in light microscope at x200. Note presence of the primordium and surrounding increments, rapid extension zone with 30-50 micron microincrements (white rectangle), and a narrow increment zone ("winter zone") extending from approx. 1.8 mm out from the primordium to the otolith margin.

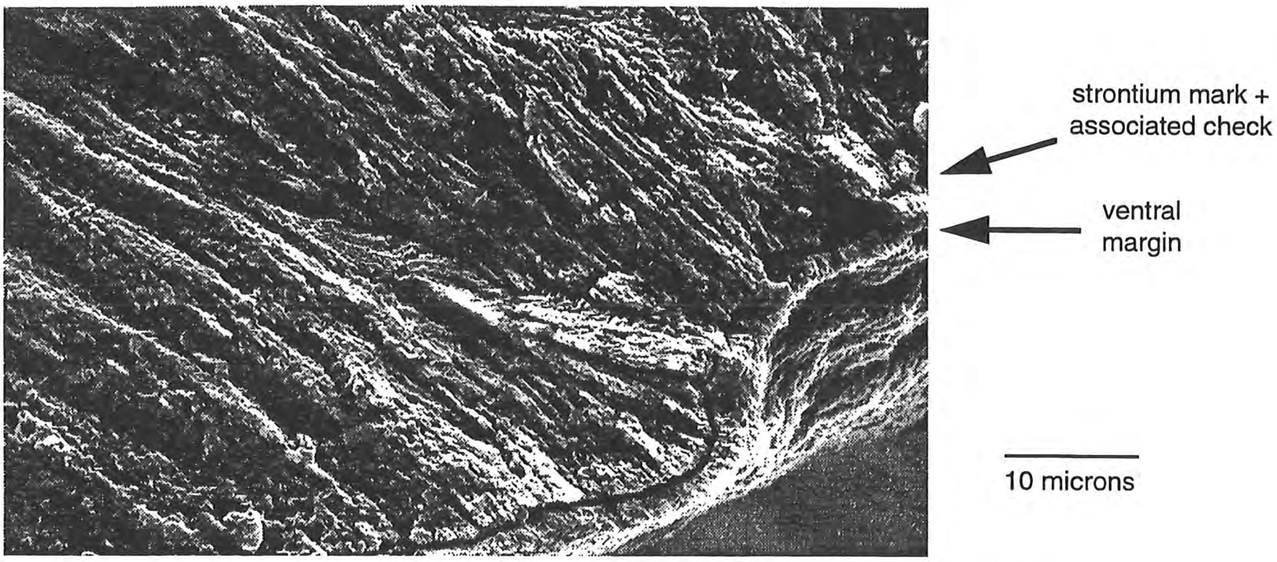


Fig. 14. Etched fractured otolith showing outermost portion of the TS counting path in summer-tagged Sr marked fish (SBT OB 162) with short time-at-liberty following marking (18 days). This fish was 87 cm FL at time of tagging. Note presence of clear "summer" microincrements prior to tagging event, the severe "check" marking the location of the strontium band, and limited growth (4 microns approx..) without clear microincrement deposition post-tagging. SEM photograph, taken at x2,200.

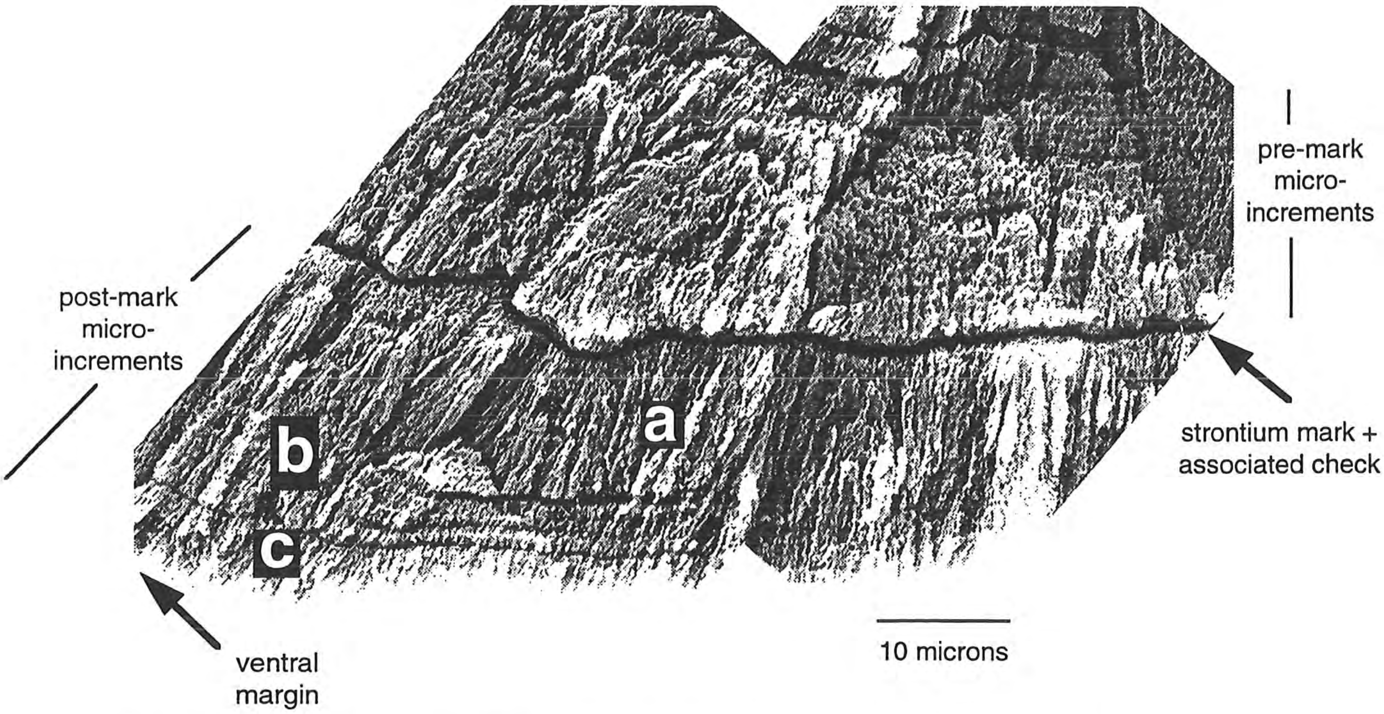


Fig. 15. Etched fractured otolith showing outermost portion of the TS counting path in summer-tagged Sr marked fish (SBT OB 29) with clear microincrements preceding the strontium mark and some observable microincrements between the mark and the margin. Estimated count for this specimen post-marking is 34 (17 in region "a", 10 in region "b" and 7 in region "c", all values approximate); this fish was 78 cm at time of tagging, and at liberty for 39 days. SEM photograph, taken at x2,000.

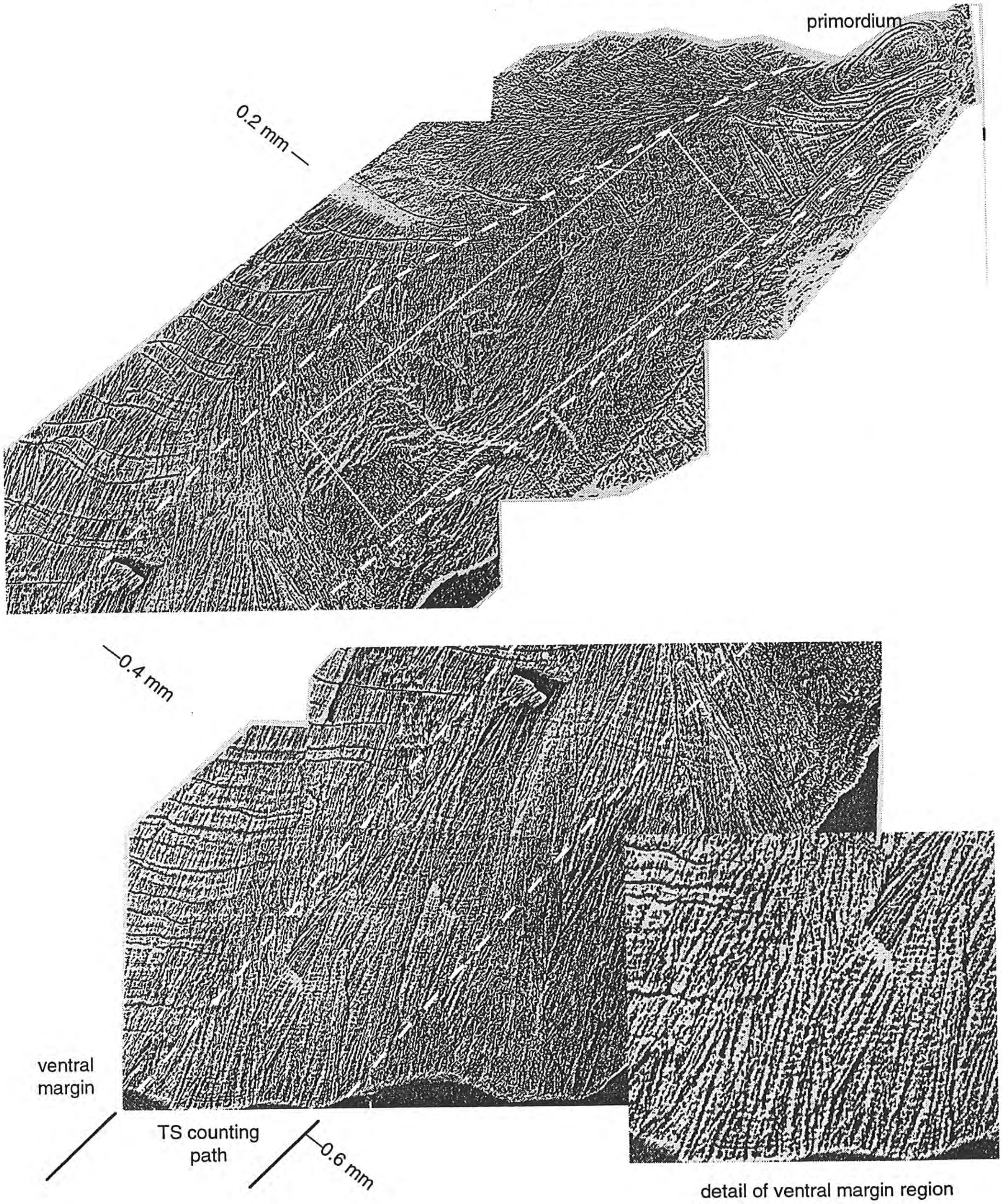


Fig. 16. Etched fractured otolith showing the complete TS counting path in a small (31 cm) SBT. White rectangle indicates approx. extent of "rapid extension zone". Note presence of clear "summer" microincrements through to the proximal margin. This fish is estimated to be 122 days old. SEM photographs, taken at x500.

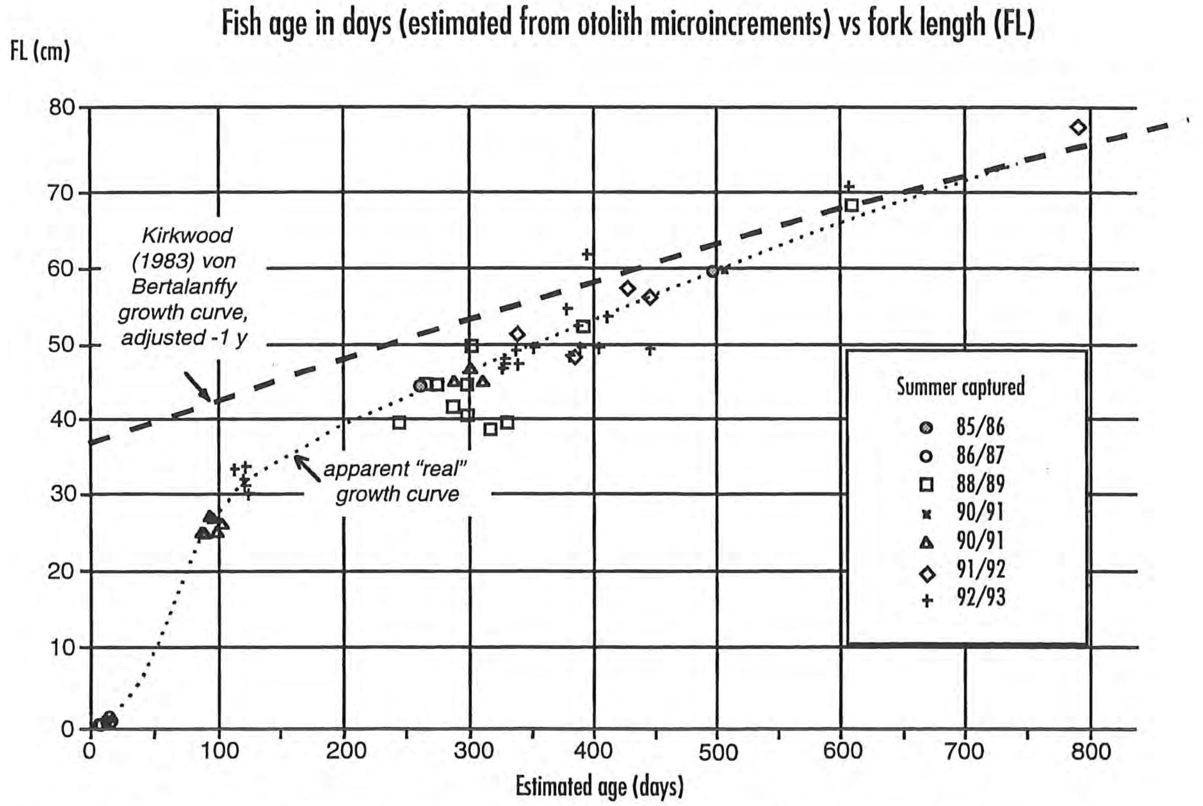


Fig. 17. Plot of estimated SBT age vs fork length for 50 SBT newly aged in this study, plus larval SBT previously aged by Jenkins & Davis (1990) (1986/87 caught fish). The von Bertalanffy growth curve shown is that from Kirkwood (1983) adjusted by one year, i.e. his "age 2"= present "age 1" (see text for more details).

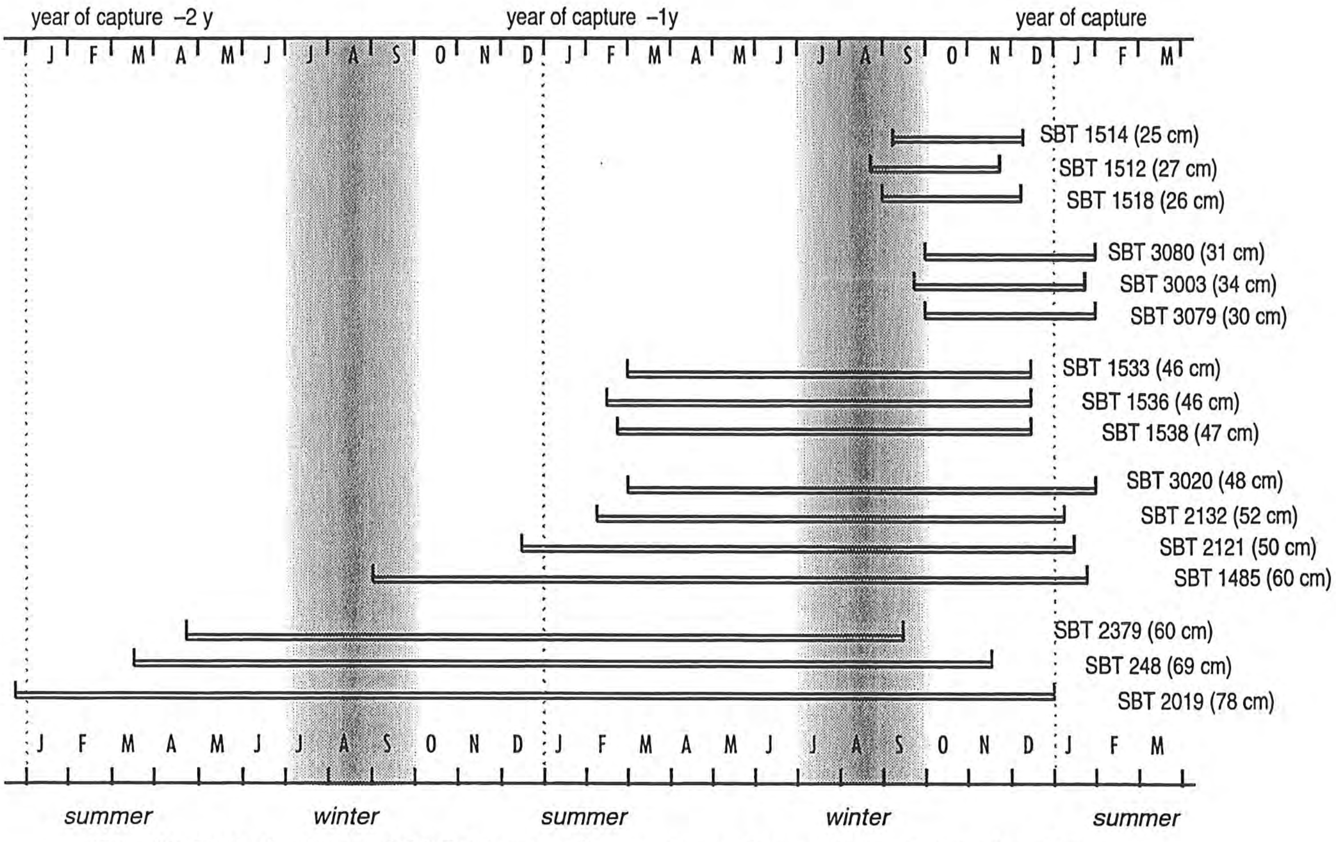


Fig. 18. Plot of estimated SBT lifespans for specimens from representative size-classes examined in this study, shown from back-calculated date-of-birth to date of recapture. Dark zones shown indicate notional winter periods in southern hemisphere (Jul-Sep).

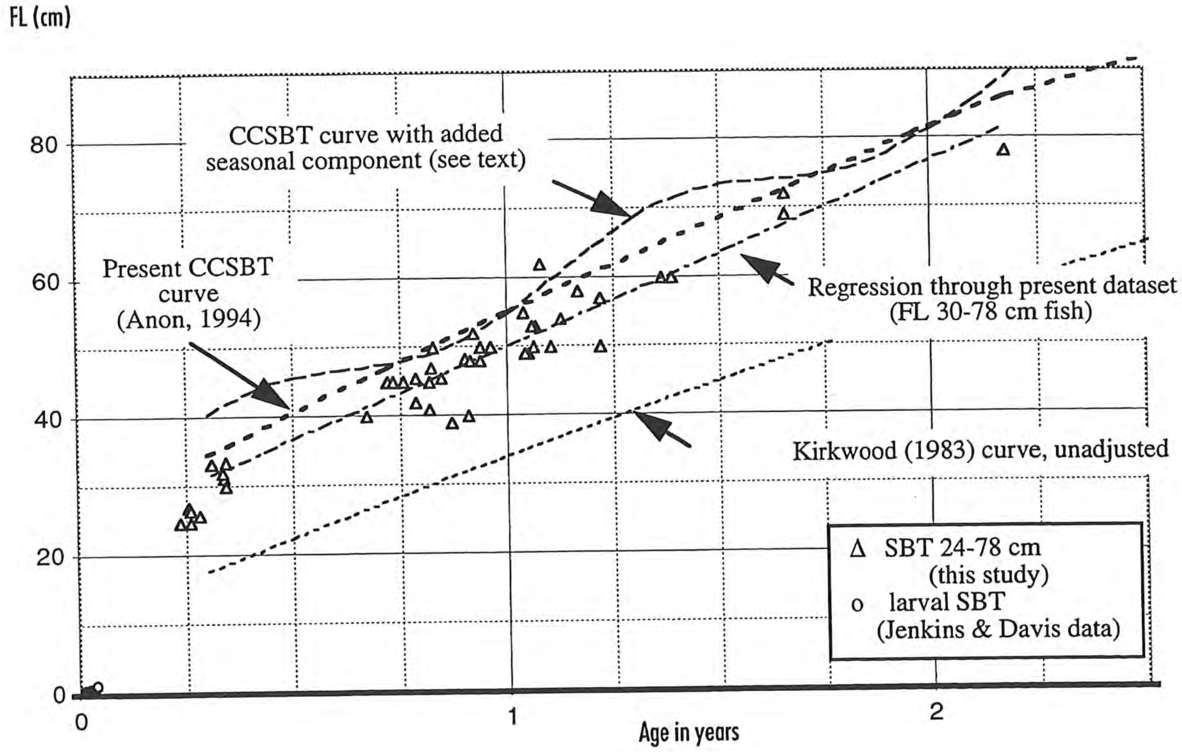


Fig. 19. Comparison of regression through present age data from microincrements of SBT FL 30-78 cm with other growth curves available for juvenile SBT. For full details, see text.

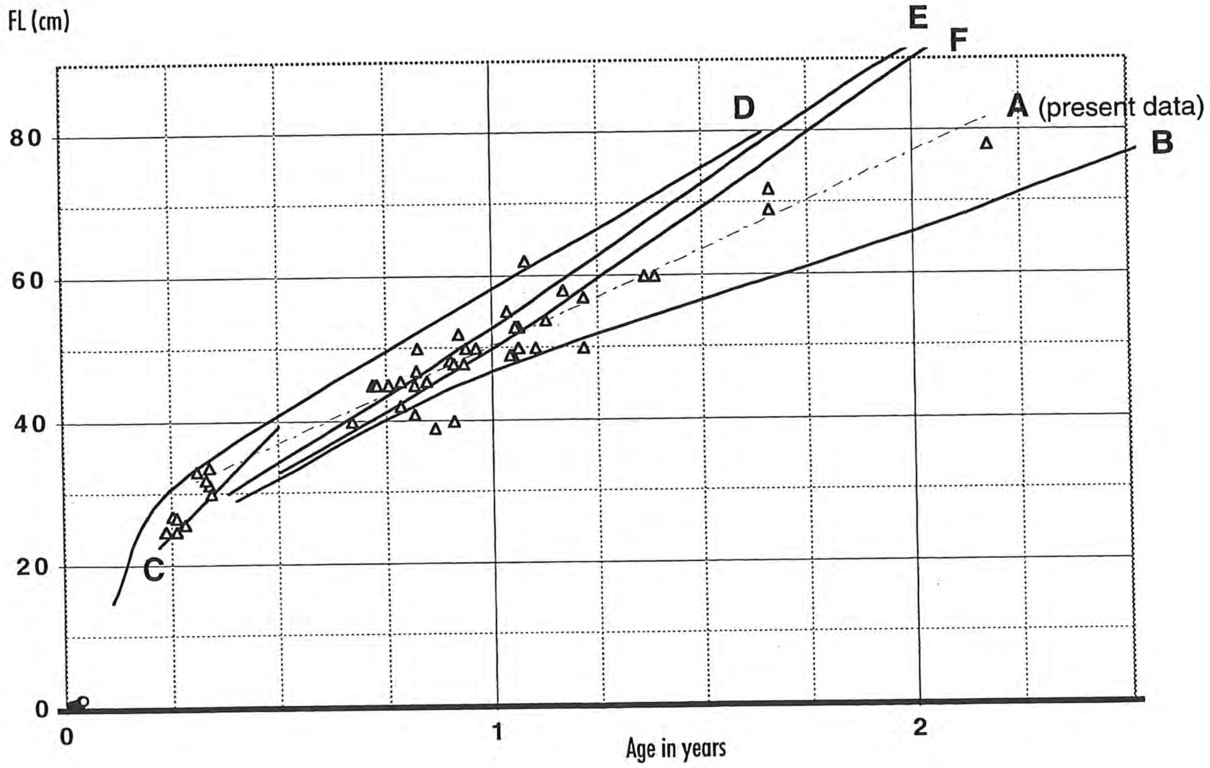


Fig. 20. Comparison of regression through present age data from microincrements of SBT FL 30-78 cm with growth curves available for other tuna species (northern bluefin and yellowfin). A = present regression for SBT FL 30-78 cm; B= Pacific northern bluefin >30 cm (Foreman, 1996); C = Atlantic northern bluefin 25-40 cm (Brothers et al., 1983); D = eastern Pacific yellowfin 15-80 cm (Yamanaka, 1990); E = Indian Ocean yellowfin (Stecquert et al., 1996); F = eastern Pacific yellowfin > 30 cm (Wild, 1986).

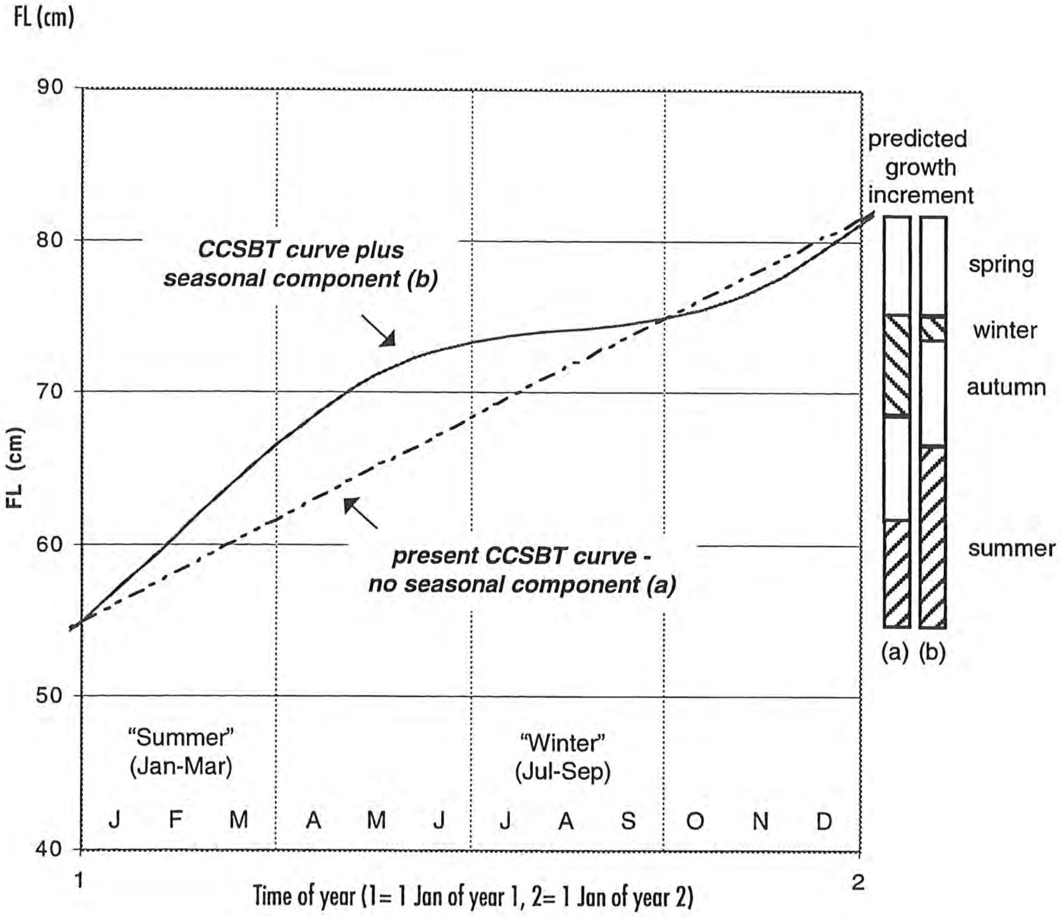


Fig. 21. Example of a standard von Bertalanffy growth curve (dashed line) for SBT with no seasonal component (curve shown is the present CCSBT curve, i.e. fitted to 55 cm at 1 January, age 1), and the same with an added sinusoidal seasonal growth component (solid line) with maximum seasonal growth centred on mid February. The amplitude of the seasonal component is selected so that growth rate approaches zero in mid-winter, and the value of T_0 for this curve is adjusted such that FL at age 1 is the same for both curves.

Hatched areas in side bars show predicted summer and winter 3-month growth increments using the standard von Bertalanffy curve (a) and the same with added seasonal component (b). "Summer" growth is 26% of the annual total with no seasonal component, or 45% with seasonal adjustment; "winter" growth is 25% of the annual total with no seasonal component, or 6% with seasonal adjustment.

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 3

Project 92/42

Use of the Bomb Radiocarbon Chronometer to Determine Age of Southern Bluefin Tuna (*Thunnus maccoyii*)

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Key words: age validation, bomb radiocarbon chronometer, carbon, otoliths, radiocarbon, southern bluefin tuna, *Thunnus maccoyii*

Abstract

The growing otoliths of fish incorporate radiocarbon in concentrations that are equivalent to that found in ambient seawater dissolved inorganic carbon. Therefore, pulses of anthropogenic radiocarbon produced by the atmospheric detonation of nuclear weapons can ultimately be detected in otoliths. This study estimates the age of large southern bluefin tuna *Thunnus maccoyii* using an age estimation procedure based on the determination of levels of bomb-derived radiocarbon in otoliths. Radiocarbon data from selected regions of southern bluefin tuna otoliths indicate that this species may reach ages in excess of 30 years. Furthermore, individuals that approach the asymptotic length are likely to be 20 years of age or older. The data agree generally with accepted models of southern bluefin growth, but show that these fish live longer than was believed previously. Comparisons between otolith section and bomb radiocarbon age estimates indicate that reading otolith sections is an effective method to estimate the age of larger southern bluefin. The presence of a significant number of individuals greater than 20 years of age in the southern bluefin population may alter estimates of natural mortality rates currently used in Virtual Population Analysis models for stock assessment of this species.

Introduction

Southern bluefin tuna (*Thunnus maccoyii*) is a highly migratory species that is found throughout the Southern Ocean north of about 60°S latitude. Southern bluefin is exploited by fishers from Australia, Japan, New Zealand, Indonesia, Taiwan, and Korea and it is the most valuable commercial finfish species in Australia with the annual Australian catch valued at more than $80 million (Kailola et al. 1993). Catch rates for southern bluefin have decreased dramatically in the last decade, presumably due to increased fishing pressure on the resource, associated with its increased value (Caton et al. 1990). International management of southern bluefin has been placed under the jurisdiction of the Convention for the Conservation of Southern Bluefin Tuna (CCSBT), a trilateral convention that includes Australia, Japan, and New Zealand as its members. The CCSBT has indicated that age estimation of southern bluefin is a high priority research area (Anon. 1994). Furthermore, the lack of validated catch-at-age data for the exploited population has introduced significant uncertainty into the Virtual Population Analysis that is the primary stock assessment tool used by the CCSBT.

Age estimation for the larger tunas including southern bluefin and northern bluefin (*Thunnus thynnus*) is problematic due to difficulties involved in the interpretation of marks on hard parts, such as otoliths and vertebrae (Hurley and Isles 1983, Prince and Pulos 1983, Prince 1985, Thorogood 1987). Furthermore, logistical problems associated with the mobility and longevity of these species make tagging studies and, concomitantly, the validation (*sensu* Kalish et al. 1995) of a "preferred" age estimation procedure, both difficult and costly. Despite the complications associated with age estimation and the related validation for the larger *Thunnus spp.*, these areas of research have a high priority in many nations that have significant fisheries for these species. In Australia research related to age estimation for southern bluefin includes a mark-recapture study using strontium chloride to mark calcified tissues (Clear et al. In preparation), investigation of conventional age estimation methods based on otoliths and vertebrae (Gunn et al. 1995), and the application of bomb radiocarbon measured in otoliths to age determination and validation (this study).

Although obvious zones are present in the otoliths and vertebrae of southern bluefin of all sizes (Gunn et al. 1995), and annual bands have been validated in the otoliths of fish up to 6+ years (133 cm LCF) (Clear et al. In preparation), there are still problems with the interpretation of zones from calcified tissues of larger and presumably older fish. In larger southern bluefin, discrepancies in the number of presumed annual increments counted in otoliths and vertebrae have lead to uncertainty over which, if either, provides a true estimate of age for mature fish. An additional complication is the lack of known age individuals and the rarity of tagged and returned fish with long periods at liberty (>10 years). Similar problems exist for age estimation of Atlantic bluefin tuna (*Thunnus thynnus*) (Lee & Prince 1995). Until recently, stock assessment of southern bluefin was carried out under the assumption that these fish can be up to 20 years of age (Collette & Nauen 1983, Majkowski & Hampton 1983, Caton et al. 1990); however, the only direct evidence for this came from one tag recovery 20 years after release of a one year old bluefin (CSIRO unpublished data).

Determination of southern bluefin longevity and validation of age estimation methods for larger individuals is required in order to increase the reliability of stock assessments for this species. Zone counts in otoliths (Gunn et al. 1995) have suggested ages well in excess of the previously accepted maximum age of 20 years, but in the absence of tag-recapture data, there has been little chance of validating these estimates.

The bomb radiocarbon chronometer (Kalish 1993, 1995a, 1995b) provides an alternative method to tag-recapture studies. The bomb radiocarbon chronometer can be used to estimate the age of individual fish and the technique is well-suited to estimating the age of southern bluefin tuna. Because there is no accepted "routine" method for age estimation of larger southern bluefin tuna, direct estimation of age on the basis of the bomb radiocarbon chronometer presents a viable alternative to test assumptions regarding southern bluefin longevity. Southern bluefin are suited to age estimation using bomb radiocarbon due to the presumed longevity of the species and the likelihood that individuals in the present population were spawned during the bomb-related increase in radiocarbon in the atmosphere and ocean (Kalish 1993).

Materials and methods

Southern bluefin otoliths (sagittae) were selected from otolith archives maintained at the CSIRO Marine Laboratories (Hobart, Tasmania). A single otolith from each pair was selected for analysis with the other otolith, when available, being retained for studies of routine age estimation procedures. The majority of otoliths selected were from large individuals as these fish are likely to be more suitable for age estimation on the basis of bomb radiocarbon due to their presumed birth date. Otoliths were obtained from large fish caught off southeast Tasmania and in the Java Sea between 1988 and 1994. Otoliths from two southern bluefin that were one year of age (55 cm FL) were also selected to assist with calibration and to provide an indication of the decrease in ocean radiocarbon since 1980. The small fish were caught off the southwest coast of Western Australia in 1985 and 1993. In this study, calibration refers to the process of establishing a relationship between surface ocean radiocarbon levels in a region and time (Kalish 1995b).

Otoliths were weighed dry and then prepared for radiocarbon and stable carbon isotope analysis. The earliest formed portions of individual otoliths was isolated with a fine, high speed drill. This was achieved by “sculpting” from the larger otolith, an otolith that was representative of a southern bluefin less than 1 year of age. During the sculpting process the position of "landmarks" such as the otolith core and zones associated with the presumed first annual increment were monitored frequently. This ensured that the sculpted otolith contained material only deposited during the early life of the fish. The final product was a single piece of otolith aragonite (Fig. 1). Sample weights ranged from about 13 to 24 mg. Otolith carbonate was converted to CO_2 by reaction *in vacuo* with 100% phosphoric acid. An aliquot of the CO_2 was used to determine $d^{13}C$ for each sample and the remaining CO_2 was converted to graphite (Lowe and Judd 1987) for analysis of radiocarbon. Radiocarbon levels in each sample were determined by accelerator mass spectrometry (AMS) at the Institute of

Geological and Nuclear Sciences, Lower Hutt, New Zealand (Wallace et al. 1987). Radiocarbon values are reported as $\Delta^{14}C$, which is the age- and fractionation-corrected permil deviation from the activity of nineteenth century wood (Stuiver and Polach 1977). Radiocarbon determinations were made via the NBS oxalic acid standard in conjunction with the ANU sucrose standard. Reported errors for the radiocarbon data are 1 standard deviation. Radiocarbon errors include both counting errors and laboratory random errors.

There are few radiocarbon data available that are suitable for the temporal calibration of radiocarbon data obtained from the earliest formed portions of southern bluefin tuna otoliths. This portion of the otolith is presumed to be deposited in the tropical and subtropical Indian Ocean, specifically in the region of the Java Sea and southeast Indian Ocean. Radiocarbon data were selected from both the pre- and post-nuclear testing GEOSECS (Geochemical Ocean Section Study) expeditions to the Indian Ocean (Bien et al. 1965, Stuiver and Ostlund 1983), studies of corals from Cocos-Keeling Island (Toggweiler et al. 1991) (Table 1), and the otoliths of two 55 cm southern bluefin tuna that were one year (Table 2) of age based on previous studies of southern bluefin age and growth (Gunn et al. 1995). These data were used to model the bomb-related increase in Indian Ocean radiocarbon during the 1960s and 70s.

For zone counts, otoliths were embedded in polyester resin and sectioned with a low-speed diamond saw. Three parallel replicate sections were taken in the dorso-ventral plane (transverse), one anterior to the primordium, one through the primordium and one posterior to it. Each section was then mounted on glass rounds and polished to thicknesses of 0.4-0.6 mm using 600 grade wet-dry paper.

Age was estimated from otolith sections by a reader that had read more than 1000 southern bluefin otolith sections (NPC). These age estimates were compared with age estimates derived from otolith radiocarbon data.

Results

Birth date estimates for individual tuna were determined directly from the otolith radiocarbon data. A second order polynomial function that describes the bomb-related increase in $\Delta^{14}C$ in the eastern Indian Ocean was established from a combination of the GEOSECS data, the Cocos-Keeling coral data, and the two small southern bluefin of known age (birth dates 1984 and 1992). Pre-bomb $\Delta^{14}C$ measured from seawater dissolved inorganic carbon (DIC) and corals, both representative of the year 1953, were not used in the estimation of the function. Radiocarbon data are not available in the relevant area between about 1963 and 1970. The function determined from these data (Fig. 2) describes a time series of $\Delta^{14}C$ that is similar to that modelled for $\Delta^{14}C$ in the southern hemisphere tropical Indian Ocean by Broecker et al. (1985). The resulting function was used to estimate the birth dates of the large southern bluefin tuna on the basis of the $\Delta^{14}C$ measured in the earliest formed regions of the individual otoliths. $\Delta^{14}C$ data measured in the sagittae of 22 southern bluefin tuna are plotted with $\Delta^{14}C$ determined in surface seawater DIC from the GEOSECS expeditions (Bien et al. 1965, Stuiver and Ostlund 1983) and from known age segments of hermatypic corals from Cocos-Keeling Island in the Indian Ocean (Toggweiler et al 1991) (Fig. 3).

Radiocarbon-based estimates of birth dates for the 20 large southern bluefin tuna range from 1958 to 1973 (Table 2). The birth date estimated for the oldest tuna in this study provides an indication of the latest reasonable birth date (i.e. the fish could be older). Because this individual was spawned prior to significant atmospheric testing of nuclear weapons, the portions of the otolith that were analysed do not contain any detectable bomb-derived radiocarbon. Radiocarbon-based birth dates from southern bluefin were also plotted with otolith radiocarbon data from *Pagrus auratus* and *Centroberyx affinis* (Fig. 4). The data from *Pagrus auratus* describe an established calibration curve for increases in radiocarbon in the temperate South Pacific Ocean off New Zealand (Kalish 1993). The data from *C. affinis* are based on age estimates derived from the reading of otolith sections combined with radiocarbon analyses and are representative of changes in ocean radiocarbon off the east coast of Australia at temperate latitudes (Kalish 1995). The tuna birth dates and corresponding $\Delta^{14}C$ values are coincident with both the *P. auratus* and *C. affinis* data until the 1970s when tuna otolith $\Delta^{14}C$ reaches higher levels than the data from *P. auratus*. In later years the tuna data agree more closely with the *C. affinis* data.

Age estimates for southern bluefin tuna were calculated on the basis of the radiocarbon-based birth date estimates and known collection dates for individual fish (Table 2) and were compared with age estimates from the counting of presumed annual increments on transverse sections of otoliths. Because pairs of otoliths were not obtained from all fish only 15 of the 22 samples analysed for radiocarbon had corresponding otolith sections. An age difference plot, where the difference between the age estimated from the otolith sections and $\Delta^{14}C$ is plotted as a function of the "bomb radiocarbon age", was used to compare age estimates (Fig. 5). Pairwise age comparisions between the section and bomb radiocarbon estimates suggest that there may be significant differences among the two methods of age estimation (two-tail t-test; df=14; P=0.051). If the bomb radiocarbon age estimates are taken as the standard, then there appears to be evidence that section ages of several of the youngest fish may be overestimates for this small sample.

When $\Delta^{14}C$ measured in southern bluefin otoliths is plotted as a function of the birth dates estimated from the reading of otolith sections several data points diverge significantly from the bomb radiocarbon curve derived from *Pagrus auratus* otoliths (Fig. 6). Specifically, three southern bluefin section ages (Sample Nos. 203, 564, 598) fall outside the curves defined by the 95% confidence limits determined for individual predicted values from the *P. auratus* radiocarbon and birth date data. The ages (birth dates) of these presumably younger fish, with greater quantities of bomb radiocarbon in their otoliths, appear to be overestimated as they fall to the left of the bomb radiocarbon curve defined by *P. auratus*.

Discussion

Objective estimates of southern bluefin tuna age are possible on the basis of radiocarbon analyses made in the earliest formed segments of otoliths. The age estimates were based on the assumption that most of the observed variation in otolith $\Delta^{14}C$ was related to differences in the birth date of individual southern bluefin. The primary support for this assumption is derived from the fact that there were rapid increases in radiocarbon in tropical and temperate oceans during the 1960s and 70s and that otolith radiocarbon is a good proxy of radiocarbon in seawater DIC (Kalish 1993).

Radiocarbon data from selected regions of southern bluefin tuna indicate that this species can reach ages in excess of 30 years. Furthermore, individuals that approach the asymptotic length are likely to be 20 years of age or older. The data agree generally with accepted models of southern bluefin tuna growth derived from tagging data and with growth curves based on zone counts in otoliths across the size range of the species (Gunn et al. 1995). A detailed comparison with current growth models was not possible because of the size range of individuals used. The estimates of length at age suggest that the estimate of L_∞ is good and, furthermore, that it is not feasible to estimate age from length for southern bluefin tuna greater than about 180 cm LCF.

Peak radiocarbon levels measured in southern bluefin tuna otoliths are higher than those measured in *Pagrus auratus* otoliths from off the east coast of New Zealand, but similar to those measured in *Centroberyx affinis* otoliths from the Tasman Sea off southeast Australia. The higher level of radiocarbon in the tuna otoliths would be expected on the basis of the relative proximity of the northeastern Indian Ocean and the temperate central South Pacific Ocean to northern hemisphere atmospheric testing. Rapid transport of seawater through the Pacific-Indian Oceans throughflow region (Fieux et al. 1994) would result in effective transport of radiocarbon from the Pacific to the Indian Ocean and, ultimately, would also result in relatively high radiocarbon levels in the tropical and subtropical Indian Ocean. The ocean off southeast Australia would also be expected to receive greater quantities of bomb derived radiocarbon due to ocean transport via the East Australian Current.

There are several possible sources of error that may affect the strict interpretation of radiocarbon in southern bluefin tuna otoliths in terms of birth dates/age. Firstly, the material isolated for radiocarbon analyses is sculpted from the whole otolith. Incomplete removal of otolith calcium carbonate deposited later in life (i.e. younger otolith material) would affect the level of radiocarbon measured in that sample. For example, many of the tuna otoliths that had relatively high radiocarbon levels (>50‰), that is relatively young fish, were estimated to be older on the basis of reading otolith sections. If sculpting failed to remove all otolith material deposited after the first year of life for fish spawned in the late 1950s or 1960s, then younger otolith material with higher radiocarbon levels would "contaminate" the sample. This would result in a radiocarbon-based age estimate that was younger than the true value. The majority of discrepancies between section age and bomb radiocarbon age indicate that the radiocarbon age is younger than the section age. On this basis, contamination of the samples with calcium carbonate deposited after the first year of life and/or a slight bias to overestimate age from otolith sections must be considered as possibilities.

The inclusion of younger otolith material in a sample believed to be representative of the first year of growth may occur due to inaccurate sculpting, however, the degree of contamination required to explain the larger age discrepancies observed is not likely. A mass balance model was used to illustrate the effect of different levels of contamination on $\Delta^{14}C$ for the two samples that showed the greatest difference between bomb radiocarbon age and section age. Sample No. 203 was estimated to be 16 years of age (birth date of 1972) on the basis of $\Delta^{14}C$ (116.2±9.4‰), whereas the otolith section age was 24 years (birth date of 1964). $\Delta^{14}C$ in the eastern Indian Ocean was estimated to be about 40‰ in 1964 (see Fig. 2), well below the value measured in Sample No. 203 (Table 2). If this fish was, in fact, spawned in 1964 and the sample was sculpted without contamination, then we would expect the sample of about 20 mg to have a $\Delta^{14}C$ of 40‰. Consider the possibility that, after sample sculpting is complete, the sample contains 18 mg of material with a $\Delta^{14}C$ of 40‰ and 2 mg of material deposited after the first year of life and with a $\Delta^{14}C$ of 50‰. In this case, the sample would have resulted in a $\Delta^{14}C$ of 40.5‰. Assume each additional 2 mg of younger calcium carbonate, that replaces 2 mg of calcium carbonate deposited during the first year of life, results in a 10‰ increase in mean $\Delta^{14}C$ of the inappropriately included material. Under this scenario, it would be necessary for the sculpted Sample No. 203 to contain 2 mg of calcium carbonate with a $\Delta^{14}C$ of 40‰ deposited during the first year of life and 18 mg with a $\Delta^{14}C$ of 130‰ to obtain a $\Delta^{14}C$ of 116‰ for a southern bluefin spawned in 1964. Given the degree of care taken with the sculpting process this extent of contamination is extremely unlikely. Furthermore, this model overestimates the probable $\Delta^{14}C$ for the contaminant and it is likely to be much lower in those instances where the sample sculpting process was imprecise. A similar model was applied to the results from Sample No. 564 where bomb radiocarbon and otolith section age estimates differed by 7 years (Table 2). In order for the correct birthdate to be 1960 ($\Delta^{14}C$ of about -18‰) for Sample No. 564, it would have been necessary for the 18.2 mg sample to contain 15.5 mg of "contaminant" with a $\Delta^{14}C$ of about 90‰. These arguments assume that, although spatial variation in surface ocean $\Delta^{14}C$ can be significant over the possible range of 1 year old southern bluefin (see below), this variation is small when compared with temporal variation in $\Delta^{14}C$ during the 1960s and early 1970s. The relatively small variation in $\Delta^{14}C$ over large (primarily meridional) spatial scales is evident in Fig. 4 where data derived from the east coasts of New Zealand (*Pagrus auratus*) and New South Wales (*Centroberyx affinis*), and the eastern Indian Ocean are compared.

The likelihood of sample contamination by otolith material deposited later in life could be reduced by sculpting smaller samples. In this study, otolith sample weights for radiocarbon analyses were maintained between 13.1 and 24.4 mg (1.6 to 2.9 mg of carbon) based on the requirement for a minimum quantity of carbon to achieve a specified level of analytical precision at the AMS facility that did the analyses. Analysis of radiocarbon by AMS has developed to the point where it is possible, at some facilities, to obtain high precision analyses on samples that contain less than 0.5 mg of carbon (4 mg of $CaCO_3$). Because the ability to analyse small samples varies among laboratories, it is important to consult with the operators of AMS facilities before submitting samples for radiocarbon analysis. It is recommended that the smallest sample size that can be analyzed with high precision (<±10‰) be used for AMS analysis in those cases where the species under investigation has relatively small otoliths.

Southern bluefin tuna is a highly migratory species and appears to travel great distances during the first year of life (Shingu 1978, CSIRO unpublished data). The exact nature of these movements is not well-defined and some individuals appear to migrate south along the western coast of Australia, whereas other young southern bluefin may move in a south-westerly direction towards the coast of South Africa. Data on southern bluefin movements indicate that one year old fish can move significant distances from the spawning grounds in the tropical Indian Ocean south of Java, between about 10°S and 15°S latitude. Southern bluefin 25 cm LCF (90-120 days old) have been collected between Northwest Cape and Freemantle, Western Australia and fish between 50-55 cm LCF (one year old) have been captured off New South Wales, Tasmania, Victoria, South Australia, Western Australia and South Africa (CSIRO unpublished data). These movements would expose individual southern bluefin to varying levels of radiocarbon while the earliest formed portions of the otolith were being deposited. In addition, the rapid increase in ocean radiocarbon levels between about 1960 and 1975 would expose individual fish to temporally varying radiocarbon.

Radiocarbon data in Stuiver and Ostlund (1983) show significant latitudinal and longitudinal variation during the 1977-78 Indian Ocean GEOSECS expedition. These data can be used as a basis for understanding the distribution of bomb carbon in earlier years. The highest concentration of bomb carbon was found in the central gyre of the Indian Ocean between about 10° S and 35°S. In the eastern section of the gyre $\Delta^{14}C$ values were up to about 140‰, but were slightly lower in the western portion of the gyre. Far greater variation in $\Delta^{14}C$ values was found with latitude. In 1977-78, maximum $\Delta^{14}C$ values of around 140‰ in southern tropical latitudes of the Indian Ocean decreased to about 28‰ (eastern Indian Ocean) and -14‰ (western Indian Ocean) at around 50°S latitude, the southern extent of the range of adult southern bluefin. The range of $\Delta^{14}C$ values was much less at the southernmost extent of the distribution of 1 year old southern bluefin at around 35°S latitude. In 1977-78, $\Delta^{14}C$ levels were about 98‰ at 35°S latitude in both the eastern and western Indian Ocean.

As juvenile southern bluefin migrate south or southwest from the tropical Indian Ocean the growing otolith will integrate radiocarbon levels over a broad temporal (months) and spatial (1000s of km) scales. Because both the path taken by individual tuna and the temporal and spatial variation in surface ocean radiocarbon over time will vary, individual otoliths will incorporate different levels of radiocarbon, regardless of the birth date of individual fish. The effect of this variation can be reduced by sculpting smaller otolith samples for radiocarbon analysis, thereby limiting the range of possible movements by young fish as they move from the spawning grounds.

Fish species incorporate different levels of carbon isotopes into the $CaCO_3$ of the otoliths and these differences may be linked to metabolic rate effects (Kalish 1991). Southern bluefin tuna otoliths have been shown to be relatively depleted in ^{13}C compared with otoliths from other non-scrombrid fishes. This fractionation of carbon isotopes in the otoliths is likely to be reflected in the incorporation of ^{14}C, however, these effects have been considered in this study as the calculation of $\Delta^{14}C$ accounts for fractionation (Stuiver and Polach 1977).

Southern bluefin tuna otoliths for this study were selected at random from large fish sampled by CSIRO researchers and collaborators. The sample is not adequate to estimate the range of ages present in the population of southern bluefin tuna, but it does suggest that a large percentage of the fish greater than 180 cm FL are at least 20 years of age and that southern bluefin can live to ages in excess of 30 years. Furthermore, the results show that reading otolith sections is an effective method to estimate the age of larger southern bluefin. The analysis of additional samples could provide data relevant to defining the age structure of larger southern bluefin tuna and more precisely define the accuracy of age estimation by the reading of otolith sections.

Acknowledgments

We thank the numerous observers who have worked at sea on longline vessels to collect samples from southern bluefin tuna. This research was supported by a grant from the Fisheries Research and Development Corporation.

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Table 1. Measurements of $\Delta^{14}C$ made on tropical Indian Ocean surface water samples or corals from latitudes comparable to locations of juvenile *Thunnus maccoyii* otolith deposition. DIC is dissolved inorganic carbon.

Latitude	Longitude	Sample type	Sample date	$\Delta^{14}C$ (‰)	Reference
12°S	97°E	hermatypic coral	July 1953	-30	Toggweiler et al 1991
12°S	97°E	hermatypic coral	July 1970	118	Toggweiler et al 1991
12°S	97°E	hermatypic coral	July 1972	129	Toggweiler et al 1991
12°S	97°E	hermatypic coral	July 1973	130	Toggweiler et al 1991
12°S	97°E	hermatypic coral	July 1974	134	Toggweiler et al 1991
12°S	97°E	hermatypic coral	July 1976	121	Toggweiler et al 1991
10°31'S	105°34'E	seawater DIC	19 Oct 1960	-16	Bien et al. 1965
18°49'S	88°33'E	seawater DIC	27 Nov 1960	-7	Bien et al. 1965
36°18'S	98°41'E	seawater DIC	29 Dec 1960	-25	Bien et al. 1965
33°14'S	108°45'E	seawater DIC	1 Jan 1961	-15	Bien et al. 1965
34°11'S	105°49'E	seawater DIC	25 Nov 1962	20	Bien et al. 1965
29°15'S	109°58'E	seawater DIC	8 Mar 1978	140	Stuiver and Ostlund 1983

Table 2. Southern bluefin tuna (*Thunnus maccoyii*) fish and otolith data. Sample weights indicate the weight of otolith material separated for individual analyses of stable carbon and radiocarbon and are representative of less than the first year of otolith growth for an individual fish. The birthdate was determined as discussed in the text. The age is the date caught minus the birthdate determined from the radiocarbon data.

Sample No.	Date caught	Fork length (cm)	Otolith wt. (g)	Sample wt. (mg)	$\delta^{13}C$ (‰,PDB)	$\Delta^{14}C$(‰)	Birth date (years, A.D.)	Age (years)	Otolith section age (birth date)
190	26/11/88	195	0.1768	19.7	-6.49	29.3±8.7	1963	25	25 (1963)
195	2/12/88	190	0.1802	17.6	-8.38	119.7±9.6	1972	16	
203	2/12/88	185	0.1578	19.2	-6.70	116.2±9.4	1972	16	24 (1964)
529	12/7/89	180	0.1465	19.1	-7.84	41.2±9.7	1964	25	26 (1963)
552	25/6/89	180	0.1523	19.8	-7.16	68.1±9.2	1966	23	26 (1963)
564	4/7/89	195	0.1695	18.2	-7.32	78.5±10.1	1967	22	29 (1960)
584	9/7/89	195	0.1851	19.5	-8.42	69.6±8.5	1966	23	
598	15/7/89	182	0.1861	12.3	-7.26	75.2±10.7	1967	22	27 (1962)
642	29/6/89	186	0.2353	24.4	-8.18	-38.8±7.1	1958	31	
753	16/6/89	190	0.1863	18.6	-7.26	55.2±9.9	1965	24	
2278	24/2/93	55	0.0135	13.8	8.53	80.0±8.6	1992	1	
4670	11/11/93	184	0.1550	13.1	-6.59	101.0±11.0	1970	23	24 (1969)
4682	18/11/93	180	0.1774	15.6	-6.79	88.2±10.8	1968	25	23 (1970)
4693	18/11/93	185	0.1684	19.6	-7.33	73.8±10.4	1967	26	27 (1966)
4698	14/11/93	201	0.1719	15.8	-7.58	50.1±9.4	1965	28	
4732	18/4/85	55	0.0140	14.2	-9.51	115.1±9.2	1984	1	
6288	6/11/94	190	0.219	16.8	-6.8	71.1±10.2	1967	27	30 (1964)
6289	3/12/94	194	0.2046	19.8	-6.8	37.2±11.9	1964	30	26 (1968)
6290	28/12/94	189	0.2835	19.8	-7.1	85.7±10.7	1968	26	28 (1966)
6291	6/12/94	196	0.2773	19.2	-7	-18.1±8.8	1960	34	34 (1960)
6292	6/12/94	196	0.2402	19.8	-7.8	73±8.6	1967	27	28 (1966)
6293	26/12/94	199	0.2093	19.9	-7.4	54.2±8.6	1965	29	29 (1965)

Figure captions

1. *Thunnus maccoyii.* Comparison among, from left to right: whole sagitta (219 mg) from 190 cm southern bluefin, sculpted sagitta (18.6 mg) from 190 cm southern bluefin , whole sagitta (14.2 mg) from 55 cm southern bluefin.

2. *Thunnus maccoyii.* Data and function used to provide a calibration for Indian Ocean $\Delta^{14}C$ values during the increase in bomb-derived radiocarbon. Data points prior to 1980 were derived from measurements of dissolved inorganic carbon in surface seawater (Bien et al. 1965, Stuiver & Ostlund 1983, Toggweiler et al 1991). Data points from 1984 and 1992 were determined from measurements of radiocarbon in whole otoliths of 1 year old southern bluefin tuna. The polynomial function is: $\Delta^{14}C = -1759667 + 1778\,(\text{year}) - 0.45\,(\text{year}^2)$.

3. *Thunnus maccoyii.* Radiocarbon data from southern bluefin tuna otoliths, Cocos-Keeling Island hermatypic coral (Toggweiler et al. 1991), and GEOSECS (Geochemical Ocean Section Study) DIC (dissolved inorganic carbon) (Bien et al. 1965, Stuiver and Ostlund 1983). The date of calcification for the southern bluefin tuna data was determined on the basis of a second order polynomial function described by the post-bomb coral and GEOSECS radiocarbon data, as well as 2 small southern bluefin tuna with birthdates of 1983 and 1991.

4. *Thunnus maccoyii.* $\Delta^{14}C$ of southern bluefin tuna otolith cores plotted against the birth date determined from radiocarbon levels. $\Delta^{14}C$ data from *Pagrus auratus* otolith cores are plotted against the true birth date (Kalish 1993) and $\Delta^{14}C$ values from *Centroberyx affins* (Kalish 1995b) are plotted against birth dates determined from reading otolith sections. For southern bluefin tuna, *Pagrus auratus*, and *Centroberyx affinis* $\Delta^{14}C$ values are based on otolith material deposited over a time period equivalent to about the first year of life. Errors are ±1 sd.

5. *Thunnus maccoyii.* Differences (years) between age estimates determined from radiocarbon data and reading otolith thin sections.

6. *Thunnus maccoyii.* $\Delta^{14}C$ values versus birth date estimates for southern bluefin tuna from otolith readers plotted with $\Delta^{14}C$ data versus birth date data for *Pagrus auratus*.

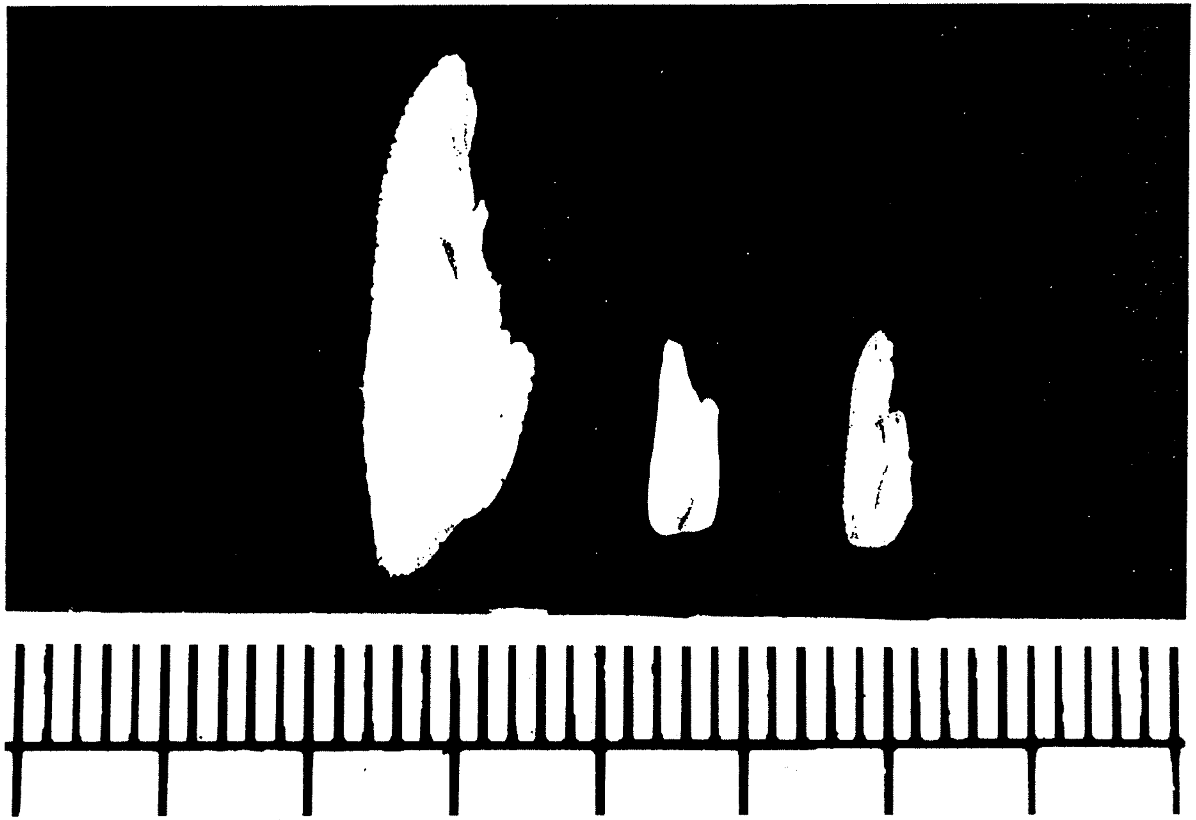


Figure 2

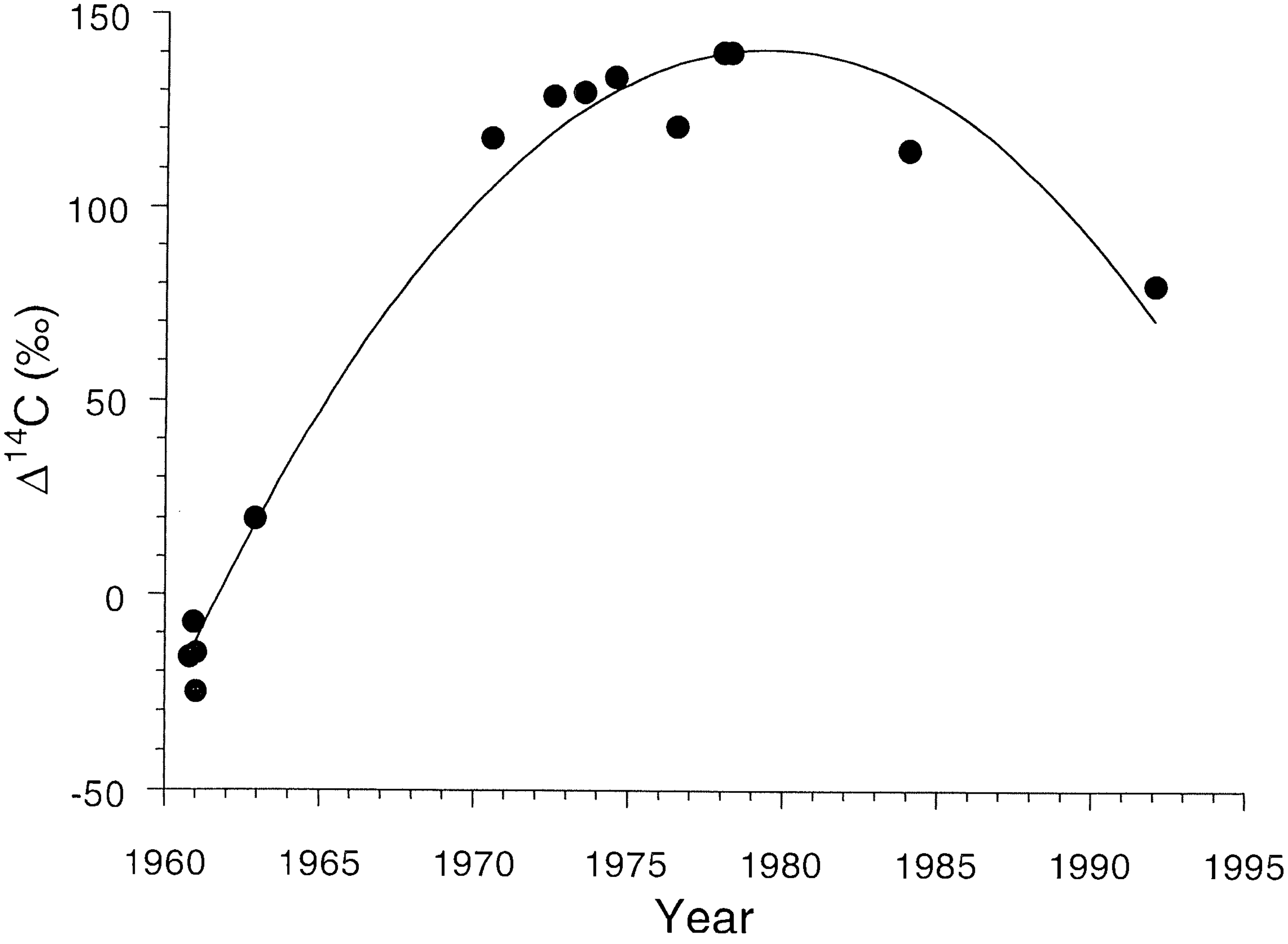
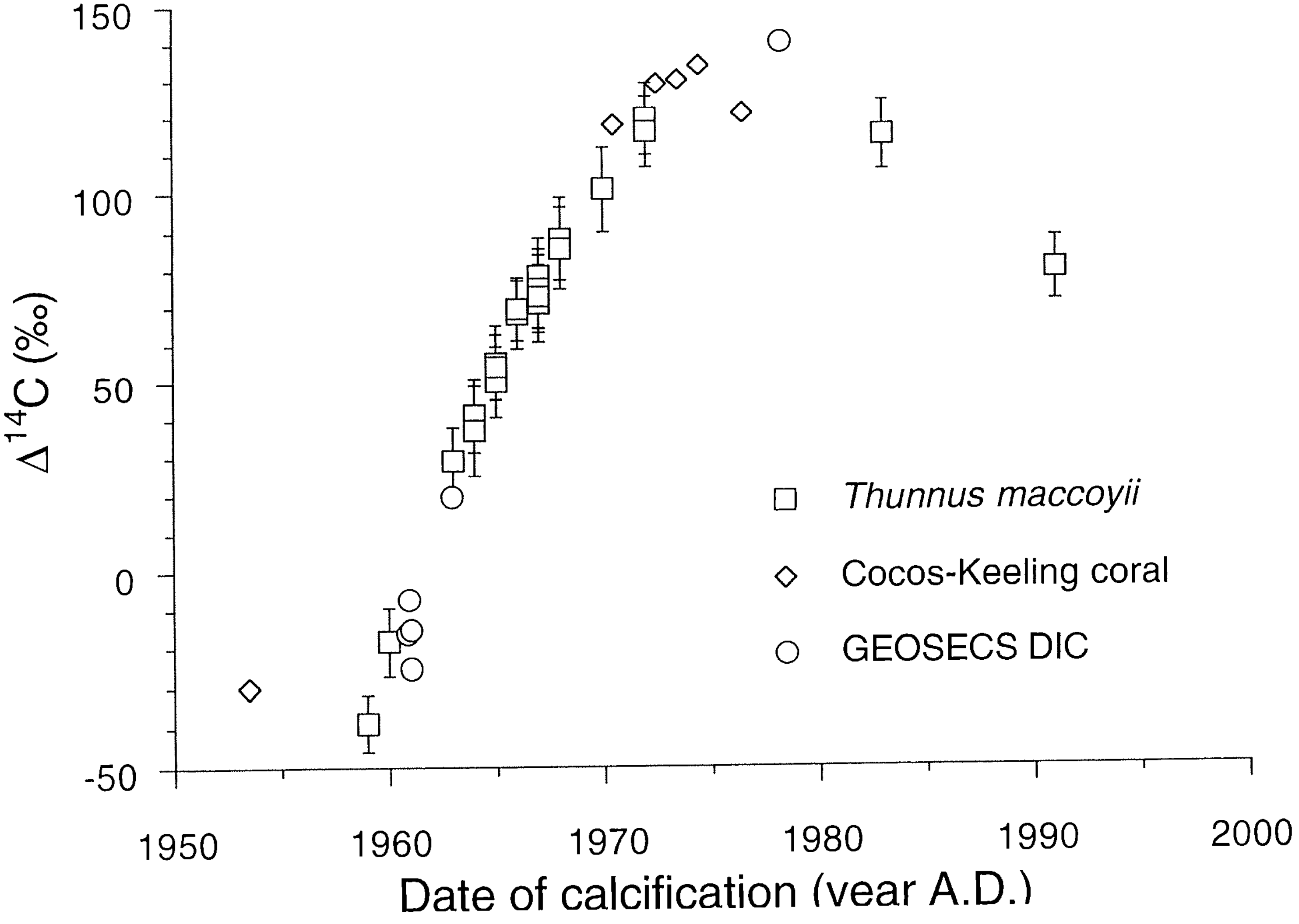
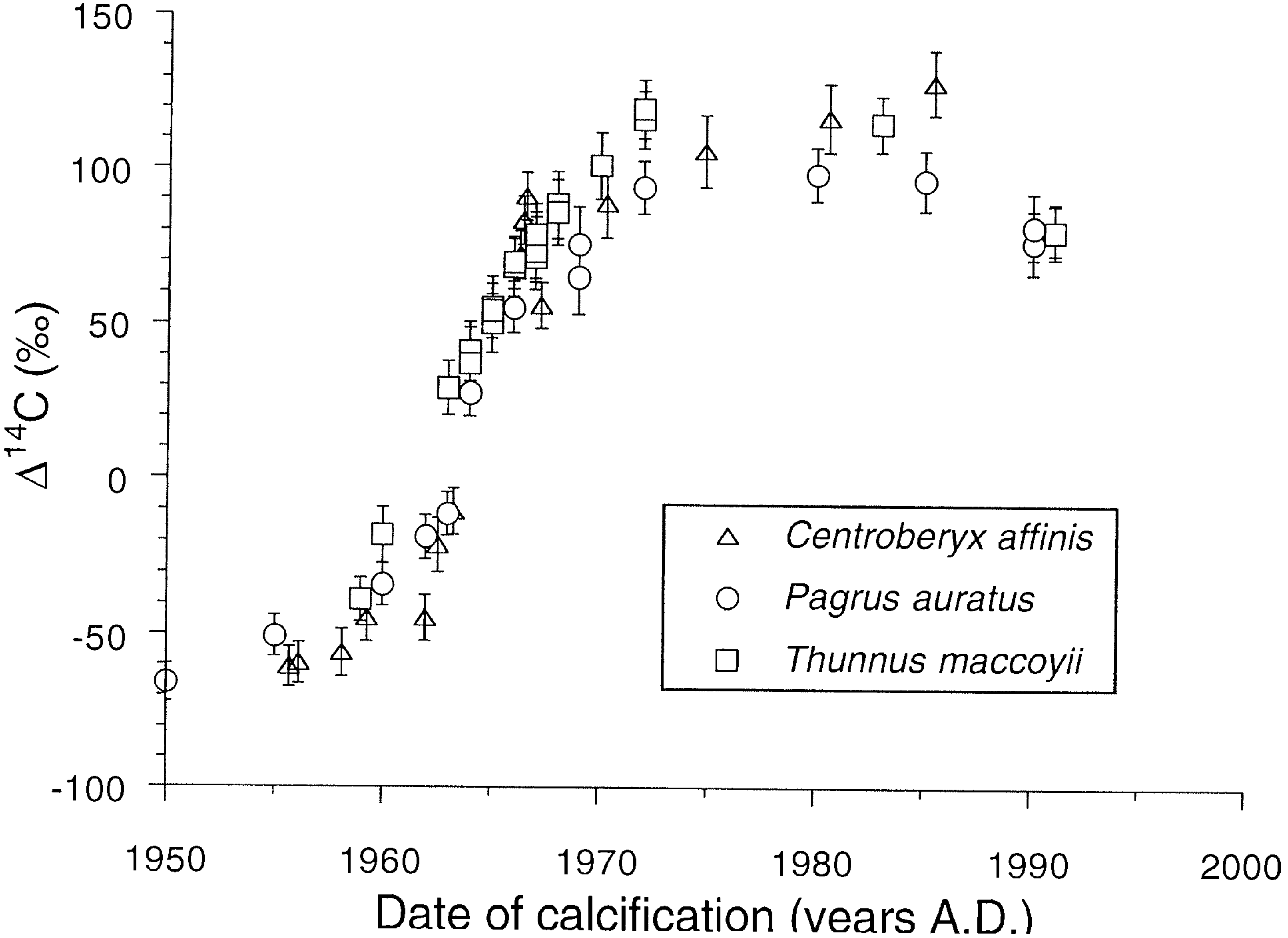

150
100
50
0
-50
Δ14C (‰)
1960
1965
1970
1975
1980
1985
1990
1995
Year

Figure 3



150
100
50
0
-50
Δ14C (‰)
1950
1960
1970
1980
1990
2000
Date of calcification (year A.D.)
□ *Thunnus maccoyii*
◇ Cocos-Keeling coral
○ GEOSECS DIC

Figure 4



150
100
50
0
-50
-100
Δ14C (‰)
1950
1960
1970
1980
1990
2000
Date of calcification (years A.D.)
△ Centroberyx affinis
○ Pagrus auratus
□ Thunnus maccoyii

Figure 5

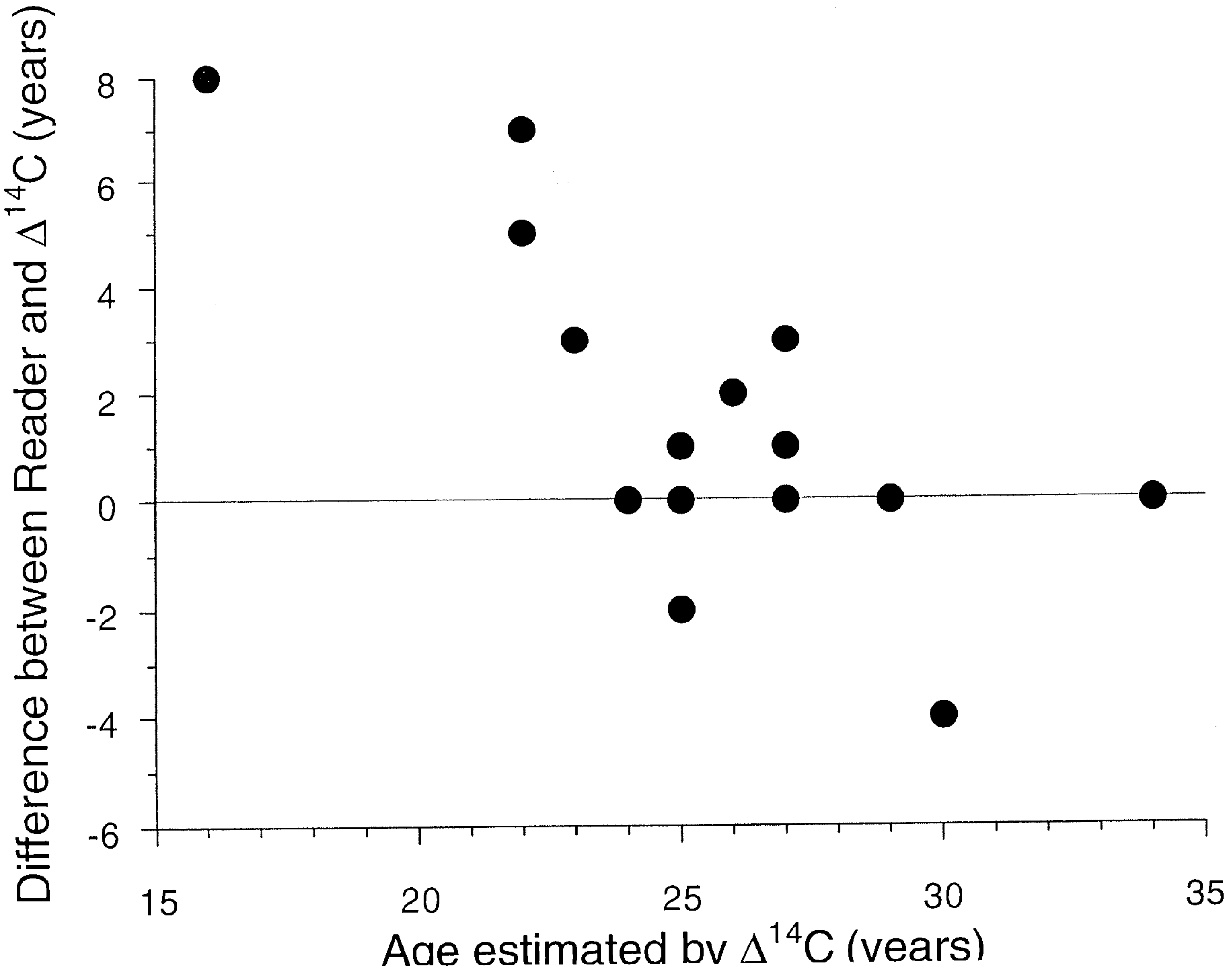
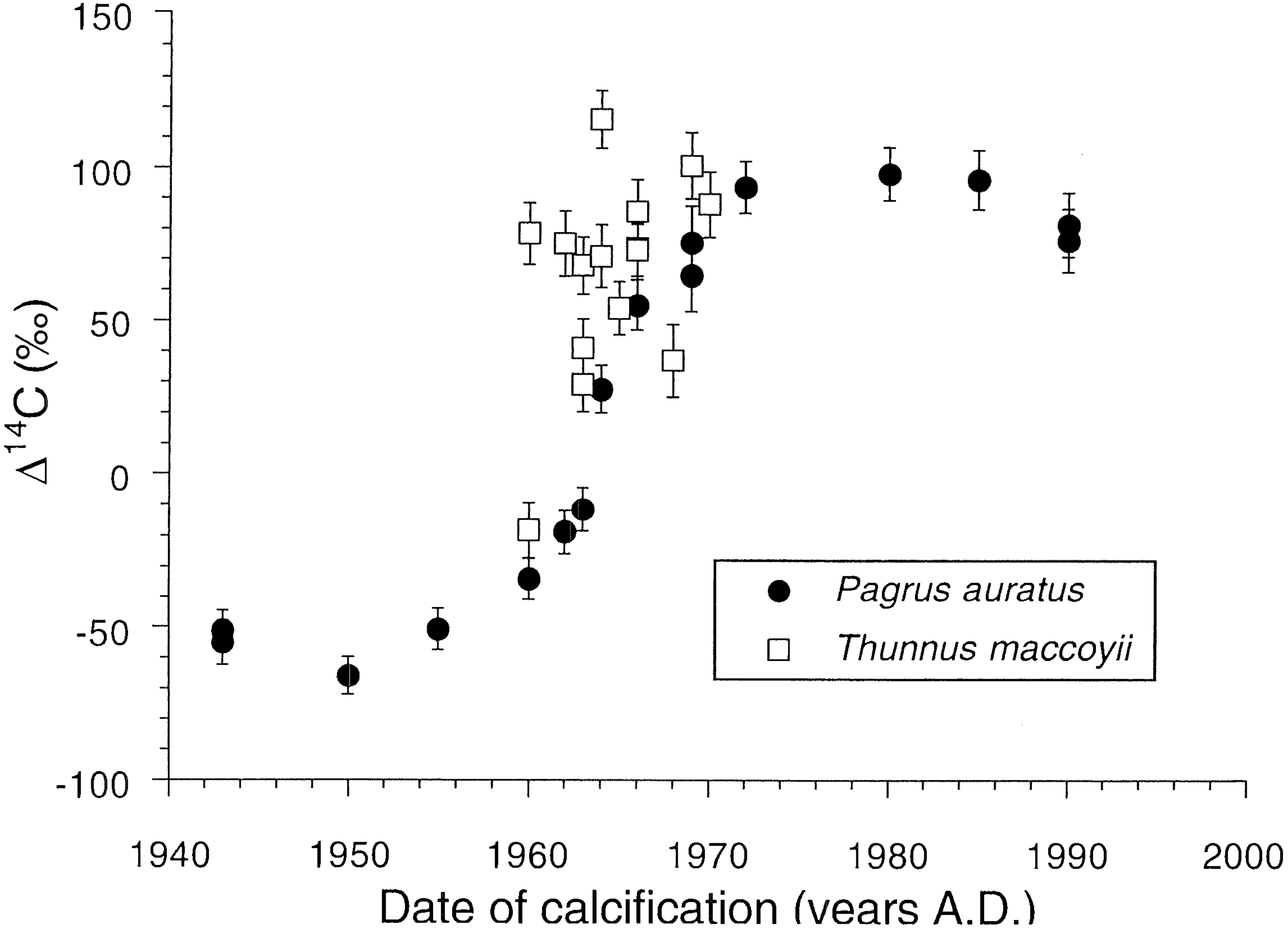

Difference between Reader and $\Delta^{14}C$ (years)
8
6
4
2
0
-2
-4
-6
15
20
25
30
35
Age estimated by $\Delta^{14}C$ (years)

Figure 6


150
100
50
0
-50
-100
$\Delta^{14}C$ (‰)
1940
1950
1960
1970
1980
1990
2000
Date of calcification (years A.D.)
● *Pagrus auratus*
□ *Thunnus maccoyii*

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 4

Project 92/42

The direct estimation of age and growth in Southern Bluefin Tuna, *Thunnus maccoyii* (Castelnau), using otoliths, scales and vertebrae.

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Key words: otolith, vertebrae, annual banding, validation, longevity, growth.

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Abstract.

Introduction.

Over the last three decades, southern bluefin tuna (SBT), *Thunnus maccoyii*, have been the target of large and very lucrative longline fisheries throughout the southern Atlantic, southern Indian and Southern Oceans, and surface fisheries around the southern coast of Australia (Caton 1991). Scientists from Japan, Australia and New Zealand agreed in 1983 that SBT stocks were in decline and as a result, catch quotas were set. In 1993, ten years after the introduction of catch restrictions, and with quota reduced to 11,650 tonnes (37.5% of the first agreed global quota, set in 1986), scientists determined that the SBT parental stock was at historically low levels ([1] Anon, 1994). Recently however, uncertainties within assessment methodologies have resulted in conflicting interpretations on the status of the stocks, a situation that promotes debate on the optimal quota levels.

The primary method used to assess SBT stocks over the past decade has been virtual population analysis (VPA) ([2] Polacheck et al., 1996). The primary input into these analyses is the catch-at-age distribution of the fishery. As validated direct ageing data is available for only the first two to three age classes, the age distribution of the catch has been estimated from the conversion of lengths and weights to ages based on growth curves derived from tagging and length frequency data (Hampton, 1991; [1] Anon, 1994). These conversions introduce a large degree of unmeasurable uncertainty into the catch-at-age distribution and tend to dampen and smooth out differences over time in the relative proportion of different year classes within the catch ([3] Hearn, 1990). Large and unmeasurable uncertainties in the catch-at-age distribution translate into uncertainties in the assessment results. Thus, an improved understanding of the age and growth of the species has been a high priority within the SBT assessment process for a number of years and the current study was developed to provide length-at-age estimates for all age classes in the population.

There have been three previous studies in which the age and growth of SBT have been estimated directly from hardparts. Robins (1963) used scales from fish of 30-80 cm fork length (FL) to estimate the age of recruitment of SBT to surface fisheries in Western Australia. Although no attempt was made to validate the readings, Robins concluded that SBT recruit at two years of age. Hynd (1965) later supported Robin's findings using length frequency data from the Western Australian fishery.

Yukinawa (1970) used scales to estimate the age of fish from 38-184 cm fork length. Although he found scales from fish larger than 153 cm very difficult to "read", Yukinawa (1970) produced a growth curve that had growth rate and L_∞ parameters very close to those subsequently derived from tagging experiments (Kirkwood, 1983). Yukinawa used a form of marginal increment analysis to demonstrate that checks in the scales of 1 to 8 year-old SBT formed annually.

Thorogood (1987) was the first to use slow growth zones on otoliths to estimate the age of SBT. Using otoliths of fish from 42-167 cm FL. (the majority were smaller than 110 cm), Thorogood's age estimates and von Bertalanffy growth parameters were in close agreement with those of Yukinawa (1970). Thorogood used marginal increment analysis to validate the annual formation of slow growth zones in otoliths from fish he assigned ages of 2-4 years.

Robins (1963), Yukinawa (1970) and Thorogood (1987) provided age estimates for only a fraction of the size and age ranges within the SBT population. The bulk of the samples they analysed were from small, immature fish, mostly caught in Australian surface fisheries. They had been unable to estimate the age of pre-recruits or the large, mature fish targeted by high-seas longline fisheries. With longline fisheries accounting for more than half of the global SBT catch, significant uncertainty remained over the age composition of this component of the catch-at-age matrix used within VPA's.

Compounding the uncertainties in the catch-at-age matrices, in the absence of a validated age-length key, estimates of many of the key life history parameters used in assessment process (e.g. natural mortality, maximum age and age at maturity) were based on conversions of length to age using the growth curves of Kirkwood (1983).

Recognising the serious inadequacies in previous work and the critical requirement within the SBT assessment process for accurate data on age and growth of SBT, we initiated a broad-ranging study with the following objectives:

1. Develop a method for sampling otoliths from sashimi-grade SBT without damaging the fish and reducing market value.
2. Conduct a large-scale sampling program on high seas-caught SBT from throughout the species size range.
3. Develop and evaluate a range of techniques for the direct ageing of juvenile and adult SBT, and compare age estimates based on vertebrae, otoliths and scales.
4. Through a large-scale mark-and-release experiment, validate annual periodicity in the formation of bands on the otoliths of SBT for as many year classes as possible.
5. Use validated methods to determine the age-at-recruitment of SBT to the Western Australian surface fishery, age at maturity, age distribution of spawners, longevity, catch-at-age distribution for one of the principal SBT fishing grounds, off Tasmania, Australia.

This is the forth paper from the study and it details the estimates of age and growth from scales, otoliths and vertebrae. These data are used to estimate longevity, age-at-maturity and age distributions of catches throughout the fishery.

Other papers from the study report on the validation of annual formation of slow growth zones in otoliths (Kalish et al., in press; [4] Clear et al. in prep.), the estimation of age of mature size classes using a bomb-radiocarbon chronometer (Kalish et al., in press) and the estimation of the size-at-age 1 and age at recruitment using otolith microstructure ([7] Rees et al., in prep.). Since 1994, the new data on age and growth produced by the study have had a significant impact on the assessment of SBT stocks ([5] Anon, 1996).

Methods and Materials

Sampling

Samples of otoliths, vertebrae and scales were collected throughout the species' range from catches by four fishing nations. Table 1 provides a summary of the collections and sizes of fish from each sampling area.

An important breakthrough in this study was negotiating permission from Japanese longline operators to collect samples of otoliths and vertebrae from their catches. SBT are a very valuable, sashimi-grade product and in Japanese sashimi markets the external appearance of a fish is an important element in evaluation of market value. Thus, it is not possible to collect otoliths from SBT using the standard method of cutting through the cranium with a knife or hack-saw to expose the semi-circular canals. To avoid external damage, we used a hole saw fitted to a cordless electric drill to remove the underside of the cranium. The sagittal otoliths lie immediately above and slightly anterior to the basi-occipital plate, which is exposed after the removal of the gill arches. To remove the otoliths, the hole saw is driven from each side of the basi-occipital plate at approximately a 45 degree angle towards the orbit. This produces a core containing the cranium and, if the axis is accurate, also the otoliths (Fig. 1).

Removing the otoliths this way affects neither the external appearance of the fish nor its value. The method is now well-accepted by Japanese fishermen and over the last eight years 6000+ sets of sagittal otoliths have been collected from fish caught by Japanese longliners. A high powered drill can be used in the same way to collect otoliths from frozen fish.

Wherever possible, scales, vertebrae and otoliths were collected from the same fish, to allow comparison of the age estimate from each structure. Fork length was measured to the nearest cm and, in a subset of samples, fish weight was measured to the nearest 0.1 kg.

In the laboratory, sagittal otoliths were cleaned, weighed to the nearest 1 mg and stored dry. Vertebrae were stored frozen and scales cleaned and stored dry.

Otolith preparation and reading techniques

Otoliths from fish less than 135 cm FL could generally be read whole. Increments on the otoliths comprised two zones: an opaque zone (assumed to be fast growth) and a narrower, translucent zone (assumed to be slow growth) which appeared dark under a dissecting microscope with reflected lighting and a black background. Using a modification of Thorogood's (1987) method, one of each pair of sagittae was burned until it turned golden brown on a 400°C hot plate. The colour change was greater in the translucent zones making them more obvious despite having lost some translucence. The first two increments deposited on the otolith were often broader and more vague than subsequent increments in which the translucent zones were discrete. Increments were most obvious on the rostral and post-rostral axes (Fig. 2) but the translucent zones could usually be followed on the otolith surface between these axes, parallel to the ventral margin.

On whole otoliths counts were made from the primordium to the margin along the rostral and post-rostral axes and, from these counts, the age was estimated. Each estimate was assigned a “readability” which indicated the number of interpretations of the increments the reader could make. Measurements were made of the otolith length, the length of the rostral and post-rostral axes and the distance between the primordium and the inside of each translucent zone (the outer margin of the zone was often difficult to identify in otoliths from small fish) along the rostral and post-rostral axes.

Otoliths from all fish greater than 135 cm FL and from some fish between 95 and 135 cm FL were sectioned following the methods of Berry et al. (1977). Four 0.3-0.4 mm thick serial transverse sections were made, which usually provided at least one section through the primordium (Fig. 3). Increments were counted under a compound microscope using transmitted light, and a confidence from 0 to 5 was recorded for each age estimate. Around 1% of attempted readings were unsuccessful, these being the estimates assigned a confidence of 0 and whole sagittae that were considered unreadable.

The appearance of the otolith sections was similar to that described previously from Atlantic bluefin tuna (Berry et al., 1977, Hurley and Isles, 1983). Both arms of the sections displayed translucent and opaque zones which appeared as alternating light and dark zones but the longer arm (medio-ventral ridge) gave consistently higher counts than the shorter arm (medio-dorsal ridge) so we routinely used the long arm for age estimates.

The appearance of the increments was not consistent along the arm, with the first 4 or 5 nearest the primordium difficult to interpret, as they were broad, diffuse and the translucent zone was not distinct. From 5 to 15 the increments became more condensed and slightly easier to read. In otoliths from larger fish the distal increments (above 20) were usually regular with distinct light and dark zones and much easier to count however, we sometimes saw “pairs” of dark zones where two dark bands were closer than normal (Fig. 4). If these bands coalesced at the margin of the section we counted them as part of the same increment otherwise we counted two increments. By focusing up and down through the section, we could generally determine if the area contained one or two increments. These may be equivalent to the bands described by Berry et al. (1977) who hypothesised that a pair of these paired bands comprised an increment, representing annual growth. We saw no evidence to support the hypothesis that two opaque and translucent zones are deposited per year and the uniformity of the increments in the distal region of the otoliths of large fish substantiated our method of counting. An age estimate of up to 10 more years results from counting each of the increments but the higher counts are validated by the close agreement between increment counts and bomb-radiocarbon age estimates (Kalish et al., in press).

Each otolith was read at least twice by the principal reader (OR1); the interval between readings being no less than 3 months. All readings were made without reference to the length or weight of the fish, its date or location of capture, or to the previous reading(s). To examine inter-reader consistency, sub-samples of otoliths were also read “blind” by two less experienced readers (OR2 and OR3) following a brief period of training during which the counting criteria employed by OR1 were demonstrated using both straightforward and “problem” otoliths.

The level of intra- and inter-reader consistency in otolith readings was quantified using the average percentage error (APE) method of Beamish and Fournier (1981).

The methods used to validate the annual periodicity of band formation in SBT otoliths have been described and discussed by [4] Clear et al. (in prep.) and Kalish et al. (in press). These studies have concluded that slow growth zones are formed once per year, characteristically during the late winter and autumn months, throughout the life of SBT.

Vertebrae preparation and reading techniques

The 35th and 36th caudal vertebrae were used for age estimation. Prior to reading, vertebrae were cleaned, dried and stained following the methods Lee et al. (1983). After staining, vertebrae from fish less than 120 cm were read whole, while vertebrae from larger fish were either cut in half, or a segment was removed to allow light to the fall on the band structure at the optimal angle. In the bulk of the samples collected, both the 35th and 36th caudal vertebrae were available and the anterior surface of the 35th was used preferentially for measurement and interpretation. Where difficulties in interpretation of bands were encountered, both anterior and posterior surfaces of the vertebrae were used to assist in identification and counting of bands.

The criteria described by Lee et al. (1983) were used for counting vertebral bands. The radius of each band (from the core to the inside of the band) and the total cone radius (from the core to the outside edge) were measured to the nearest mm. As SBT vertebrae are not round in cross section, the radial measurements were made on a set axis on the medio-lateral internal surface of the cone.

All vertebrae were read at least twice by the principal reader (VR1), with replicate readings conducted blind after intervals of at least 4 weeks. The APE of replicate readings by VR1 was estimated using the method of Beamish and Fournier (1981). A sub-sample of vertebrae were read by a less experienced reader (VR2) following training in the counting criteria used by VR1. APE's between the readings of VR1 and VR2 were then estimated to measure inter-reader consistency.

Scale preparation and reading techniques

Scales were removed either from under the pectoral fin (fish up to 80 cm) or from the caudal peduncle (some fish over 80 cm). In the laboratory, after cleaning and drying, between 6 and 10 scales were mounted between glass slides. These were then examined under a profile projector, which throws a magnified image onto a ground-glass screen.

Annual marks (annuli) were identified following the techniques of Ricker (1971). The most useful criteria for identification of annuli were the crowding of the circuli to form a dense band and the association of annuli with lateral breaks on individual circuli. On the clearest scale from each batch examined, the oral radius of each annulus was measured directly from the ground glass screen.

Results and Discussion

Otoliths

Relationship between otolith size and fish length and age.

The relationship between otolith size and fish size was examined by measuring the total length and weight of the left or right sagitta of 551 fish, ranging from 30-216 cm fork length. Otolith length was linearly related to fish length (Fig. 5); $r^2 = 0.951$ (although a second order polynomial provided a marginally better fit $r^2 = 0.959$), while otolith weight and fish length had a curvilinear relationship (Fig. 5 - cubic polynomial fit $r^2 = 0.903$). Although the goodness of fit was high in the latter, there was considerable variation in the weight of otoliths of fish over 160 cm.

Otolith weight was also strongly correlated with fish age (Fig. 5), although the variance in otolith weight-at-age makes otolith weight an imprecise predictor of age in SBT, particularly at otolith weights greater than 125 mg and above an age of 10-12 years. Although there is clear evidence that otoliths do continue to grow in weight (size) throughout the life of SBT, from approximately 10-12 years of age, the rate of increase in otolith weight is slower than in younger fish.

Validation of annual formation of bands in otoliths

The annual formation of bands in SBT otoliths has been validated by [4] Clear et al (in prep.) using a large-scale mark and recapture experiment and Kalish et al (in press) using bomb radiocarbon chronometry. These studies have demonstrated that the bands we have counted, for age classes 1-6 in whole otoliths, and for age classes 6 and above in sectioned otoliths, are formed annually, throughout life.

Precision of age estimates

APE's among replicate readings by OR1 were low; 2.59% was the mean for all age classes combined (n=1048). There was no relationship between fish age and the level of precision of estimates by OR1 ($r^2 = 0.036$). A subsample of otoliths were read blind by two other readers, OR2 and OR3. OR2 received two week's training from OR1, while OR3 received limited training and was essentially left to read the otoliths blind on the basis of agreed counting criteria and some brief discussion of a small sample of otoliths. OR2 made three replicate readings of 95 otoliths, all from large fish. The APE for these readings was 4.21%. OR1 then read these otoliths blind and the APE among the two reader's age estimates was 3.92%. OR3 read 30 otoliths on two occasions, separated by 12 months. The APE between these replicate readings was 8.47% while the APE between OR1 and OR3 for this subsample was 9.44%.

The very low APE's compare favourably with estimates of precision for other tunas (Lee and Prince, 1995; Foreman, 1996) reaching similar ages. The APE's achieved on blind counts indicate that the counting criteria developed by OR1, and subsequently learned by OR2, allow repeatable counts of the number of slow growth zones in both whole and sectioned otoliths. With the validation that these bands are formed annually, the counting criteria provide a reliable method of estimating age using SBT otoliths. The comparatively high intra- reader APE for OR3, and the level of discrepancy between OR3 and OR1 demonstrate the difficulty untrained readers have in using SBT otoliths to estimate age.

The timing of translucent, or slow growth, band formation.

The majority of the 1131 otoliths examined in this study were sampled during the southern hemisphere winter from high seas' longline catches. Thus, there was little scope for examining the seasonality of band formation for most age classes. However, there were sufficient samples of two-year-old fish to examine the change in marginal increment width (i.e. the increment between the completion of the translucent or slow growth band and the edge of the otolith) over a twelve month period (Fig. 7). Although we had no samples from June or July, the significant fall in marginal increment width between May and August suggests that the slow growth band forms during the late winter. This is slightly different from the findings of Thorogood (1987), who concluded that the peak in marginal increment was in June-July, followed by a slow growth period from August to November. The difference appears to relate to what was being measured rather than changes or differences in either the period of slow growth or otolith structure. Thorogood measured the marginal increment as the growth between the outside edge of the translucent zone and the outside edge of the otolith, while our measurements were taken from the inside margin of the translucent zone to the outside edge. Thus, if the slow growth period began in June-July, using Thorogood's method one would expect this to be the peak in marginal increment while it would be the minimum point in our data. As this is what was observed, both data sets indicate that the slow growth band in 2 year old fish is deposited over the period during which water temperatures off Southern Australia are lowest.

A translucent zone was observed on the margins of otoliths from most fish caught in June to October. Opaque zones on the otolith margins were smallest in fish caught in November and, from the increase in the growth of the opaque zone of the marginal increment, we estimated the period between February and April to be the time when the opaque zone is deposited most rapidly and hence, the period of fastest otolith growth.

Age estimates

Estimates of age were made from the otoliths of 1,131 fish, ranging in fork length from 26-216 cm. Mean and standard deviations of the length-at-age for age classes 1-34 inclusive, and ages 36, 39 and 41, are given in Table 2. The oldest fish within our samples was 41 years old.

It is clear from these data that SBT can live well in excess of 30 years. This finding is in agreement with the recent work of Kalish et al. (in press) in which bomb-radiocarbon chronometry determined that SBT caught in the late 1980's and early 1990's were spawned in the late 1950's and early 1960's.

Prior to this study and the related work by Kalish et al. (in press), 20 years was thought to be the maximum age of SBT. This estimate was based on the recapture of a single tagged fish, 18 years after its release as a two year old . As so often happens in population dynamics and assessment, in the absence of any data to the contrary, the 20-year longevity has been used for over a decade as the basis for estimates, or assumptions, on the natural mortality rates of SBT used in tuning VPA's. Our new data effectively doubles the longevity estimate for the species and suggests that natural mortality rates are significantly lower than previously estimated. The new longevity estimates have forced an adjustment of natural mortality estimates used in the assessment of SBT stocks by the CCSBT Scientific Committee and recently this body has begun consideration of age-specific natural mortality rates in the species. We suggest this is an important topic for further investigation and that the validation of age estimates from throughout the life span of SBT provide a means of estimating natural mortality directly from data on the population demographics.

Sexual dimorphism in length-at-age.

The otolith data provides some evidence of sexual dimporhism in length-at-age of SBT. Although females appear to live as long as males (Fig. 8), length frequency data collected by high-seas observers indicate that fish larger than 180 cm (Fig. 9a) are more likely to be male than female; in fish above 185 cm, the ratio of males to females in greater than 2:1. A similar pattern is evident in the sex ratio of large fish for which we have otolith samples (Fig. 9b). Unfortunately, only a small proportion of our otoliths were sampled from fish of known sex and, as a result, it is not possible to compare statistically for individual age classes the mean length-at-age for the two sexes. If the data are aggregated into ten-year-class groupings, males have a significantly higher mean length-at-age for in both the 20-29 and 30-39 year-old categories; mean differences between female and male being -6.262 cm ($t = -2.482$, $p = 0.0183$, DF = 33) and -6.879 cm ($t = -2.142$, $p = 0.0461$, DF =18), respectively. There was no significant difference in the 10-19 year-old class.

Similar dimorphism has also been described in the growth rates of the Atlantic bluefin tuna, *Thunnus thynnus* (Butler et al., 1977; Hurley et al., 1981; Hurley and Isles, 1983). The lower growth rates of females in the two bluefin species are most likely due to the combined effects of high fecundity (Farley and Davis, in press - SBT; Baglin, 1982 - ABT), long reproductive life - in SBT this can be in excess of 20 years ([5] Gunn et al., 1996b) and very long migrations from feeding grounds in temperate waters to spawning grounds in the tropics (Farley and Davis, in press - SBT; Clay, 1991 - ABT). Sexual dimorphism in size-at-age has not been recorded in yellowfin, *Thunnus albacares* (Wild and Foreman, 1980) or albacore, *Thunnus alulunga* (Labelle et al. 1993). These two species have very different life histories to the bluefins; they are relatively short lived and thus have significantly shorter reproductive lives and while migratory they are not known to undertake long spawning migrations annually.

Growth

As there was insufficient data with which to examine the hypothesis that growth in males and females is different, a von Bertalanffy growth curve was fitted to length-at-age data pooled from both sexes (Fig. 10). The von Bertalanffy growth parameters are given in Table 5, where they are compared to estimates for previous studies of growth in the species. The growth estimates within the literature are based on two kinds of data; Anon (1994), Kirkwood (1983) and Murphy (1977) used tagging or length frequency data, or a combination of both. The other studies used hard parts in the direct estimation of age; Yukinawa (1970) and Shingu (1978) from scale annuli, and Thorogood (1987) from band counts of whole otoliths. There is significant variance in the L_∞ and k terms among the studies, although the estimates of [1] Anon (1994) are very similar to those produced by our study. Some of the variance is likely to be due to the very limited range of fish size within many of the data sets, e.g. Shingu's (1978) growth estimates were based predominantly on fish less than 110 cm fork length and 4 years of age. Although large, the data sets from tagging programs in the 1960, 80's and 90's include very little useful data for fish over 12 years of age. From 45,000 releases in the 1960's, there have been only six returns with reliable length data for fish at liberty for more than eight years. Similarly from 10,000 releases in the 1980's there have been only nine returns of fish at liberty for more than eight years. With data sets restricted to varying portions of the early phases of life, during which growth is fastest, it is not surprising that estimates vary among studies, nor that our estimates, based on ages from 1-36 years, are different. Given the large sample sizes examined and their coverage of all age classes, we believe that the growth parameters derived from the current study are likely to represent a realistic appraisal of growth in a species.

Recent analysis of tagging experiments conducted since the 1960's suggests that the growth rate of two and three year-old SBT increased significantly between the 1960's and 1980's-90's ([1] Anon, 1994). This change is reflected in the differences in estimates of "k" between the studies of Yukinawa (1970), Shingu (1978), Murphy (1977) and Kirkwood (1983), all of which used samples or tagging data collected during the 1960's, and those from our study and [1] Anon (1994), which are based on either samples or tagging data collected during the 1980's and 1990's. The estimates of k for the 1960's range from 0.128 - 0.146, compared with 0.185 and 0.180 from this study and Anon (1994), respectively.

Thorogood's (1987) estimate of 0.108 for k, based on samples collected during the 1980's is difficult to explain. Thorogood (1987) does not show any data on individual age estimates, and thus, it is impossible to determine why his growth parameters are so radically different from all of the earlier studies, and from our own. Thorogood (1987) did not age any pre-recruit fish and did not use daily increment counts to verify the first slow growth band in his otoliths. He appears to have accepted the estimate of 2 years as the age at recruitment (Robins, 1963; Hynd, 1965) and allocated an age of 2 years to fish 42-50+ cm fork length - the smallest size class he examined. [6] Gunn et al. (1995) and [7] Rees et al. (in prep.) have since demonstrated on the basis of daily increment counts that Robins (1963) was in error and that 40-55 cm new recruits are one year old rather than two. Similarly the analysis of scales in the current study suggests the first "annuli" read by Robins (1963) and Yukinawa (1970) is a false check.

Thorogood (1987) also states that in developing a method to estimate age from otoliths both transverse and longitudinal sections were examined, but that "these showed no greater clarity of banding than did whole otoliths". This is a perplexing result as our study has clearly demonstrated that in fish older than 6 years the banding patterns on whole otoliths become very difficult to impossible to interpret, and that the only way to resolve the outer bands on otoliths from larger - older fish is to section them. There is evidence from a studies of many fish species that counts on whole otoliths are useful only for the younger age classes, and that sectioning most often reveals many bands not visible on whole otoliths (McFarlane and Beamish, 1995). We suggest that Thorogood (1987) consistently underestimated age in the otoliths of larger fish he examined, and that as a result his growth function is erroneous.

Vertebrae

Relationship between vertebral radius and fish size

Across the size range of fish examined, there was a significant and positive linear correlation between fork length and the radius of the 35th vertebra ($r^2 =$ 0.974). However, despite this highly significant correlation, the variance in the relationship between fish length and vertebral radius at sizes larger than 160 cm was much higher than in smaller fish. The slopes of the regressions between fish length and vertebral radius for fish greater and less than 160 cm FL are also significantly different; the reason being a slowing in the growth of vertebrae over the size of 160 cm (Fig. 11), at least along the axis being measured. It appears that at larger sizes the outer edge or margin of the vertebrae curls out from the centrum rather than continuing to grow along the essentially flat growth plane followed at smaller sizes.

Similar growth patterns have been reported in the vertebrae of Atlantic Bluefin (Lee et al., 1983), and the close spacing of bands has provided acute problems in age estimation for larger size classes in the species (Mather and Schuck, 1960; Rodriguez-Roda, 1964; and Caddy and Butler, 1976).

Validation of annual formation of bands in vertebrae

With the objective of determining the periodicity of vertebral band formation, the vertebrae of fish marked with strontium chloride were examined to determine whether strontium marks had been deposited in the calcite matrix. Unfortunately, no strontium marks were detected on the vertebrae examined, and thus no direct validation of annual band formation was possible.

However, as we had been able to validate using strontium marking ([4] Clear et al. in prep.) and bomb radiocarbon chronometry (Kalish et al., in press) that counts of the translucent slow growth zones in otoliths provide an accurate estimate of annual age, and we had a large number of vertebrae and otoliths collected from the same fish, we have used the comparisons of estimates for the two structures to examine the periodicity of band formation in SBT vertebrae.

If the bands we counted on vertebrae were deposited annually throughout life, one would expect the otolith and vertebral counts to be the same for all ages. Across the range of ages within the population, this would result in a linear relationship, with a slope not significantly different from one, between age estimates from the two structures. The data from otoliths and vertebrae from 226 fish (45 to 194 cm) examined show clearly that the bands on vertebrae do not form every year throughout life (Fig. 12). The relationship between age estimates from otoliths and vertebrae is linear for the first 9-11 year classes, up to a size of approximately 157-160 cm FL (Vertebrae age = 0.106 + 1.008 Otolith age, $r^2 = 0.935$), indicating that in this early phase of life a pair of fast and slow growth zones are deposited alternatively once each year on both otoliths and vertebrae. However, at ages greater than 9-11 (i.e. fish > 157-160 cm FL), counts of vertebral banding significantly underestimate the age of fish, with the discrepancy between the two readings and hence the error in the vertebral counts being greatest at the largest sizes.

APE's between vertebral and otolith age estimates indicate the scale of the error (Fig. 13). For age classes 1-11 the APE is 2-6% (i.e. in the same order as the APE's between replicate otolith readings on the same specimen by our most experienced reader), but in age classes 12-41 the APE's rise linearly from 6-36%.

Vertebrae have been used in the estimation of age in other *Thunnus* species. Labelle et al (1993) compared the estimates of age and growth based on vertebral ring counts of South Pacific albacore (*Thunnus alalunga*) with those derived from tagging and length-frequency analyses. Although no direct validation of the periodicity of ring formation was attempted, von Bertalanffy growth parameters calculated from each of the independent data sets were similar, prompting Labelle et al. (1993) to conclude that their assumptions of annual formation of vertebral rings were valid. South Pacific albacore attain much smaller maximum size than SBT ($L\infty = 121$ cm FL), and live for only 14 or so years, so it may be that their relatively fast growth and high natural mortality prevents them from reaching the stage at which vertebral growth is so slow that ring structures become too difficult to interpret, as reported for Atlantic bluefin tuna (Mather and Schuck, 1960; Rodriguez-Roda, 1964; and Caddy and Butler, 1976). Labelle et al. (1993) provide no data on the relationship between vertebral radius and fish size, so it is not possible to discern whether the growth of South Pacific albacore vertebrae slows to the same extent as in Atlantic bluefin tuna (Lee et al. 1983) and SBT.

Although the growth of vertebrae in Atlantic bluefin tuna slows significantly at sizes above approximately 200 cm FL, making difficult the estimation of age in what are classed as “giants”, Lee and Prince (1995) demonstrated close agreement between the “known” age of tagged-and-recaptured Atlantic bluefin and the counts of both vertebral rings and otolith slow growth zones for fish up to 13 years of age and 267 cm FL. The agreement between vertebral and otolith age estimates, and the validation that ring and slow growth formation on both structures up to this age is very similar to the agreement we have found for SBT.

The similarities between ABT and SBT go further in that after approximately the age of 10-12 the estimates of size-at-age derived from the two structures diverge and counts of otolith slow growth zones (Hurley and Isles, 1983) produce much higher estimates of longevity than counts of vertebral rings. In both SBT and ABT, the maximum age estimated from otoliths is in the order of twice that estimated from vertebral ring counts.

Age and growth estimates from vertebrae

As discussed above, the estimates of age based on vertebral banding appear valid for age classes 1-10, but age is underestimated after this point. The maximum age estimated from vertebrae was only 20 years, for three fish ranging in size from 189-196 cm FL; less than half the maximum age estimated from otoliths.

Predictably, the mean size-at-age estimates from otoliths and vertebrae are very similar for the first ten age classes (Table 2), but for vertebral ages 11-20 the mean size at age gets progressively higher than the corresponding otolith-based ages.

Similarly, the relationship between length and age estimated from vertebrae (Fig. 14) is significantly different from that derived from otoliths. The vertebral data does not reach an asymptotic length, an observation in contrast to both the otolith data from this study and data from tagging studies conducted since the 1960's ([1] Anon, 1994).

In conclusion, bands on vertebrae do not provide accurate estimates of age for SBT over the age of approximately 10 years (approximately 160 cm FL). This corresponds with the age and size at which SBT reach sexual maturity (Farley and Davis, in press; [5] Gunn et al., 1996b). As this is also the size at which the growth plane of vertebrae appears to change (see Fig. 12), it seems most likely that the onset of sexual maturity has an influence on the growth patterns of vertebrae.

Scales

Counting criteria, counting precision and age estimates.
Using the counting criteria of Ricker (1971), the success rate in identifying and counting annual growth checks from the 6 to 10 scales examined per specimen decreased sharply from almost 100% for 40 cm fish to less than 1% for 180 cm fish. The major reasons for this were significant increases in the number of regenerated scales in larger fish (scales in which the central region is devoid of circuli), and the progressive piling up of the circuli on the scale margin as fish size and age increases. As a result of these difficulties, we believe that scales are only useful for estimating age in SBT up to 3-4 years old.

Yukinawa (1970) also found that scales became progressively more difficult to read as size increased, although he was confident enough in estimates of ages up to 8 years to use them in the derivation of a von Bertalanffy growth curve. Yukinawa's growth parameters were significantly different from those derived in this study (Table 5), perhaps due to changes in the growth rates of fish since the 1960's ([1] Anon, 1994) but also due to differences in the estimate of size-at-age 1. Yukinawa (1970) estimated the age of new recruits to the Western Australian fishery fish to be 2+ years. However, our readings of scales, vertebrae and otoliths, indicate these fish are one, not two, year olds. We examined scale samples collected at the same time and the same location as those used aged by Yukinawa and found no significant difference between the structure of these and the scales we collected in the 1990's. A small percentage of the scales we examined had a faint check inside the first annuli that may have been interpreted by Yukinawa (1970) as the first annuli. However, as it is not present in all fish, it is difficult to understand why Yukinawa (1970) did not mention difficulty in interpretation of this first check.

There was a significant relationship between age and the repeatability of age estimates on blind replicate readings by our scale reader. Within the subset of scales from fish aged as 0+ to 3+ re-read to examine consistency in applying the selected ageing criteria, discrepancies were small for fish with none or one annuli, and increased to a rate of 14-16% disagreement for fish with two or three annuli (Table 3).

For year classes 1-3, estimates of age from counts of scale annuli provided mean size-at-age estimates very similar to those derived from otoliths and vertebrae (Table 2). However, above this age, difficulties in interpretation of scale structure and small samples sizes did not allow reliable estimates. The relationship between fish size and age estimated from scales (Fig. 15) suggests that for fish larger than 100-110 cm FL counts of “annuli” on scales significantly underestimate age. Although this is significantly smaller than the size at which Yukinawa (1970) found age impossible to estimate, at a similar size he was able to age only 20-40% of scales he examined.

Validation of annuli in the scales of 2 year-old fish

To verify that the annuli we were reading were deposited once per year, the marginal increment between the last formed ring and the scale margin was recorded on a monthly basis for a sample of 2 year old fish (Table 4). A zero marginal increment was recorded once a year in October, a similar situation to that found by Yukinawa (1970). This is also the time of year that slow growth zones on otoliths are laid down.

In summary, we were able to demonstrate that scales lay down a check once a year for at least the first two-to-three years. Yukinawa (1970) found that these checks were annual for the first eight years of life. However, as scales become progressively more difficult to use for estimating age as size increases and otoliths lay down annual slow growth zones throughout life we believe the latter to be the best structure for estimating age in SBT.

Catch-at-age and age at first spawning

The distribution of ages within the catch of the Japanese longline fishery that operates off the east and south coasts of Tasmania from May-August each year was estimated using otoliths collected from a random sample of the 1988-93 catches (Fig. 16). The age of each fish was used to back-calculate the cohort to which it belonged, allowing fish caught in different years but belonging to the same cohort to be pooled. From these data, it is clear that a significant proportion of the catch, and presumably also the SBT population, is older than 25 years, indicating a relatively low level of natural mortality for the species, at least in mature fish.

The distribution of cohorts within the Tasmanian fishery during the early 1990's shows the dominance of fish spawned since 1984, the year in which quotas were imposed on the SBT catch. Since 1984, the numbers of juvenile fish taken in surface fisheries off Western and southern Australia has declined markedly, allowing these fish to survive and recruit into the longline fisheries. The effect of the very high catches of one-to-three year old fish in the Australian surface fisheries can also be seen clearly in the disproportionately low representation of year classes spawned between 1971 and 1983. Despite being considerably older, there are 50% more fish remaining in the population from the 1964-73 cohorts than from the 1974-83.

The otolith-based age data, and the preliminary age-length keys produced by this study have recently been used in examining the age composition of the catches in other high seas fisheries. Fig. 17 compares the age distributions of 1995 catches by Indonesian longliners on the SBT spawning grounds with the age distribution of catches by Japanese longliners off the spawning grounds in the Southern, the southern Indian and South Atlantic Oceans. The catch-at-age distributions were calculated using the mean and standard deviations of length-at-age estimated from otoliths and the length frequency distributions of the catch. From these data we see that the age distribution of catches throughout the Japanese fishery are similar to those in the Tasmanian fishery (Fig. 16) with the bulk of the catch (in terms of numbers of fish) consisting of fish less than 12 years old. The signal of year classes from the mid-1970's to mid-1980's being underrepresented in the population that is obvious in the otolith data shown in Fig. 16 tends to be dampened within the ages derived from conversions from length to age using an age-length key. However, it is still clear that these age classes are less abundant throughout the population than would be expected from a constant rate of natural mortality.

The distribution of ages in the Indonesian longline fishery, which operates on the SBT spawning grounds in the area south of Java and Bali, suggests that the current estimates of age at maturity in SBT may be too young. There are very few fish less than 13 years of age in these catches, and the majority are older than 20 years. Whether the Indonesian fishery, which targets yellowfin and bigeye tuna and only catches SBT as an incidental by-catch ([8] Davis et al., 1996), provides a representative sample of the SBT spawning stock needs further clarification ([6] Gunn et al., 1996b). However, it seems more than likely that the age at first maturity is significantly older than 7 years, the figure used in assessments prior to this study, and also higher than the age adopted by the 1994 CCSBT Scientific Committee ([1] Anon, 1994) which concluded that "while there was a fair bit of variability, the mode (in size of fish on the spawning ground, based on conversions of lengths to ages using the growth curves accepted by the CCSBT) appears to be roughly at age 10 and age 8 is not a bad estimate of where the 50% (maturity) level is". [9] Davis (1995) estimated the mean size at maturity from ovarian development to be 157 cm FL. Based on the otolith-based age estimates from this study (see Table 2), this size corresponds to an age of approximately 12 years.

All in all, the new data on age produced by this study suggest that a number of the basic population parameters used within the CCSBT assessment process have been in error. Table 6 provides a summary of the key changes to these parameters. The species certainly lives much longer than we thought previously, leading to the conclusion that it also has much lower natural mortality rates than those used throughout the 1980's and early 1990's to tune the VPA's that provide the basis of annual stock assessments. The age at recruitment appears to be a year younger than we thought while the age at first maturity may be as high as 5-6 years older than previously accepted.

All of these new perspectives are now being integrated into the CCSBT assessments. It is to hoped that the availability of more accurate age data, and the potential for validated catch-at-age matrices as the basis for VPA's will significantly decrease the levels of uncertainty currently surrounding interpretation of SBT assessment outputs.

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Tables

1. Collection details for samples analysed. Catch location was allocated using SBT statistical areas.
2. Length at age for otoliths, scales and vertebrae.
3. Intra-reader precision in age estimates from scales from two blind readings.
4. Marginal increments from the last formed annuli to the scale edge - all fish aged as 2 year olds.
5. Von Bertalanffy growth curve parameters from the present study compared with those from the literature on SBT growth.
6. A list of key population parameters altered as a result of the new age data provided by this study.

Figures

1. The hole-saw drilling technique used to extract otoliths from SBT without adversely affecting the external appearance or market value of the fish.
2. A whole, burnt sagittal otolith from a 98 cm FL SBT, caught in January, showing three slow growth zones.
3. A transverse section of a sagitta from a 180 cm FL SBT, showing alternating translucent and opaque zones along the medio-ventral ridge (longer arm) and the medio-dorsal ridge (shorter arm). As the medio-ventral ridge consistently had more slow growth zones clearly evident, we routinely used the long arm for age estimates.
4. "Pairs" of slow growth zones on the outside margin of the medio-ventral ridge of a large sagitta. If these bands coalesced at the margin of the section we counted them as part of the same increment otherwise we counted two increments. By focusing up and down through the section, we could generally determine if the area contained one or two increments.
5. a. Otolith length as a function of fork length (n = 591).
   b. Otolith weight as a function of fork length (n = 551).
6. Otolith weight as a function of age estimated from counts of slow growth zones (n = 561).
7. Changes over a 12 month period in the width of the marginal increment in the sagittal otoliths of 2-year old SBT (n = 61).
8. The relationship between length and age in male (n = 100) and female (n = 67) SBT.
9. a. The length distributions of male and female SBT in the catches from all areas in 1990-1995 (Data collected by Australian Commonwealth Observers in the AFZ and by Japanese and Australian observers on vessels involved with the RTMP).
   b. The length distribution of male and female SBT for which we estimated ages in this study.

10. The relationship between length and age estimated from counts of otolith slow growth zones - data from both sexes pooled (n = 1131). Superimposed on these data (in open circles) is the von- Bertalanffy growth curve developed by the CCSBT for use in its 1994 assessments ([1] Anon, 1994).
11. The relationship between fork length and the total cone radius of the 35th vertebrae of SBT. The two regressions are for fish less < 160 cm FL (n = 296) and > 160 cm FL (n = 84).
12. A comparison of age estimates from otoliths and vertebrae collected from the same specimen (n = 226).
13. The APE between vertebral and otolith age estimates conducted on samples collected from the same specimen, plotted as a function of the otolith-based age.
14. The relationship between fork length and age estimated from vertebral band counts (n = 561).
15. The relationship between fork length and age estimated from scale annuli counts (n = 581).
16. The distribution of cohorts within the SBT catch of the Japanese longline fishery off Tasmania 1988-93, estimated from age estimates of a random sample of otoliths collected from the catch. Superimposed on these data are the annual catch of 1-3 year-old SBT by the Australian surface fisheries from 1950-1992 (open diamonds).
17. a. Age composition of the 1995 SBT catch by the Japanese on the high seas fishing grounds in the Southern Oceans and the 1995-96 Indonesian catches on the SBT spawning grounds.

b. Relative abundance of year classes in the 1995 SBT catch by the Japanese on the high seas fishing grounds in the Southern Oceans and the 1995-96 Indonesian catches on the SBT spawning grounds.

Table 1. Sample details

Fishing Area	LCF (cm)	Otoliths	Vertebrae	Scales
1	< 50	-	-	-
	50 - 100	-	-	-
	100 - 150	-	-	-
	150 - 200	148	-	-
	> 200	5	-	-
2	< 50	17	-	16
	50 - 100	5	-	-
	100 - 150	-	-	-
	150 - 200	-	-	-
	> 200	-	-	-
3	< 50	58	19	49
	50 - 100	396	132	340
	100 - 150	36	5	22
	150 - 200	-	10	-
	> 200	-	-	-
4	< 50	-	-	-
	50 - 100	-	-	58
	100 - 150	-	-	2
	150 - 200	-	-	-
	> 200	-	-	-
5	< 50	-	-	-
	50 - 100	-	-	-
	100 - 150	-	-	-
	150 - 200	-	-	-
	> 200	-	-	-
6	< 50	-	-	-
	50 - 100	-	-	-
	100 - 150	-	-	-
	150 - 200	-	-	-
	> 200	-	-	-
7	< 50	-	-	-
	50 - 100	25	-	30
	100 - 150	208	186	49
	150 - 200	137	122	5
	> 200	3	-	-
8	< 50	-	-	-
	50 - 100	6	7	1
	100 - 150	26	21	-
	150 - 200	52	54	-
	> 200	-	-	-
9	< 50	-	-	-
	50 - 100	1	-	-
	100 - 150	15	5	9
	150 - 200	5	3	3
	> 200	-	-	-
10	< 50	-	-	-
	50 - 100	-	-	-
	100 - 150	-	-	-
	150 - 200	-	-	-
	> 200	-	-	-

Table 2. Mean and Standard Deviations of the mean length-at-age estimated from otoliths, vertebrae and scales.

	Otoliths			Vertebrae			Scales		
Age class	Mean fork length (cm)	SD	n	Mean fork length (cm)	SD	n	Mean fork length (cm)	SD	n
0	30.5	2.37	10				37.3	6.32	14
1	52.0	7.03	155	52.7	5.23	65	51.8	5.27	156
2	77.6	8.13	175	79.5	7.14	45	79.6	10.00	222
3	95.8	5.78	218	95.6	6.09	69	95.1	10.46	112
4	112.7	6.29	84	110.5	7.14	37	103.9	10.51	37
5	122.8	3.64	38	119.4	4.82	46	115.3	21.54	19
6	129.4	3.99	40	129.8	5.34	50	124.3	25.02	13
7	139.5	4.27	38	137.4	4.55	19	112.8	16.26	4
8	147.5	4.85	11	143.4	5.64	46	154.5	23.74	4
9	147.3	4.04	25	152.0	7.44	27			
10	156.1	4.14	19	159.7	7.87	21			
11	156.8	4.52	17	160.3	5.11	19			
12	159.6	4.70	11	163.1	3.84	20			
13	166.3	3.80	11	172.1	6.90	26			
14	166.8	4.60	9	175.9	4.76	20			
15	169.5	7.01	11	176.1	6.00	20			
16	173.1	6.29	22	177.8	5.26	13			
17	179.5	8.62	15	184.1	3.24	7			
18	177.3	7.46	15	185.0	4.80	5			
19	179.8	7.43	20	186.7	2.31	3			
20	179.9	5.04	9	193.0	3.61	3			
21	178.0	6.63	24						
22	178.7	5.97	15						
23	183.9	6.91	14						
24	178.6	8.68	19						
25	181.8	7.42	14						
26	183.9	13.62	14						
27	183.7	6.04	14						
28	186.6	10.30	16						
29	183.5	10.06	8						
30	185.0	6.31	11						
31	187.1	6.71	8						
32	189.7	15.82	6						
33	191.2	7.40	5						
34	191.2	6.65	6						
35									
36	186.5	10.61	2						
37									
38									
39	187.0	-	1						
40									
41	184.0	-	1						

Table 3. Intra-reader precision in the age estimates from scales on two blind readings.

	2nd Reading				
1st reading	Age 0	Age 1	Age 2	Age 3	Age 4
Age 0	18	1			
Age 1	1	141	2		
Age 2		7	49	1	
Age 3			6	43	2

Table 4. Marginal increments from the last formed annuli to the scale edge - all fish aged as 2 year olds.

Increment (mm)	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec
0										1		
.05										1		
0.1											1	
0.15	1										1	
0.2	2									2	4	
0.25	1											3
0.3	2										1	4
0.35	2	2									2	
0.4			2	1					1			2
0.45	2	2	1	1							1	1
0.5		4	1		1				1		1	
0.55		1	1						2			
0.6		1	1									
0.65			2		3				2	1		
0.7				4		1						
0.75			1	3	1				1			
0.8				1	1				1	1		
0.85					1							
0.9				1								
0.95												
1.0					1							
1.05									1			
1.1			1									
1.15												
1.2					1				1			

Table 5. Von Bertalanffy growth curve parameters from the present study compared with those from the literature on SBT growth.

	Type of data used for estimation	L_∞	k	t_0
This study	Otolith band counts, ages 1-41 years	183.18 (S.E. .523)	0.185 (S.E. .003)	-0.923 (S.E. .038)
Anon (1994)	Tagging and length data ages 1-12 and >12	183.9	0.180	-1.322
Thorogood (1987)	Otolith band counts, predominantly for ages < 6	261.3	0.128	-0.157
Kirkwood (1983)	Tagging and length data predominantly ages < 9	207.6	0.128	-0.394
Shingu (1978)	Scale annuli, ages <4	225.5	0.140	-0.011
Murphy (1977)	Tagging data, predominantly ages <8	180.8	0.146	0.011
Yukinawa (1970)	Scale annuli, ages <8	209.7	0.135	0.040

Table 6. Key population parameters resolved by the study, compared with the assumptions and estimates previously used in CCSBT assessments.

Population parameter	**Prior to this study**	**New Estimate**
Age at recruitment to surface fisheries in WA	2 years	1 year
Age at recruitment to high seas longline fisheries	5-6 years	4 years
Age at maturity	7 years	10-12 years
Maximum Age	20 years	40+ years
Natural Mortality	Constant	Non-linear, likely to be very low post-maturity

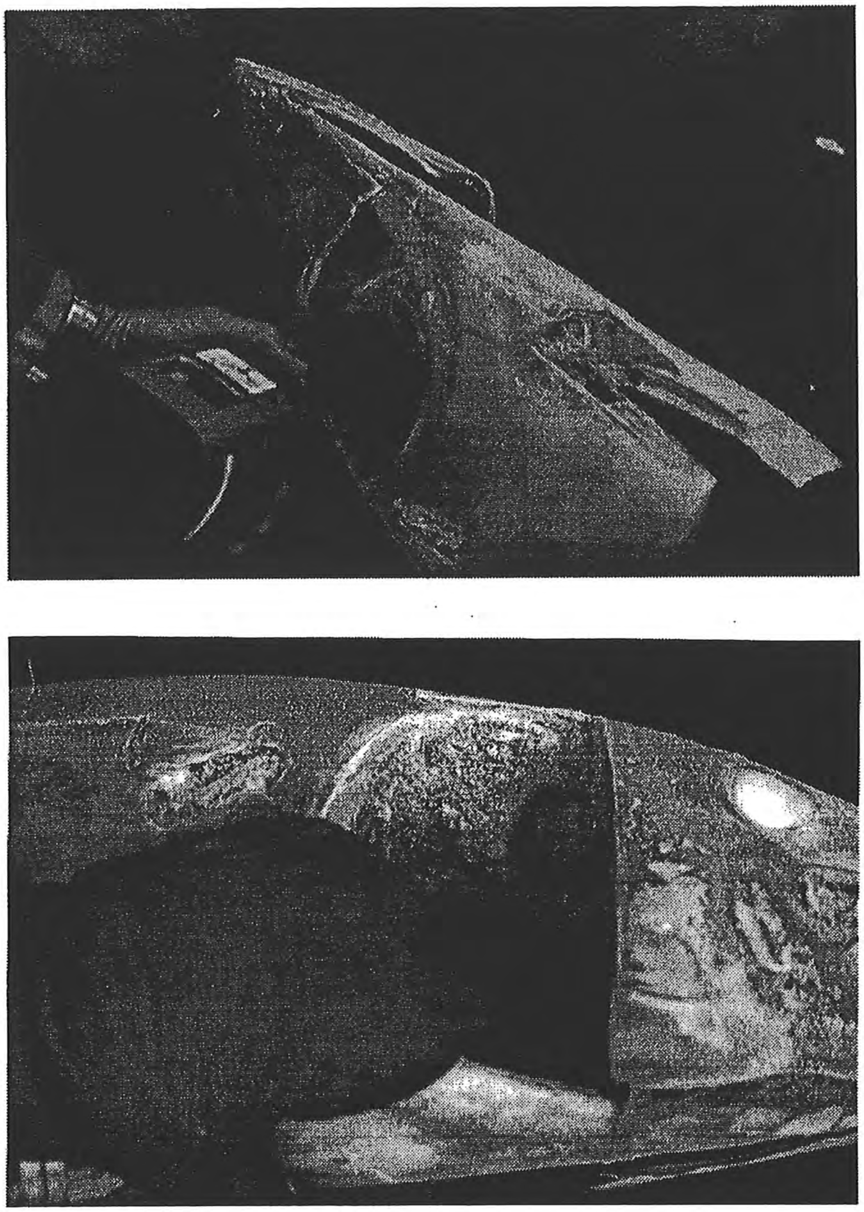

Fig 1

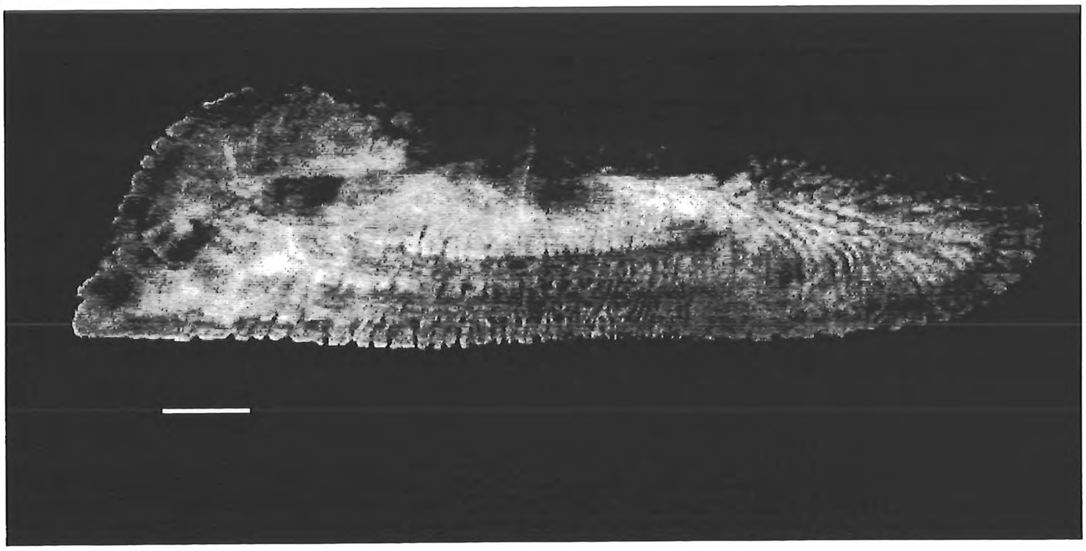

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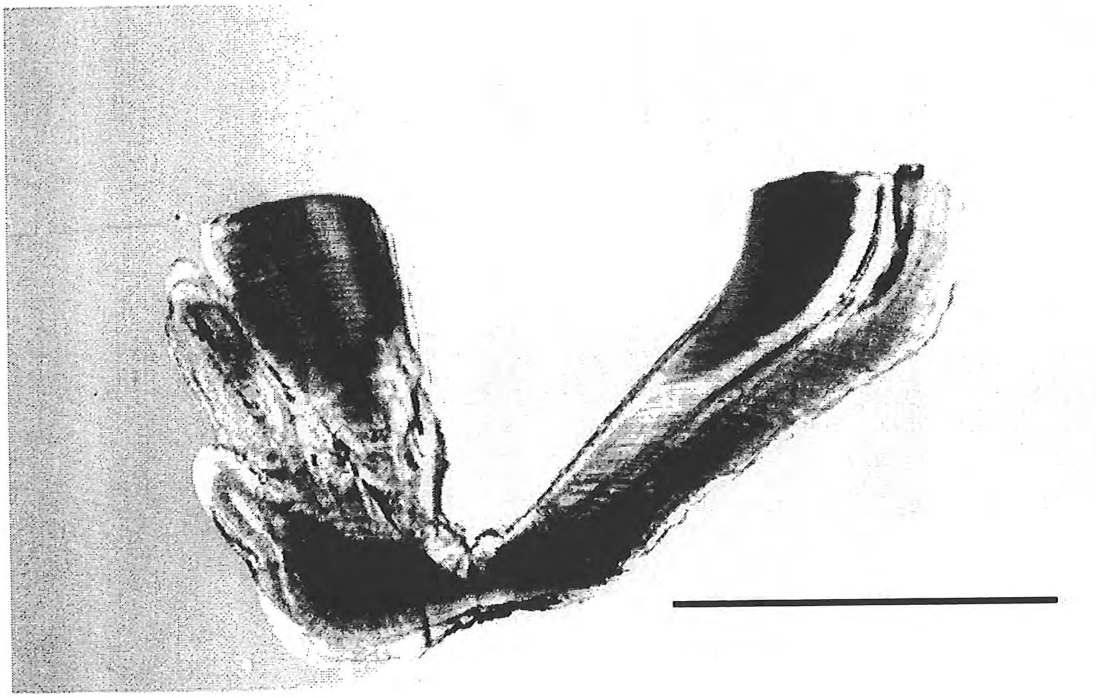
Fig 2



Scale Bar : 1 mm

Fig 3

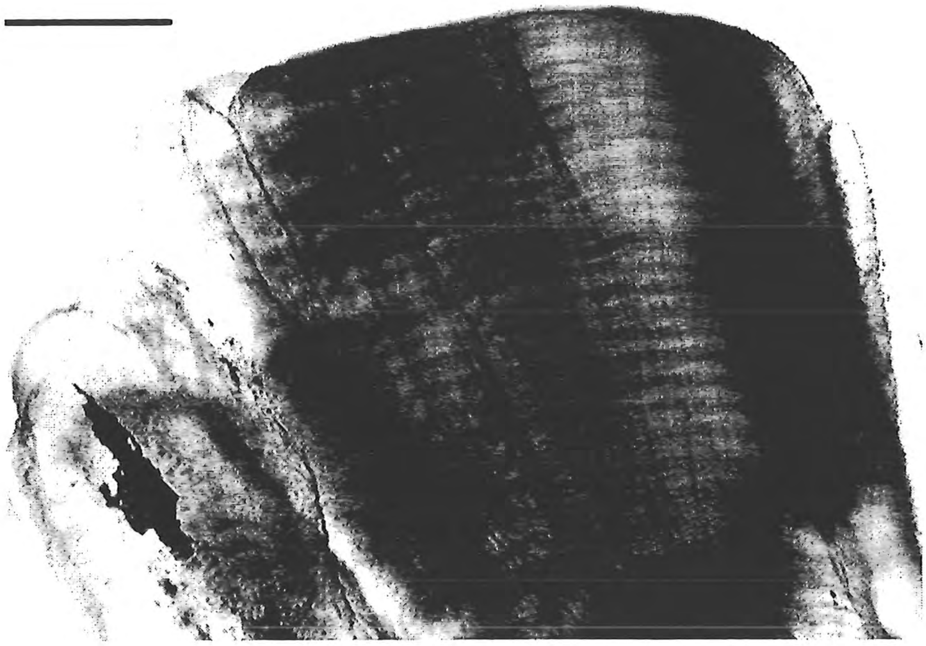


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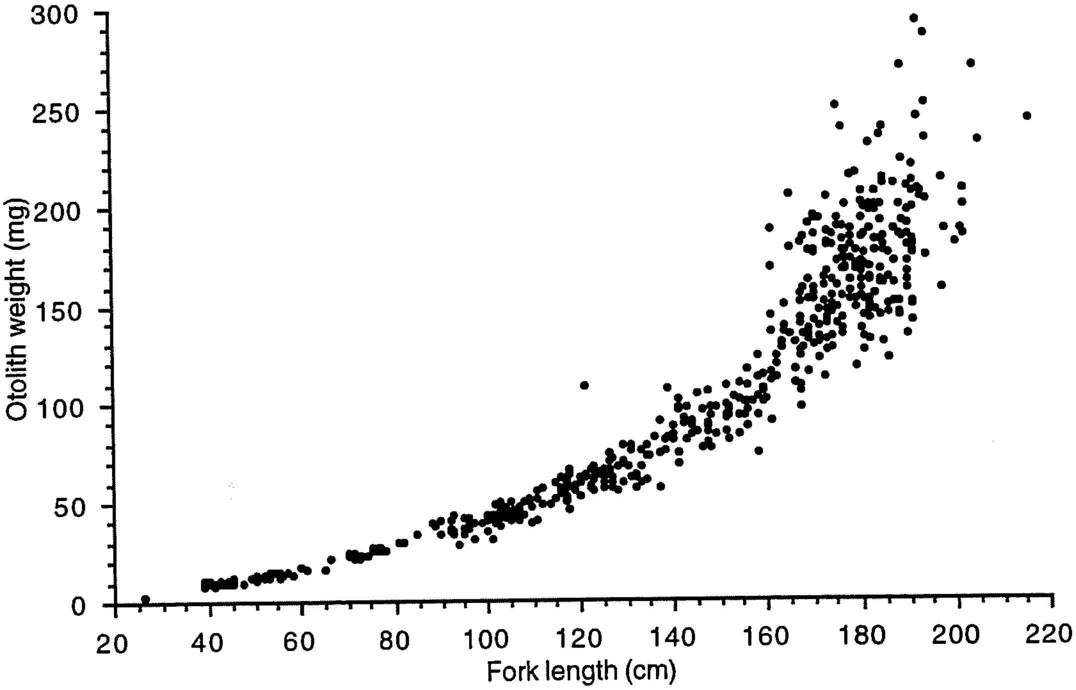
Fig 4

a)


Otolith weight (mg)
300
250
200
150
100
50
0
20
40
60
80
100
120
140
160
180
200
220
Fork length (cm)

b)

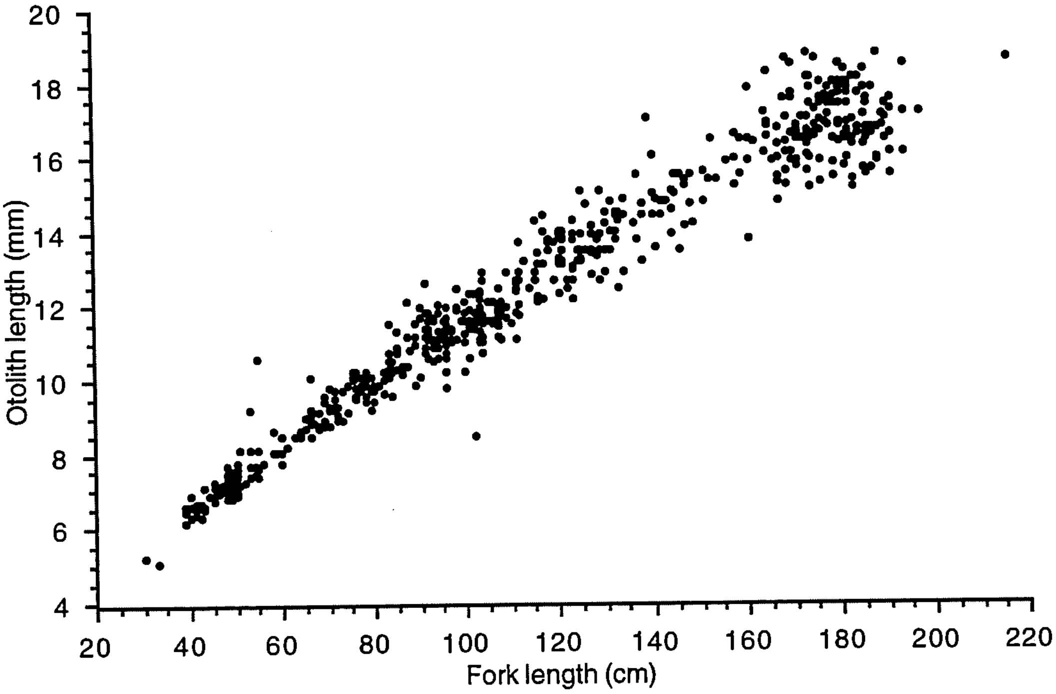

Otolith length (mm)
20
18
16
14
12
10
8
6
4
20
40
60
80
100
120
140
160
180
200
220
Fork length (cm)

Fig 5

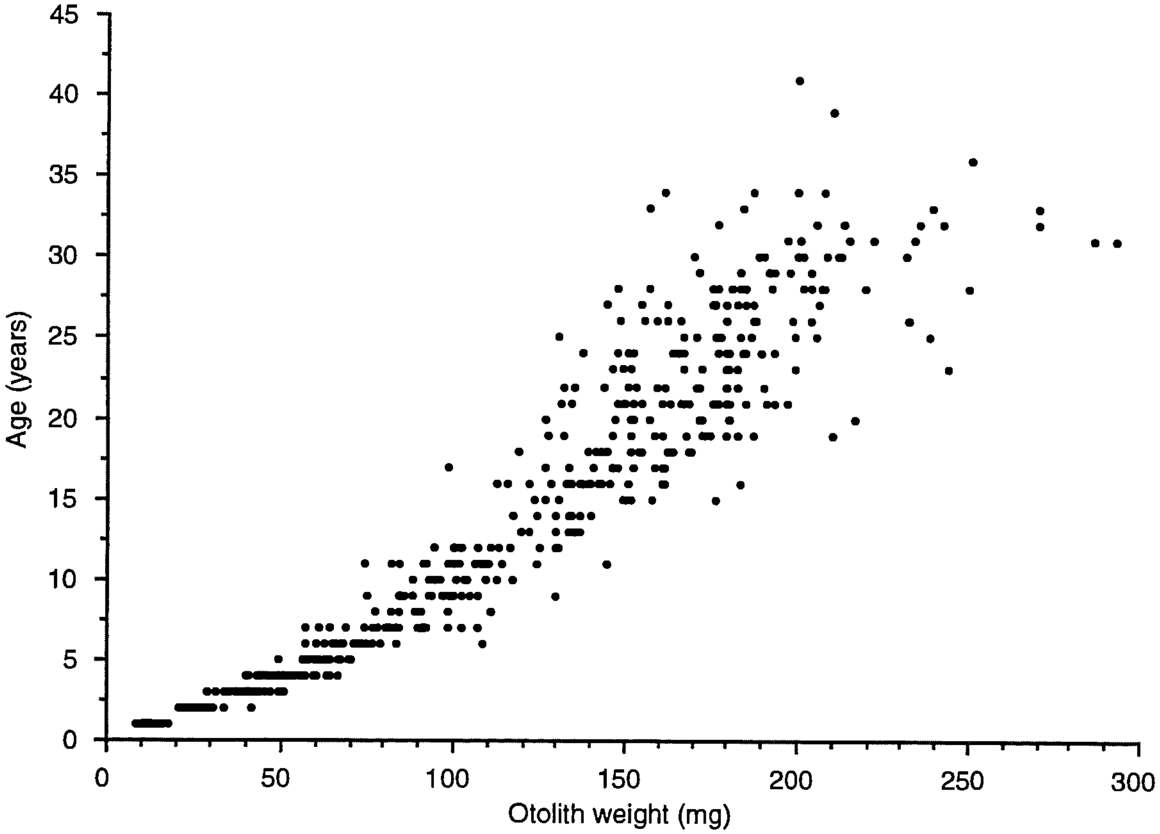

45
40
35
30
25
20
15
10
5
0
Age (years)
0
50
100
150
200
250
300
Otolith weight (mg)

Fig 6

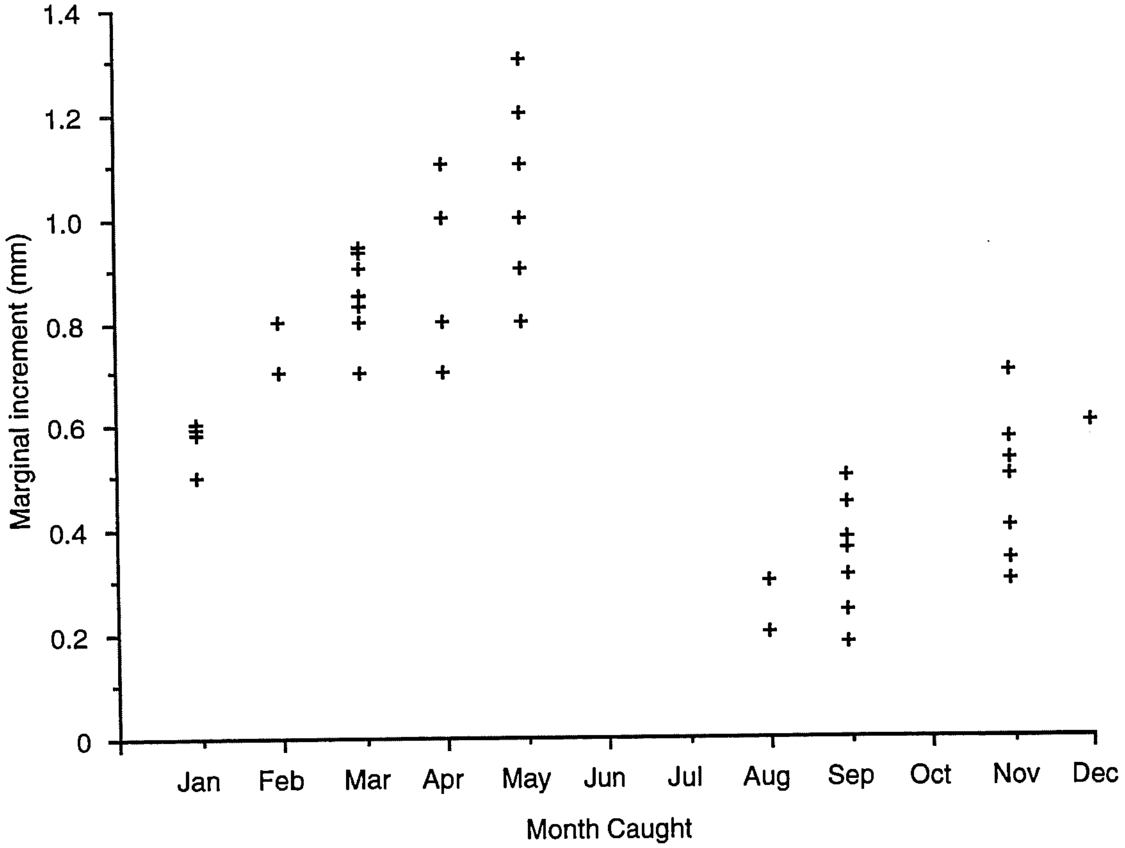

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
Marginal increment (mm)
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month Caught

Fig 7

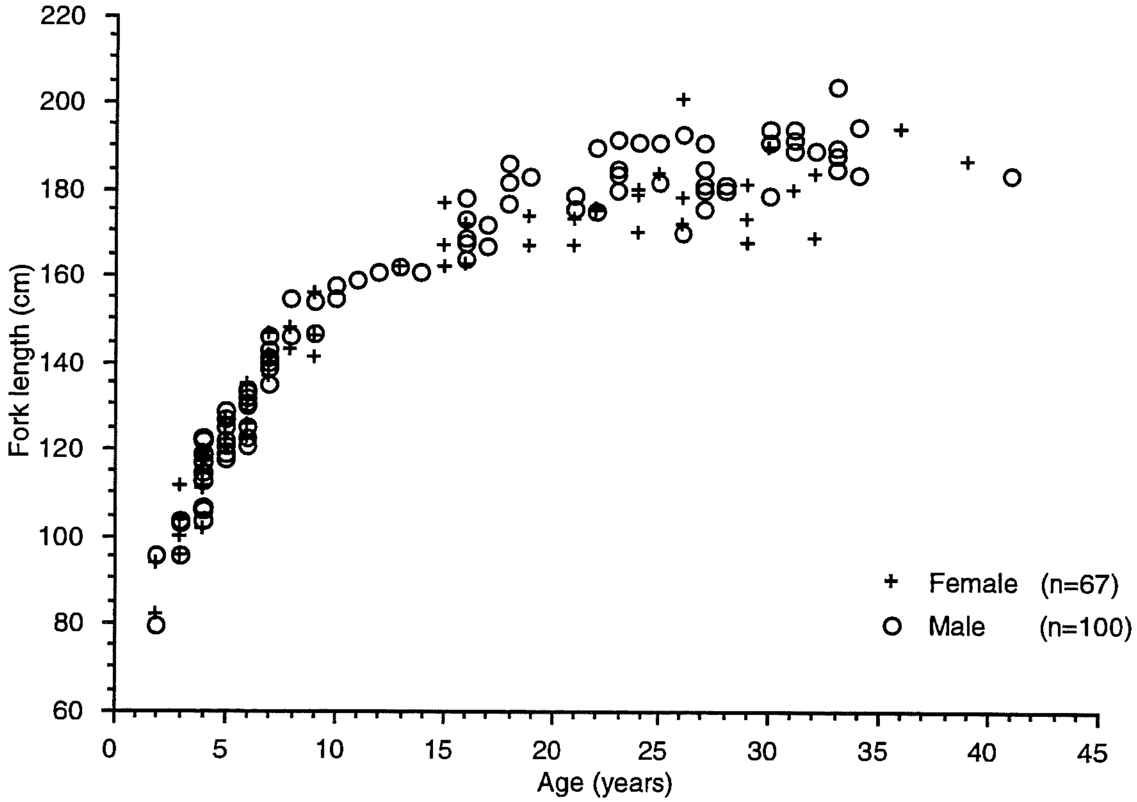

220
200
180
160
140
120
100
80
60
Fork length (cm)
0
5
10
15
20
25
30
35
40
45
Age (years)
+ Female (n=67)
O Male (n=100)

Fig 8

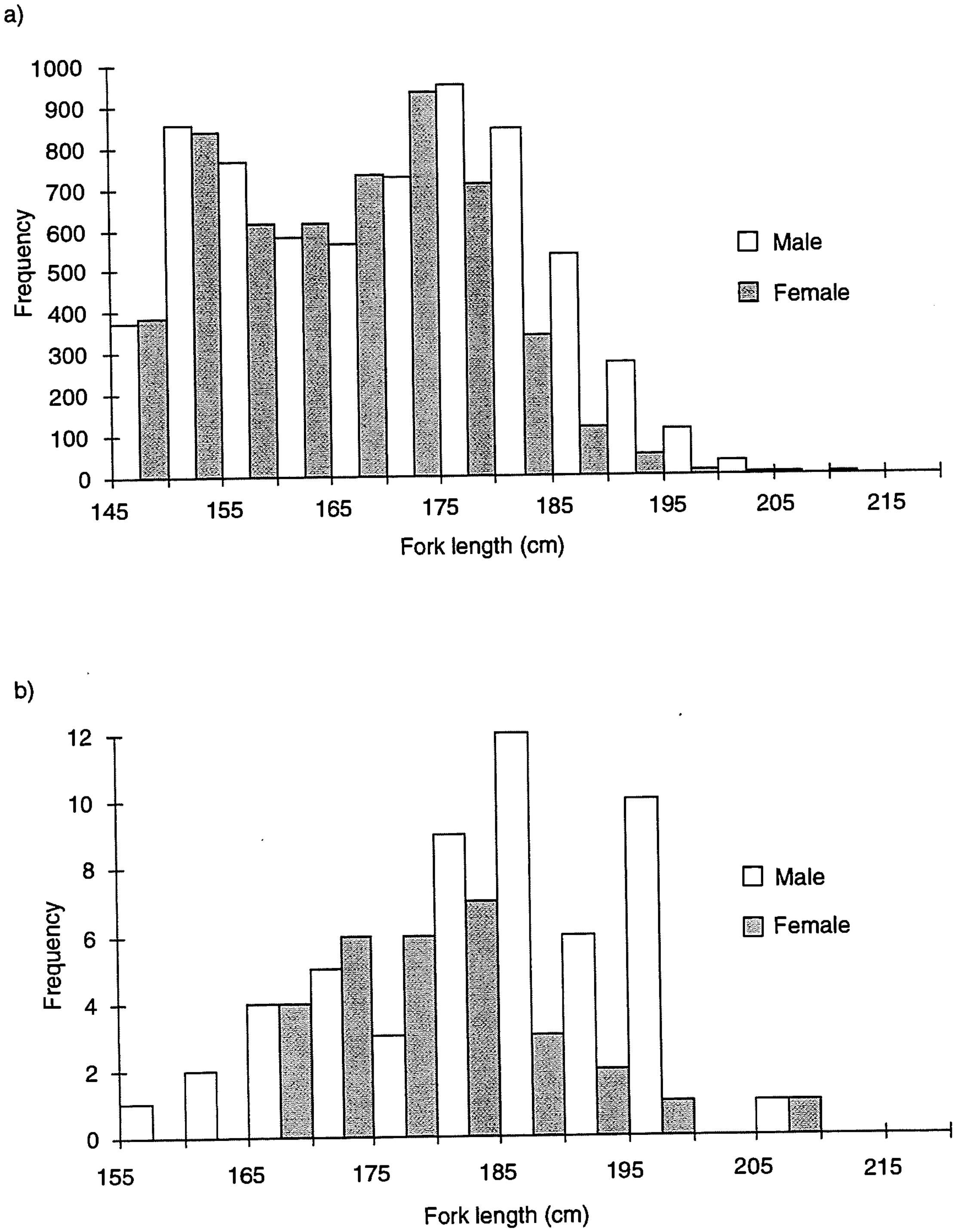
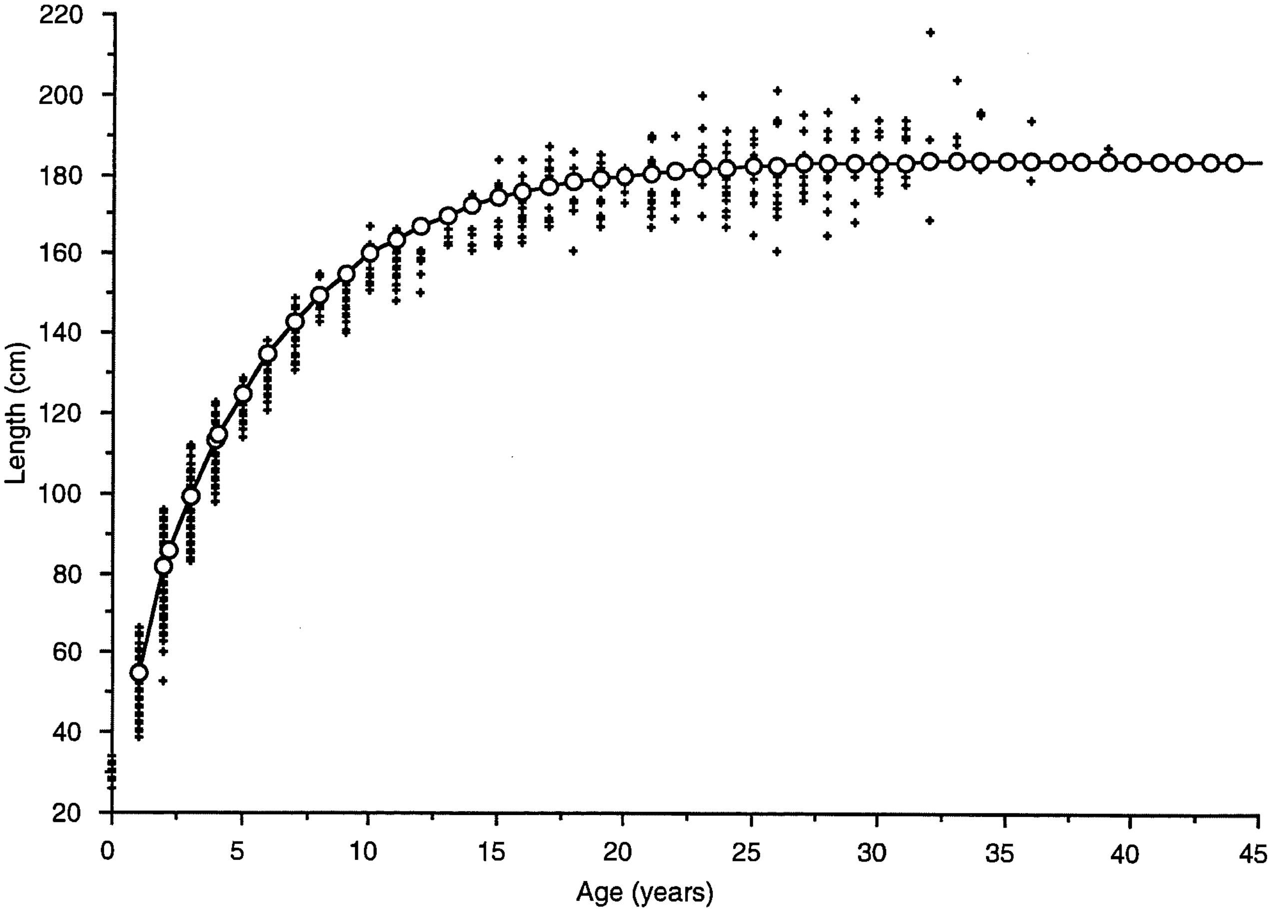

a)
1000
900
800
700
600
500
400
300
200
100
0
Frequency
145
155
165
175
185
195
205
215
Fork length (cm)
☐ Male
▨ Female
b)
12
10
8
6
4
2
0
Frequency
155
165
175
185
195
205
215
Fork length (cm)
☐ Male
▨ Female

Fig 9.



220
200
180
160
140
120
100
80
60
40
20
Length (cm)
0
5
10
15
20
25
30
35
40
45
Age (years)

Fig 10

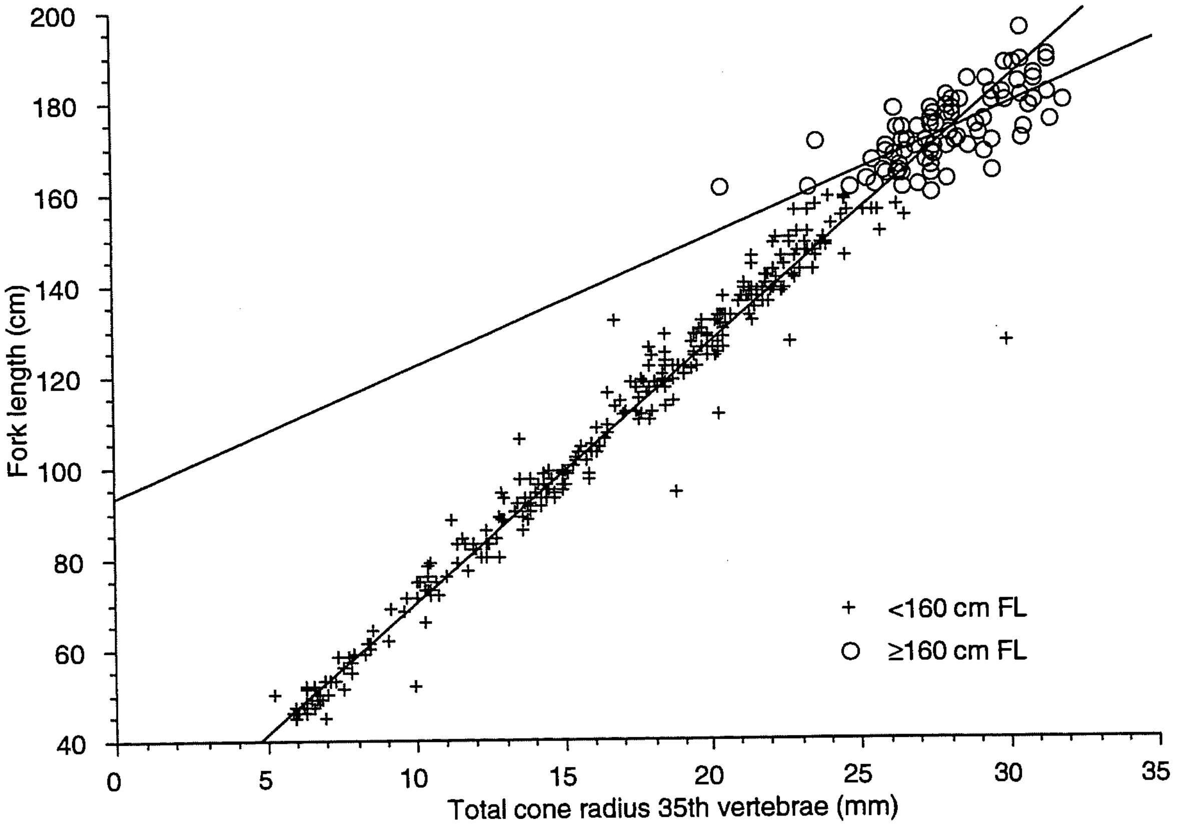
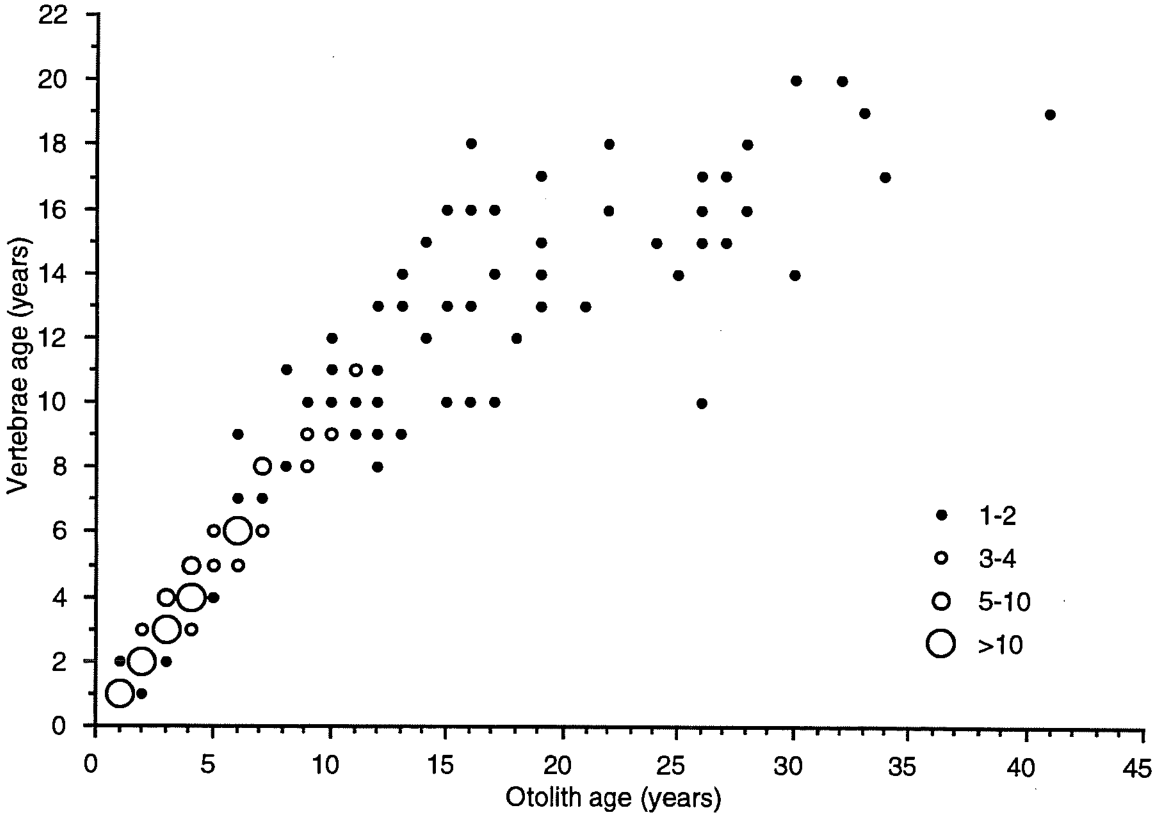

200
180
160
140
120
100
80
60
40
Fork length (cm)
0
5
10
15
20
25
30
35
Total cone radius 35th vertebrae (mm)
+ <160 cm FL
○ ≥160 cm FL

Fig 11


Vertebrae age (years)
22
20
18
16
14
12
10
8
6
4
2
0
Otolith age (years)
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5
10
15
20
25
30
35
40
45
1-2
3-4
5-10
>10

Fig 12

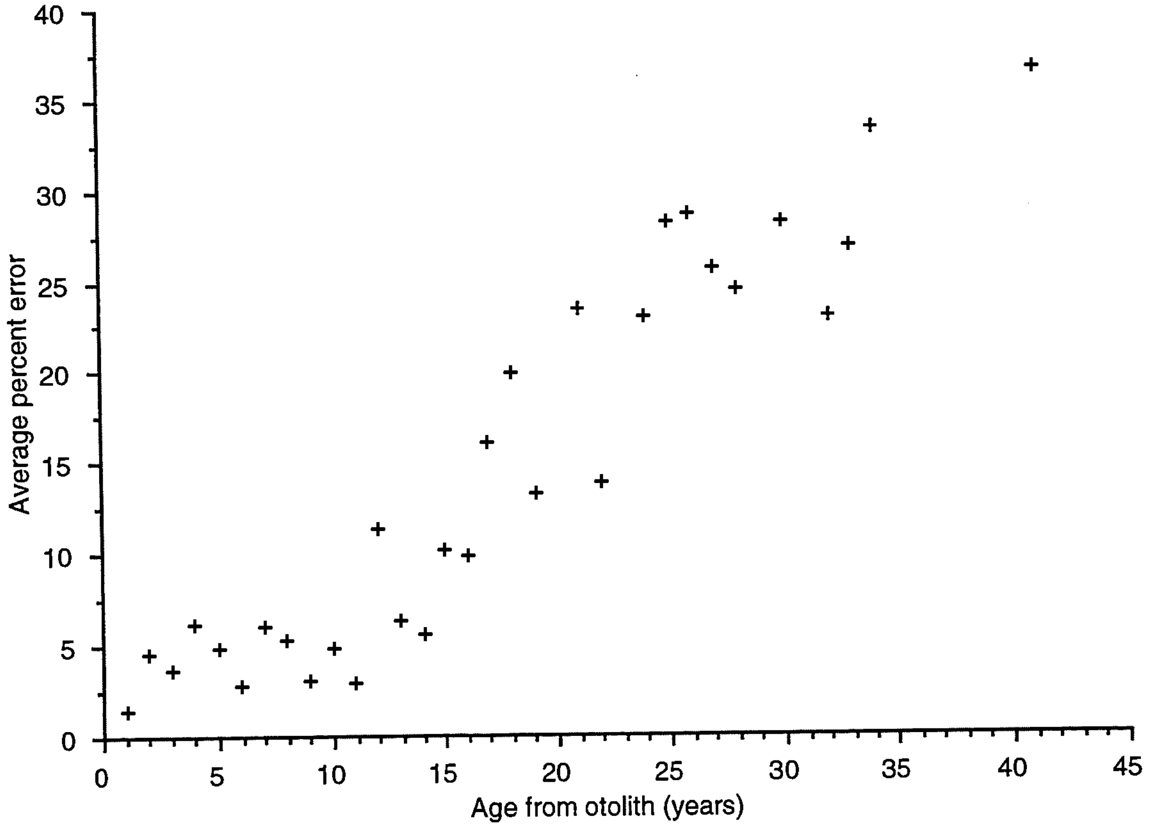

Average percent error
0
5
10
15
20
25
30
35
40
Age from otolith (years)
0
5
10
15
20
25
30
35
40
45

Fig 13

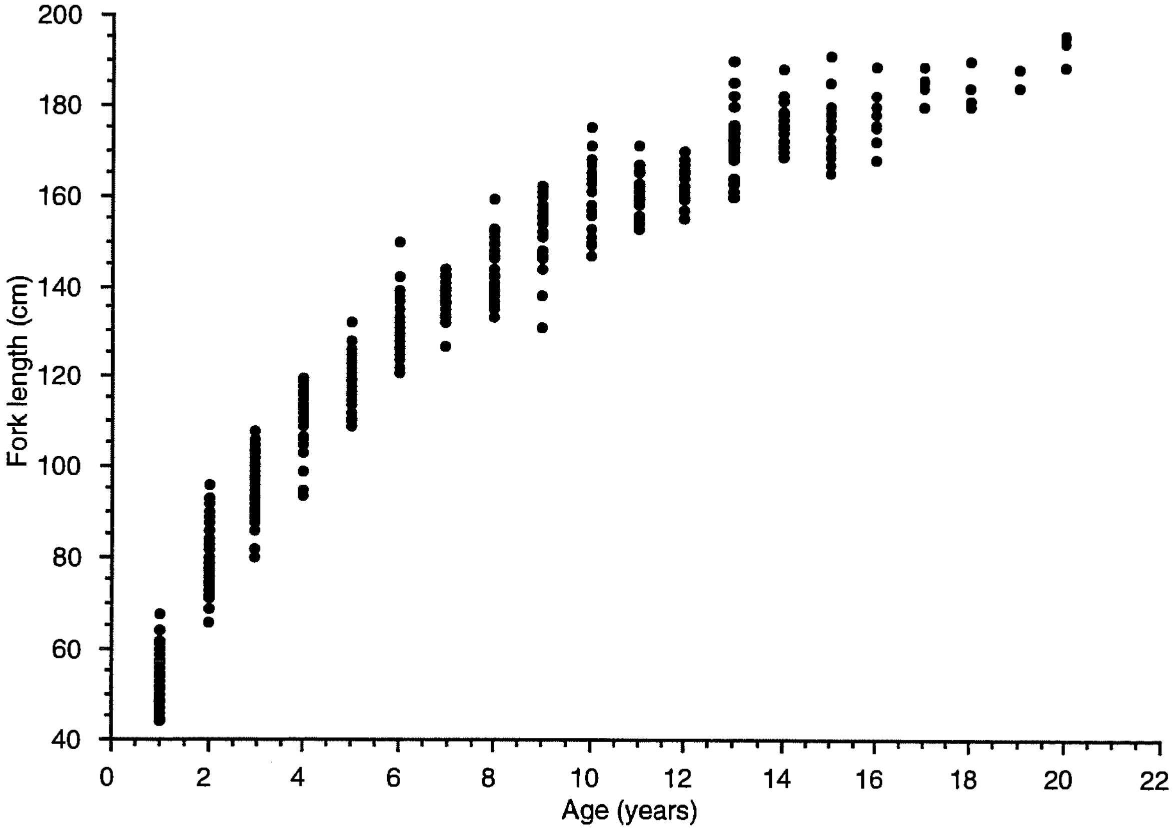

200
180
160
140
120
100
80
60
40
Fork length (cm)
0
2
4
6
8
10
12
14
16
18
20
22
Age (years)

Fig 14

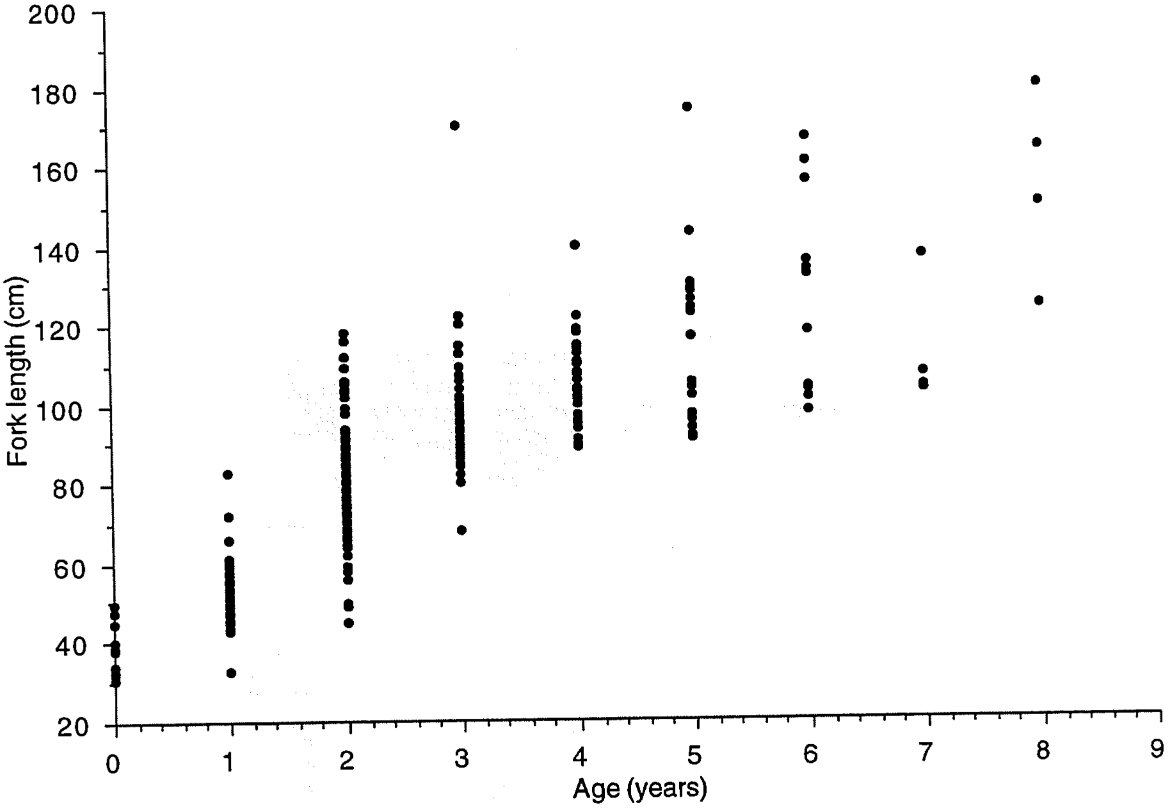
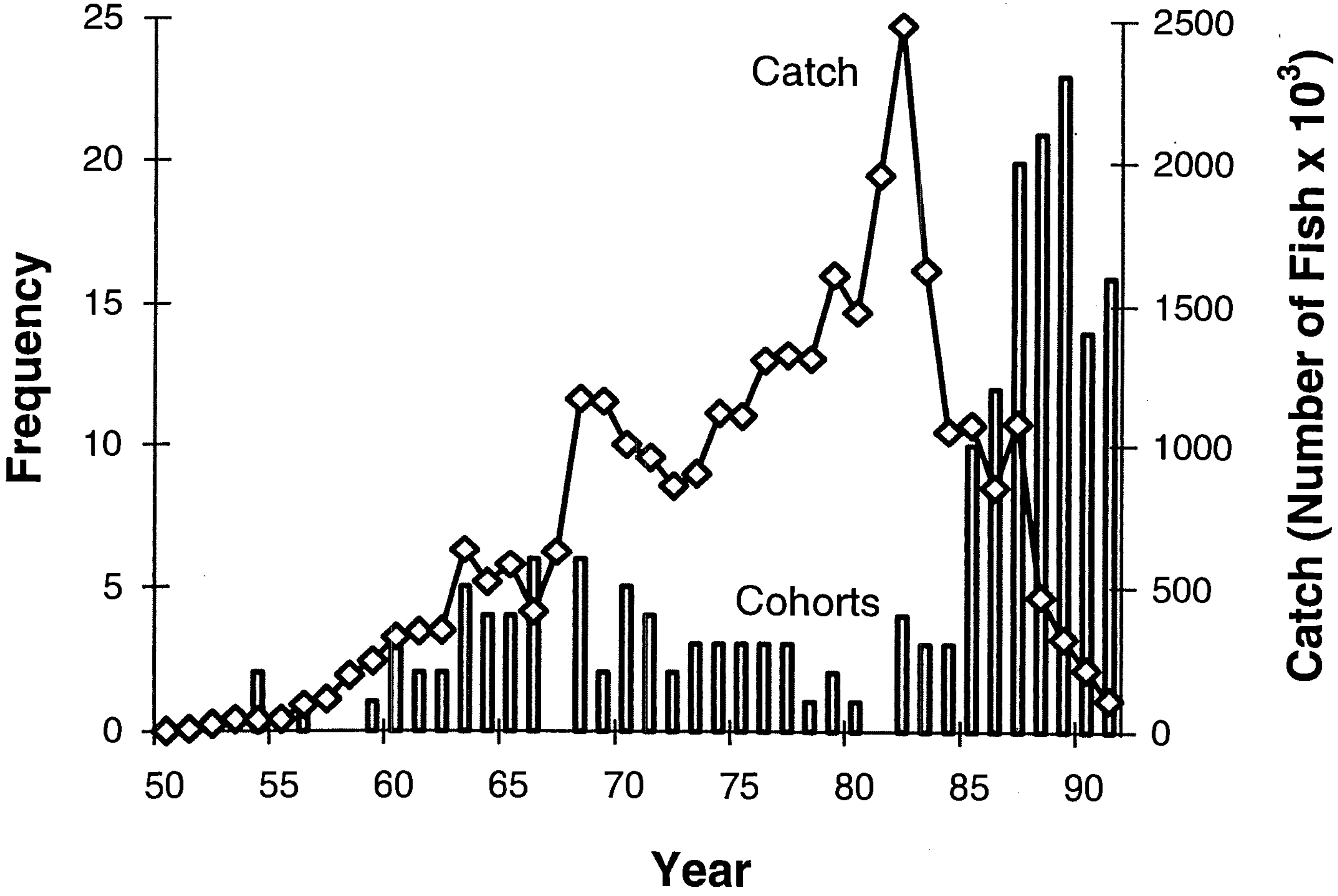
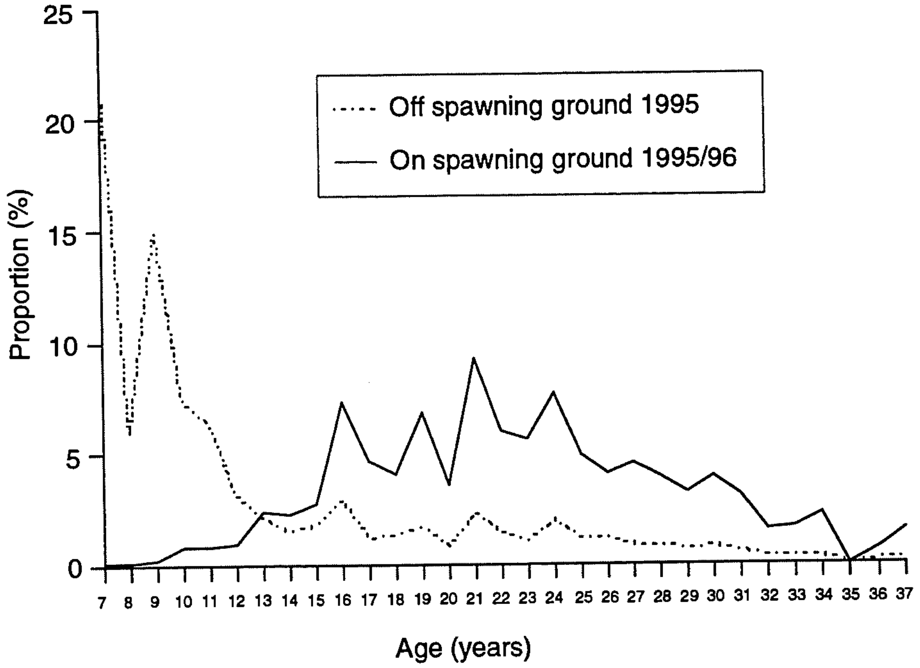

200
180
160
140
120
100
80
60
40
20
Fork length (cm)
0
1
2
3
4
5
6
7
8
9
Age (years)

Fig. 15

Fig 16

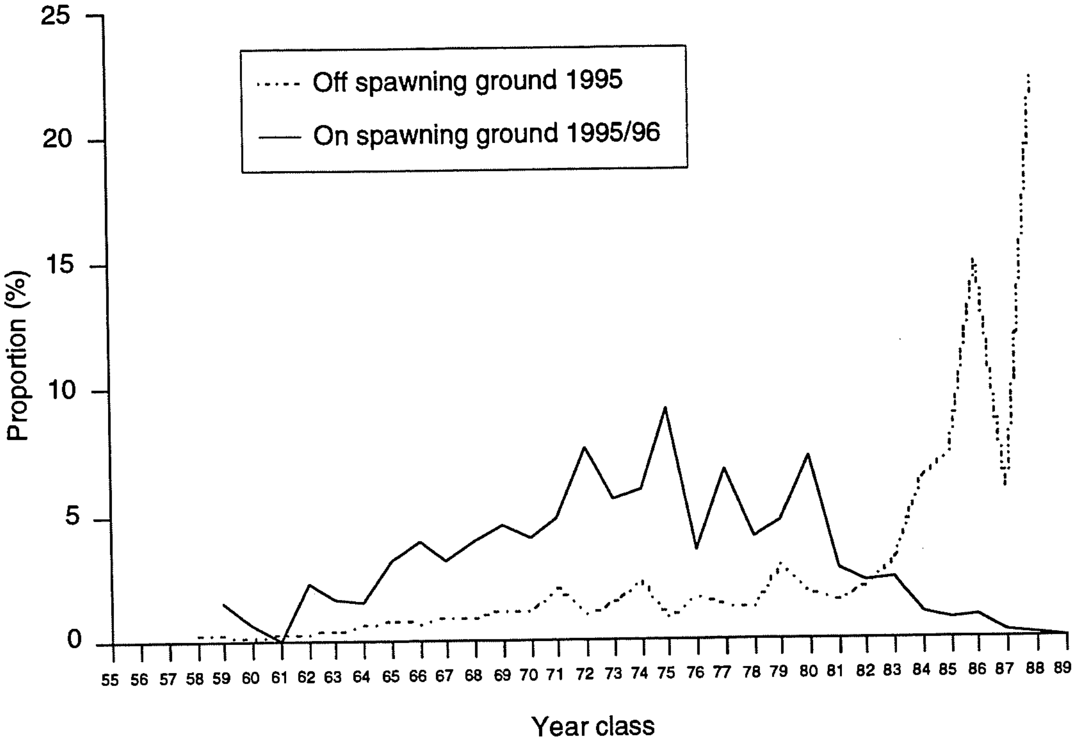

Catch
Cohorts
Frequency
0
5
10
15
20
25
Catch (Number of Fish x 10^3)
0
500
1000
1500
2000
2500
Year
50
55
60
65
70
75
80
85
90

a)



Off spawning ground 1995
On spawning ground 1995/96
Proportion (%)
25
20
15
10
5
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37
Age (years)

b)



Off spawning ground 1995
On spawning ground 1995/96
Proportion (%)
25
20
15
10
5
0
55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89
Year class

Fig. 17

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 5

Project 92/42

CCSBT/SC/96/10

PRELIMINARY ESTIMATES OF THE AGE STRUCTURE OF THE SBT SPAWNING STOCK

(INCLUDING A REVISED ESTIMATE OF THE AGE AT FIRST SPAWNING)

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Introduction

Two of the major sources of uncertainty in the current assessment of the southern bluefin tuna resource are the natural mortality rate for the mature / spawning component of the stock and " a basic inconsistency in the catch and effort data between 12+ group and the 4-11 year olds, as examined with present VPA model" (Report of the 1996 CCSBT Workshop on VPA and CPUE modelling). To resolve both of these problems, accurate data on the age structure of the spawning component of the SBT stock is needed. With adequate coverage of the population, these data would provide the basis for reduction in the degree of uncertainty surrounding the natural mortality rates of the spawning stock. They would also assist in improving our understanding of the dynamics of the "plus" group catches and effort relative to the apparent inconsistencies.

Until recently, estimates of the age structure of the SBT catch and population were derived from the conversion of lengths to age using growth curves based on tag return and length frequency data. This approach can not be used to derive estimates of the age structure for the older component of the population because there is a very large overlap in the size range of animals at different ages (i.e. size for larger fish is not a good predictor of age). Hence, there has been considerable uncertainty surrounding both the longevity of the species and the demographics of the 12+ component of the population.

Recent CSIRO research has developed techniques to age SBT throughout their size range (Gunn et al 1995). This work has also validated the annual formation of bands in otoliths throughout the life of SBT (Kalish et al. in press, Gunn et al. 1996). These advances overcome the historical reliance on tag return and length frequency data for estimation of age structure. Initial estimates of age for large fish indicate that the maximum age of SBT is in excess of 40 years, a longevity indicative of a relatively low natural mortality rate, at least at larger sizes. There is also strong evidence that a significant proportion of the SBT population is in excess of 25 years old (Gunn et al , 1996).

With the ability to directly and accurately estimate the age of mature SBT, it is now possible to examine some of the key uncertainties surrounding the age structure of the SBT population. This work has begun only recently and this report provides data from analyses conducted over the last three months.

As a first step, our priority has been to determine the age distribution of fish caught by the Indonesian longline fishery on the SBT spawning grounds, from which we can estimate the current age distribution of the SBT spawning stock. This report provides our preliminary estimates based on relatively small sample sizes. We emphasise that the results should be taken as indicative rather than conclusive. However, as the techniques have been validated, and the otoliths analysed were taken at random from larger samples collected from throughout the fishery, there is good reason to suggest the preliminary results will hold true when a more thorough analysis is completed.

The objectives of the preliminary study were to:

1. Estimate and compare the distributions of ages from samples of otoliths collected from fish caught by the Indonesian longline fishery over three spawning seasons - 1993-94, 1994-95 and 1995-96.
2. Use the distributions of ages within the otolith samples to estimate the age distribution of populations on the spawning grounds and determine whether there are significant differences between years in these distributions.
3. Compare the distributions of ages of fish caught on the spawning grounds with those caught on feeding grounds in the Southern Ocean.

Methods and Materials

1. Otolith samples and age estimation

a. Samples from the spawning ground

One hundred and fifty otoliths were selected from the CSIRO otolith archives from samples collected on the SBT spawning grounds over three spawning seasons; 47 from 1993-4, 54 from 1994-5 and 49 from 1995-6. The samples were collected from throughout the Indonesian fishery (see Davis et al., (1996) for the spatial distribution of SBT catches between October 1992 and June 1996) and otoliths from the 1994-95 and 1995-96 seasons were selected at random from the full size range of SBT sampled (Fig. 1). The otoliths analysed from the 1993-94 catches were stratified by size category. The sex of fish from which otoliths were sampled is not known.

b. Samples from off the spawning ground

As a component of a project on the development and validation of techniques for the direct estimation of age in SBT, Gunn et al. (1996) used otoliths to estimate the age of 261 SBT caught by Japanese vessels on the high-seas fishing grounds at latitudes south of 30°S. These are referred to in Figs. 1&2 as fish from the "feeding grounds". These otoliths were extracted by observers from fish sampled at random from the catch. The catches sampled were made between 1988-95 at locations in the Tasman Sea off Tasmania (n=163), the SE Indian Ocean (n=95) and the SW Indian Ocean (n=1). Two otoliths were from unspecified positions in the Indian Ocean. The age estimates are presented without weighting for catch or effort levels in each of the regions from which samples were collected.

c. Sample preparation and age estimation methods

In preparation for ageing, otoliths were embedded in polyester resin and a transverse section was cut through the primordium and ventral edge of the otolith. This is the optimal plane of sectioning when estimating age in large SBT (Gunn et al., 1995) and other *Thunnus* species (Hurley and Isles, 1983). Four sections were cut from each otolith and these were mounted on microscope slides with additional resin.

In transverse section, banding is visible on the two major growth axes of the otolith; the medial distal (short) axis and the medial lateral (long) axis. The proximal region (closest to the primordium) of both axes is the most difficult to interpret. However, problems with interpretation of banding in sections was overcome during earlier work using otoliths from small fish of validated age (Gunn et al., 1995). The distal region of the section is easier to interpret as the bands are more regular and well defined. Age was estimated for each otolith by counting the number of alternating opaque and translucent bands on the medial lateral axis. The annual formation of these bands has been demonstrated using mark-and-recapture experiments (Clear et al. Unpublished report. In: Gunn et al. 1996) and bomb-radiocarbon chronometry (Kalish et al. in press).

Otoliths were read blind on three occasions by one reader (R1) and twice by an independent reader (R2), and a comparison of the average percent error (APE) was made within readings and between readers using methods described by Beamish and Fournier (1981). Briefly, the calculation of APE is simple and is defined as follows:
N fish are aged; R is the number of times each fish is aged. Let X_{ij} be the *i*th age determination of the *j*th fish

(1) Let $X_j = \frac{1}{R}\sum_{i=1}^{R} X_{ij}$

(X_j is the average age calculated for the *j*th fish)
Then,

$$(2) \quad \frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}$$

is the average error in estimating the age of the *j*th fish, as a fraction of the average of the age estimates. Multiplied by 100 it becomes the APE for the *j*th fish, and

$$(3) \quad \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|X_{ij} - X_j|}{X_j}\right]$$

is the index of the average error and multiplied by 100 it becomes the Index of APE.

2. Size distribution of the Indonesian and Japanese catches on and off the spawning grounds

Davis et al. (1996) describe the methods used to sample the lengths of SBT caught by Indonesian longline vessels fishing on the SBT spawning grounds. All length data from the spawning grounds used in this report were collected in the 10-15° S, 110-120° E block within CCSBT Statistical Area 1. For the purposes of this report we shall refer to this block as Area 211. It is worth noting that the samples analysed for this report were collected from throughout the fishery's range in Area 211.

Japanese longline catches within the 15-20° S, 110-120°E block in CCSBT Statistical 1 are referred to as Area 311 catches.

The length distributions of Japanese catches off the spawning grounds, used in calculation of age distributions from these areas, comprise length frequency data summed across all CCSBT Statistical Areas other than Area 1. These data have not been weighted according to effort.

Results and Discussion

Direct estimates of age of SBT caught on the spawning grounds

The otoliths from the Indonesian samples were similar to those from SBT caught throughout the range of the fishery. The precision of age estimates used in this study was high; intra- and inter-reader APE's were 4.21% and 3.92%, respectively.

The distributions of sizes and ages of samples collected from the Indonesian SBT catch during the 1993-94, 1994-95 and 1995-96 spawning seasons are shown in Figures 1 and 2, respectively. Within the age distributions there are a number of noteworthy features.

First, although the range of ages is similar among seasons - 15-34, 10-34 and 16-36 years for 1993-94, 1994-95 and 1995-9, respectively - there is variation in the numbers of fish in the younger age classes. We believe this variation is an artefact of the small sample sizes analysed, rather than an indication of interannual variation in age distribution of the spawning stock. The samples consisted of approximately 50 fish, spread over 20+ year classes, so it is not surprising to see under-representation of year classes that may not be fully recruited to the spawning stock. It seems likely that with larger samples the range of ages within the distributions will more consistent across years.

Despite the minor differences in representation of younger age classes, the overall distributions of ages among the 1994-95 and 1995-96 samples were not significantly different (Kolmogorov-Smirnov: $\chi 2 = 6.125$, DF = 2, p=0.0935), suggesting that the age distribution of the spawning stock does not vary from year to year, at least over short time scales. Unfortunately, the 1993-94 sample could not be used in pairwise comparisons as the otoliths aged were not selected at random. Random samples for this season will be analysed in the future to allow for this comparison. Once again, the power of the interannual comparison is limited by the small sample sizes analysed, and it is possible that with larger sample sizes, and the greater resolution they provide, more subtle differences will become apparent.

Use of direct age estimates to determine the age distribution of SBT catches on the spawning grounds.

The distributions of ages of SBT catches on the spawning grounds during the 1994/95 and 1995/96 spawning seasons were estimated using length frequency data collected by Davis et al (1996) and an age-length key based on a combination of the direct estimates of age of fish caught on and off the spawning grounds (spawning ground data derived from this study, off spawning ground data from Gunn et al. 1996). Fish caught on the spawning grounds during the 1993/94 spawning season were not used in the development of the age-length key because of problems detected in the length measurement of those fish.

Given the small sample sizes available from the spawning ground, and the inherent difficulties in testing for differences between distributions that are inadequately sampled, we have assumed that there are no significant differences in the age-length relationship between fish caught on the spawning grounds and fish caught off the spawning grounds. This seems reasonable given the acceptance of the single stock hypothesis for SBT, as there seems no reason to expect fish caught on the spawning ground to be growing differently from those sampled off the spawning grounds. This would only be the case if there is significant spatial heterogeneity in age and growth within the population. In this case, if different components of the population spawned at different times of the year, or in locations that may be sampled differentially by the Indonesian catch monitoring program, then one might see difference in an age-length relationships between the spawning and feeding grounds.

The age-length key used for the estimation of ages in this study is based on age estimates of 411 fish from ages classes 7-43 years (Gunn et al. 1996). This sample size is sub-optimal, and in the future larger samples will be used to formulate an otolith-based age-length key for estimation of age distributions of the population.

The distributions of the estimated ages of fish caught on the spawning grounds, derived from conversions of lengths to ages using the otolith-based age-length key, are presented in Figure 3. The distributions for the two seasons covered in our samples are very similar and there is no indication of the expected progression in the modal lengths by one year. This is not surprising however, since a common age-length key was used for both seasons and there was little difference among seasons in the length distributions of catches

The lack of sensitivity in the conversions of lengths to ages suggests that if we are going to use direct ageing data to examine trends in the age distribution of the spawning stock - an approach that would provide powerful support to the VPA assessments - we will need to age a greater proportion of fish in each season. With adequate samples, year-specific age-length keys could be used to estimate year-specific age distributions for the spawning stock.

Notwithstanding the limitations of the data, there is some, albeit weak evidence of a decrease in the proportion of older fish and a slight increase in the proportion of younger fish in 1995/96 over the previous season (Fig. 4).

Estimation of the size at which SBT first spawn

It is clear in both seasons for which we have data that age groups 7-15 are poorly represented in the spawning population, at least as it is represented by the Indonesian samples (Fig. 3). This observation is in conflict with previous estimates of the mean size and age at first maturity, on the basis of which we could expect to see fish from ages 8 and above well represented in the spawning stock. The 1994 CCSBT Scientific Workshop (Anon, 1994) concluded that "while there was a fair bit of variability, the mode (in size of fish on the spawning ground, based on conversion of lengths to ages using the growth curves accepted by the CCSBT) appears to be roughly at age 10 and age 8 is not a bad estimate of where the 50% (maturity) level is".

On the basis of ovarian development, Davis (1995) estimated the mean size at first maturity to be 157 cm - essentially the same length estimated by Anon (1994). If one converts length to age using the growth curve based on tagging and length frequency data employed throughout the CCSBT assessments, then both Anon and Davis estimate the mean age at maturity as 8-10 years. If one uses the age-length key of Gunn et al. (1996), based on direct estimation of age from otoliths, then the age at maturity is 1-2 years older, depending on the birth dates and accordingly assigned ages.

The discrepancy between the observed and expected representation of 7-15 year-old fish in our samples from the spawning grounds could be explained by one or a combination of factors:

1. The spawning fish sampled by the Indonesian catch monitoring program are not representative of the whole spawning population.
2. The younger fish are poorly represented because cohorts making up that part of the population are very low in abundance.
3. The currently accepted mean length and age at first maturity has been underestimated.

As this is clearly a very important issue, we discuss below the data supporting or refuting each of the explanations and attempt to reach an estimate of the age at which SBT first spawn, rather than the age at which they mature.

1. Fish measured and sampled through the Indonesian catch monitoring are not representative of the whole spawning population.

The vast majority of SBT sampled by the Indonesian catch monitoring program come from area 211,in the northern half of the SBT spawning grounds. Others are collected as far north as the Banda Sea. In some species of fish it has been observed that larger fish within the population tend to migrate longer distances than smaller fish. If this was the case in SBT, and the smaller and younger SBT did not travel as far north on their spawning migration as the larger and older fish, then small fish would preferentially spawn in area 311, south of Area 211. Under this regime, one could expect very different length frequency distributions in catches from the two areas.

Unfortunately, as very few Japanese vessels fished in area 311 during the period 1994-96 while CSIRO have been monitoring Indonesian catches in area 211, there are insufficient length data to enable comparison between the two areas for this period. However, we can compare the length frequency distributions between areas 211 and 311 using historical Japanese data.

In the three years for which there were sufficient SBT measured in the two areas, there is no evidence to suggest that small fish occurred in larger numbers in area 311 than in area 211 (Fig. 5). Therefore, unless there has been a dramatic shift in the behaviour or demographics of SBT in recent years, one would not expect significantly different length frequency distributions in the 1990's.

To examine the evidence for behavioural or demographic changes on the spawning grounds, Fig. 6 shows the length distribution of Japanese catches in areas 211 and 311, pooled over 5 year periods. For the period 1990-95, the length distribution of the Indonesian catches is superimposed on the Japanese distributions.

The first point to note is that throughout the 25 years of data presented there are no obvious differences in the length distributions of Japanese catches in areas 211 and 311. There is no evidence in these data that smaller fish do not migrate as far north as the larger fish, and thus that catches in area 211 are not representative of the spawning population as a whole.

Within the Japanese data there is evidence that the mean size of fish has been getting larger over the last two and a half decades ; in 1970-74 it was 160.95 cm, 1975-79 - 161.54 cm, 1980-84 - 162.44 cm, 1985-90 - 165.78 cm and in 1990-94 (the last year for which we have Japanese data) - 169.88 cm. Thus, over the 25 or so years the mean size of fish caught by Japanese longliners on the spawning ground has increased by almost 9 cm. A similar trend has been observed in the population off the spawning ground where Polacheck et al. (1995) document a significant change in the proportion of fish more than 20 years old in the plus group; from 1965-75 this component of the stock fell from 13% to 7% of the plus group, remained constant until 1983 and since then it has risen to now constitute 31% of the plus group.

Unfortunately, Japanese data for Areas 211 and 311 from the 1990's consists of only 145 measurements. Nevertheless, the distribution of lengths in this sample is significantly different to that from the Indonesian catch, for which the mean length is 181.17 cm. Fish less than 160 cm are essentially missing from the Indonesian catches, while fish greater than 190 cm are missing from the Japanese catches. Why there should be such a significant difference in lengths of catches from the same area is a mystery.

The length measurements made by the Indonesian monitoring program are taken using standard equipment and regular checks are made to ensure data quality and validity. Following these checks, a discrepancy of approximately 5 cm was detected in lengths of SBT measured on the spawning ground during the 1993-94 spawning season, the discrepancy was obvious in the length-at-age estimates for these fish and the data was excluded from all analyses. Significantly, the length-at-age relationships for all subsequent samples from Indonesia have not been different from those for fish caught off the spawning grounds.

If the measurements taken on Indonesian fish are not in error, and the catch is being taken from the same area as Japanese catches, then possible explanations for the differences include :

1. Length measurements from the Japanese catch are biased. This is unlikely as the Japanese length data for the 1990's is based on direct measurement of length of fresh fish, on board the vessels, rather than conversions from weight to length. RTMP observers have examined the accuracy of length measurements by Japanese crews and found these to be unbiased, e.g. Fig. 7 shows the length distribution of catches in Area 8 for 1995, as measured by RTMP observers and by crews. There is close agreement between these two data sets.
2. The Indonesian catch, taken throughout the season by dozens of boats is a better sample of the fish on the spawning ground than the few fish caught by Japanese fishery training vessels. Although, as described above, there are obvious differences between these two data sets, it is significant that two major modes within the limited Japanese data set

- at approximately 177 cm and 185 cm - appear as the strongest modes in the Indonesian data. Although not present in the limited Japanese data from area 211, fish larger than 190 cm are represented in Japanese commercial catches in area 8 in 1995 (Fig. 7), as they are throughout catches on the feeding grounds. This suggests that these larger size classes may simply be underrepresented in the small samples taken by the Japanese training vessels in Area 211. However, there are also large numbers of fish from 160-170 cm in the Area 8 catches, fish that do not appear to be caught by the Indonesians.

3. There is a possibility that the 160-170 cm. size class of SBT is being misidentified as bigeye by those monitoring the Indonesian catch. The fishery targets bigeye with SBT being only a small bycatch. This explanation seems unlikely however, given the respective value of the two species and the fact that all of the product is sold as sashimi on the Japanese market. Nevertheless, the identification of tunas in the Indonesian factories will be verified as part of this year's catch monitoring program.

In conclusion, it seems likely that the Japanese length distributions from catches made in the spawning ground in the 1990's do not represent the whole spawning stock length distribution. There remains an inconsistency between the Japanese and Indonesian data sets in the representation of fish between 150 and 165 cm. The possibility that data from the Indonesian catch monitoring program do not represent the whole spawning stock length distribution can not be discounted, although given the large sample sizes, the geographic coverage of sampling and the consistency between years, it is very difficult to understand why this would be the case.

There is strong evidence to suggest that the average size of fish on the spawning ground has increased significantly over the last 25 years; by at least 9 cm (based on Japanese data, and perhaps as much as 20 cm based on Indonesia data).

2. Younger fish are poorly represented in the Indonesian samples because cohorts constituting that part of the population are low in abundance.
To resolve this explanation it is necessary to compare catch-at-age distributions for catches on and off the spawning grounds (Fig. 8). From these it is clear that in the off-spawning ground catches, and thus, presumably, the population as a whole, there are significantly less 12-15 year-old fish than there are 7-11 year-olds. When these data are used to estimate cohort strength (Fig. 9), it is apparent that cohorts spawned after 1984 are much more abundant than those spawned before 1984. This is not surprising given the significant reduction in catches of 0-4 year-old fish following the introduction of catch quotas in 1984.

Although the low relative abundance of 12-15 year old fish off the spawning grounds would explain their low abundance on the spawning ground, the high relative abundance of 7-11 year-olds off the spawning grounds suggest that if they were mature that they should be caught in much greater numbers than they apparently are by the Indonesian fishery.

3. The currently accepted mean length and age at first maturity has been underestimated.
Given the relatively strong representation of 7-11 year old fish in catches off the spawning grounds (Figs. 8 & 9) , and their poor representation on the spawning grounds, it seems likely that these age classes do not contribute significantly to the spawning stock. This suggests strongly that the age at first spawning is older than estimated in the past.

General Discussion and Conclusions

The preliminary data covered in this report significantly impact on our understanding of the SBT population. We believe with larger sample sizes and continued monitoring of the Indonesian fishery on the spawning ground that it will be possible to further clarify the status and dynamics of the spawning stock and the demographics of the population as a whole. With adequate samples it should also be possible to estimate natural mortality rates for the "plus" group.

There is strong evidence that the size of fish on the spawning grounds has increased over the past decade or so. Whether this is a function of reduction in the numbers of small fish in the spawning stock, or a real change in the size-at-age is unclear. If the latter is the case, then it would indicate that maturity in SBT is related to age rather than the size of fish - a hypothesis first considered at the 1993 Trilateral Assessment workshop.

Given our preliminary finding that previous estimates of the age at first maturity may be too low, a first priority of future work will be to examine this question. On the basis of the data available, 12-13 years seems a more appropriate estimate of mean size at first spawning than the previous estimate of 10-11 years for the age at first maturity (Davis 1995), or the estimates of 7-9 years used in the 1993 CCSBT workshop . Why there should be a discrepancy between estimates based on age composition of catches on the spawning ground and estimates of maturity based on ovarian development is not clear. However, it suggests that many of the fish less than 12 years old that appear on the pre-spawning grounds in pre-spawning condition do not migrate north onto the spawning grounds, or contribute to the "parental biomass".

If one accepts that the size and age distributions of the spawning stock are adequately represented by the Indonesian catch monitoring program data, then it is apparent that the spawning stock has yet to feel the full impact of poorly-represented cohorts spawned between the mid-1970's and mid-1980's. As there is a significant relationship between fish size and fecundity (Batch fecundity = $(4.78242 \times 10^{-17}) \times \text{Length}^{7.530}$ - Farley and Davis, in prep), and 12-20 year olds are generally smaller than older fish, the weak representation of the mid-1970's to mid-1980's cohorts in today's spawning stock will likely have an increasing negative impact on the "population fecundity" over the next decade. On the other hand, it appears that the 1985-89 cohorts are well represented in the population

(Fig. 8), and thus there are good prospects for increases in the size of the spawning stock as these fish mature over the next over the next 5-7 years.

As more direct age estimation is conducted and data on the relative abundance of cohorts within the spawning stock becomes available, it should be possible to examine trends in the relative contribution to the parental biomass of these poorly represented cohorts. In combination with the VPA's and CPUE analyses currently used within the CCSBT assessments, accurate data on the catch-at-age distributions of the spawning stock will provide a much-needed improvement in our understanding of the way in which the SBT population has responded to the catch levels of the past decade.

Future research on the age structure of the SBT population

Following the completion of CSIRO's major research project on developing and validating age estimation methods for SBT (Gunn et al. 1996), the scope now exists for both routine age estimation in support of the VPA- assessment process and for targeted studies examining specific questions regarding the age structure of the population.

1. Routine estimation of the SBT catch-at-age.

Since 1990, CSIRO has been co-ordinating the collection of otoliths from SBT catches throughout the range of the fishery. With funding from the Australian SBT Management Advisory Committee, an SBT Hardpart Archive was established in 1992. The Archive is supported by a data base and all otoliths in the collection are cleaned and ready for processing. Each year collections by Australian AFZ and RTMP observers and CSIRO scientists add approximately 1000+ otoliths to the archives. The otoliths are sampled from fish selected at random from the catch. The archiving process stalled in 1995-96 as a result of lack of funds. However, observers have continued to collect otoliths.

Any routine age estimation program would need significant support from CCSBT countries. SBT otoliths require careful preparation before estimates of age can be made, and readers require thorough training before the precision and accuracy of estimates are high. In conjunction with the Australian Central Ageing Facility at Queenscliff, Victoria, CSIRO has prepared an indicative budget for curating, archiving and ageing 1000 SBT per annum. On the basis of each otolith being read twice by at least two readers, the cost would be in the order of $50-60K per annum.

2. Demographics of the SBT population.
The data presented in this report are the first use of otolith-based age estimates in the study of SBT population demographics. The early indications are that these kinds of data will be very useful in the examination of trends in abundance of year classes, and in determining the response of cohorts to exploitation levels.

Over the next three years CSIRO are proposing to examine in more detail the age structure of the SBT spawning stock, using material collected from the Indonesian catch monitoring program, and where these are available otoliths collected from other fishing on the spawning grounds

A second line of investigation will be the examination of spatial heterogeneity in the demography of the SBT population. We plan to examine whether Japanese catches in the SW Indian/SE Atlantic, SE Indian and Tasmanian fisheries, comprise similar mixes of cohorts, and compare these with the Taiwanese and Indonesian catches in the Indian Ocean, and the Australian and New Zealand fishery catches in the Pacific. The data from these comparisons would add valuable information to the assessment of the impact of various catch and effort levels on the population as a whole, but most importantly the spawning stock.

The projects outlined above are currently under development and proposals for work to be conducted from 1997-2000 will be submitted later in 1996. Input from the 1996 CCSBT Scientific Workshop in Hobart would be most welcome.

Future monitoring of the Indonesian catch on the SBT spawning grounds.

CSIRO is proposing to continue monitoring of the Indonesian fishery, with resources from the Australian Fisheries Management Authority's research funds. A priority over the next 12 months will be to examine possible biases in SBT length data and the investigation of possible mis-identification of small SBT as bigeye. Once again, it should be emphasised that this is considered unlikely, but as the data are critical to our understanding of the spawning stock, it is essential to validate all sources.

As an understanding of the dynamics of the spawning stock is seen as a high priority by the CCSBT, it may also be worth considering an experimental fishing program on the spawning grounds, to collect length data that could be compared with the Indonesian data, as well as valuable biological samples.

Acknowledgements

The collection of otoliths in Indonesia was supervised by Sofri Bahar. Australian observers collected otoliths throughout the Japanese high seas fishery as part of the AFMA AFZ and CSIRO RTMP observer programs and we are grateful for their unstinting efforts in difficult conditions. The skippers and crews of Japanese longline vessels made their catch available for sampling and their support is gratefully acknowledged. We also thank Tom Polacheck of CSIRO for his valuable input into the discussion of these data. The Australian Southern Tuna Management Advisory Committee provided the funds to analyse the otoliths collected by the Indonesian catch monitoring program.

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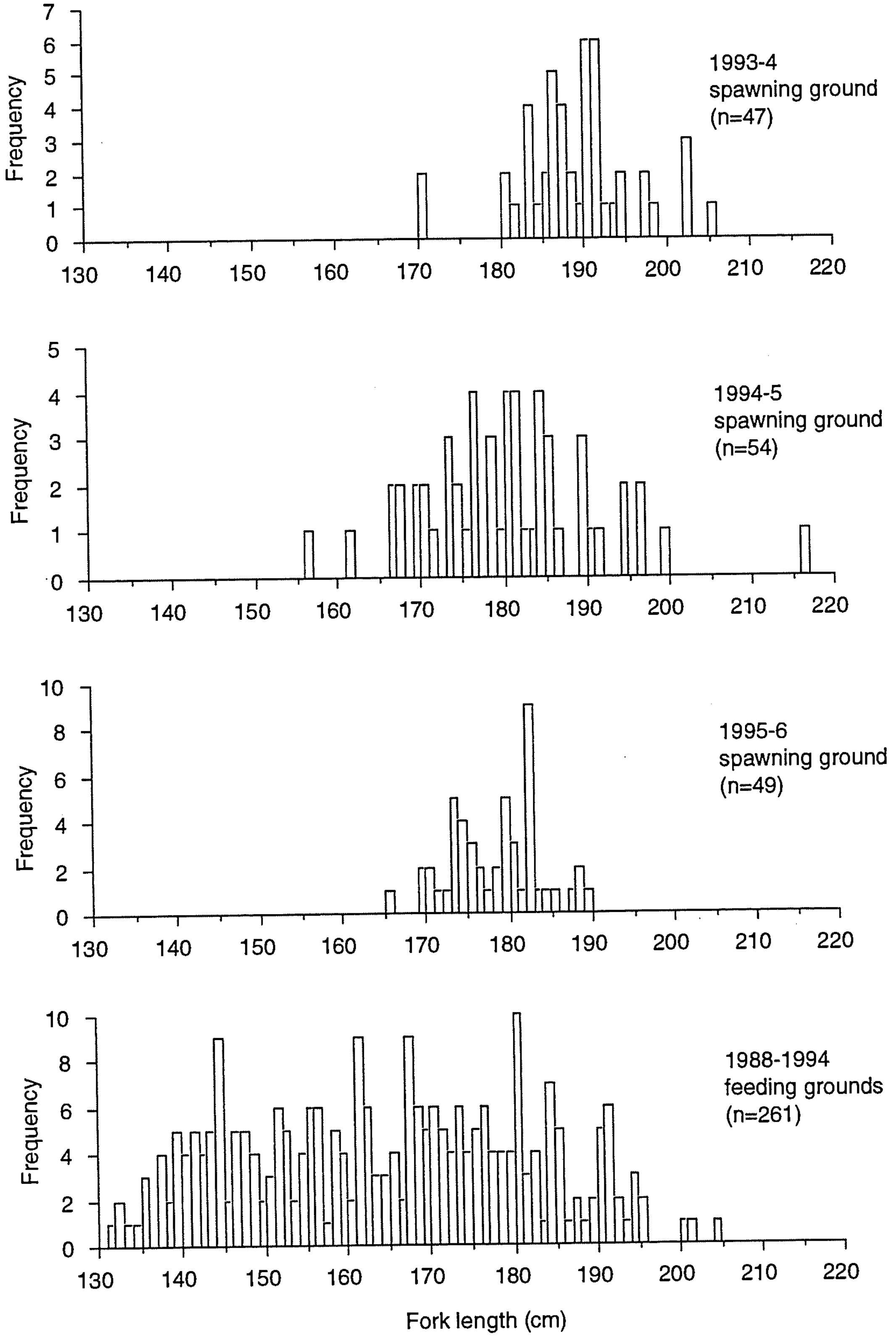

Fig. 1. Length distributions of fish from which ages were estimated using otoliths.

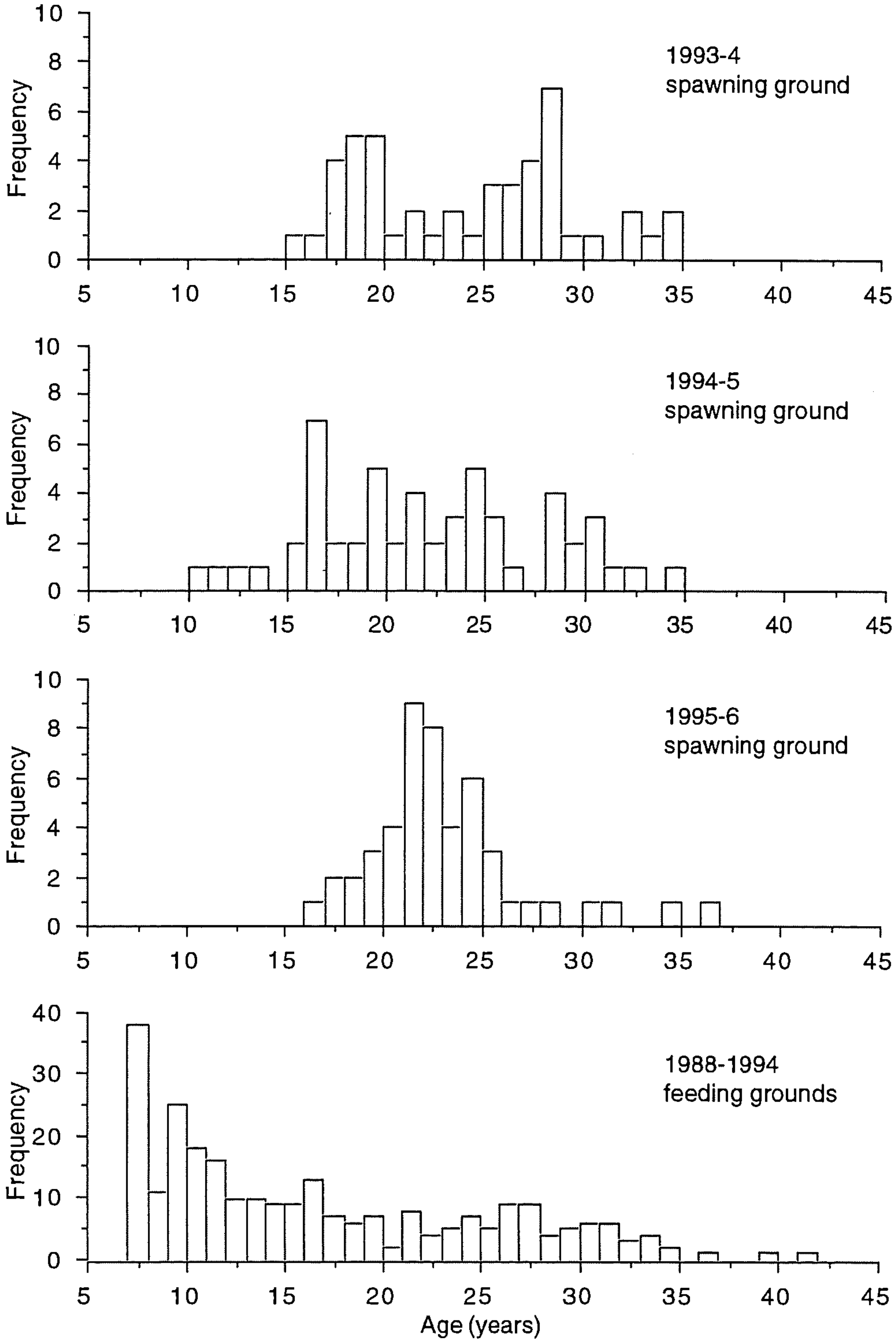

Fig. 2. Estimates of age distributions for SBT caught on the spawning ground during the 1993-4 (a), 1994-5 (b) and 1995-6 (c) spawning seasons, and SBT older than 7 years old caught on the southern ocean feeding grounds for 1988-94 (d). Note feeding ground ages truncated at age 7 due to dominance of <7 year olds in data

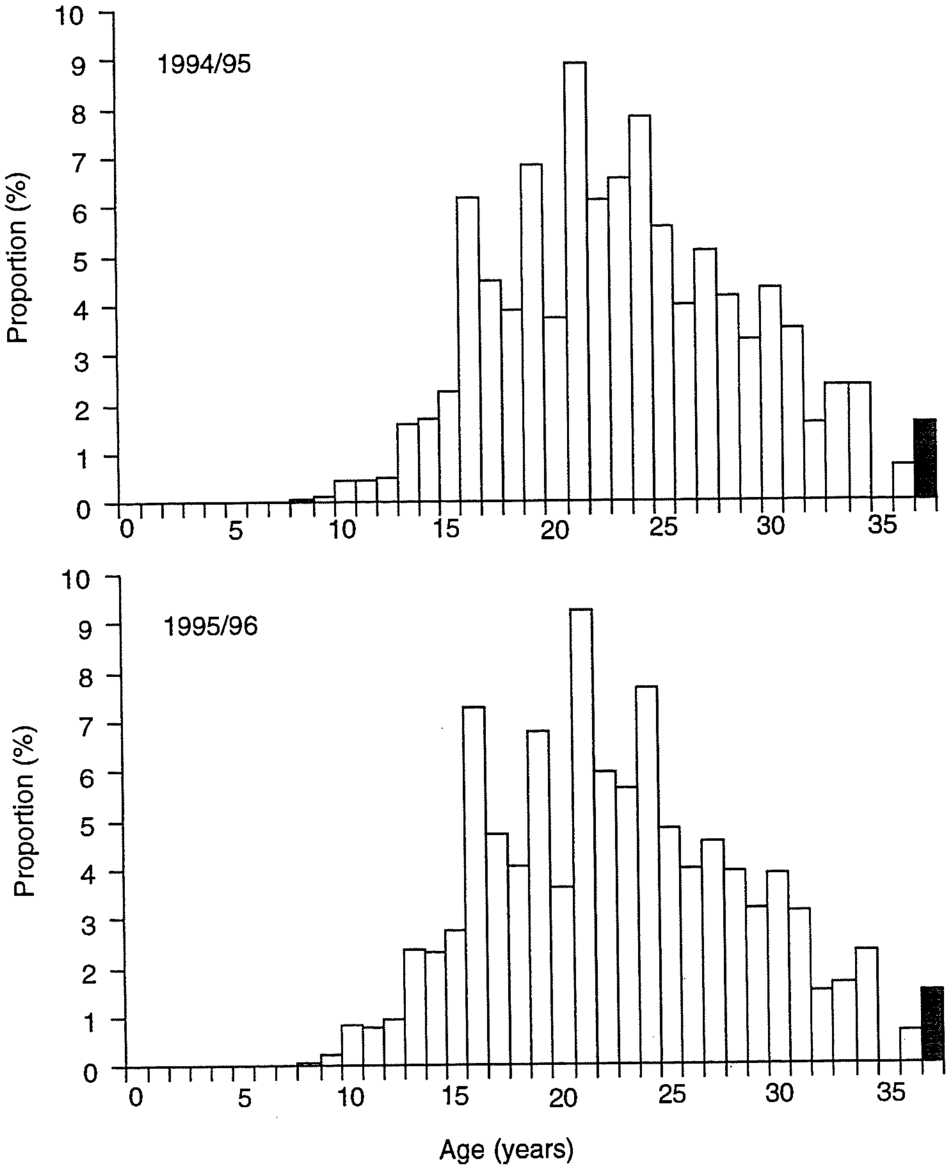

Fig. 3. Age distribution of SBT on the spawning grounds during the 1994/95 and 1995/96 spawning seasons. The shaded bars corresponds to the 36+ age group.

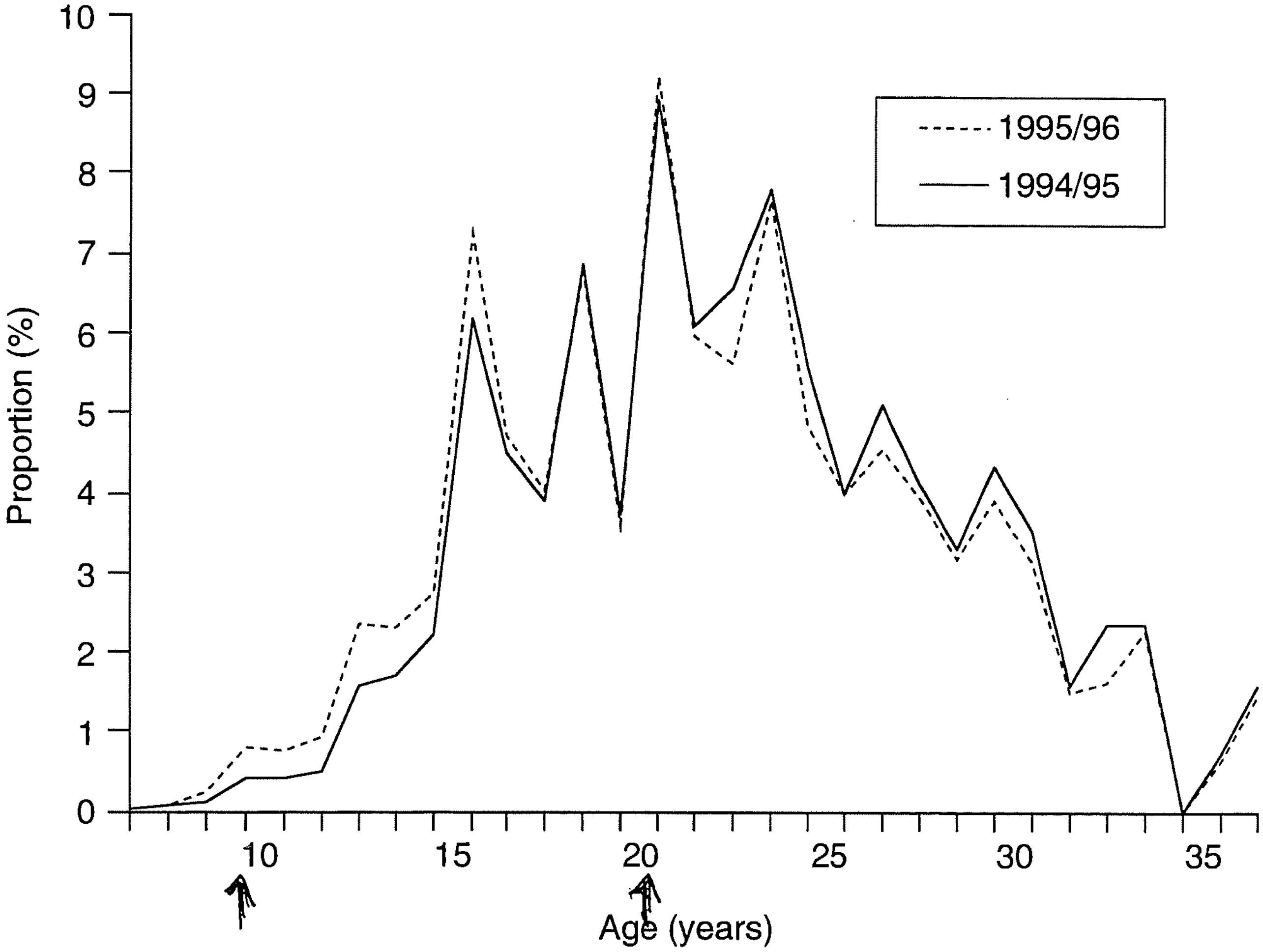

Fig. 4 Age distribution on the spawning grounds during the 1994/95 and 1995/96 spawning seasons.

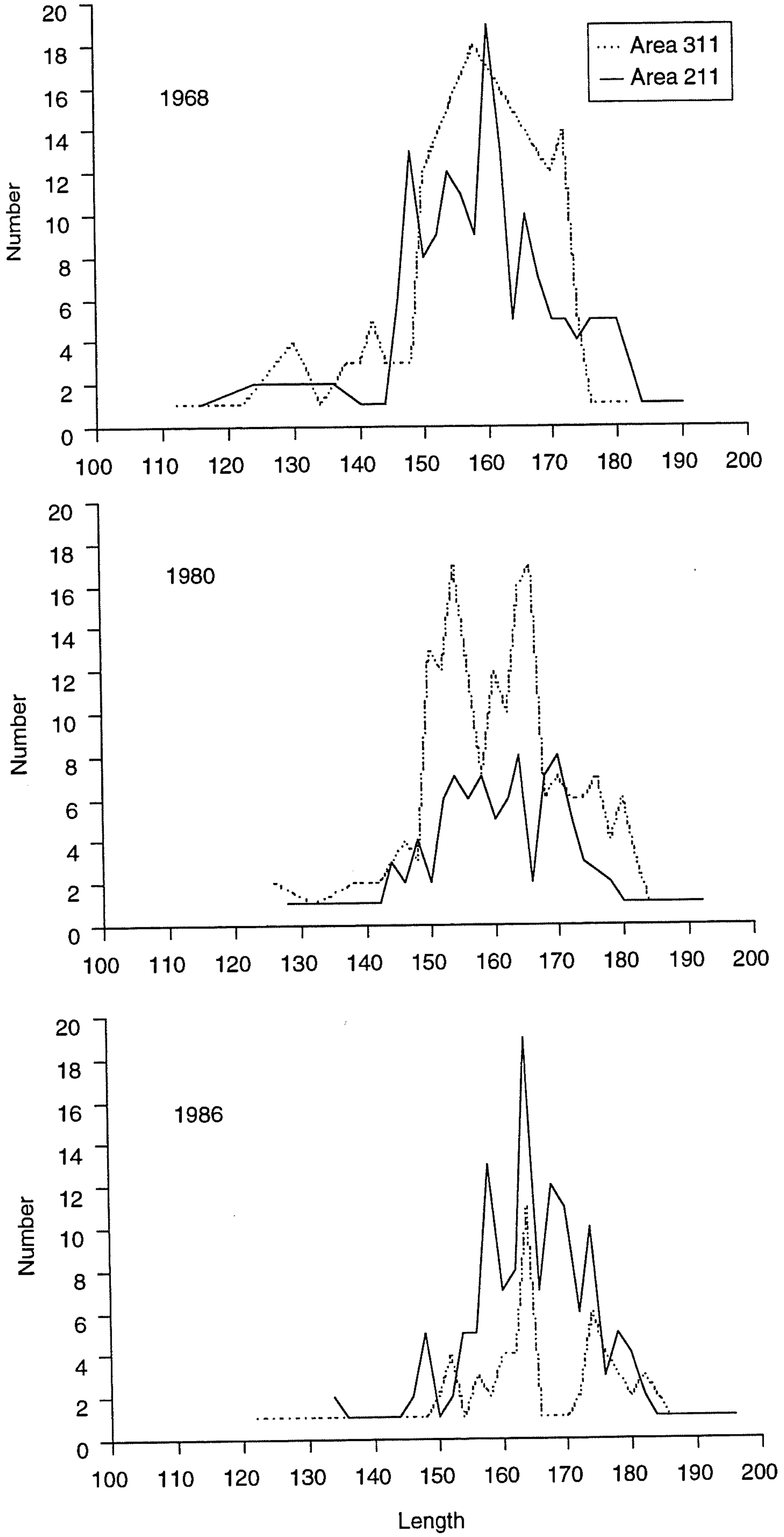

Fig. 5. Length frequency of SBT caught by Japanese longliners in areas 211 and 311 in 1968, 1980 and 1986.

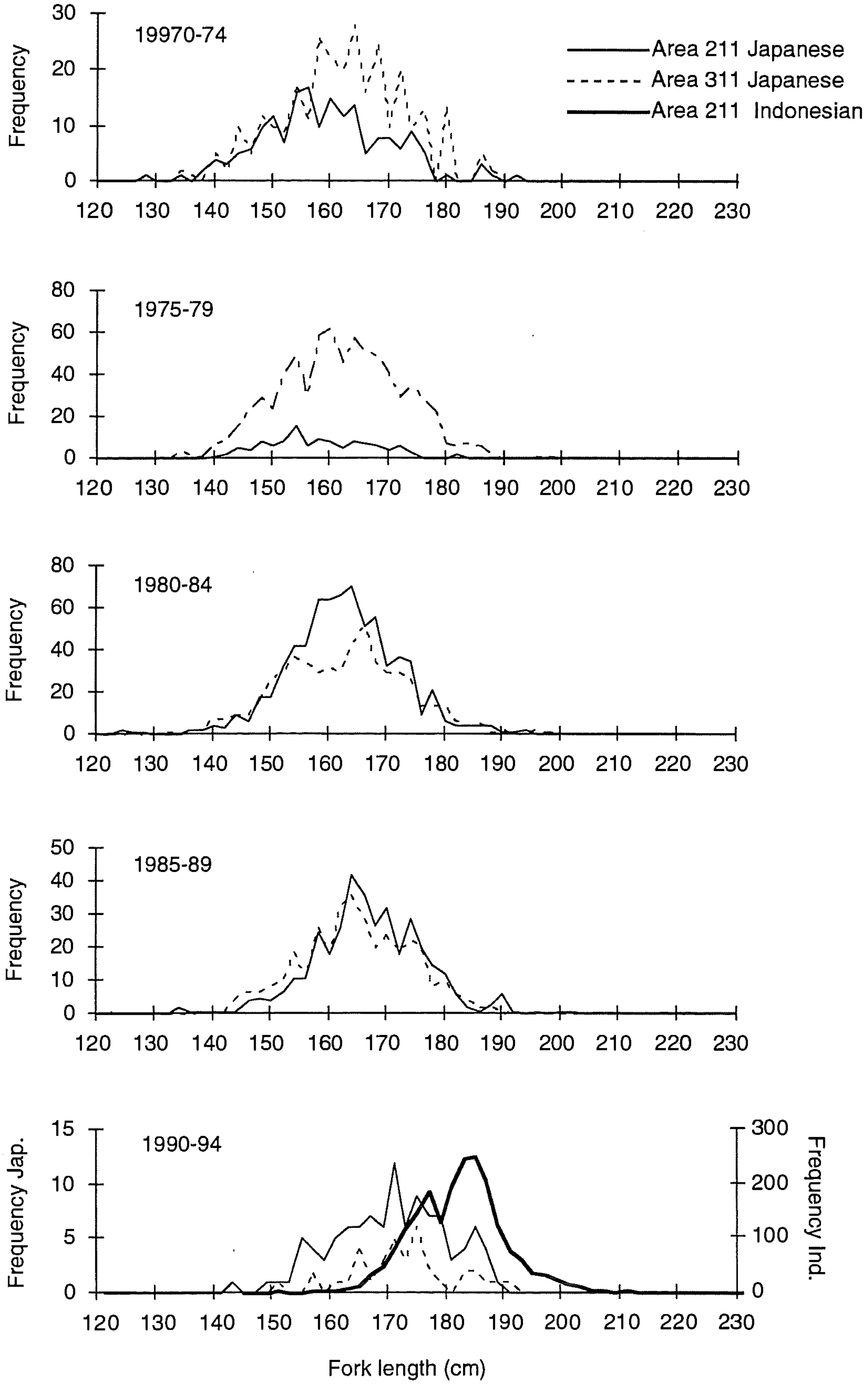

Fig. 6. Length frequency distributions of SBT caught by Japanese longliners (a-d); and Japanese and Indonesian longliners (e) in areas 211 and 311.

Draft Agenda 7
CCSBT Scientific Committee meeting
26 August - 5 September 1996
Hobart, Australia

1. Meeting establishment
    1.1 Welcome from Chief of CSIRO Division of Fisheries
    1.2 Appointment of Chair and rapporteurs
    1.3 Adoption of the agenda
    1.4 Documents

2. Country reports
    2.1 Japan
    2.2 New Zealand
    2.3 Australia
    2.4 non-Commission countries

3. New biological information on SBT

4. Interpretation of CPUE and other indices of abundance

5. Population assessments

6. Major sources of uncertainty in population assessments

7. Projections

8. Major sources of uncertainty in assessment and projections

9. Recommendations as appropriate concerning the conservation, management and optimal utilization of SBT.

10. Research recommendations

11. Report of the Ecologically Related Species Working Group

12. Rules of Procedure

13. Other business

14 Adoption of report

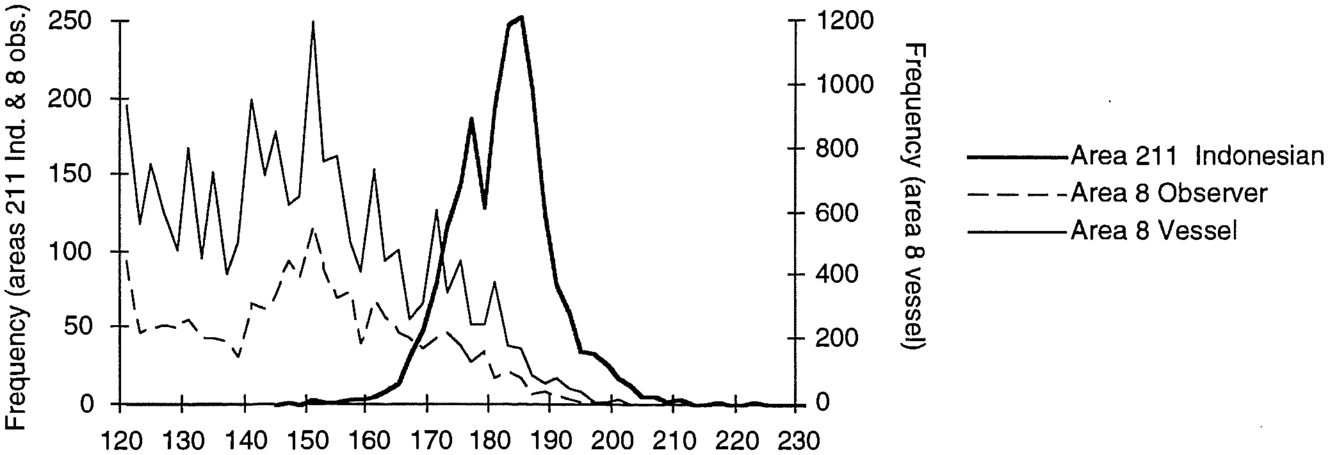

Fig. 7. Comparison of length distributions of SBT caught by the Japanese longline fishery in 1995 within area 8 with those caught by the Indonesian fishery in 1994-96 within area 211. The Japanese data includes lengths taken by the vessel and by RTMP observers.

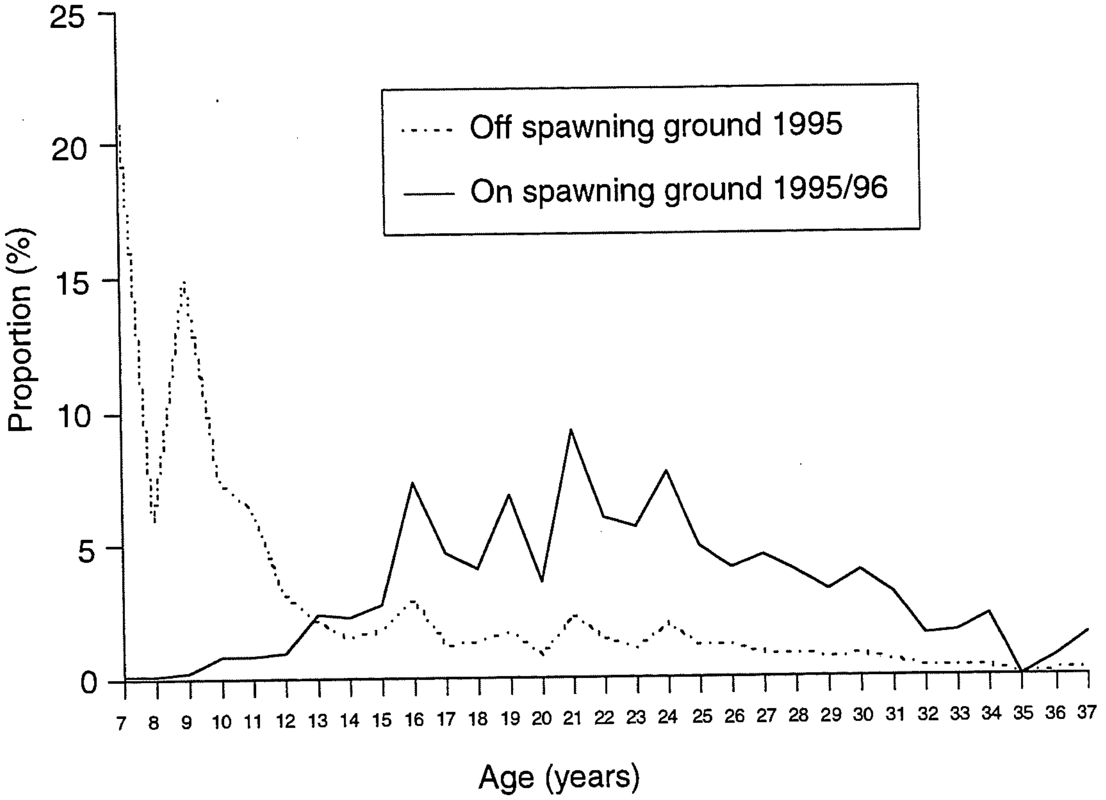

Fig. 8. Age distribution on the spawning grounds during the 1995/96 spawning seasons, and age distribution of Japanese catch data in 1995.

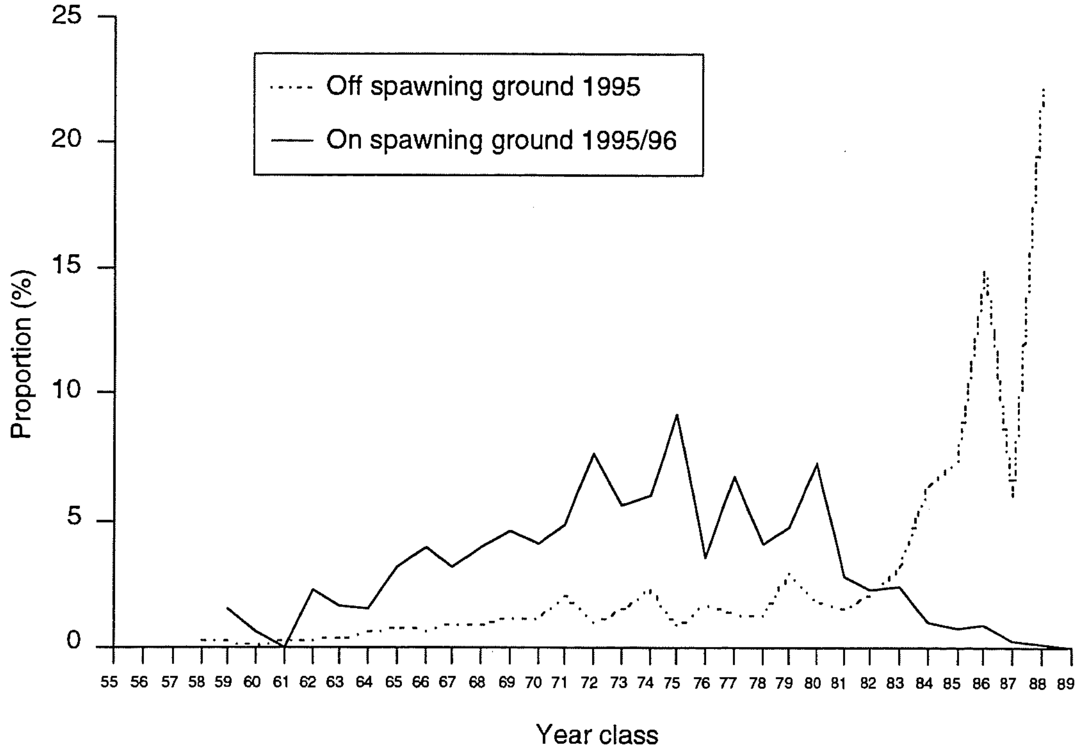

Fig. 9. Relative abundance of year classes on the spawning grounds during the 1995/96 spawning season, and off the spawning ground in 1995 (based on Japanese catch data).

The direct estimation of age and growth of Southern Bluefin Tuna

Appendix 6

Project 92/42

CSIRO
AUSTRALIA

CSIRO - Division of Fisheries
GPO Box 1538
Hobart TAS 7001

FISHERIES RESEARCH & DEVELOPMENT CORPORATION RESEARCH GRANT

Final Audited Statement of Receipts and Expenditure

As at 12 September 1996

Name of Grantee: CSIRO - Division of Fisheries.
Title of Project: The direct estimation of age and growth of southern bluefin tuna.

CSIRO Project No: DF24HMBFS - - YR
FRDC Project No: 92/42

FRDC Funds:-

1992/93	$108,893
1993/94	$80,925
1994/95	$72,445
	$262,263
Received to date:	$226,042

Receipts:	**1992/93**	**1993/94**	**1994/95**	**1995/96**	**1996/97**	**Total**
July - December	$54,446	$20,232	$36,223			$110,901
January - June	$54,447	$60,694				$115,141
TOTALS	$108,893	$80,926	$36,223	$0	$0	$226,042

Less Expenditure:	**Overall Budget**	**1992/93**	**1993/94**	**1994/95**	**1995/96**	**1996/97**	**Total**
Salaries	$181,883	$51,390	$66,678	$64,864	($377)	$0	$182,555
Travel	$25,980	$6,983	$9,377	$7,117	$1,806	$0	$25,283
Operating	$41,100	$8,089	$16,498	$14,888	$4,673	($18)	$44,130
Capital	$13,300	$6,138	$0	$4,158	$0	$0	$10,296
TOTALS	$262,263	$72,600	$92,553	$91,027	$6,102	($18)	$262,264
Cash Balance:		$36,293	$24,666	($30,138)	($36,240)	($36,222)	($36,222)

Comments: Final 50% payment of 1994/95 funds ($36,222) is still to be made subject to project completion, receipt of final report and this audited acquittal statement.

Minor overspend from 1995/96 transferred to CSIRO accounts in 1996/97.

Prepared by responsible officer:

Greg Lyden
External Grants Officer
12-Sep-96

Certified by responsible officer:

Peter Green
Finance Manager
12-Sep-96