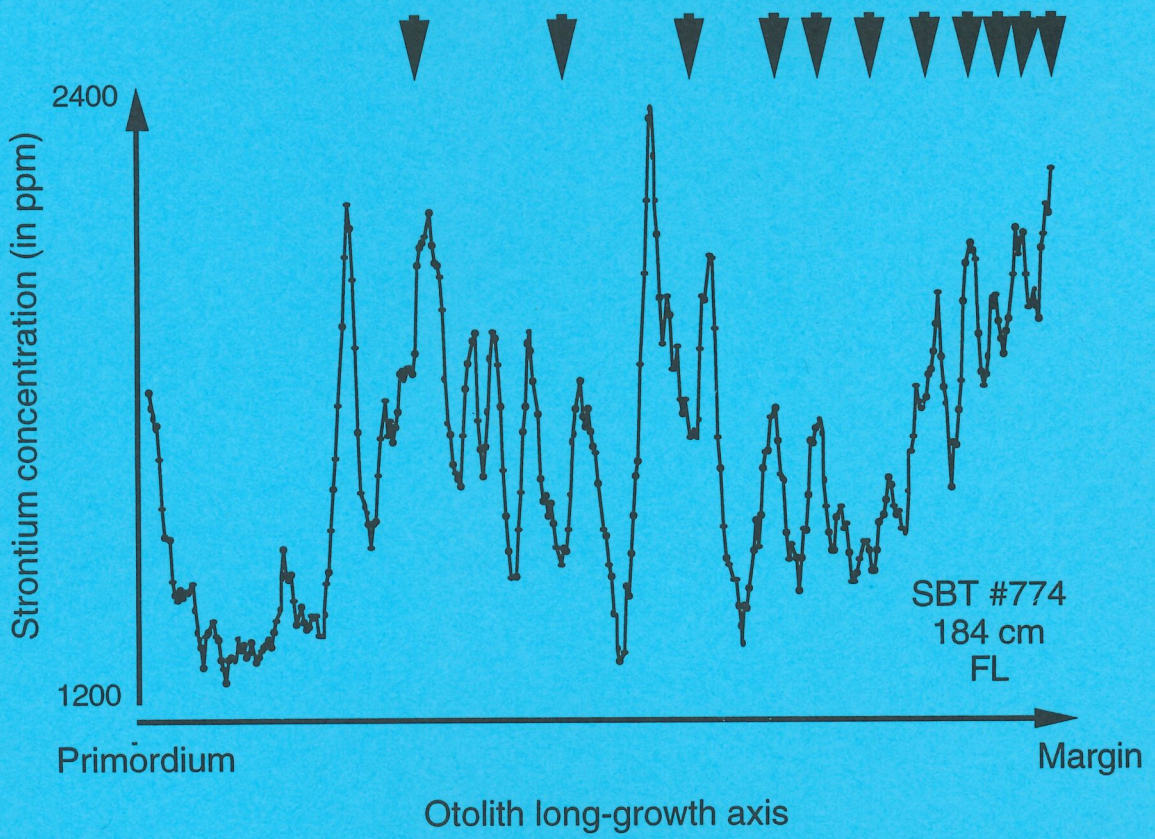
April 1994

FINAL REPORT TO
FISHERIES RESEARCH AND DEVELOPMENT CORPORATION

GRANT #1983/30

AGE DETERMINATION AND ASSESSMENT OF VARIATION IN LENGTH-AT-AGE OF MATURE SOUTHERN BLUEFIN TUNA BY MEANS OF ANALYSIS OF OTOLITH AND VERTEBRAL COMPOSITION



CSIRO AUSTRALIA
DIVISION OF FISHERIES

CONTENTS

1. NON-TECHNICAL SUMMARY

Patterns of ontogenetic variation in the composition of the otoliths of southern bluefin tuna (SBT), Thunnus maccoyii, strongly suggested that the concentrations of some elements (and particularly strontium) varied episodically, and probably annually. This suggested that counting the numbers of peaks and troughs in strontium concentrations, as measured across the entire growth axis of a vertebra or otolith, could be an accurate and precise way to determine the ages of fully mature SBT. This suggestion was tested by 1) determining experimentally the extent to which otolith composition varies seasonally, 2) comparing ages estimated using otolith composition with those estimated for the same fish by conventional hard-part aging, 3) determining the extent to which different observers examining the same data would agree on the age of a given specimen (which is a measure of the inherent imprecision of the otolith chemical approach), and 4) attempting to validate annual cycles of deposition by measuring changes in the composition of otolith margins for samples collected over an 18 month period.

The results indicate that most of the elements we measure in SBT otoliths do not vary in concentration as a direct response to changes in, for example, water temperature. As well, differences among individuals in the response patterns, even when they are held until identical conditions, are extremely high. The implication is that physiological or genetic differences among individuals are at least as important as environmental factors in determining otolith composition, and that attempting to derive any simple means of aging that will apply across all individuals may not be possible. At the same time, ontogenetic patterns in otolith composition in SBT are more complex than those of any other species we have thus far examined, which makes it difficult to interpret consistently the patterning for purposes of determining ages. In part because of these high levels of variability, and in part because we had considerable difficulty getting samples for mature SBT year-round (due to the seasonal nature of the fishery), the attempted validation proved inconclusive. This could mean that the basic hypothesis is wrong, that is, that Sr concentrations do not vary annually. However,. when we compared a growth curve based on the assumption that the cyclic deposition is annual with one independently derived from population parameters, we found a very close fit. This suggests that the hypothesis of annual cycles of deposition is correct, but that proving it is very difficult in SBT due to their mobility and the high levels of individual variability. This further suggests that the technique may prove successful when applied to more sedentary species for which age determination is still uncertain, such as redfish.

2. BACKGROUND

At the time of this application for funding was made, strategies for the management of SBT were based primarily on Virtual Population Analysis (VPA).

The precision and accuracy of VPA depends in part on the accuracy of age determination, particularly for older, mature fish. In SBT, for purposes of these analyses, all large individuals were pooled into a single cohort irrespective of their true age. This pooling of year-classes and the resultant loss of precision was necessary due to the lack of any reliable and validated method for aging mature SBT. Conventional aging techniques, based on counting optical "annuli" in otoliths, were poorly validated for the species, not validated for mature fish, and appeared unlikely to be useful for these fish, due to problems of resolving closely spaced "annuli" near the otolith margin. Thorogood (1987) had demonstrated a new method of aging SBT using burnt otoliths and expressed confidence in resolving up to 9 annuli, but again, difficulty in resolving annuli near the otolith margin of older fish limited the applicability of this technique. Methods of direct age determination using age-related banding in other calcified structures (including vertebrae, scales, and fin-spines) have been investigated for SBT and for the closely related northern bluefin tuna, Thunnus thynnus , but none were considered suitable for older fish (Hurley and Iles 1982, Thorogood 1987, Yukinawa 1970).

Prior to our work, several studies had revealed episodic, ontogenetic variations in the incorporation of chemical constituents (most notably strontium) in the calcified structures of teleosts (Radtke and Targett 1984) and elasmobranchs (Cailliet and Radtke 1987). In both groups, these variations were suggested to be "annuli". In our preliminary studies, in which we investigated whether the composition of SBT otoliths could be used as a means of stock discrimination (FIRDC Final Report #1987/15), we noted the presence of conspicuous episodic variations in composition in otoliths from both small and very large SBT. Moreover, the number of these episodes, when counted along the entire growth axis, was similar to the expected age of each fish, based on its size and previous estimates of the growth rates for the species. These cycles of compostional change were evident even at the margin of otoliths of fish >170cm F.L.

The current study examines in detail the possibility that these episodic variations in composition are annual, and the potential that they can be used to determine the ages of even very large SBT. On the basis of literature reports, we also conducted a feasibility study on vertebrae, to determine if they might be a better suited structure for age determination

3. PROJECT DETAILS

OBJECTIVES

1. To determine whether mature SBT can be aged by counting the number of regular variations in the composition of their hard parts, by testing the hypothesis that such variations derive from annual cycles in deposition along the margins;

2. To compare growth rates, maximum ages and precision of age estimates as determined by microanalysis with published values based on traditional aging techniques; and

3. To quantify variation in length-at-age for mature SBT.

PERSONNEL

R.E. Thresher	Ph.D.	(50)	Project Supervisor
J. Gunn	B.Sc. (Hons.)	(50)	Data analysis/Field Operations
C. Proctor	B. Sc. (Hons.)	(50)	Microprobe Operations
T. Carter		(100)	Specimen Preparation

4. TECHNICAL RESULTS

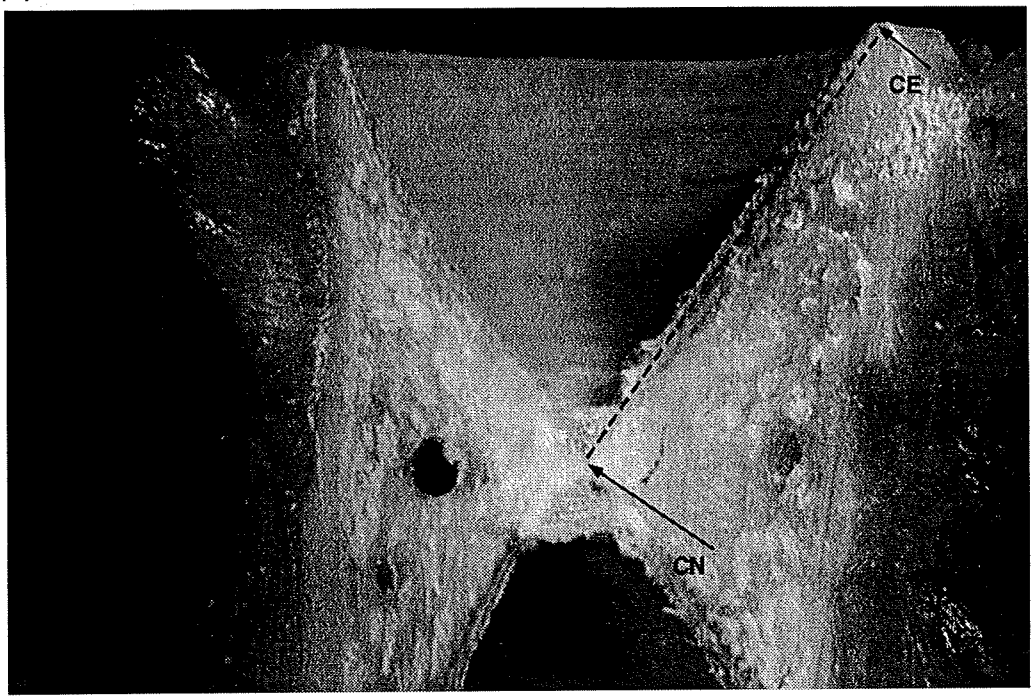
4.1 COMPARISON OF OTOLITHS AND VERTEBRAE AS STRUCTURES SUITABLE FOR AGE DETERMINATION

One of the original objectives of this project was to investigate ontogenetic variability in chemistry across sections of SBT vertebra, in conjunction with similar investigations for SBT otoliths. The investigation encompassed three principal questions 1) Can SBT vertebrae be adequately prepared for electron probe microanalysis (EPMA) ?, 2) Are there regular ontogenetic variations in the concentrations of one or more constituent elements ?, and 3) If such variations are evident, do they derive from annual cycles and hence could be used as a means of aging large, mature fish ?

Growth bands on the surface of vertebral centra have been used as means of aging in many elasmobranch (Cailliet et al. 1983, Stevens 1975, Thorson and Lacy 1982) and some teleost species, including several scombrids (Berry et al. 1977, Johnson 1983, Prince et al. 1985). However, concerns had been expressed over the reliability of growth band (annuli) counts on vertebrae of large scombrids because, with age, inter-band distance progressively decreases in the outer margin region to the extent that individual bands become irresolvable (Lee et al. 1983).

Cailliet and Radtke (1987) used EPMA to analyse ontogenetic variability in Ca and P concentrations across centra of elasmobranchs, revealing regular, apparently annual cycles that were easily discerned even at the margin. In the light of their results, we attempted to develop a method for preparation of SBT vertebrae for EPMA and conduct trial analyses.

(a)



(b)

Figure 4.1.1 (a). SBT vertebra centrum that has been sectioned, exposing centrum nucleus and centrum surface layer (CSL). GL = growth lines on centrum surface. Dotted line illustrates scan path of trial EPMA analyses along exposed face of centrum surface.B. Incident light micrograph of longitudinal section of SBT vertebra prepared for EPMA. White dots highlight size and location of trial EPMA analyses along centrum surface layer (CSL). V = deep vacuole, R = resin. Scale bar = 100 microns.

METHODS

The 35th vertebrae (in the caudal region) was chosen for the trials following recommendations in earlier studies of scombrid vertebrae used for age examination (Berry et al. 1977). In addition to the advantage that the centrum of this vertebrae was reported to possess clear growth banding, it presented the least number of problems for sampling given that the caudal region is usually removed and discarded during the post-capture processing of the fish. 35th vertebrae were dissected from caudal sections taken off immature SBT (56 - 100cm F.L.) and larger, mature fish (150 - 170cm F.L.). Following removal of all adhering tissue, vertebrae were dried in an oven at 60°C overnight.

We expended considerable time and effort in attempts to prepare a section from the vertebral centrum that would allow EPMA across the centrum surface, as per Cailliet and Radtke (1987). However, we encountered two problems, which we have still not been able to overcome. First, EPMA requires a flat, highly polished surface. However, even relatively small vertebrae from immature SBT are concave, to the extent that we could not produce a single section that encompassed the full growth axis and was flat enough for analysis. Surface curvature was an even bigger problem in centra from large SBT. Second, thrre are numerous ridges and grooves on the surface of centra, which form the growth markings, This relief degraded signals from the EPMA to the point they were unreliable. Attempts to polish down this surface relief while retaining the integrity of the surface layer proved fruitless.

Longitudinal sections from the vertebrae were prepared (Fig.4.1.1a) which included the full growth axis, centrum nucleus to margin. These sections were ground and polished, to the level of the centrum nucleus, using the otolith procedures described in Gunn et al. (1992). EPMA trial analyses were conducted along the edge of these sections within the surface layer. Analyses were conducted for Ca, Na, Sr, K, S and Cl. Beam conditions were as follows: 15kV accelerating voltage, 25nA beam current, 14 μm beam diameter (beam power density = 2.4 $\mu W\ \mu m^{-2}$), with a total acquisition time of ~4 min per analysis.

RESULTS AND DISCUSSION

Difficulties were experienced with locating programmed line scans (as were employed for otolith life-history scans - see Sect.4.2 - Methods) along the surface layer of the sectioned centra for two reasons: 1) because of the curvature of the centrum surface, but more importantly, 2) because of an abundance of deep vacuoles in the bone structure which extend into this surface layer (Fig. 4.1.1b). In life-history scans across SBT otolith sections, up to 5% of analyses require omission because of surface irregularities such as pits and cracks that result in X-ray absorption artifacts. The vacuoles in the surface layer of centra affected >25% of point analyses, thereby significantly reducing the quality of ontogenetic

information that could be obtained. Full life-history scans could be conducted without such information loss, but only by avoiding vacuoles through a manual stage shifting procedure or by programming every individual point location. Both these methods were considered impractical.

Even if these specimen related problems could have been overcome, the distance from centrum nucleus to margin, which reaches 30mm in mature SBT, would make the time and expense required for EPMA full life-history scans prohibitive. A conservative estimate, based on 50µm point spacing for the first two-thirds of the scan and 25µm spacing for the latter third approaching the margin (as growth becomes increasingly compacted), is 53 hours of probe time to complete one 'mature' vertebra. This compares to a maximum of 24 hours to complete a full scan of an otolith section from a similar sized fish. If analyses were restricted to fewer 'key' elements (eg. Sr, S and Ca) these times would be considerably less, however, vertebrae would still consume twice the amount of probe time to that of otoliths. Taking this time/expense factor into consideration, and the fact that otoliths were yielding ontogenetic information with comparatively fewer specimen preparation and analytical difficulties, EPMA analyses of vertebrae were discontinued.

4.2 ENVIRONMENTAL SENSITIVITY OF ELEMENTAL COMPOSITION OF SBT OTOLITHS

The assumption that underlies our analysis is that otolith composition varies seasonally due to environmental factors. This assumption was tested by examining the otoliths of fish held for eight months in sea-cages off South Australia. We hypothesized that if elemental concentrations were environmentally sensitive, then the portion of the otolith deposited prior to caging would differ among individuals, whereas after caging, when all fish were held in identical conditions, elemental composition would converge among individuals and follow a common ontogenetic pattern. If this common pattern was the result of seasonal changes in environmental conditions, we hypothesized a close correlation between, for example, daily variations in water temperatures at the sea-cage and ontogenetic variation in elemental composition at the outer portion of the otolith.

Direct tests of the effects of environmental variation on otolith composition are uncommon and not entirely consistent. To date, studies have focused on two sets of compositional data: isotope ratios (typically oxygen), and elemental concentration. The former are overall consistent with changes in oxygen isotope ratios that correlate with changes in water temperature (Devereaux 1967, Degens et al. 1969, Mulcahy et al. 1979, Radtke 1984 a & b, Radtke et al. 1987, Kalish 1991a, Iacumin et al. 1992). The results of studies involving elemental data are more variable. Several field studies suggest Sr/Ca ratios are affected strongly by water temperature (Radtke and Targett 1984, Radtke and Calliet 1984, Radtke 1987, Radtke and Morales-Nin 1989, Radtke et al. 1990) and salinity (Radtke et al. 1988, Kalish 1990, Secor 1992). However, several other studies (Kalish 1989, Gallahar and Kingsford

1992, Sadovy and Severin 1992) report the effects of temperature on concentrations of several otolith micro-constituents, including Sr, as weak or non-existent. In a previous study (FRDC Final Report #1987/15), we found no evidence that major shifts in habitat significantly affected the micro-constituent composition of otoliths in juveniles of jackass morwong, with the possible exception of a slight affect on Sr concentrations. On that basis, we concluded that environmental affects on otolith composition at the >100 ppm level were slight or heavily buffered by physiological mechanisms.

Kalish (1989) suggested that at least some portion of the variable results of the experimental studies can be attributed to effects of stress, which can affect rates of Sr incorporation in skeletal structures. Numerous studies indicate that laboratory conditions can markedly affect otolith microstructure (see Campana and Neilson 1985), which suggests similar artefacts in elemental deposition. For this reason, there is considerable scepticism about the applicability of small-scale laboratory studies for investigating the effects of environmental variability on otolith composition.

The current study, involving fish held in sea-cages, was undertaken to test experimentally the effect of environmental conditions on otolith composition, in an environment which would minimise the artefacts induced by laboratory conditions.

METHODS

Access to sea-caged SBT was made available by agreement with the Australian Tuna Boat Owner's Association (ATBOA) and the Japanese Overseas Fishery Co-operation Foundation (JOFCF).

Juvenile SBT were caught off South Australia, and transferred immediately to sea-cages (for description of collecting methods and sea cages, see Krueger 1991). On 14 May 1991, with the assistance of Mr Kevin Williams (WW Fisheries Consultants), 20 SBT were double-tagged externally and injected with strontium chloride, at a dosage of 3 ml of a 1gm/ml stock solution of $SrCl_26H_2O$ (= 60 - 70 mg/kg Sr/fish, assuming the average weight at stocking of 15kg). $SrCl_2$ injection induces a conspicuous mark on the otolith, which can be used to determine that portion of the otolith deposited after individuals were placed in the sea-cage. Three tagged fish died within a week of tagging, which was attributed to 'shock' effects of catching and handling. Fish were harvested from the cage at regular intervals from mid-October to mid-December. Tagged individuals were routinely caught during these harvestings, such that only 3 of the original 20 tagged SBT remained in the cage until the final harvesting on 17 December 1991. At capture, all fish were measured (fork length), weighed, and then decapitated. Frozen heads were sent to the CSIRO Division of Fisheries, Hobart, for removal and processing of otoliths.

Water temperatures and samples for nutrient and salinity measurements were collected on a near-daily basis (no samples or data taken on Sundays). Water samples were frozen after collection, and held frozen until analysis. Temperatures taken both during the morning and afternoon on most days. Procedures for nutrient analyses (silicate, nitrate/ite and phosphate) follow Airey and Sandars (1987) and the CSIRO Marine Laboratory Hydrochemistry Manual (in prep). Measurements were made using a Technicon segmented flow autoanalyser. Salinity was measured using a YEO-KAL inductively coupled salinometer.

Induced Sr-rich bands in the otoliths were identified using a Robinson backscattter detector on a Phillips SEM at 30kV, and confirmed by subsequent probe analyses. Sr marks show as a bright band, the result of a significant difference in atomic number relative to the surrounding otolith material (see Fig. 4.2.1). Confirmation that bright marks were Sr-rich came from subsequent electron probe analyses.

Electron Probe Microanalysis (EPMA) – Life-history scans

Procedures for embedding and sectioning SBT otoliths, and for preparing the otoliths for EPMA are described in Gunn et al. (1992). Only sagittae otoliths were used in this study, primarily because of their larger size and longer growth axis, but also because the lapilli and asterisci are too fragile to withstand preparation for such analyses. Prior to embedding, a scaled diagram of the distal surface of each otolith was made in order to guide subsequent sectioning. The otolith was then fixed upright on its ventral edge to the base of an embedding mould with a drop of 5-min Araldite. The mould was then filled with a harder-setting resin (Araldite D). After hardening, the otolith was sectioned using a diamond-edged saw blade (350 μm thick) on a rotary saw (larval otoliths were embedded in resin but not sectioned). Grinding to the plane of the primordium was done by hand using 2400 grade silicon carbide wet/dry paper. Final polishing was done using progressively finer grades of diamond paste (6, 3 μm) and aluminium oxide powder (Linde B) on a lapping machine. After polishing, the section was ultrasonically cleaned and stored in a moisture-free environment. Prior to EPMA, the section was heated on a hot-plate at 80°C for 10 minutes to remove any residual moisture, and then coated with a 250-300 Å (measured by colour on brass) coat of carbon, using a sputter coater, and then stored under vacuum until insertion into the probe.

The following is a description of the methodology we routinely employed to analyse SBT otoliths with EPMA. Separate details are provided throughout this report where analytical procedure differed from these 'routine' conditions.

The procedures used to analyse otolith composition are detailed in Gunn et al. (1992). The electron probe microanalyser used was a Cameca "CAMEBAX" fitted with three wave-length dispersive detectors. The concentrations (weight-fractions) of Na, K, Ca, S , and Cl were calculated based on the count rates measured for their respective K_α lines, and for Sr, the L_α line. S and Cl were measured on

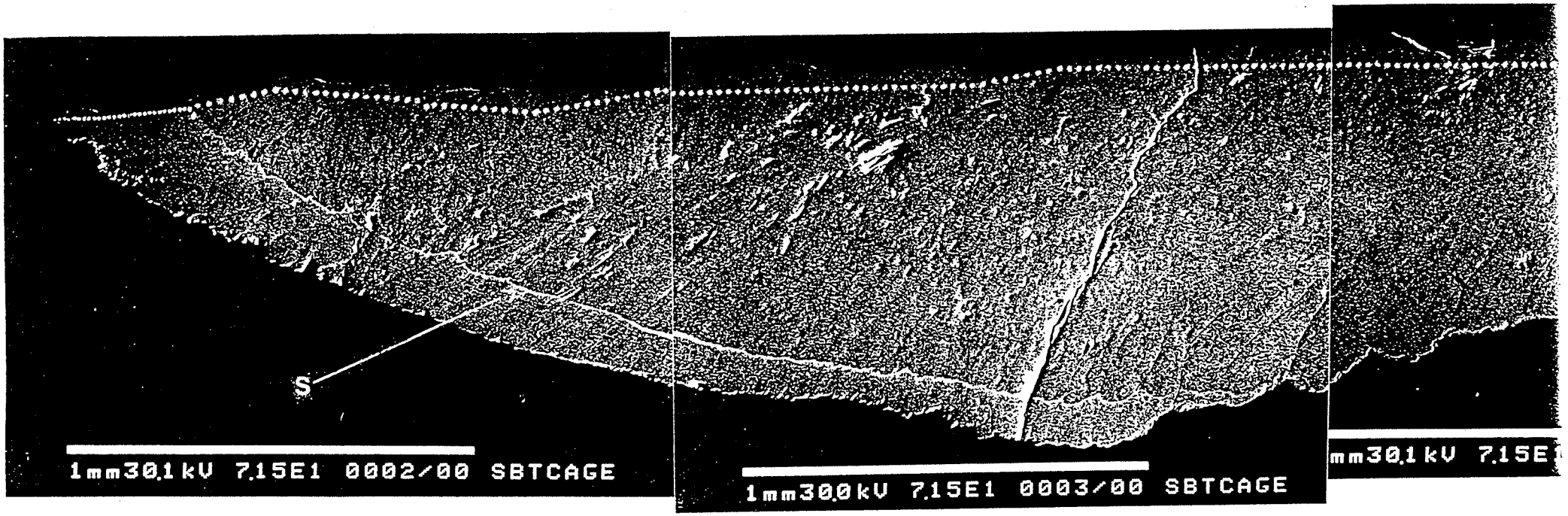


Figure 4.2.1. SEM image of otolith section from a caged juvenile SBT, enhanced using back scattering mode. S = High Sr band induced by strontium chloride injection at the beginning of the caging period. White dots are points scanned using EPMA.

Spectrometer 1 (PET), K and Ca on Spectrometer 2 (PET), and Na and Sr on Spectrometer 3 (TAP) Matrix corrections were made using the "PAP" (Pouchou and Pichoir 1984) matrix conversion software supplied by Cameca. Minimum detection limits and confidence intervals (see Gunn et al. 1992) for the concentration estimates are based on equations provided by Ancey et al. (1978). Our standard beam conditions were: 15kV accelerating voltage, 25nA beam current, 14µm 'defocused' beam diameter (beam power density = 2.4 $\mu W\ \mu m^{-2}$), 25µm spacing between points, with a total acquisition time of 3 min 42 sec per point.

Ontogenetic variation in composition was assessed using EPMA by analyzing a series of points along the longest growth axis of each otolith section - a "life-history scan". The finished section of the SBT sagitta exposes an uninterrupted growth axis, through which we ran a mapped series of programmed scan lines that tracked the slight curvature of the axis (Fig.4.2.1). We duplicated this axis as closely as possible in each specimen, in order to maximise comparability of the data sets. The life-history scan line for each fish ran from the primordium to the posterior ventral tip of the otolith.

EPMA conducted on otoliths from the sea-cage fish followed a modified procedure. Analysis points from primordium to the Sr-rich mark were made with the standard beam conditions detailed above, but analysis points from Sr-rich mark to posterior margin of the section were made on a finer spatial scale under the following conditions : 15kV accelerating voltage, 16nA beam current, 10µm beam diameter (beam power density = 3.1 $\mu W\ \mu m^{-2}$), and 12.5µm spacing between points.

RESULTS

Environmental Data

Environmental conditions at the sea cage are depicted in Figure 4.2.2. Salinity data were not collected daily, unfortunately, and silicate values after day 126 are all zero, apparently due to poor specimen fixing or partial thawing of the frozen sample.

Overall, temperature varied over an approximate 7 C degree range and reflected the expected seasonal changes in water temperature, whereas salinity was relatively constant throughout the experimental period, increasing gradually near the end of the study. Of the three nutrients measured, nitrates were generally low throughout the study, phosphates were highly variable, but declined steadily, and silicates showed evidence of peaks and troughs during the early part of the study. As noted, however, there is no silicate data for the last half of the study.

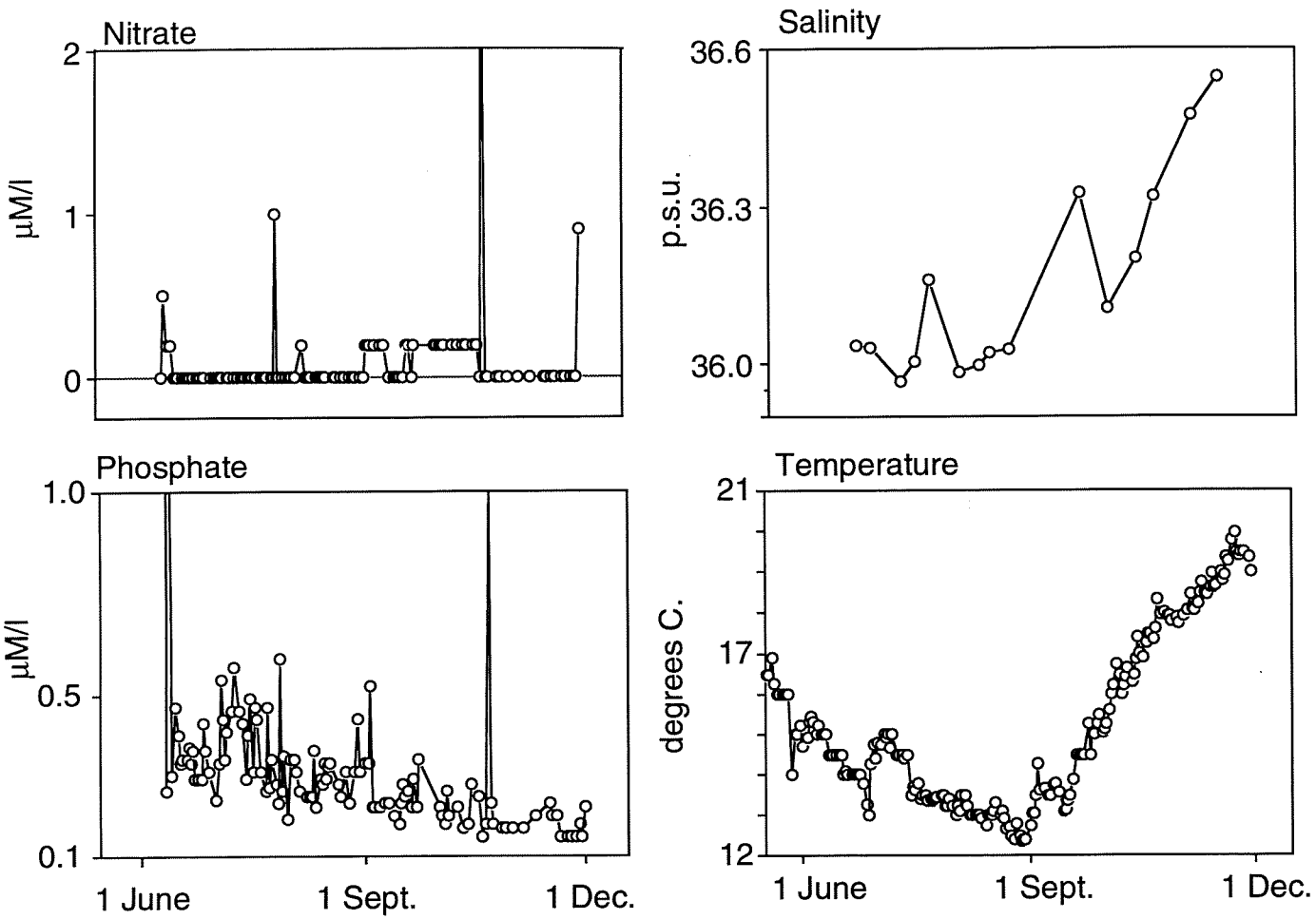


Figure 4.2.2. Changes in environmental conditions during the sea cage experiment,. For sampling details, see text.

Otolith Composition

Otoliths from five SBT were analysed, with a full life-history scan conducted on each specimen (Fig. 4.2.3). As noted above, points were more closely spaced near the otolith margin, as this was the area of greatest interest in terms of environmental correlates of composition.

The effect of sea-caging on otolith composition was tested for elements by comparing ontogenetic variation pre- and post-caging. We hypothesized that, if elemental concentrations were environmentally sensitive, otolith chemical profiles that showed variability prior to caging would converge and follow the same ontogenetic pattern after caging. Results of these analyses are shown on Figures 4.2.3 for the five micro-constituents. Ca was not plotted, as its concentration in otoliths varies only slightly.

In general, there is little or no evidence of consistent convergence in elemental concentrations after introduction of the fish into the cages. Sr concentrations are extremely high after the tagging, and show evidence of a slow, exponential decay to pre-injection levels. Sr concentrations at the otolith margin are higher than those pre-injection, despite the seven month intervening period. Despite this, there is some indication of commonality among the five individuals examined, with regard to Sr trajectories. Superimposed on the exponential decline is a common pattern of relatively increasing concentrations of Sr late in the caging period, followed by a decline close to the margin..

Sulphur is unusual in the elements examined in that there is evidence both of convergence following caging and of parallel post-cage variation. The pattern is unclear, however, in that there appears to be two groups of individuals, each following a different post-caging ontogenetic trajectory (Figure 4.2.4). One group, consisting of T9, 12 and 15, show a broad early peak in S concentrations, which then decline rather precipitously to the otolith margin, with all three showing a minor, secondary peak in concentrations at about 8.4 mm from the otolith primordium. The second group, consisting of T10 and T11, show evidence of a single sharp peak in S concentrations at about 8.3 mm, followed by a rapid decline and then a slight rise close to the otolith margin.

Environmental Correlates of Otolith Composition

Of the microconstituents examined, only two show evidence of parallel variation after caging. We conclude, therefore, that only these two are likely to be directly environmentally sensitive. Neither, however, unambiguously correlate with any of the five environmental variables measured. Evaluation of the sulphur data are confused by the presence of two, very different sets of individuals. One set, involving three fish, show a sulphur trajectory similar to that of measured phosphate levels, which suggests a productivity link of some kind. The second set

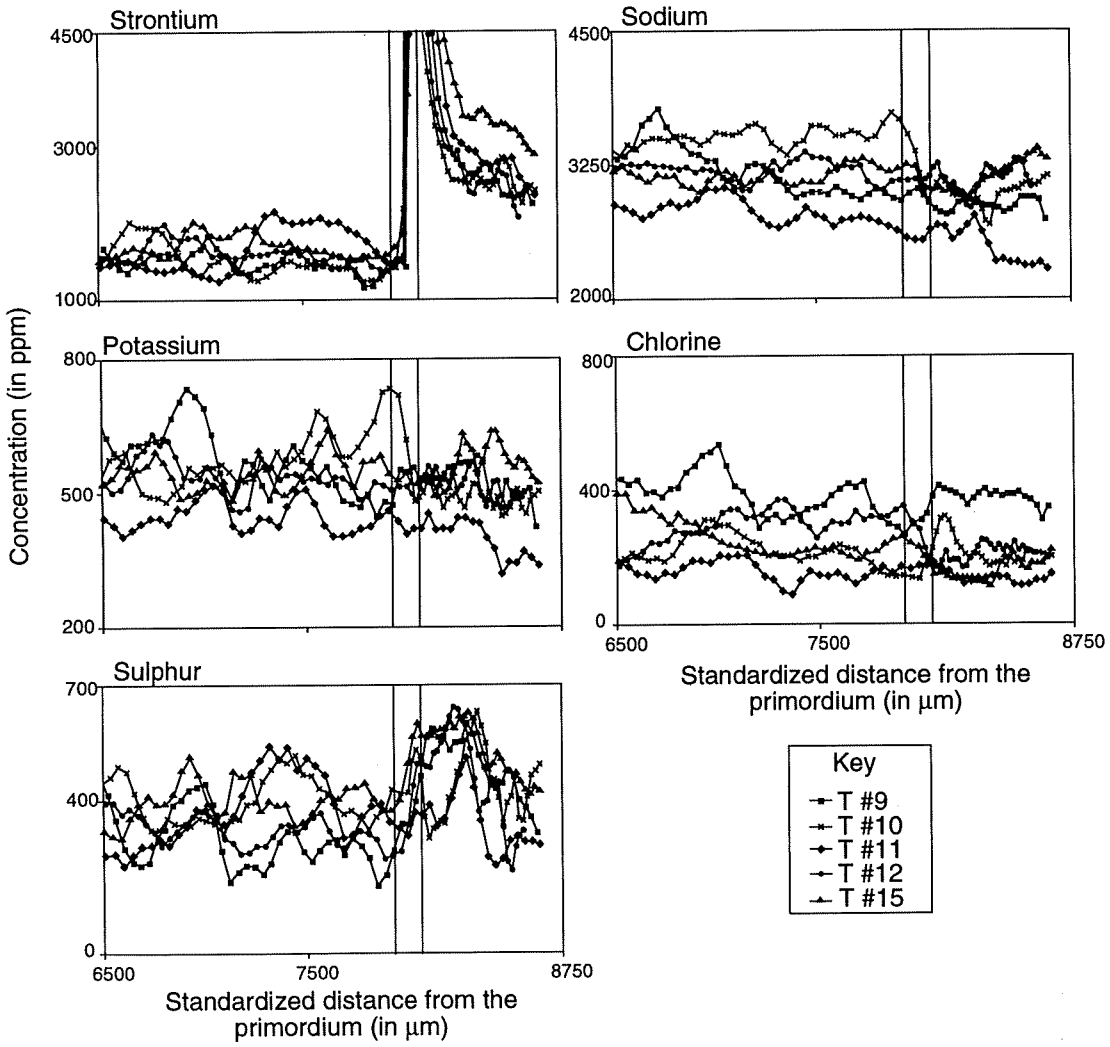


Figure 4.2.3. Life history traces of five elements for five juvenile SBT held in sea cages. Paired vertical line indicates otolith radius at the beginning of the caging period, as indicated by the induced increase in Sr concentrations.

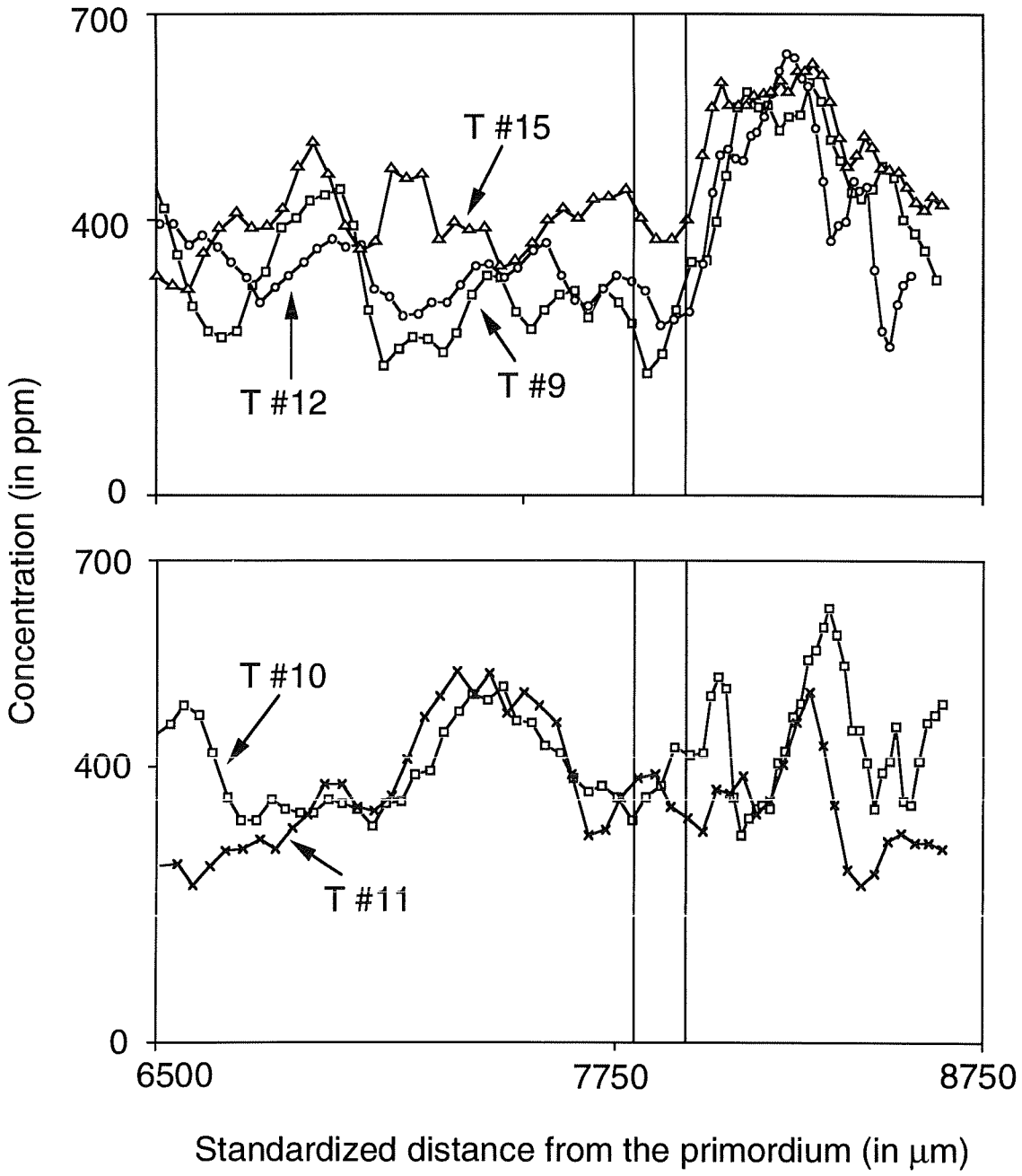


Figure 4.2.4. Life history traces of S for five juvenile SBT held in sea cages, grouped on the basis of common patterns of ontogenetic variation.

of individuals, however, show quite a different S trajectory, which does not obviously correlate with any measured environmental variable. Sr concentrations are conspicuously distorted by the effects of tagging, but relative changes (i.e. after factoring out the exponential decay) suggest a trend inversely correlated with water temperatures. Sr concentrations, in general, show a relative peak late in the caging period, when water temperatures were lowest, and the continue to decline towards the margin, which is consistent with increasing water temperatures shortly before the fish were harvested.

DISCUSSION AND CONCLUSIONS

Environmental variations in the sea-cages were consistent with expectations based on normal seasonal variation in the SA inshore environment. Consistently low nitrate/nitrite levels suggest the cages were well flushed. Phosphate (and perhaps silicate) levels, in turn, are consistent with an expected winter peak, followed by a decline in spring/summer as the nutrient is utilised by phytoplankters.

Three fundamental conclusions can be drawn from the analyses of otolith chemistry: (1) there are high levels of individual variability in the concentrations of most elements; (2) few (and perhaps none) of the measured elemental concentrations unambiguously vary as a result of measured water parameters; and therefore (3) a simple and direct reconstruction of the environmental history of SBT from otolith chemistry is not justified, and may not be possible. In all three instances, these results confirm those we collected earlier on a temperate shelf fish, jackass morwong (*Nemadactylus macropterus*), and indicate that otolith composition is likely to be determined by a complex interaction of physiology, genetics and the environment. On that basis, we suggest that previous attempts to use variations in otolith elemental chemistry to reconstruct the environmental histories of fishes are premature and unjustified.

Nonetheless, the data also suggest that some reconstruction of life-history features is possible, but perhaps only using a few elements and only where physiological features are taken into account. The convergence of individuals in the cage onto a common set of sulphur trajectories suggests strongly that this element, at least, is responding to something common among the fishes in the cage. At the same time, however, that these individuals converge of two different sets of trajectories indicates that this environmental response is neither simple nor direct, but rather is mediated by some individually specific characteristic. Exactly what this characteristic is we cannot yet say. Sulphur concentrations in otoliths have been suggested to be growth rate dependent (slow growth = high sulphur concentrations), based primarily on the observation that sulphur is bound into the protein portion of the otolith and that protein concentrations overall increase during periods of slow growth. To the extent that this hypothesis is correct, then it appears that the two sets of SBT in the cages grew at two different rates. Again, why this is so is difficult to determine. The sulphur plots for the three-fish group

are overall similar to the phosphate/silicate trajectories in the cage, which could be attributable to three mechanisms: 1) water column nutrient levels directly affected SBT growth rates, 2) slow SBT growth resulted in high nutrient levels, and 3) both nutrient levels and growth rate are independent responses to a third, forcing variable. Of the three, the last is the most likely. It is difficult to determine how phytoplankton production in the cage would directly affect SBT growth rates, given that the fish were fed, and the prospect that slow growth rates increased nutrient concentrations (perhaps through increased rates of defecation), while feasible, is inconsistent with the overall low nitrate/nitrite levels. It is more likely that both the tuna and the nutrient levels were responding independently to seasonal variability in growth conditions, as reflected best in the water temperature data. Again, this does not explain why the fish would show two different sets of growth trajectories.

Interpretation of the strontium data are difficult due to the prolonged effect of the tagging. Previous studies, cited earlier, suggest Sr as the element most likely to be influenced by environmental variability. Hence our inability to assess it accurately is somewhat frustrating. Nonetheless, what analyses we can do, by extracting Sr trajectories after factoring out the exponential decay, are consistent with at least some degree of dependence of Sr incorporation on temperature.

We tentatively conclude, therefore, that 4 of the 6 elements that can be measured using EPMA show little or no indication of environmental sensitivity. These elements are Ca, Na, K and Cl. None are likely to be of use as a means of aging SBT as a result of seasonal variations in composition. Sulphur is problematical, given evidence of high levels of individual variability. Nonetheless, it may show some indications of seasonal patterning, even if comparing individuals may be difficult. Finally, although the data on strontium concentrations are distorted, they suggest that Sr offers the best prospect for an element whose concentrations in otoliths is likely to vary as a response to water temperature, and hence which might be expected to vary seasonally.

4.3 VALIDATION: MARGINAL ANALYSIS OF OTOLITH COMPOSITION AND COMPARISON WITH INDEPENDENTLY DERIVED GROWTH TRAJECTORIES

Examination of variations in Sr concentrations along the growth axis of otoliths of large SBT suggest regular episodic variations, which we hypothesized to reflect annual environmental cycles. If this hypothesis is correct, then (1) the episodic variation should be consistent among individuals, reflecting exposure to a common environmental cycle, and (2) the variation should be capable of being tracked during seasonal changes in otolith deposition, in a manner analogous to traditional 'marginal validation' of density bands in conventional aging by means of hard parts. These predictions were explored by assembling Sr and S life-history scans for SBT over a wide range of sizes and collected throughout the year, and by

comparing the patterns in these scans and, particularly, changes in marginal otolith composition.

METHODS

Four sets of samples were used to compare patterning in the ontogenetic variability in the composition of SBT otoliths: (1) small (25 cm FL) juveniles caught by long-line off the west coast of Australia, (2) large juveniles taken from the commercial catch off southern Australia, (3) large juveniles taken from the commercial catch off South Africa, and (4) adults taken from the commercial catch off SE Australia. Sample locations are depicted in Figure 4.3.1, and details of the samples are given in Table 4.3.1. The western Australian juveniles were caught by trolling off the RV Shoyo Maru in March 1990. The southern Australian juveniles were collected at points all along the southern Australian coast during 1988 - 1990. The large adults were taken by commercial long-liners working off the SE Australian coast, also during 1988 - 1990.

Otoliths from the majority of adult fish were extracted immediately following capture. Otoliths extracted from freezer stored specimens (-60°C, max. 20days) included those from the South African fish and from a small number of the Australian fish. After extraction, each otolith was cleaned of adhering tissue using fine forceps and a soft bristled brush in millipore-filtered distilled water. Otoliths were then dried in an oven at 40-45°C for a minimum of 6 hrs, after which they were stored in polyurethane capsules within a dessicator.

Procedures used for preparing the otoliths for EPMA and conducting life-histroy scans are detailed in Sect. 4.2 - Methods. The only deviation from the standard procedure was that the final plane of section was not always exactly at the level of the primordium. The sections were prepared to the plane exposing the maximum length in growth axis to the posterior ventral margin.

Otoliths used for the margin analyses were a subset of sagittae sampled by Australian Fisheries Service Observers operating onboard Japanese longlining vessels in the Australian Fishing Zone, off the east coast of Tasmania and were from large mature SBT of 160 - 180 cm F.L. A small number of otoliths were sampled from fish caught in the spawning region south of Indonesia; fish which had been in freezer storage (-60°C).

Margin EPMA analyses were conducted as close to the otolith section edge as possible, at the posterior end, using the following beam conditions: 15kV accelerating voltage, 8nA beam current, defocused to 8μm diameter (beam power density = 2.4μW μm^{-2}), with a total aquisition time of ~ 6 min per analysis. S was measured on Spectrometer 1 (PET), Ca on Spectrometer 2 (PET), and Sr on Spectrometer 3 (TAP).

Table 4.3.1. Capture details of *T. maccoyii* analyzed in this study

Code	Location		Date Caught	Number of fish	Size range (F.L. cm)
Larvae					
L:	Lat.16°43'S	Long.115°8'E	16 Jan 87	4	<1
	Lat.16°30'S	Long.115°51'E	28 Jan 87	6	<1
Immature (<140cm FL.)					
WA:	Lat.21°50'S	Long.114°30'E	17 Dec 87	5	all 24
	Lat.24°45'S	Long.112°37'E	9 Dec 90	3	all 25
	Lat.29°49'S	Long.114°27'E	13 Dec 90	3	27 - 28
	Lat.33°11'S	Long.114°48'E	16 Dec 90	2	44, 44
O:	Lat.34°10'S	Long.121°55'E	11 Dec 83	1	56
	Lat.33°0'S	Long.131°0'E	10 Mar 88	4	56 - 61
	Lat.34°49'S	Long.134°42'E	7 - 8 Apr 88	3	75 - 85
	Lat.43°3'S	Long.148°4'E	12 Jun 88	2	125, 134
	Lat.34°50'S	Long.134°40'E	18 Mar 90	2	98, 98
	Lat.38°0'S	Long.151°0'E	4 - 8 Jul 90	4	134 - 138
	Lat.37°58'S	Long.151°15'E	5 Jul 90	1	131
S:	Lat.42°0'S	Long.23°0'E	20 - 25 Jul 90	2	111, 124
	Lat.44°0'S	Long.25°0'E	20 - 25 Jul 90	4	122 - 131
	Lat.44°0'S	Long.3°0'E	20 - 25 Jul 90	2	126, 139
Mature (>140cm FL.)					
A:	Lat.43°3'S	Long.148°4'E	12 Jun 88	1	179
	Lat.44°48'S	Long.145°35'E	28 Nov 88	2	175, 180
	Lat.45°8'S	Long.145°17'E	4 Dec 88	1	165
	Lat.44°0'S	Long.147°10'E	13 - 14 Apr 89	2	177, 178
	Lat.43°0'S	Long.148°0'E	14 - 16 Jul 89	2	178, 178
	Lat.15°0'S	Long.114°0'E	15 Oct 89	2	176, 184
	Lat.43°10'S	Long.148°10'E	5 - 6 Apr 90	2	175, 177
	Lat.37°58'S	Long.151°15'E	10 - 12 Jul 90	2	175, 178

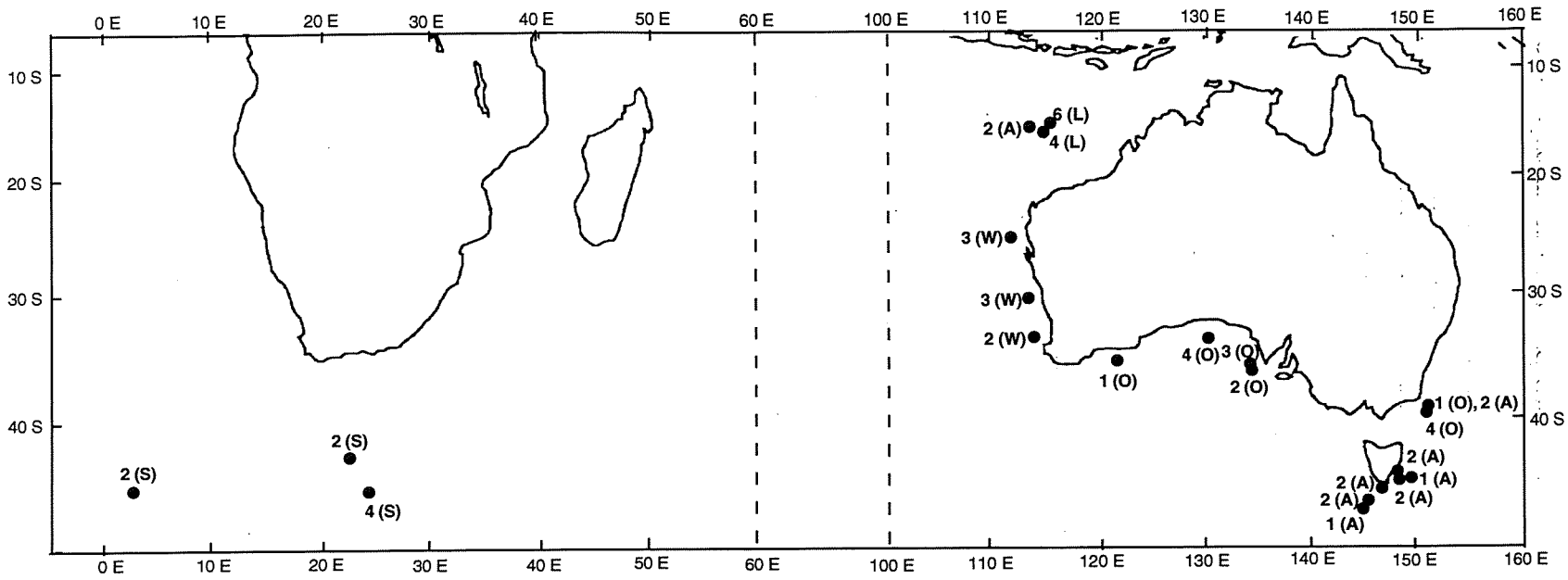


Figure 4.3.1. Locations at which SBT were collected for use in this study.

Sections prepared from sagittae of juvenile SBT result in a relatively standard section shape that varies little from section to section (see Figs. 4.2.2 and 4.3.2a), and the 'end-point' of these sections at the posterior margin is obvious. By contrast the shape at the margin of sections from sagittae of older, mature SBT is often more irregular and the decision on where to locate a representative margin analysis is more difficult. When this was the case, up to three margin analyses were done on each section (Fig.4.3.2b), and a mean margin concentration obtained.

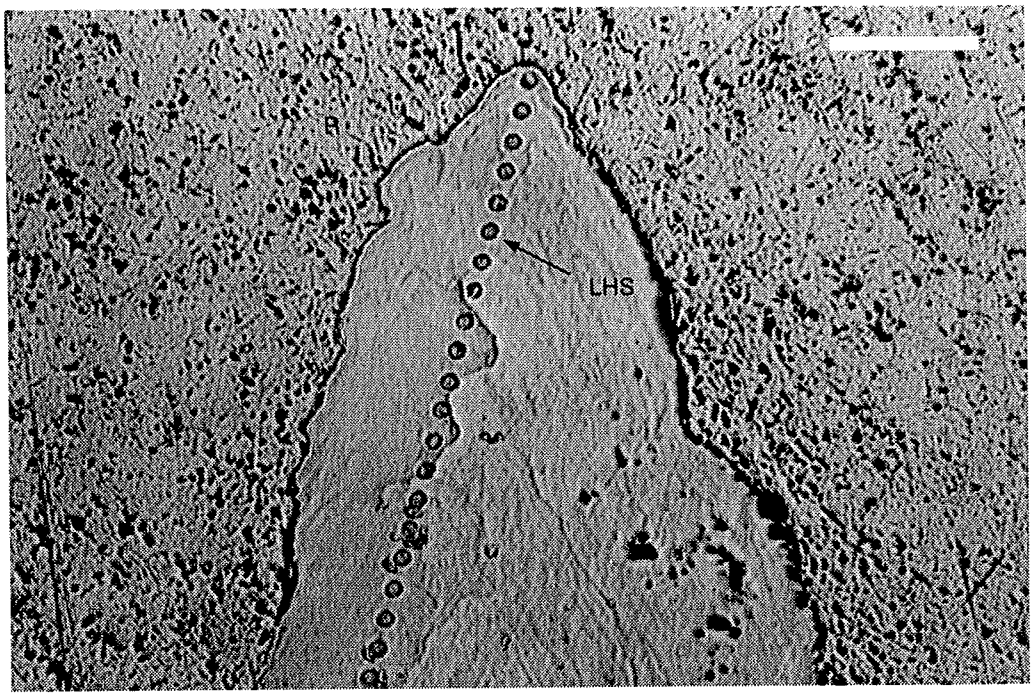
RESULTS

Patterns of individual variation in life history traces

Life-history traces of Sr and S for SBT divided by size class are depicted in Figures 4.3.3 and 4.3.4. Although both elements show indications of regular patterning, particularly for smaller fishes (< 100 cm), the patterning is overall less clear-cut in S than in Sr. Nonetheless, for both elements there are clear indications of a size-correlated (and hence presumably age-correlated) pattern of elemental deposition, which can be tracked through the smaller size classes at least. Sr concentrations start off high close to the otolith primordium, then decline steadily as the fish grow to a length of approximately 25 cm. Thereafter, for both elements, there is an indication of a peak in relative concentrations approximately 100 points (2.5 mm) exterior to the otolith primordium. For Sr, there are indications of additional peaks at approximately 150-200 points (3.75 - 5.0 mm) and approximately 225 points (5.75 mm) exterior to the primordium. Beyond this radius, the patterning is highly variable among individuals and no clear trends, other than a gradual increase in mean Sr concentrations, is readily apparent. The discernable peaks (albeit highly variable in character) appear to occur at approximate fish sizes of 50-60 cm FL, 80-90 cm FL and 110-120 cm FL.

The extent of individual variability is illustrated by examining representative life-history traces for fish randomly drawn from the sample in the 120-150 cm size group (Figs. 4.3.5 and 4.3.6). The plots indicate three points. First, both S and Sr concentrations are highly variable in all specimens. Second, most, if not all specimens, show a broad peak in Sr and S concentrations between 100 and 150 points exterior to the primordium, but the presence of the second and third peaks, at approximately 175 and 225 points, is much more variable. And third, the broad peaks are themselves constituted by a variable number of much narrower peaks, of highly variable height and width. Detailed examination of Peak #1 in individuals >70 cm FL suggests that, modally, two or three narrower peaks constitute the broad peak, though as many as 5 or 6 narrow peaks is not uncommon. The result is that the underlying, broad peaks are often ill-defined and their limits subjective. We estimate that only slightly more than half of the specimens examined would be classified as having 'clearly defined' broad peaks and troughs in the Sr plots. The proportion of clear episodic variation in S plots is much lower.

(a)



(b)

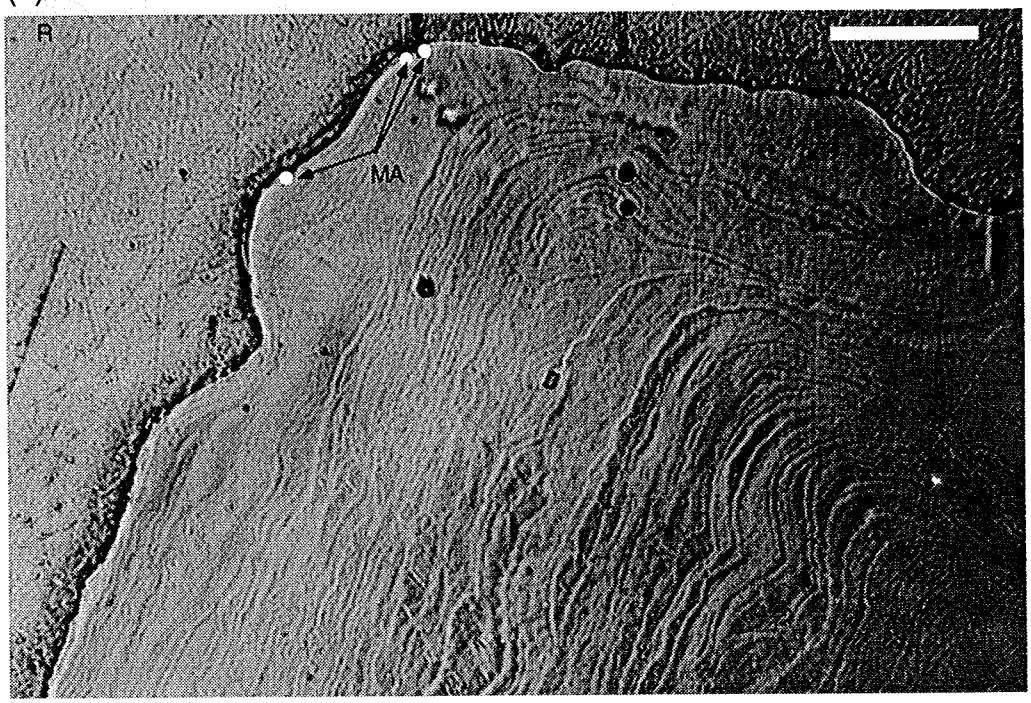


Figure 4.3.2. (a) Incident light micrograph of posterior section of otolith from a juvenile SBT. LHS = 'life history scan, EPMA analyses leading to the posterior margin. R = resin. Scale bar = 100 microns. (b) Incident light micrograph of posterior margin of otolith from a mature (FL 175 cm) SBT. MA = margin probe analyses. Note the broader shape of the margin relative to that of the juvenile. The farthest left of the three analysed points is a 'shoulder' analysis. R = resin. Scale bar = 100 microns.

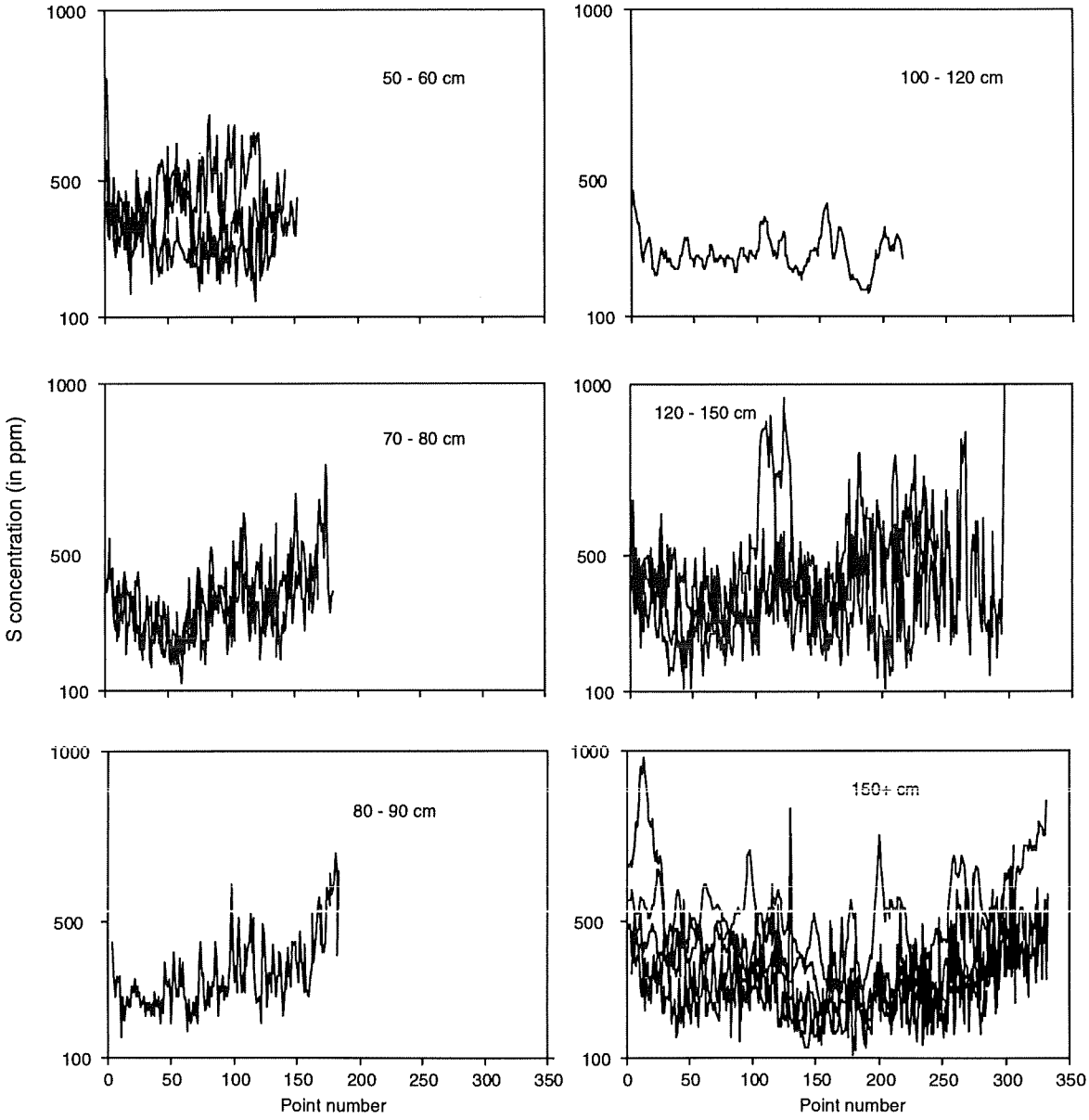


Figure 4.3.3. Ontogenetic variation in the concentration of S as measured along the main growth axis of the otoliths of SBT, grouped by size class.

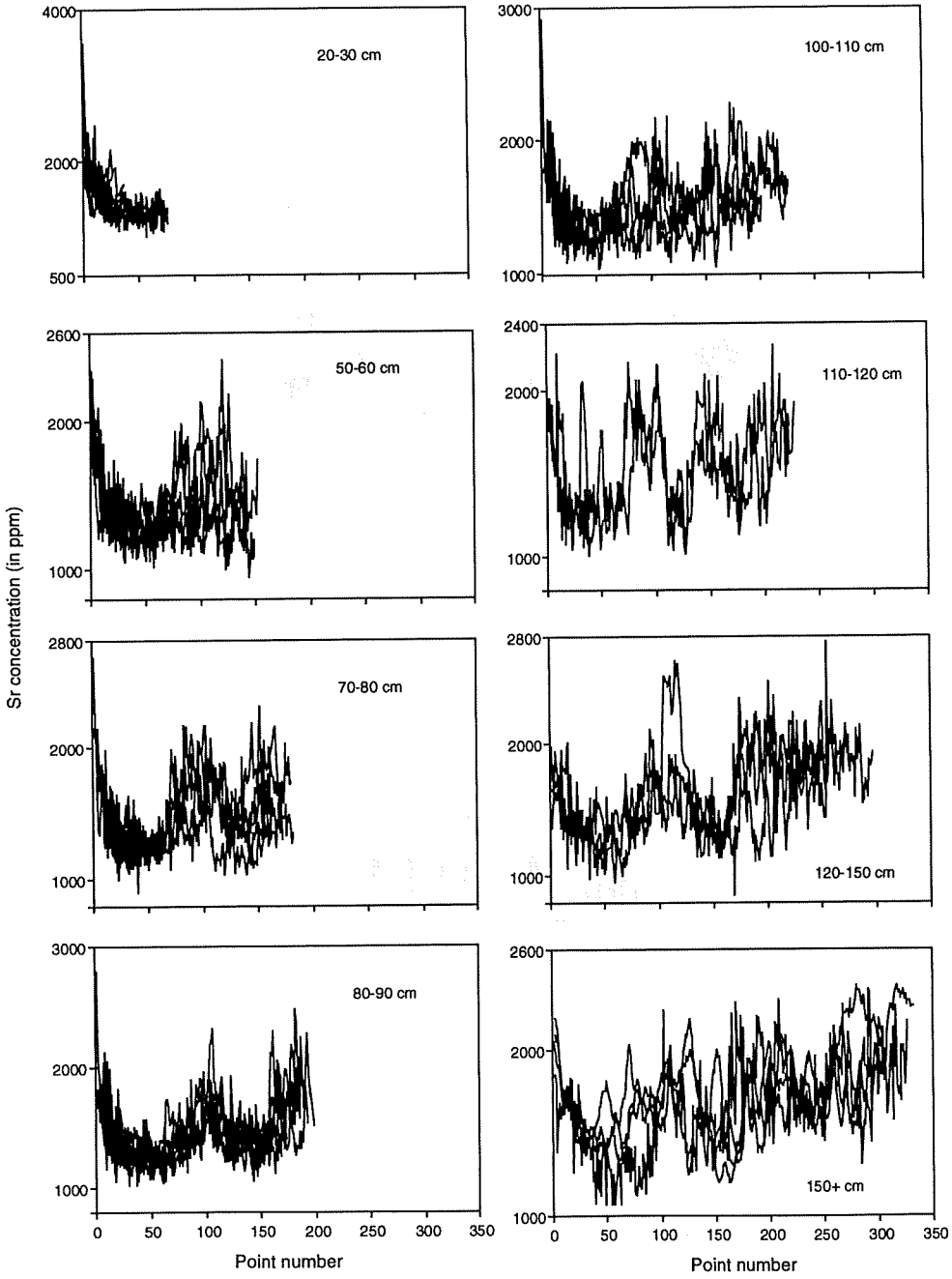


Figure 4.3.4. Ontogenetic variation in the concentration of Sr as measured along the main growth axis of the otoliths of SBT, grouped by size class.

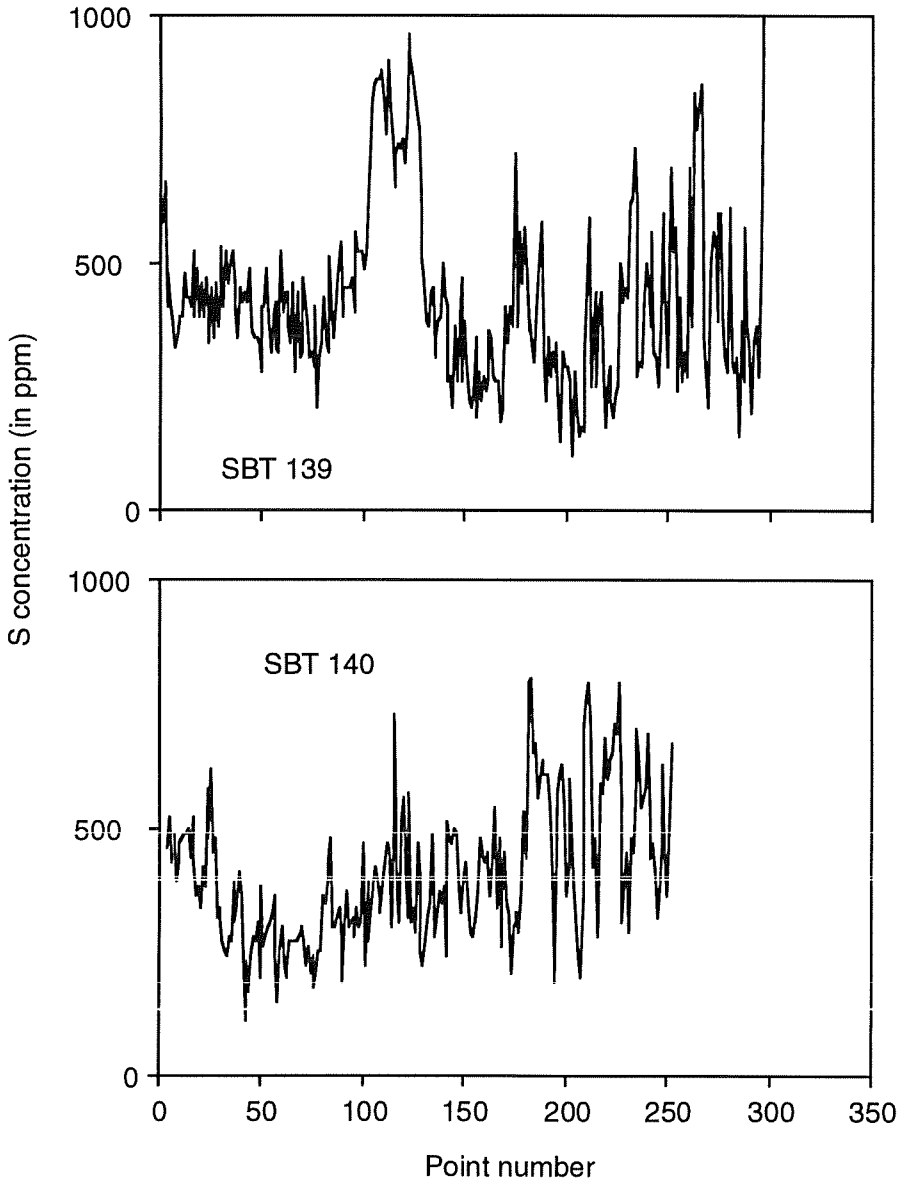


Figure 4.3.5. Individual variations in the ontogenetic pattern of suphur concentrations along the inner section of the main growth axis of large (120-150 cm FL) SBT

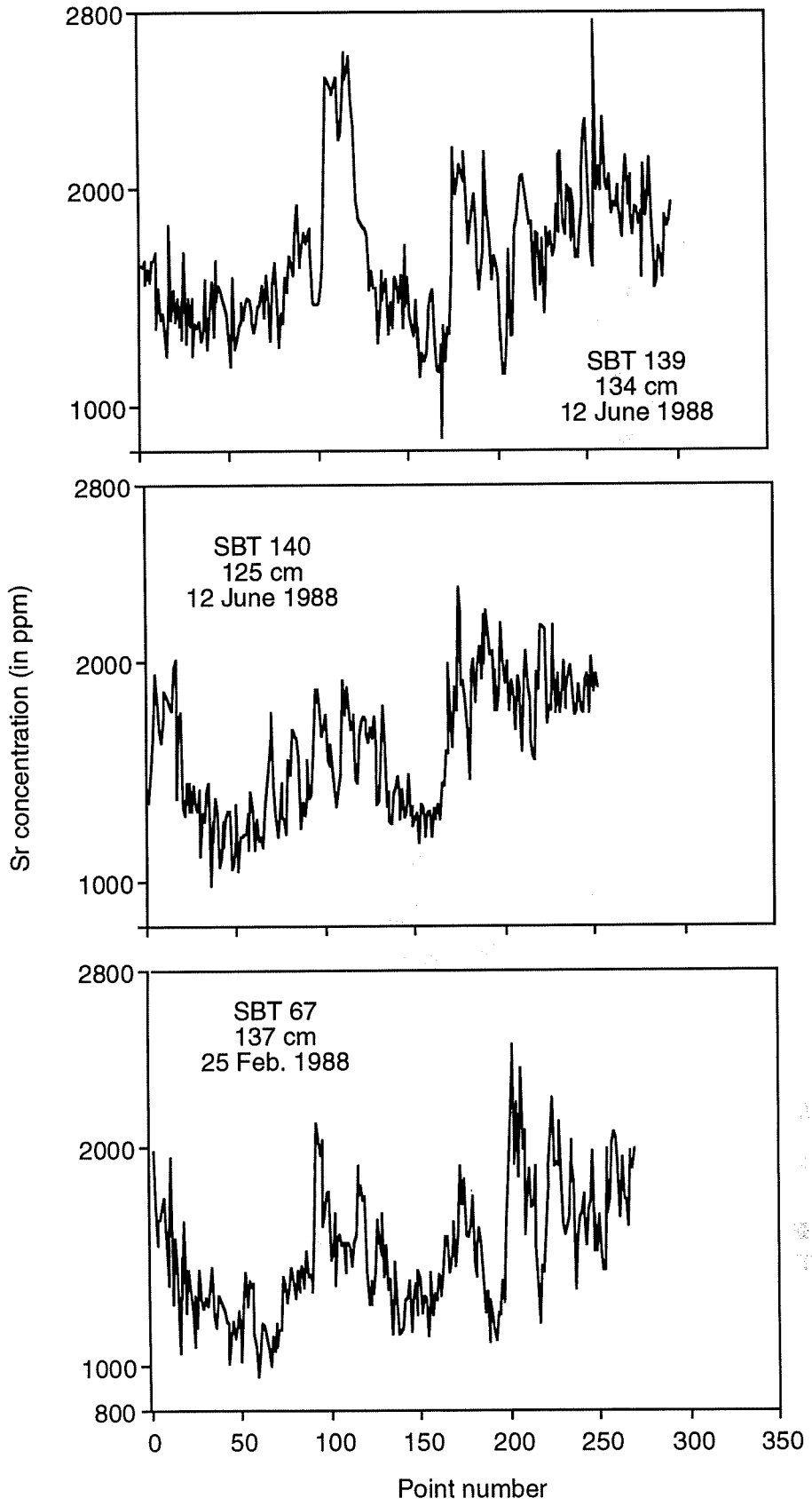


Figure 4.3.6. Individual variations in the ontogenetic pattern of strontium concentrations along the inner section of the main growth axis of large (120-150 cm FL) SBT

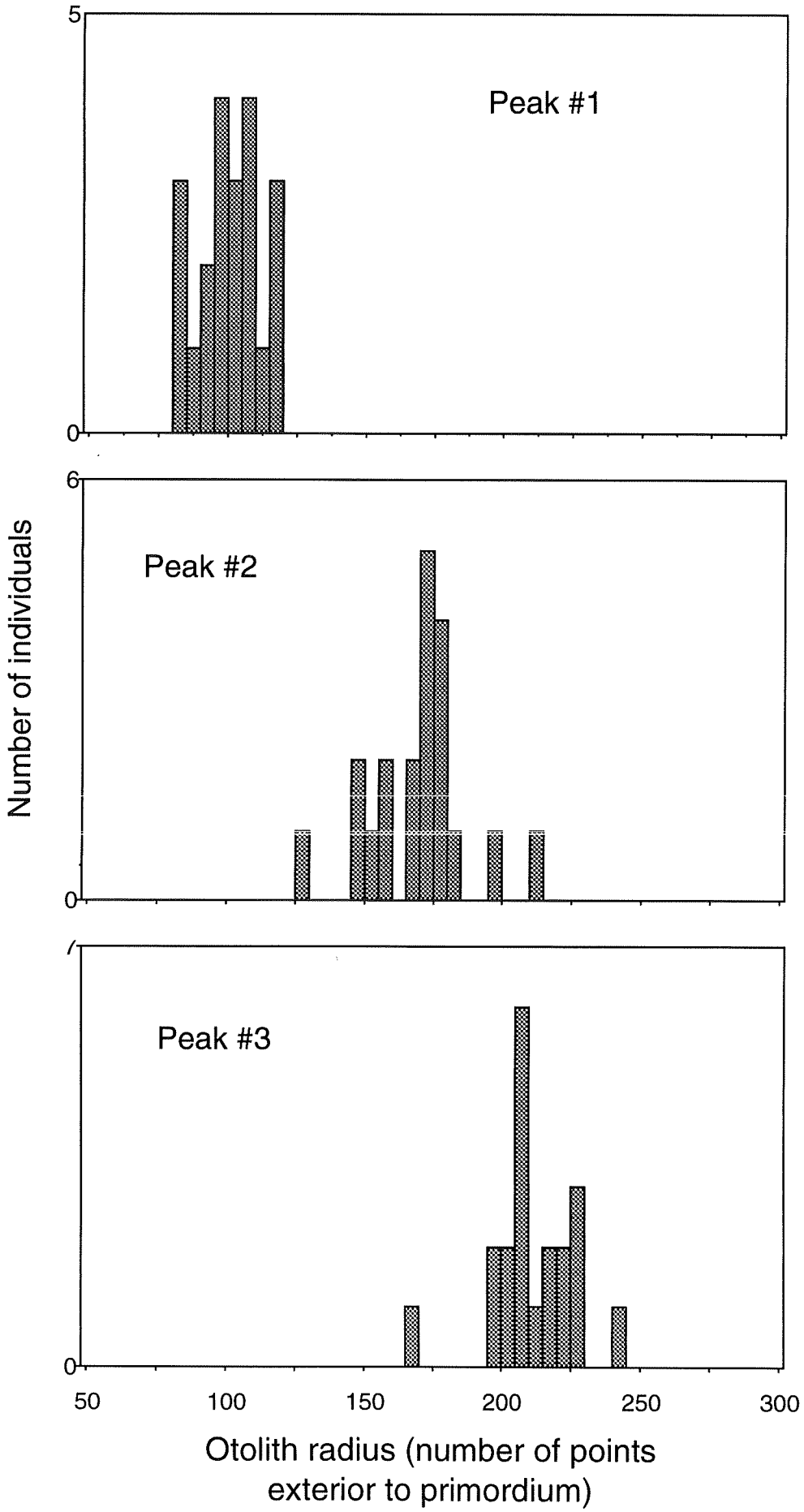


Figure 4.3.7. The distribution of the radii along the long growth axis of major peaks in Sr concentrations. Location of the peak is estimated to the nearest 5 μm by visual inspection of the life history scan.

The distribution of the approximate mid-points of the broad peaks is also relatively variable among individuals (Fig. 4.3.7). Modally, the interior-most peak centres on approximately point # 100, but can vary among individuals between points #75 and #125; peak #2 centres on point #175, varying from point #125 to #215; and peak #3 is centred at approximately point # 215, varying among individuals from #170 to #240. The position of one peak correlates relatively poorly among individuals with the position of the other two peaks, suggesting the factors responsible for their formation are independent. Among individuals, the radius of peak #1 correlates significantly with that of peak #2, but only at an R^2 of 0.29; the radius of peak #1 does not correlate significantly with that of peak #3.

An indication of the temporal nature of the peaks described above was sought by comparing trajectories of individuals collected in different seasons. Unfortunately, the limited seasonal extent of the fishing period for juvenile SBT off Australia limited the power of the comparison to intervals of less than four months. Nonetheless two comparisons were possible based on samples we examined; for 50-60 cm FL individuals collected in early summer (December) and late summer (March)(Fig. 4.3.8) and 70-90 cm individuals caught in December and in April (Fig. 4.3.9). Both comparisons suggest a slow progression in the development of the broad peaks, with little obvious and consistent differences among groups of individuals collected 3 and 4 months apart.

Marginal Analysis

The temporal pattern for the development of cycles in elemental deposition was examined formally through analysis of the otolith margins for individuals collected throughout the year. Specimens for this analysis were extremely difficult to obtain, due to the seasonal nature of the separate fisheries for juvenile and adult SBT. No juveniles were available for winter periods, which restricted marginal analysis to adult samples only. For the adults, very few specimens were available for the period from spring to early autumn, i.e. during the summer period. The few adults we were able to obtain during the spring/summer period were caught off the Indian Ocean spawning ground.

Sr plots for the outermost section of the otoliths of adults collected during the study are shown in Figure 4.3.10. For comparative purposes, we also show plots for juveniles collected in February, in the absence of adult specimens from late summer and early autumn. The plots suggest and extremely variable pattern of Sr deposition. Most individuals show indications of highly episodic patterns of Sr deposition, this pattern does not follow an obvious annual cycle. If anything, the plots suggest two periods during the year when Sr concentrations reach local peaks - in early winter (June) and in spring (October).

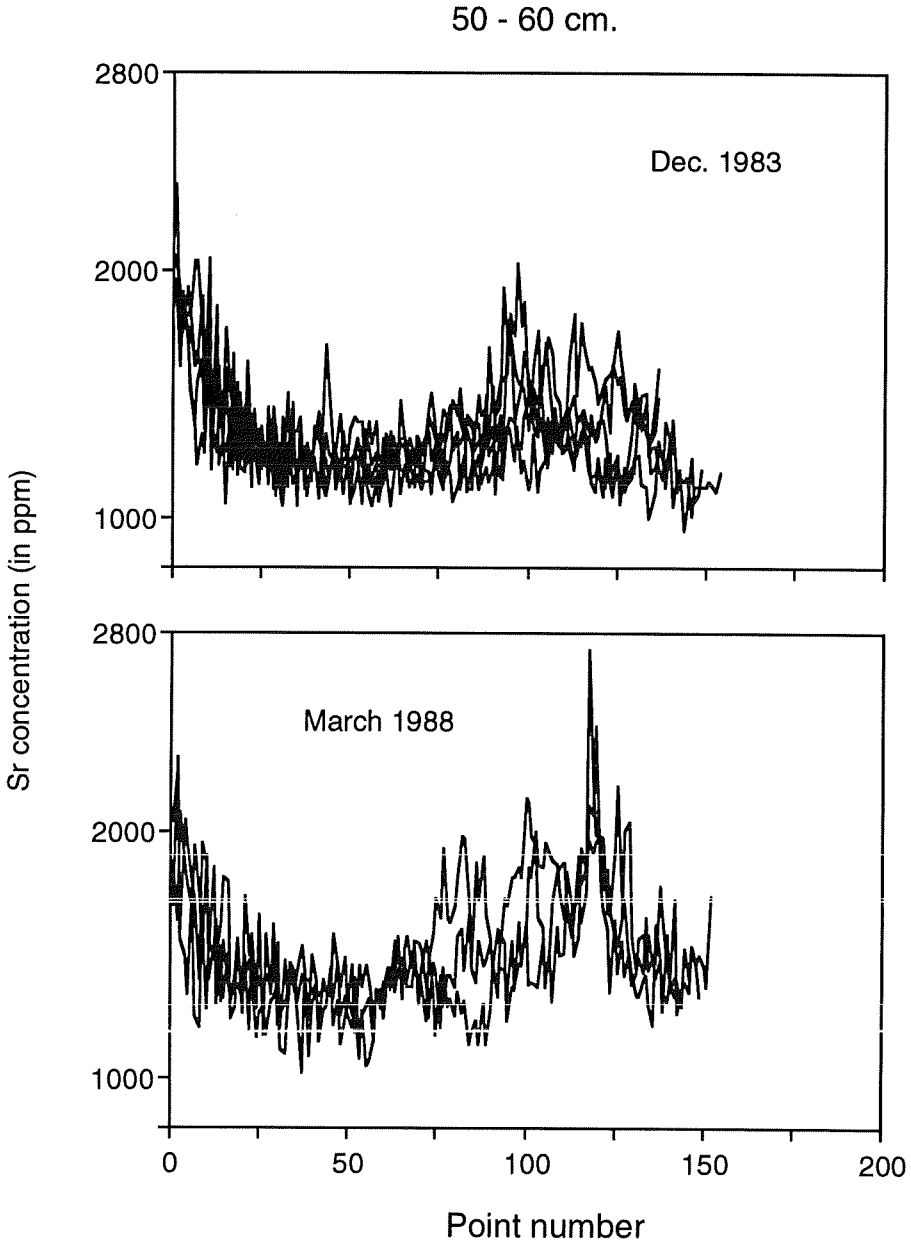


Figure 4.3.8. Comparison of Sr life history traces for small (50-60 cm. FL) SBT collected in summer (December) and early autumn (March).

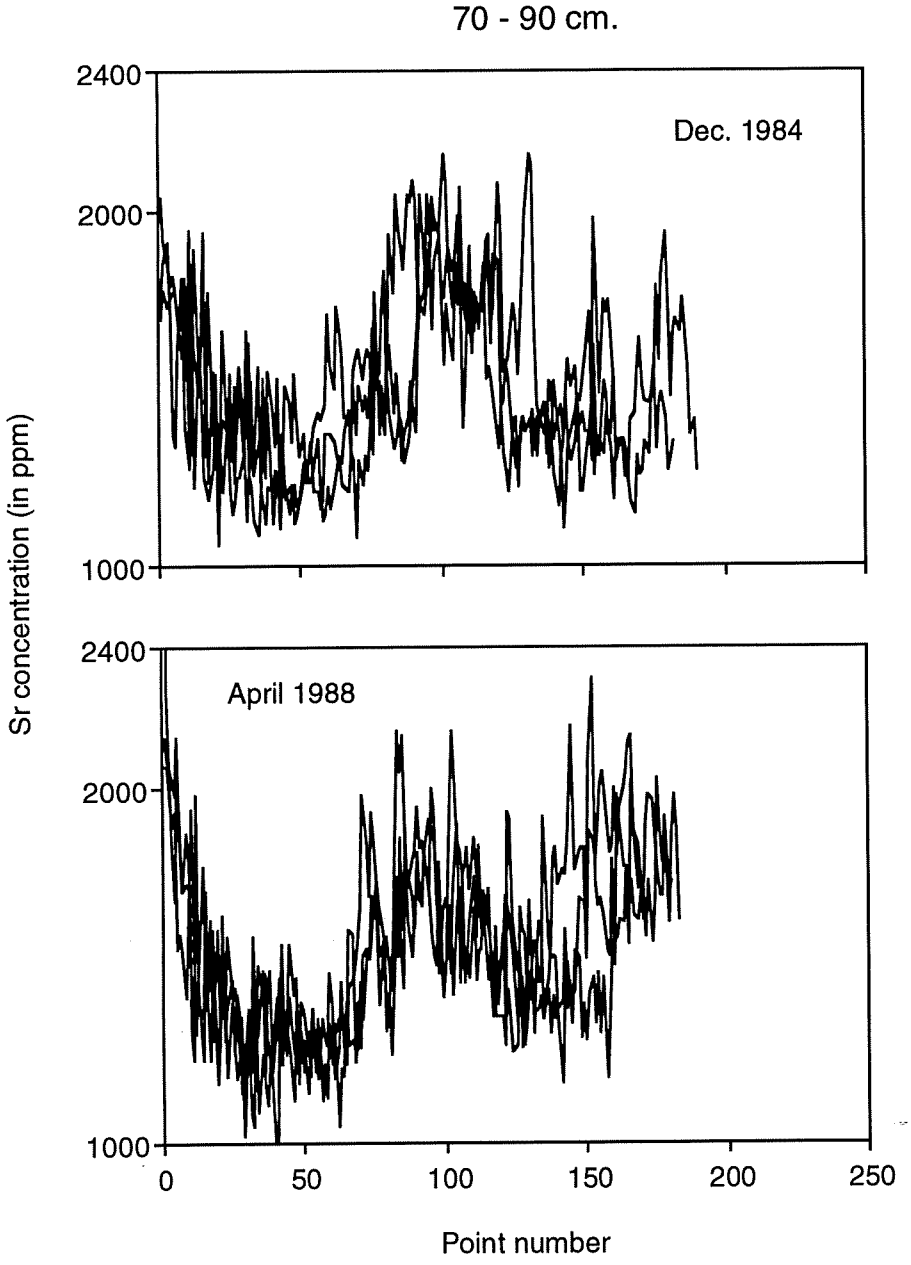


Figure 4.3.9. Comparison of Sr life history traces for 70-90 cm. FL SBT collected in summer (December) and early autumn (April).

Single-point margin analyses

Absolute concentrations for Sr and S at the margin of otolith sections plotted against the date of capture (Fig.4.3.11) do not show any obvious periodicity that is suggestive of an annual cycle. Expressing the concentrations of these elements as a ratio to Ca, as has been common practice in other studies of chemical variability in calcified structures, results in identical plots. The mean concentrations are representative of fish caught close to eachother (although not necessarily from the same school) with respect to space and time (max. 5 days between capture dates). There is some indication of cycling during the period April 90 - July 90 (Sr and S appear inversely correlated), but no such pattern is evident during the corresponding period in 1989. Seasonal inactivity by the Japanese long-lining fleet meant that there were two periods for which no otolith samples were available (Dec.88 - April 89 and Nov.89 - March 90) and these wide gaps in the time series, in combination with the small number of fish in each sample, greatly reduced our ability to identify cyclic patterning via this method.

Variability was encountered in Sr (mean range 184 ± 130 SD.ppm, n=30) and S (mean range 118 ± 85 SD.ppm, n=30) concentrations between 'replicated' margin analyses on all specimens where replicates were taken, but these levels of variability were generally within the calculated measurement errors (using formulae of Ancey 1978, see Gunn et al. 1992) for analyses of these elements conducted under the chosen beam conditions. However, on some otoliths, even analyses conducted relatively close to eachother showed variability in excess of measurement errors (Sr max. diff. 530ppm, S max. diff 310ppm). The shape of the posterior region of the section is considered a factor contributing to this variability. Analyses on the margin at the 'shoulder' of the posterior region (Fig.4.3.2b), in general, produced significantly lower Sr (mean = 16.7 ± 7.9% SD. diff., n=12) and S (mean = 33.5 ± 22.5% SD. diff., n=12) concentrations to those conducted on the margin at the most posterior point of the section. Although analyses at both these locations were true margin analyses, in that they were taken at the extreme edge of the otolith, the number of growth bands from which chemical information is obtained within each point analysis would have varied with variability in degree of growth band compaction along the margin edge.

Slope at margin analysis

With the inherent difficulties associated with using the absolute concentrations of 'single-point' margin analyses for inter-otolith comparisons, we then investigated a relative measure; that being the slope of Sr life-history scans at the margin. The basis of these analyses were a total of 47 life-history scans on otoliths from SBT of 91 - 225cm F.L. (conducted under the standard life-history scan procedure detailed in Sect.4.2 - Methods) and our initial efforts were directed towards 'categorising' the margin of each scan by assessing whether the slope at the margin was 'on a rise', 'on a fall', or 'static'. Even after profiles had been smoothed using a 5 - point running

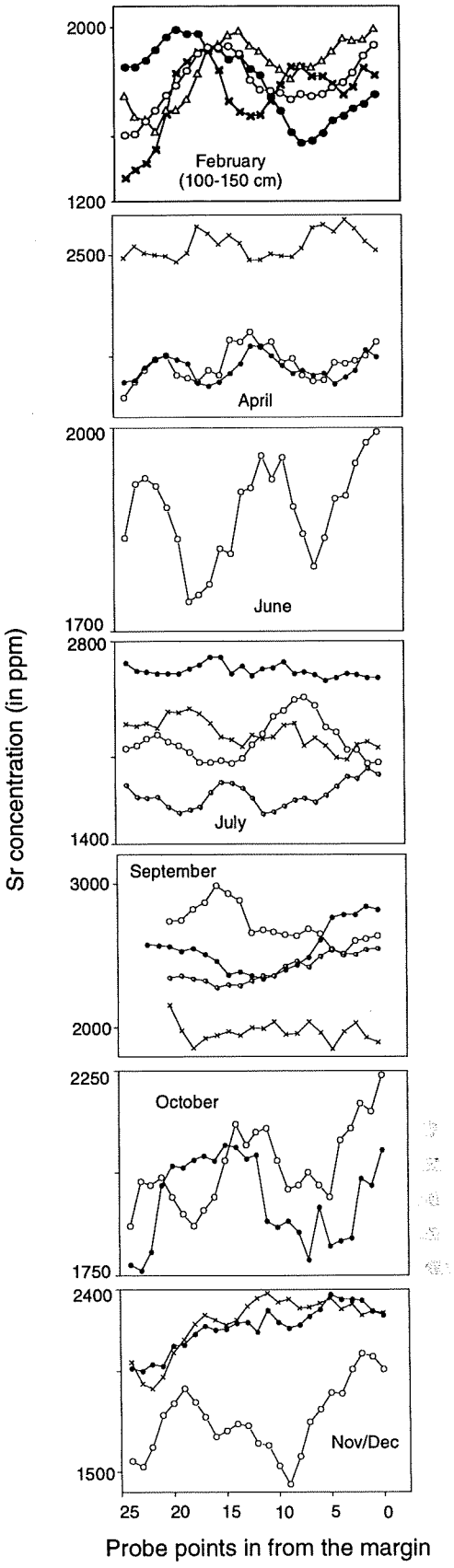


Figure 4.3.10. Outermost section of the Sr life history traces of large (>150 cm FL, except for February) SBT collected throughout the calendar year.

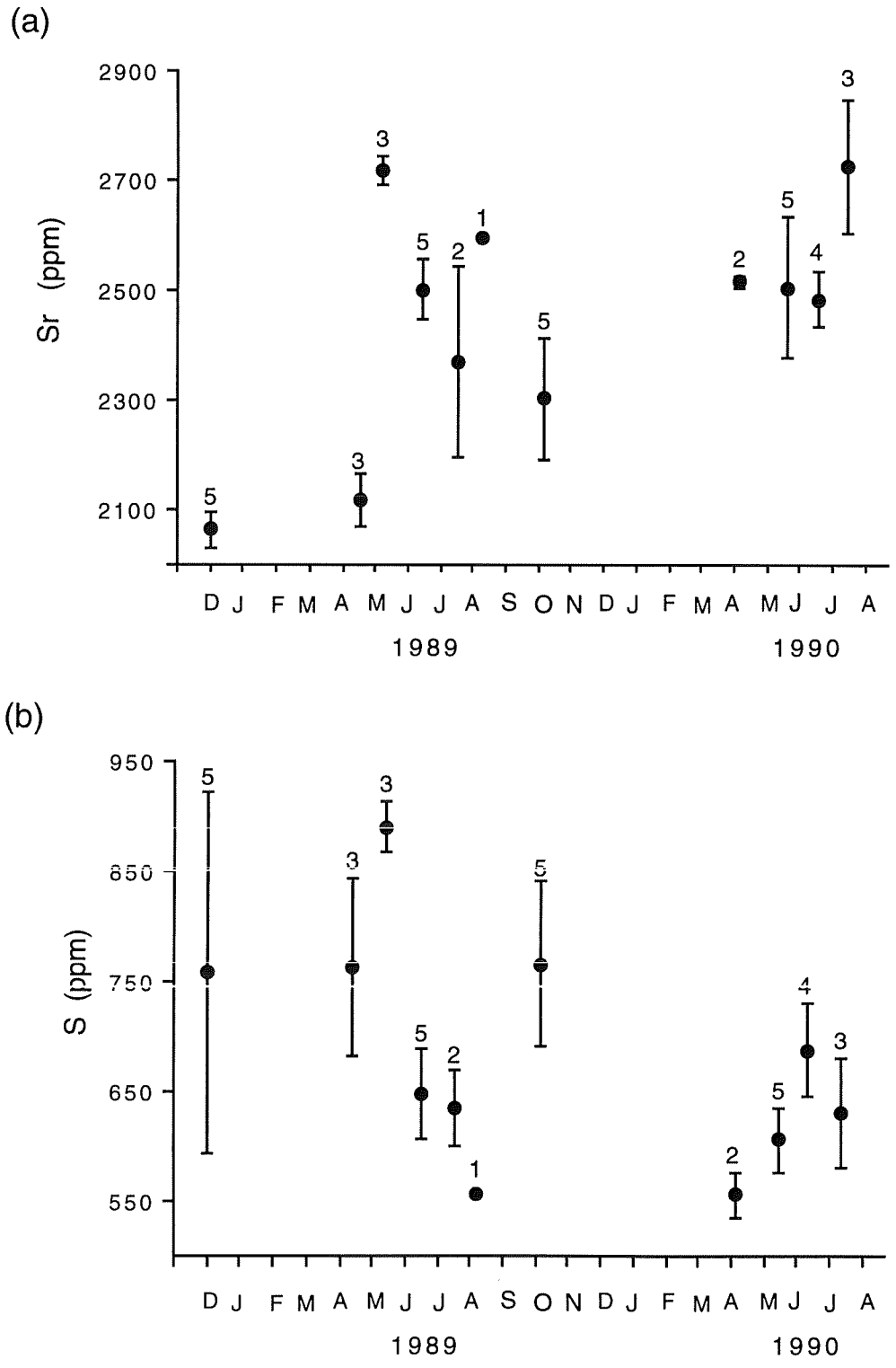
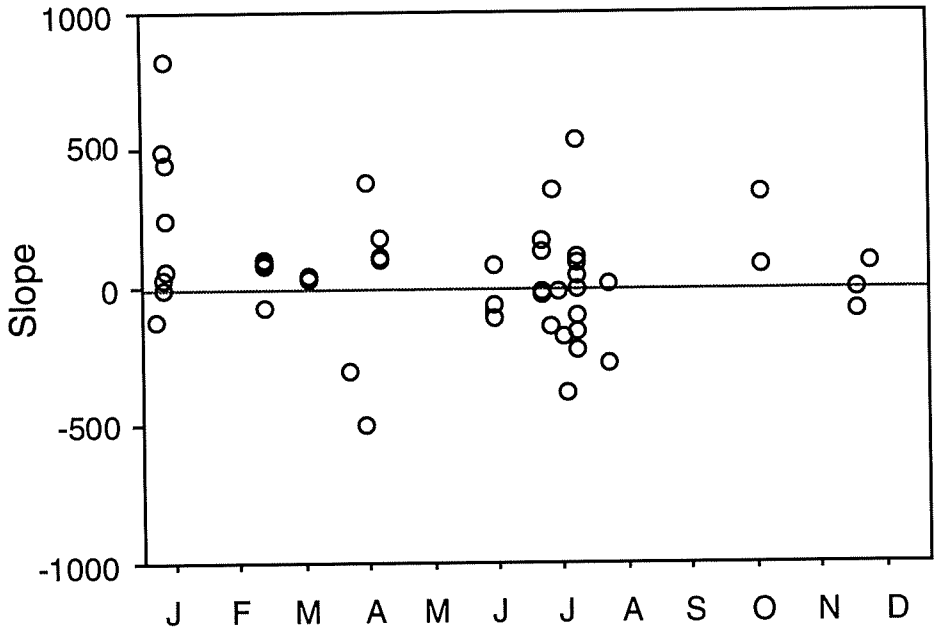


Figure 4.3.11. Absloute concentration of Sr (a) and S (b) at the otolith margin plotted against date of capture. Means (± 1 SE) are of fish pooled on the basis of being caught close together in time and space. Numbers refer to sample sizes.

(a)



(b)

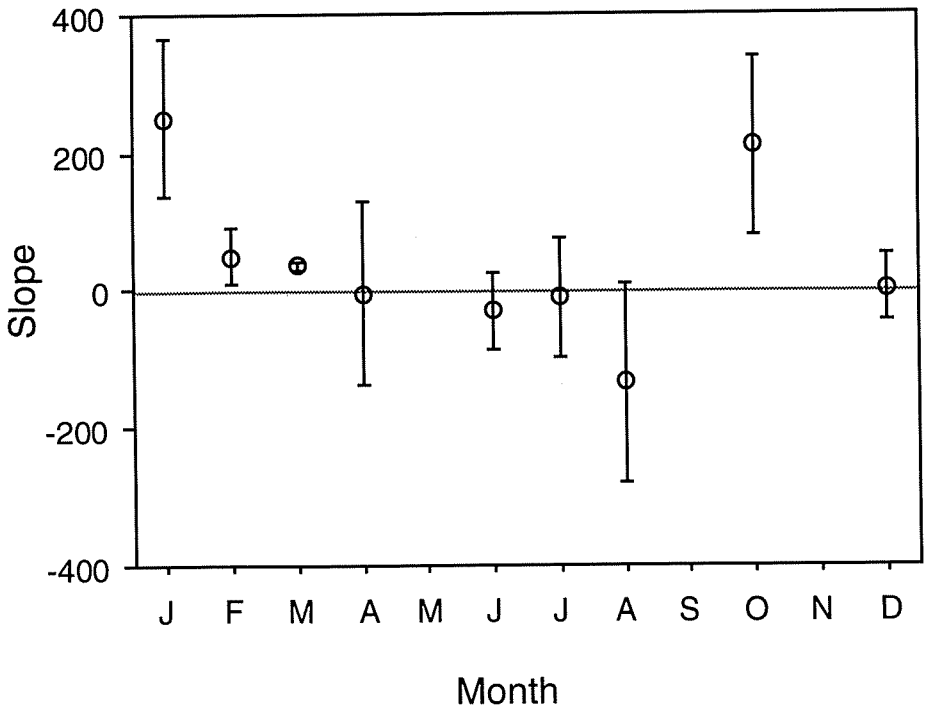


Figure 4.3.12. Slope of Sr concentration plotted against date of capture. a. Individual fish; b. Fish pooled by month of capture (error bar = ±1 SE).

mean, small scale variability made assigning a category of peak - trough position difficult, and required too high a level of subjective assessment. Our slope analysis then changed to a direct measure, that being the gradient and concentration difference (in ppm) between the first and second points in from the margin (25μm apart, centre to centre). This slope measure, plotted against date of capture, did not reveal any apparent cyclic patterning (Fig.4.3.12a) .When individual fish were pooled with respect to month of capture, the mean of the slope measure (Fig.4.3.12b) showed a weak and unconvincing cyclic trend. Gaps in the time series, again, the result of the absence of fishing activity and hence otolith collection at certain times of the year, reduced the potential of this method for identifying seasonal cycles.

Comparison with alternative aging studies

An alternative means of assessing the significance of the episodic variation in Sr concentrations is to assume the peaks are annual and compare length-at-age plots derived from this approach with those estimated using alternative approaches. Several length-at-age plots for SBT have been developed; these were reviewed and up-dated at an international workshop on SBT held in Hobart in 1994. For purposes of this analysis, the Workshop-derived length-at-age plot for the 1980's [WS94(80s)] will be taken as a standard against which to compare the EPMA-based length-at-age. The WS94(80s) trajectory is basically similar to other variations on the SBT growth curve, within the limits of the resolution likely to be achievable using microprobe-based data.

For the microprobe-based assessment, specimens were divided into three groups, based on where they were obtained: a small sample of juveniles was obtained off South Africa, another set of juveniles and adults were obtained from the Japanese fish markets by J. Thorogood (see Sect. 4.4) and the remaining individuals were caught for this study off the Australian coast. Two methods were used to assess ages. First, all peaks and troughs along the main growth axis were counted, with the observer subjectively deciding on the number of spacing of 'broad peaks'. Second, it was assumed, based on the comparative analyses above, that the section of the otolith axis between the primordium and point #225 constituted three broadly defined, annual peaks; the observer counted only peaks and troughs exterior to point #225 and added 3 to this total to derive total age. Further based on the comparison above, it was assumed that fish were approximately 25 cm at age 0, 50-60 cm at age 1, 80-90 cm at age 2, and 110-120 cm at age 3 (Fig. 4.3.4).

Length-at-age plots for the two approaches to microprobe-based age estimation are similar (Fig. 4.3.13). Variability about the trend line is higher for the unconstrained aging approach, which presumably reflects observer difficulty in defining for any one individual the limits of the first three broad Sr peaks. In both plots, estimated lengths-at-age for specimens obtained by Thorogood are much lower than those

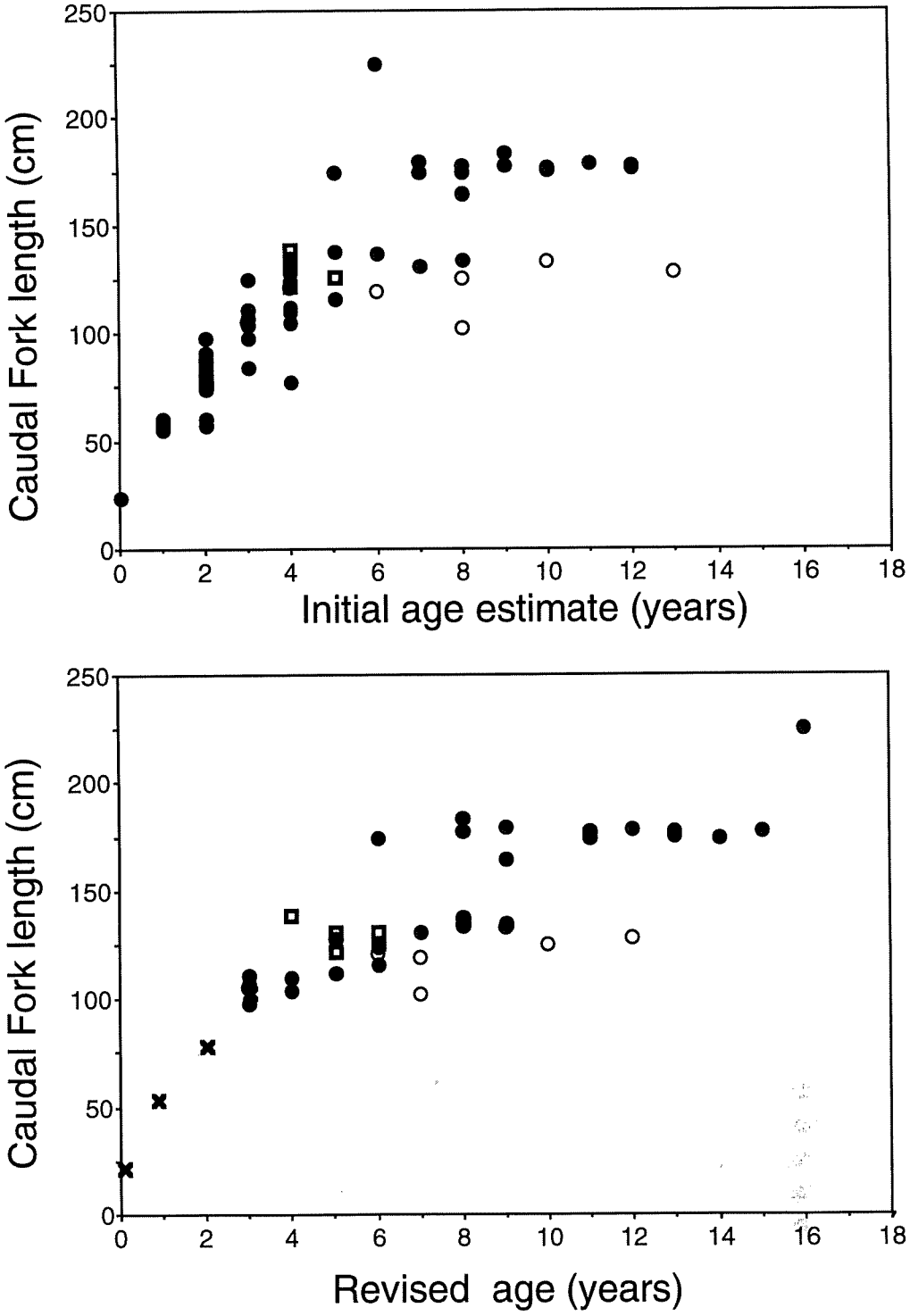


Figure 4.3.13. Length at age for SBT as estimated from Sr life history scans and the assumption that Sr peaks form annually. a) based on 'blind' reading of life history scans; b) based on assumed age three for peaks out to 125 m from the primordium and counting peaks exterior to that position. Solid circles = Australian samples; open circles = Thorogood samples; open squares = South African samples.

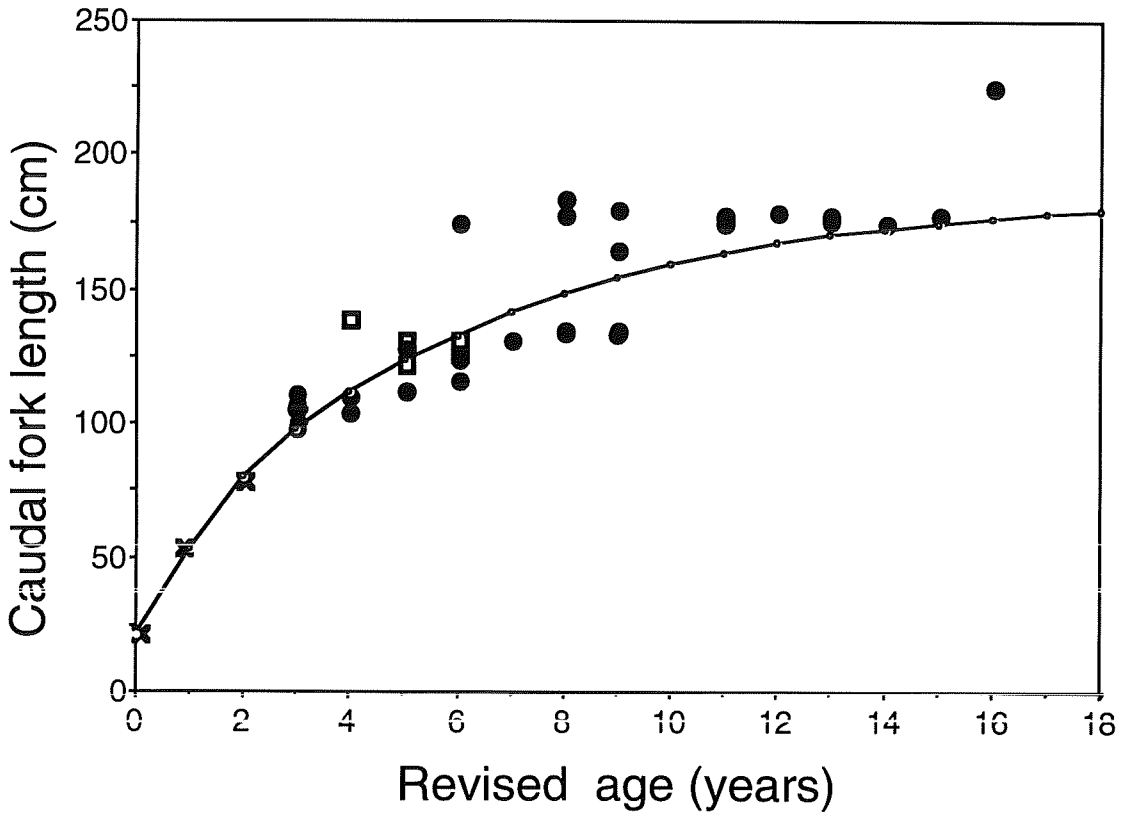


Figure 4.3.14. Comparison between length-at-age as determined by probe microanalysis and assumed annual deposition of Sr peaks, and growth curve derived from analysis of tag-recapture data and population analysis (continuous line). x = mean length of 1,2, and 3 plus fish. Open squares = South African Juveniles; solid circle = Australian samples.

derived for the South African and Australian juveniles (which are not significantly different) collected for this study.

If we exclude the Thorogood material from the analysis, there is a very good match between lengths-at-age estimated from the microprobe-based data with that derived by the 1994 SBT workshop from modal analysis of size-frequency distributions and tag-and-recapture data (Fig. 4.3.14). Estimated lengths at ages 0 - 3 years are almost identical to those derived in WS94.

DISCUSSION

If we were to set an 'ideal' suite of criteria for margin validation of annuli in otolith chemistry life-history scans, irrespective of the species concerned, we would include the following: 1) Regular (at least monthly) samples of otoliths should be obtained over a continuous period of at least 1 year, but preferably 2 years. 2) Fish sampled should be from one year class to minimise interannual variability. 3) Fish within a sample should be from the same geographic area, to minimise the probability of regional variability. 4) Otolith sections should have a 'standard shape' and clearly defined area on the margin that will afford a high level of standardisation (section to section) for the positioning of single point analyses or multiple point scan lines. For inter-section comparisons, margin analyses should integrate over the same number of growth bands (ie. integrating chemical information from an equal number of days, weeks or months pre-capture) in each section.

Our attempts to validate the observed cycles in variability within life-history scans across SBT otoliths as 'annuli', through time series analyses of variability at the margin, proved inconclusive, largely because our data set did not match the above criteria in several respects.

Firstly, gaps in the time series, resulting from unforeseen seasonal shortages of otolith material, were a significant limiting factor in both data sets. Although our samples spanned a 19 month period, the gaps over the early summer - early autumn periods (Nov. - March) when fishing activity ceased, limited the observed month-to-month variability to only 4 - 6 month periods, and any pattern of peak - trough - peak through time in Sr and S concentrations could not be followed.

Secondly, samples for the single point margin analyses were, by necessity, restricted to otoliths from SBT of 160 - 180cm F.L., mature fish of unknown age and undoubtedly variable year class. The limited availability of otoliths did not allow for further narrowing of the chosen size class. Samples for the slope-at-margin analyses included an even wider range of fish size, and hence even more variable year class spread. Our samples for the single-point margin analyses did match the third criterion, in that all fish, with the exception of the one 'spawning ground' sample of October '89, were caught in the same location (east coast Tasmania).

However, again due to limited samples, the slope-at-margin analyses required pooling of fish caught in different regions.

The number of fish-days (immediately prior to capture) from which chemical information was sampled by our margin analyses would very likely vary considerably between specimens. Our limited ability to rigorously standardise on the position of both single point analyses and life-history scans, as a consequence of the irregular shape of otolith sections from the large, mature SBT, probably accounts for much of the intra-sample variability in margin concentrations and margin slope characteristics respectively. Furthermore, the high level of variability between replicate analyses on even the same margin highlights the difficulty in obtaining a single representative margin concentration for inter-otolith comparisons in this species, at least by EPMA.

Beyond these technical problems associated with margin analysis, it is also clear that there is a great deal of short-term (small spatial scale) variability in Sr concentrations. As noted earlier, what we currently interpret as a relatively broad 'first peak' in Sr values, at fish sizes of approximately 50-60 cm FL., consist of anything from one actual peak spanning 50-60 probe points to as many as 5 or 6 very narrow peaks, of differing width and amplitude. Without real-time monitoring data (which may now be achievable with the successful deployment of archival tag technology), the time intervals involved in each of these narrow peaks is difficult to ascertain precisely. Based on our estimates of otolith growth (from the daily increment counts), many of the narrower peaks probably constitute periods of only a few weeks or a month. Given the range of environmental conditions accessible to a fish as mobile as an SBT, we think it likely these peaks and troughs represent forays into different sets of water masses for purposes of, for example, feeding. As well, the factors that determine Sr concentrations in otoliths are far from well defined. Although we assume, for the basis of this analysis, that Sr concentrations are principally a function of seasonal changes in water temperature, it is unlikely that this is the sole factor involved, or even that water temperature is recorded in an unbiased fashion in the otolith record. A strong consensus developed at a recent workshop on otolith chemical analysis, held in Hobart in 1992, was that the search for a unitary cause for variations in otolith composition was probably fruitless (Thresher et al., in press).

The multivariate nature of the factors determining Sr concentrations is also likely to be the principal difficulty associated with our marginal analysis of otoliths from mature fish. In part because the objective of the study was to assess the utility of otolith composition as a verifiable means of aging mature SBT, our marginal analysis focused on these larger individuals. In retrospect, the very high levels of short-term variability in otolith composition would have made marginal analysis of the juveniles even more difficult that that encountered in the adults. For the adults, however, examination of life-history traces suggested relatively smooth and well developed peaks in Sr concentrations which, in theory, we should have been

able to validate relatively easily as annual. Our inability to do so, we suspect, derives from the small sample sizes of adults we were able to analyse (particularly for the spring-summer period) and the very real possibility that Sr peaks in concentration at different times of the year for different individuals. Although statistical analysis of the data are difficult, because of the uncertain time base, there are indications throughout the data set of 'double peaks' in Sr concentrations annually for at least some individuals. The pattern is far from uniform, but appears persistently in the data. Such a double peak is consistent with the seasonal comparison we could develop, which suggested peaks in Sr concentrations in early June and October. The October sample was for fish collected on the Indian Ocean spawning ground, and may reflect a change in otolith composition resulting from development of fish into spawning conditions. Kalish (1992) found evidence of peaks in Sr associated with spawning in two temperate fish species, though the extent to which these could have been coincident due to low winter spawning temperatures is not yet clear. Nonetheless, we speculate that spawning individuals (and perhaps only females, based on some of our unpublished data) may peak in Sr concentrations at a different time of year than non-spawning fish, and may often show double peaks in years when they do spawn.

Hence our principal conclusion from this analysis is that SBT represent a far from ideal species on which to test the hypothesis that Sr peaks represent annual markers in fish otoliths from which ages can be determined. Comparison of our results (the length-at-age plot) with that developed at the 1994 SBT Workshop suggests that this hypothesis remains very likely valid, if exceptionally difficult to verify in a highly mobile fish species for which a year round supply of specimens is difficult to obtain.

4.4 COMPARISON OF CONVENTIONAL HARD-PART AND MICROPROBE-BASED AGE ESTIMATES, AND EVALUATION OF THE PRECISION OF MICROPROBE-BASED ESTIMATES

METHODS

Three putative aging techniques were compared: conventional aging using the 'Thorogood method" (Thorogood 1987), conventional aging by means of traditional methods, and aging based on the number of episodic variations in Sr concentrations, as indicated in life-history traces using EPMA. The initial intent was to use all three approaches on the same otolith, and thereby provide a direct comparison of the results. This proved impossible to carry out. Because of the way he prepared the material (burned, embedded and glued to a microscope slide), the otoliths aged by Mr. Thorogood were useless for subsequent EPMA and of questionable use for independent verification of ages using conventional techniques. The only comparison remaining was to undertake parallel analysis using 'sister' otoliths, that is, the other half of the otolith pair. Unfortunately, Mr. Thorogood had very few such complete pairs, and some of these were broken and

hence not suitable for age estimation. Ultimately, we had 44 pairs of otoliths to work with. These were divided roughly in half, with 24 used for a comparison with conventional aging, and 20 used for EPMA. Conventional aging, as well as Thorogood's techniques, rendered the otoliths unsuitable for EPMA.

Otoliths were first read by J. Thorogood, as part of a contract arrangement with CSIRO.

Conventional aging was done by the Central Aging Facility (CAF) of the Marine Science Laboratories (Victorian Department of Conservation and Environment), using a subsample of Thorogood's prepared otolith sections and the 'matching' half of the pair. Each otolith was independently aged by three experienced staff. No ancillary (e.g. size of fish) data were provided with the otoliths, so that CAF read the otoliths 'blind'. CAF staff were at liberty to prepare the otoliths for examination using any technique they thought might improve the accuracy of age estimation.

Procedures used for preparing the otoliths for EPMA and conducting life-histroy scans are detailed in Sect. 4.2 - Methods.

In order to assess the precision of age estimates made based on life-history scans, three observers, each familiar with the methodology, were asked to independently 'age' the specimens based on the Sr life-history scans. All of the Thorogood samples were examined, along with a number of additional samples, for a total sample size of 90. Apparent ages of the specimens ranged from 0+ to approximately 13+. Although all three observers had been involved in extensive prior discussions involving aging by means of Sr scans, no set of decision rules was developed or uniformly agreed to prior to independent scoring, i.e. each observer made his own 'best guess' based on the pattern of Sr peaks and troughs. As well, one observer made independent age estimates based on patterning of the Sr and S plots.

RESULTS

Independent readings of Thorogood's otoliths by experienced otolith aging staff produced highly variable annuli counts (Table 4.4.1). For most otoliths, the counts were different to those obtained by Thorogood. Differences were as great as 17 (3 vs. 19 annuli seen). Differences among the CAF staff were also considerable, though they tended to be more internally consistent than they were, as a group, with the Thorogood estimates. The CAF staff classified many of the sections prepared by Thorogood as "unreadable" or "number of annuli uncertain". On a scale of 1 to 5 (1=perfect, 5 =- impossible to read), the material prepared (and read) by Thorogood was graded as uniformly '4'.

Spec.No.	Length (cm)	Thorogood	MSL(1)	MSL(2)	MSL(3)
F6	56	3	9	11	10
F9	57	2+	8 - 9	19	?
F15	61	4	-	10+	8 (?)
F8	76	3+	?	?	?
F5	77	5	7	5	5
F1	78	5	4 (?)	6	4
F2	80	3	5 (?)	6	?
F4	82	5	6	10	8
F17	90	6	-	?	6 - 7 (?)
F3	93	5/6	4	4 (?)	5 (?)
F174	104	5	-	9+ (?)	?
F214	105	4	-	?	?
F239	105	5+	-	7	6 - 7 (?)
F307	105	5	-	10 (?)	7 (?)
F331	105	4+	5(?)	8+	4 - 6
F462	107	5	?	?	?
F719	108	4	?	?	?
F755	110	5	6+	7	?
F241	117	4+	-	7	?
F736	117	7	7 (?)	6+	6+
F35	118	7	-	15+	9+ (?)
F704	119	?	?	?	?
F203	122	5/7	-	7	7
F284	128	6+	-	8+inner	?

Table 4.4.1. Comparison of SBT age estimates for the same specimens done independently by four observers (J. Thorogood and three CAF (MSL) scientists, based on examination of hard parts.

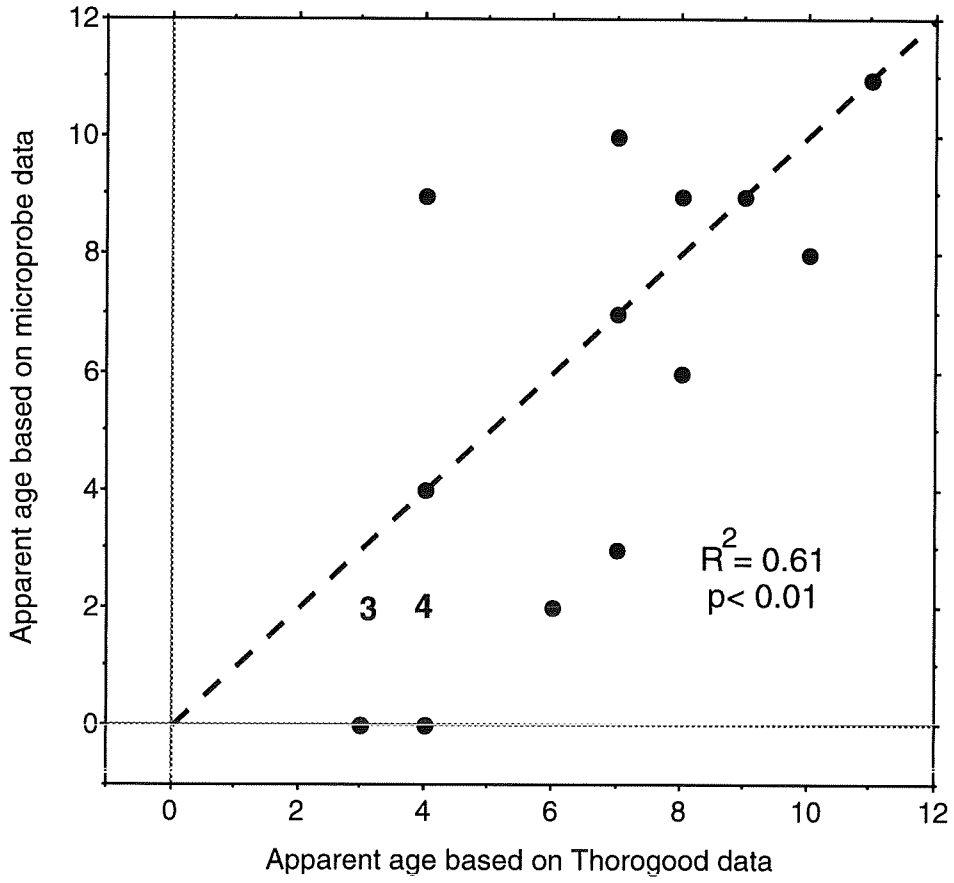


Figure 4.4.1. Comparison of ages for SBT as estimated by J. Throgood and as estimated by an independent observer based on counting Sr peaks in the sister otoliths to those prepared and examined by Thorogood.

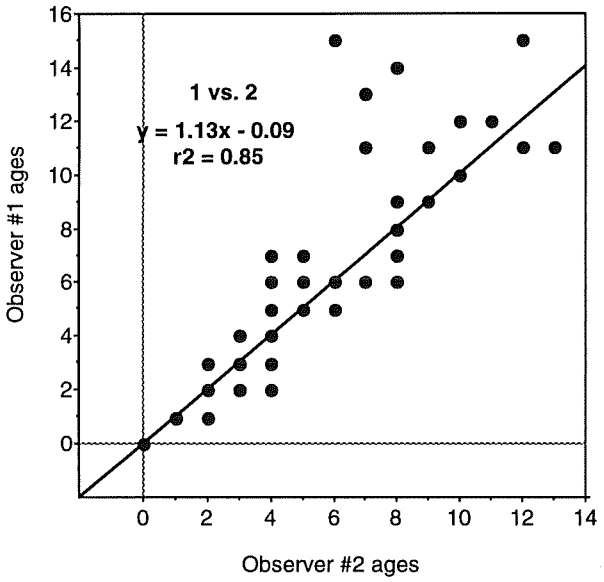
Techniques used by CAF staff to improve accuracy of age determination included acid dipping, transverse sections, and baked transverse sections. None of these techniques yielded satisfactory results, though there was a suggestion that burning followed by a transverse section showed the most promise.

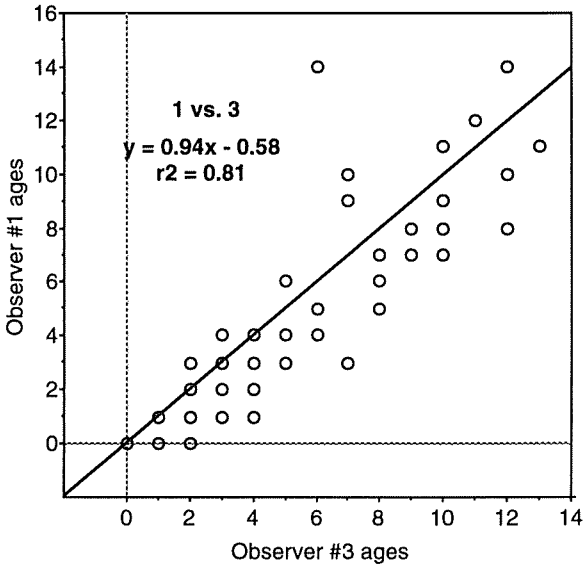
Comparison of the microprobe-based age estimates and those determined by Thorogood is depicted in Figure 4.4.1. Overall there is a significant relationship between the two techniques, indicating they are likely to both the 'reading' the same structure. Several points can be drawn from the analysis, however.

First, Thorogood's technique consistently estimates the ages of small fish as higher than we estimate using EPMA. Fish that we are virtually certain to be 0+ (based on the EPMA scan and counts of daily growth increments), Thorogood ages as 3 and 4 years. Examination of the EPMA scans suggests the source of the discrepancy is the frequent, multiple spikes in Sr concentrations early in life that superimpose on the longer time scale annual cycle. If these spikes manifest as incremental structure, their multiplicity and irregular width and darkness (height in Sr concentration) could cause considerable difficulty in discerning the underlying annual cycle. Note that this appears to be a general problem in conventional aging; at the onset of our work, 25 cm juvenile SBT were widely considered 1+, whereas our data indicated very quickly that they were young-of-the-year. This tendency to apparently over-age young fish is evident in most of the fish Thorogood estimates as less than about 6 years old. Overall, the slope of the type 2 regression between the two data sets is significantly greater than 1.0, reflecting this bias at low ages.

Second, although there is broad agreement between the two techniques, particularly for older fishes, the variance is high, and mis-matches are spectacular for a few individuals. One individual Thorogood estimates to be 4 years old, for example, we would have great difficulty concluding to be less than 9 years old. Similarly, a fish Thorogood estimates to be 6+ appears to us to fit closely the patterning seen in 2 year old fish. Again the primary difficulty appears to be confusing irregularly occurring sub-annual peaks with the underlying, presumed annual cycle.

Comparison of estimated ages from Sr plots made by three independent, but experienced observers also suggests considerable difficulty of resolving differences between annual and sub-annual structures. Although age estimates correlate highly among the three observers for the 90 specimens examined (Fig. 4.4.2), differences among observers was high, particularly for the larger, and apparently older specimens. One observer consistently estimated ages lower than those of the other two, both in terms of an overall mean lower age (intercept of the regression <0) and an increasingly lower age estimate for older fishes (slope <1). Similar difficulties arose in comparing age estimated based on Sr and S plots. Again, although the sets of two age estimates correlate highly, the scatter was wide





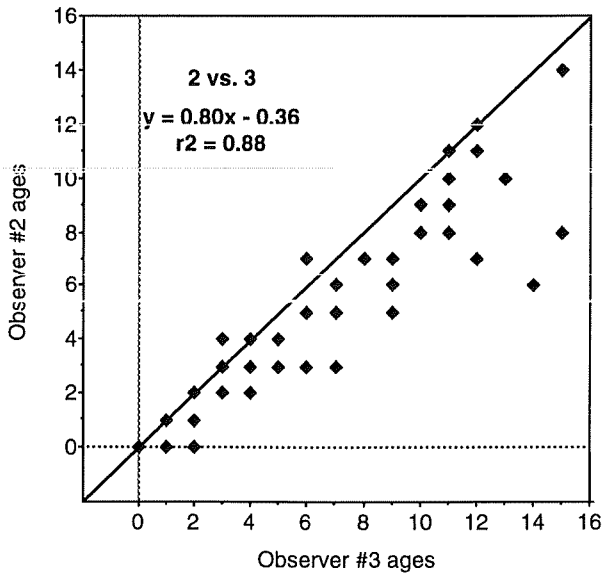


Figure 4.4.2. Correlations between ages estimated by three independent observers, based on 'blind' evaluation of Sr life history traces of juvenile and adult SBT.

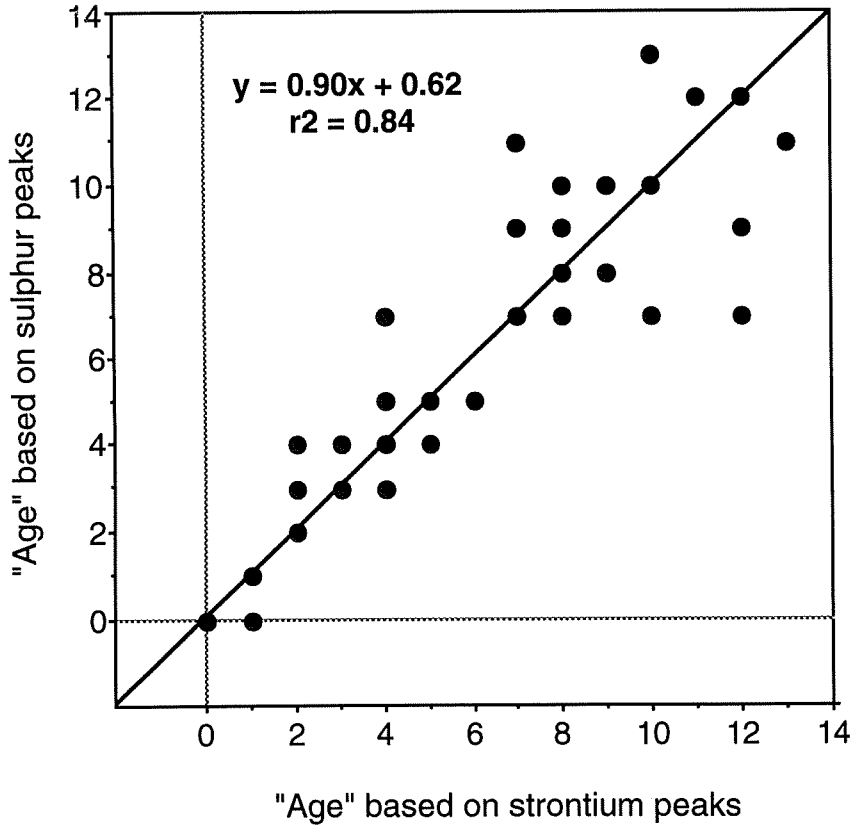


Figure 4.4.3. Comparison of ages estimated by the same observer based on counting the number of discrete S and Sr peaks in the life history scan.

enough that confidence intervals about any given age covered about 50% of the possible range (Fig. 4.43).

DISCUSSION

There remains considerable uncertainty about age estimation in SBT. Thorogood's technique appears to provide results consistent with other aging procedures (Thorogood 1987), but the basis for his procedure is not fully clear. Attempts to duplicate his results by independent 'blind' readers largely failed, but this failure may simply be the result of 'inexperience' in applying Thorogood's criteria for identification of annuli. Prior schooling of the readers by Thorogood himself may have led to greater consistency in the age estimates.

A direct comparison between the conventional age estimates by the CAF and the microprobe-based results was not possible, for reasons outlined above. However, a comparison with Thorogood's data was possible, and are surprisingly consistent. That is, results of the two studies correlate, which suggest that they are both responding to similar underlying structure in the otoliths. The occasional large discrepancies between the two techniques can also be accounted for by noting the highly irregular pattern of Sr deposition early in life, in what appears to be sub-annual structuring of the otoliths. The key question regarding aging, however, is not whether the older otolith growth shows more regular structure than early otolith growth, as this is very likely in any case. Rather, the question revolves around questions of accuracy and precision. In that regard, the match between Thorogood ages and microprobe-based ages is not very compelling. Of the 22 specimens examined, only 4 were aged identically by the two techniques, and differences between the two techniques ranged as high as 5 years. In the worst case, specimens estimated by Thorogood to be 4+ were aged by the microprobe as ranging from 0+ to 9+. We conclude therefore that even though both techniques indicate larger fishes are older, the poor match between them provides little support for either alone or the two in concert to provide precise age estimates.

The relatively low precision of microprobe-based age determination is also indicated by the high level of inter-observer differences. The practical advantage of microprobe-based aging lies in its ability to produce highly repeatable, accurate age estimates. However, even a set of experienced observers who had previously discussed the technique in detail could not agree on a common set of age estimates without very specific decision rules, which reflects the complexity of the ontogenetic patterning of Sr concentrations. In a post hoc discussion of the comparisons, the three observers agreed on most major peaks and troughs. But, there was considerable disagreement as to separation of closely adjacent peaks (do two peaks close together indicate a bimodal year, or two years separated by slow growth?), particularly close to the margin (i.e. later in life), when the peaks are small relative to the high background levels of Sr. Hence, without validation data on the basis of which firm decision rules can be established, we conclude that Sr

life-history traces in SBT are sufficiently complex that the technique does not produce unambiguous (highly precise) age estimates.

Cross comparisons with the second, potentially environmentally sensitive element - sulphur - does not improve the precision. A reasonable expectation, if both Sr and S are responding to the same pattern of seasonal changes in water temperature, is that life-history traces for the two elements will suggest the same ages. In practice, this proved not to be the case. Although age estimates based on the two elements correlate highly, differences between them were often substantial. In the worst instance, specimens aged based on the pattern of S peaks and troughs to be 7+ ranged in estimated ages, based on the Sr patterns, from 4+ to 12+, even though both estimates were done by the same person. Again, the principal difficulty is the complex nature of both the Sr and S life-history traces and the difficulty of resolving the status of closely adjacent peaks. As well, cross comparison of the two life-history traces for each individual indicated that for a large minority of the peaks and troughs, those for one element did not closely match those for the other (i.e. there were often peaks in Sr concentrations that were not matched by an equivalent S peak, and vice versa). This implies a different physiological basis for deposition of the two elements, which are decoupled enough to produce alternative possible ages for the individuals examined.

5. GENERAL DISCUSSION

This proposal was driven by a perceived need for developing a means of aging mature SBT that could be validated and that offered precision not yet achievable using conventional aging techniques. The hypothesis that variation in otolith composition could provide such an aging procedure was stimulated by observations of life-history scans along axis of SBT otoliths, in which well defined peaks in, particularly, Sr concentrations could be easily seen and enumerated. The high spatial resolution that can be achieved with the electron beam, on the order of 5-10 μm, further suggested that the technique would resolve annuli near the otolith margin which are difficult to separate by eye, that is, by conventional hard part aging.

In practice, we have been unable to validate an annual cycle of elemental deposition in SBT otoliths. There are several reasons for this failure.

First, our experimental studies suggest that the concentrations of most of the elements we routinely detect in SBT otoliths are not deposited as a direct or simple function of an environmental signal. Of the six elements we measure, only strontium and sulphur show any indication of the degree of environmental sensitivity that would lead to their potential use as in aging studies.

Second, the same experimental series also suggested very high levels of individual variability in the pattern of otolith composition. For individuals held in as nearly an identical set of conditions that we could practically hope for, differences among their chemical patterns were extreme for virtually all of the elements measured and showed little sign of converging in the cage environment. Again, of the six elements measured, only Sr and S showed indications of convergence, but in S at least convergence was to two, very different trajectories. This suggest that physiological factors interact strongly with the environment to determine otolith composition, and, in the case of S at least, would result in the annual pattern of deposition differing among sets of individuals, and possibly between years in the same individual. This level of individual variability renders difficult any attempt at validation based on an assumed unitary response by the population to environmental forcing. If there is a broad period over which an annual peak might be distributed, even if each individual does peak only once annually, then verifying cyclic deposition based on data inevitably pooled across individuals is, at best, difficult, and may not be possible.

A third possibility, of course, is that in SBT episodic patterns of elemental deposition are not annual. This is, to a large extent, certainly true for juveniles. Unlike some other species we have examined, such as jackass morwong, the pattern of variation in Sr concentrations in SBT is extremely complex. This is particularly so during the juvenile stages, where the spacing, amplitude and number of peaks and troughs in Sr concentrations vary immensely among individuals, even though broad patterns can still be discerned in the variability. Discriminating between annual peaks in concentrations, and those that are as high, but apparently formed during some short duration event (feeding migrations?) is often very difficult, even when using a relatively simple set of decision rules. This difficulty is reflected in the disagreement among experienced 'readers' when examining the same set of life-history data; although more than half of the specimens examined would relatively simple to interpret, a very large minority were so complex that alternative interpretations of the scans were possible. Hence, without a more rigidly defined set of decision rules, the precision of age estimates made using EPMA of SBT is probably little better than can be achieved using high quality, conventional techniques.

The available data are nonetheless still consistent with a broad conclusion that the relatively simple peaks and troughs deposited in the outer sections of the otoliths, i.e. post-maturation, form more or less annually. The very regular spacing of the peaks suggests a periodic deposition pattern and the close match between a length-at-age plot generated by assuming annual deposition and that independently produced by the 1994 SBT workshop suggests that this underlying period is a year. The critical phrase, however, is that the peaks form 'more or less' annually. For reasons discussed above, the assumption that Sr concentrations blindly track annual variations in water temperature is probably not valid for SBT (and may not be completely valid for other species). There are two reasons for this.

First, because of their mobility it is not entirely certain that mature SBT routinely experience a simple, annual cycle of water temperatures. Data on the seasonal changes in the distribution of mature fish are still sparse, but movement in an around the southern ocean provides relatively easy access by the adults to water temperatures that could range annually from a relatively constant signal (e.g. if fish were tracking isotherms) to warm summer/cold winter and vice versa. SBT are very different from a 'standard' fish, which is relatively site-attached and which therefore experiences the seasonal changes in water temperature at whatever site it inhabits. Although micro-scale movements to track preferred environmental conditions are quite common in fishes, they are often not of such significance as to over-ride the larger magnitude seasonal cycles.

Second, it is also not entirely certain that Sr concentrations depend primarily on water temperature. The evidence to support this hypothesized 'bio-thermometer' in otoliths is mixed, with several studies reporting an apparently very close relationship between the two variables and others finding no support for it at all (see discussions in Thresher et al., in press). The extent to which the correlation between water temperature and Sr concentrations differs among species is not known, but could be substantial. As well, Sr concentrations have also been demonstrated to vary depending upon other environmental variables, such as salinity (Secor 1993). Reproductive activity (and specifically gametogenesis) has also been implicated as a possible determinant of Sr concentrations in otoliths (e.g. Kalish 1991b). The highly specialised physiology of the tunas, including 'brain heating', makes it dangerous to generalize from studies on 'normal' fishes to them, given the evident impact of physiological factors on otolith chemistry.

Finally, the possible association between spawning periods and a peak in Sr concentrations may be worth pursuing. Individual SBT caught in the Indian Ocean in October, which we needed to hope to assemble a complete annual cycle for marginal analysis, show surprisingly high peaks in the Sr concentrations. As noted above, Kalish (1991b) speculated that such peaks form during gametogenesis and could potentially be useful in reconstructing the reproductive history of an individual fish. Our as yet unpublished experimental studies to test the effects of reproduction of otolith chemistry suggest other, more specific markers (though also the now usual high levels of individual variability). Our data also suggest the effect is sex-specific; markers of gametogenesis are much more pronounced in females than in males (which makes considerable sense, given the differences between the sexes in reproductive investment). Applying these ideas to SBT, the peak in Sr concentrations evident near the margin of otoliths of fish caught in the Indian Ocean immediately prior to spawning could reflect a 'gametogenesis peak', or could as well reflect cold waters in the southern ocean (from which the fish presumably recently migrated). More importantly, if a gametogenesis peak, then we would expect its occurrence to be sex specific and hence highly variable. The nature of the sampling in the Indian Ocean (specimens provided courtesy of two Japanese fishing schools) precluded data on the sex of the specimens, so at this

stage we cannot factor this potentially important variable into our analyses. Current work, in which samples are being obtained from the Indonesian fishery, could well overcome this problem. If Sr variations, either singly or as 'double, annual peaks' can be used as an index of spawning frequency in SBT, then resolution of such could prove useful both in assessing the reproductive potential of the species and in resolving remaining uncertainties about age determination using the microprobe data.

6. IMPLICATIONS AND RECOMMENDATIONS

1. Age determination by means of enumerating peaks and troughs in elemental concentrations across the growth axis of an otolith is not simple in SBT, even if the underlying hypothesis of annual cycles of deposition is correct. Without additional work, and particularly a more extensive set of samples of adults collected throughout the year, verification of the annual cycles and development of decision rules to distinguish between annual and non-annual peaks and troughs in deposition will be difficult for SBT. A more direct test of the potential of the technique can be achieved through the combined use of an injected otolith chemical marker such as $SrCl_2$ and archival tag technology, which should be encouraged.

2. Ontogenetic variability in elemental composition is more extreme in SBT than in other species we have thus far examined. This may reflect the highly mobile nature of the animal, and hence its ability to move rapidly between very different sets of environmental conditions. For this reason, we suggest that SBT are far from an ideal species against which to test the broader potential of age determination by means of enumerating elemental peaks across otoliths. We recommend that the technique also be tested on a more conventional fish species, such as one of the SEF quota species for which age determination is also uncertain. In particular, jackass morwong might be suited given the large amount of data we already have on the species (though not collected year-round, as we specifically sought to minimize impacts of seasonal migrations on our estimates of population structure) or redfish.

3. Length-at-age plots derived from our data (based on the assumption that the relatively simple peaks in Sr concentration evident in outer section of SBT otoliths in fact form annually) match very closely a length-at-age plot recently independently derived from analysis of population parameters. The extreme similarity of results from application of two very different techniques lends credibility to the species mean growth curve.

7. ACKNOWLEDGMENTS

We thank I. Harrowfield, C. MacRae and P. Rummel for their assistance in probe microanalysis and discussion of results, and J. Kalish and N. Manning and for their advice on the preparation of material for analysis. We particularly thank J.Collett, T. Davis, M. Excel, W. Hearn, B. Jeffriess, H. Kono, H. Nakano, A. Penney, S. Tsuji, the Australian Fisheries Service Observer program, the Funakawa and Uwajima Fisheries High Schools, the Japanese Far Seas Fisheries Research Laboratory, the South African Sea Fisheries Research Institute, the Tuna Boat Owners Association of Australia, the Federation of Japan Tuna Fisheries Co-operative Associations, and the National Federation of Fisheries Co-operative Associations for their invaluable assistance in obtaining the specimens on which this study is based. We also thank K. Chang, G. Critchley, B. Jeffriess, K.Williams, the Australian Tuna Boat Owners Association, and the Japanese Overseas Fishery Co-operation Foundation for providing specimens and assistance for the sea-cage study.

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FISHING INDUSTRY RESEARCH AND DEVELOPMENT COMMITTEE
APPLICATION FOR GRANT
1989/90

1. PROJECT TITLE

Age Determination and Assessment of Variation in Length-at-age of Large Southern Bluefin Tuna by means of Analysis of Otolith and Vertebral Chemical Composition.

2. KEYWORDS

Aging, Southern Bluefin Tuna, Microcomposition, Otoliths

3. OBJECTIVES

1. To determine whether SBT can be aged by counting the number of regular variations in the composition of their hard parts, by testing the hypothesis that such variations derive from annual cycles in the deposition of different elements along the margins;

2. To compare growth rates, maximum ages and precision of age estimates as determined by microanalysis with published values based on traditional aging techniques; and

3. to quantify variation in length-at-age for large adult SBT.

4. JUSTIFICATION

Current management strategies for Southern Bluefin Tuna are based on Virtual Population Analysis. The precision and accuracy of VPA depends in part on the accuracy of age determination, particularly for older fishes. In SBT, for purposes of analysis all large individuals are pooled into a single cohort irrespective of their true age. This pooling of year-classes and the resultant loss of precision in VPA is necessary due to the lack of any reliable and validated method for aging mature SBT. Conventional aging techniques, based on counting optical "annuli" in otoliths, are poorly validated for the species, not validated for mature fish, and unlikely to be useful for these fish, due to problems of resolving closely spaced "annuli" near the otolith margin.

For the last 18 months, we have been investigating whether the composition of SBT otoliths and, particularly, the way this composition changes as each fish

develops can be used as a means of stock discrimination. Specifically, we sought to determine what proportion of the high seas population of large adults is based on juveniles that migrate through and are vulnerable to the Australian shore-based fishery. This work, supported by FIRC grant 87/15, is still in progress. However, results to date indicate that juveniles collected of Western and South Australia have a consistent overall pattern to their otolith composition and that most, but apparently not all, large adults exhibit such a pattern. The results of this work will be detailed in a forthcoming final report to FIRDC. Along with its primary objective, the microanalysis study had as an important secondary objective the determination of whether there was evidence of seasonal variation in the relative deposition rates of specific elements, such as strontium, sodium and calcium, on the basis of which age might be determined. This secondary objective was initiated for two reasons. First, we suspected that annual variations in composition would be more conspicuous, more easily counted and statistically more robust than variations in optical density. And second, because of the fine spatial resolution possible with the electron microprobe, we also suspected that closely spaced seasonal cycles of composition near the margin of an otolith would be more easily resolved than optical annuli counted by traditional means.

In fact, we have found conspicuous episodic variations in otolith composition in both small and very large SBT (see figures). The number of such variations in an otolith seems to be consistent with the expected age of a fish based on its size and previous estimates of the growth rates of SBT, and appear to be far more easily resolvable. Cycles of compositional change are typically so clear that, if they are indeed annual, it should be possible to determine age to fractions of years.

We propose to examine and document these episodic variations in greater detail than is possible in the current grant and to test the hypothesis that they result from consistent, annual physiological or environmental cycles. If we demonstrate, as we currently strongly suspect, that these variations are annual in nature and can be counted to determine the ages of large adult SBT, we further propose to 1) determine the ages of as large a sample of such fishes as is available and time permits, from as broad a geographic range as possible, in order to 2) generate a validated length-at-age plot for SBT and 3) determine variance in length-at-age for fishes over a range of ages. We will provide these data to the appropriate population dynamacists in order to refine existing VPA-based assessments of SBT stocks.

5. PROPOSAL IN DETAIL

(A) PLAN OF OPERATION

ANALYSIS OF COMPOSITION

Analysis of microcomposition will focus primarily on otoliths, for which we have developed a protocol of specimen preparation that results in a line of points analysed along a single growth axis from the primordium to the margin. We have experimented with sectioning vertebra, but have not yet developed fully satisfactory procedures. Vertebrae also present problems in that their carbonate matrix differs substantially from the aragonitic matrix typical of otoliths, and there are resultant difficulties in developing appropriate calibration standards. We will, nonetheless, persevere on vertebrae, on the basis that for large fishes they may be easier to obtain than otoliths.

As a result of our current work, we have developed a strategy for the fine scale chemical analysis of SBT otoliths (beam conditions, dwell times, order of analysis) that provides an acceptable trade-off between sensitivity and probe time (which is expensive). These procedures will be used in the proposed work. Analysis will be conducted on the Cameca Wave Dispersive Microprobe, at the CSIRO Division of Mineral Products, with whom we have developed a good working relationship.

We also propose to conduct experimental trials using a higher resolution system. Work overseas on the composition of tuna hard parts has involved analysis based on the proton microprobe, rather than the wave dispersive electron microprobe. The proton probe is several orders of magnitude more sensitive than the electron microprobe, and produces data with a significantly improved signal to noise ratio. It is, however, less readily available than electron microprobes and, on an hourly basis, is more expensive to run. As the proposed study may lead to routine use of elemental analysis for age determination in fishes, we propose to conduct a short series of comparative runs using the two machines, in order to determine cost-effectiveness and resolution of variability in the elements of interest. Arrangements for access to a proton microprobe are currently being finalized with the CSIRO Division of Exploration and Geoscience, in North Ryde, N.S.W.

VALIDATION

We will test the hypothesis that the ratio of elements incorporated into an SBT otolith varies seasonally by determining the marginal composition of otoliths collected at different times of the year. The procedure in analogous to measuring the width of the marginal increment in order to validate optical annuli, though the analytical procedures are more complex.

At present, sampling coverage of large adult SBT is not adequate to evaluate growth and age variability in these fish. The mobility of the adults makes it particularly difficult to obtain an adequate time series for analysis of seasonal variations in marginal composition. The otoliths on hand are too few in number (to date, from all sources we have only about 40 pairs of otoliths from fish larger than 100 cm) and spread over too broad a geographic range for statistically robust validation. Consequently, in order to validate these SBT directly, we will need to obtain more material. In a two year period, we should be able to analyse otoliths for about 150 large fish. These otoliths will be obtained in two ways. First, we will continue to seek otoliths from fishes landed at Australian ports or collected by observers on foreign vessels. Over the last year, we have obtained approximately half of our otoliths for large fish by such means, and anticipate a continued trickle of material. These samples are of considerable value for our studies, particularly with regard to stock discrimination, but alone are unlikely to be adequate for rigorous analysis of seasonal changes in composition. Hence, we also propose to deal directly with the Japanese fisheries, by arranging with Japanese fisheries biologists for collection of additional material from the Japanese markets. Obtaining the necessary cooperation of the Japanese will require 1) at least one, and optimally two trips to Japan, and 2) completion of work on samples previously provided to J. Thorogood for conventional aging. Regarding the latter point, we are also very interested in a direct comparison of our results with those based on conventional age determination. If it is essential that such age determination be done prior to obtaining additional cooperation from the Japanese, we will undertake to age at least the material they have already provided, assuming it can be obtained from J. Thorogood and Mr. Thorogood is not actively involved in its analysis. If by the second year of the proposed study no other independent work on conventional aging of SBT has been carried out, we will incorporate into the renewal a short-term (3 to 6 month) study to do such work, based on the samples we will have obtained by then. If this becomes necessary, we will second into the program someone already very familiar with conventional aging methodologies.

Finally, we will also attempt validation using immature fishes, for which we can obtain adequate sampling coverage. This procedure will satisfy minimum requirements for testing whether seasonal changes in marginal composition occur in the species and will also provide detailed data on the nature of these changes, their detectability and perhaps their causes. It is unsatisfactory, however, to the extent that it still requires conclusions be extrapolated to larger fishes for the latter to be aged.

(ii) Facilities Available

CSIRO Marine Laboratories, Hobart provide office and general laboratory facilities and a full back-up of computing, technical and library services. Optical microscopy, scanning and transmission electron microscopy of otoliths and vertebrae will be done at the Marine Laboratories. X-ray microanalysis will be done at the CSIRO

Division of Mineral Products, in Melbourne, on a Cameca Camebax Wave Dispersive Microprobe. Proton microanalysis will be done at the CSIRO Division of Exploration and Geosciences, in North Ryde.

(B) SUPPORTING DATA

Evidence for seasonal variations in the composition of hard parts of marine animals is extensive in the scientific literature, though little of this literature deals with fishes. Validated seasonal cycles of composition have been documented for animals ranging from corals to molluscs, are based primarily on temperature-dependent processes of physical chemistry, and hence are likely to be present in all organisms undergoing seasonal cycles of water temperature, at least. Nonetheless, independent validation of seasonal cycles of composition are essential for fishes, in general, and SBT in particular, in order to ensure accuracy of the technique for age determination.

As noted above, for the last 18 months, we have been engaged in a detailed study of the use of otolith microchemistry as a means of stock discrimination in SBT and Jackass Morwong. The techniques we have developed for analysis of fish otoliths are state-of-the-art, and have been discussed extensively with the few other scientists engaged in similar studies elsewhere in the world. One member of our research team made an overseas trip in 1988 specifically to discuss development of analytic techniques for fish otoliths, and subsequently one American biologist visited our laboratory in Hobart to further discuss the work. Technical publications on the analytic procedures we have developed are in preparation.

6. RESEARCH PRIORITY

The proposed study is directly relevant to FIRDC priorities regarding innovative research for aging fishes, and will bear on our continuing work on the use of compositional information for stock separation. The results of the work, in the form of a validated aging method for SBT and data on variations in length at age, will have bearing in FIRDC priorities in fish resource assessment.

Moreover, the proposed study has considerable potential for developing into a far more accurate, more precise and better validated method of age-determination in fishes than is currently available. Along with SBT, we have documented similar episodic variations in composition, very reminiscent of annuli, in Jackass Morwong. Preliminary analysis strongly suggests annual cycles of deposition in Morwong. Equally striking, and consistent fluctuations in composition are also evident in the few juvenile Orange Roughy we have examined to date. If we can validate compositional changes in SBT otoliths as a means of age determination, then it would suggest an enormous potential to extend the technique for age determination to a range of other species, including perhaps those normally

considered difficult to age, e.g., deep-water and tropical species. Further, the enhanced resolution of chemical annuli near the edges of otoliths, compared with optical annuli, could markedly improve accuracy of age determination for mature fish in numerous species. Although the costs in terms of access to the analytic machines and man-power requirements for preparing otoliths for analysis makes it unlikely that microanalysis will ever become the routine method for aging large numbers of specimens in any species, it can provide a validated baseline of known age specimens against which other, less precise aging techniques could be calibrated.

7. TRANSFER OF RESULTS TO INDUSTRY

A summary of the work will be published in Australian Fisheries, and technical data derived from it will be provided directly to scientific personnel involved in stock assessment of SBT.

8. PREDICTED COMMENCEMENT AND COMPLETION DATE

Commencement Date 1 July 1989 Completion Date 30 June 1991

9. REQUESTED BUDGET

Requested Budget	$ 1989/90	$ 1990/91
Salaries	35,959	35,959
Operating Expenses	38,500	41,000
Travel	15,566	17,730
Capital Items	0	0
Total	90,025	94,689

10. FUNDS SOUGHT FROM OTHER SOURCES

Supplemental funding for the work in Japan will be sought from Japanese-Australian Collaborative Funds. The amount to be requested has not yet been determined, and will be based on the success of our initial trip to Japan and on whatever is deemed necessary to obtain a suitable sample size of high seas fish.

11. FINANCIAL CONTRIBUTION OF APPLICANT

	1989/90	1990/91
(a) Salaries of Existing CSIRO Staff (including Overhead)		
1 Research Scientist (50% of time)	42170	42170
1 Experimental Scientist (50% of time)	33368	33368
1 Experimental Scientist (50% of time)	30191	30191
Total Salaries	105729	105729
(b) Operating Expenses	Nil	Nil
(c) Capital Cost of Equipment Available		
Leitz Orthoplan Microscope and Associated Image Analysis		
Equipment	55000	55000
Leitz Laborlux D Transmitted/ Incident Light Compound Microscope	10000	10000
Electron Microscope Unit	275000	275000
X-Ray Microanalysis Unit (Division of Mineral Products)	750000	750000
Total Capital	1090000	1090000

12. DETAILED BUDGET

Item	Requested Budget $ 1989/90	Indicative Budget $ 1990/91
SALARIES		
Technical Officer (1 Max, to be appointed, to assist in preparation of otoliths and vertebrae for microanalysis)	31324	31324
Sub-total	31324	31324
Super-annuation	4195	4195
Leave Loading	440	440
Total Salaries and Wages	35959	35959
OPERATING EXPENSES		
Cost of Operating X-ray Microprobe (based on estimate provided by Division of Mineral Products)	25000	25000
Cost of Operating Proton Microprobe (based on estimate provided by Division of Exploration and Geoscience)	5000	10000
Purchase of low power objectives and phototube for Leitz Laborlux D compound microscope (required for critical point polishing of large surfaces such as those of adult SBT otoliths.	2500	0
Expendable Supplies for preparation of material for analysis, collecting specimens and computing	6000	8000
Total Operating Expenses	38500	41000

TRAVEL EXPENSES		
Three trips to Melbourne each year, each of 14 days duration (per diem based on Melbourne rate of $101/day).	5238	5238
Two trips to Sydney in the first year, three in the second, each of 14 days duration (per diem based on $116/day).	4328	6492
Two trips to Tokyo, Japan (one in each year), each of two weeks duration (per diem based on Tokyo rate of $150/day).	6000	6000
Total Travel expenses	15566	17730
CAPITAL ITEMS	Nil	Nil
TOTAL REQUESTED BUDGET	90025	94689
ESTIMATED INCOME	Nil	Nil

13. ORGANIZATION

Division of Fisheries,
CSIRO Marine Laboratory, G.P.O. Box 1538,
Hobart Tasmania 7001
Telephone: (002) 206 222 Telex: 57182
F.R. Harden Jones, Ph.D., Chief

14. PROJECT SUPERVISOR

Ronald E. Thresher
Principal Research Scientist
Division of Fisheries,
CSIRO Marine Laboratory, G.P.O. Box 1538,
Hobart Tasmania 7001
Telephone: (002) 206 222 Telex: 57182

15. STAFF

		% of Time on Project
R.E. Thresher	Ph.D. - Project Supervisor	(50)
J. Gunn	B. Sc. (Hons.)	(50)
C. Proctor	B. Sc. (Hons.)	(50)
Technical Officer	(to be appointed)	(100)

16. ADMINISTRATIVE CONTACT

G. Thill
Divisional Administrative Officer,
Division of Fisheries,
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Fig.1

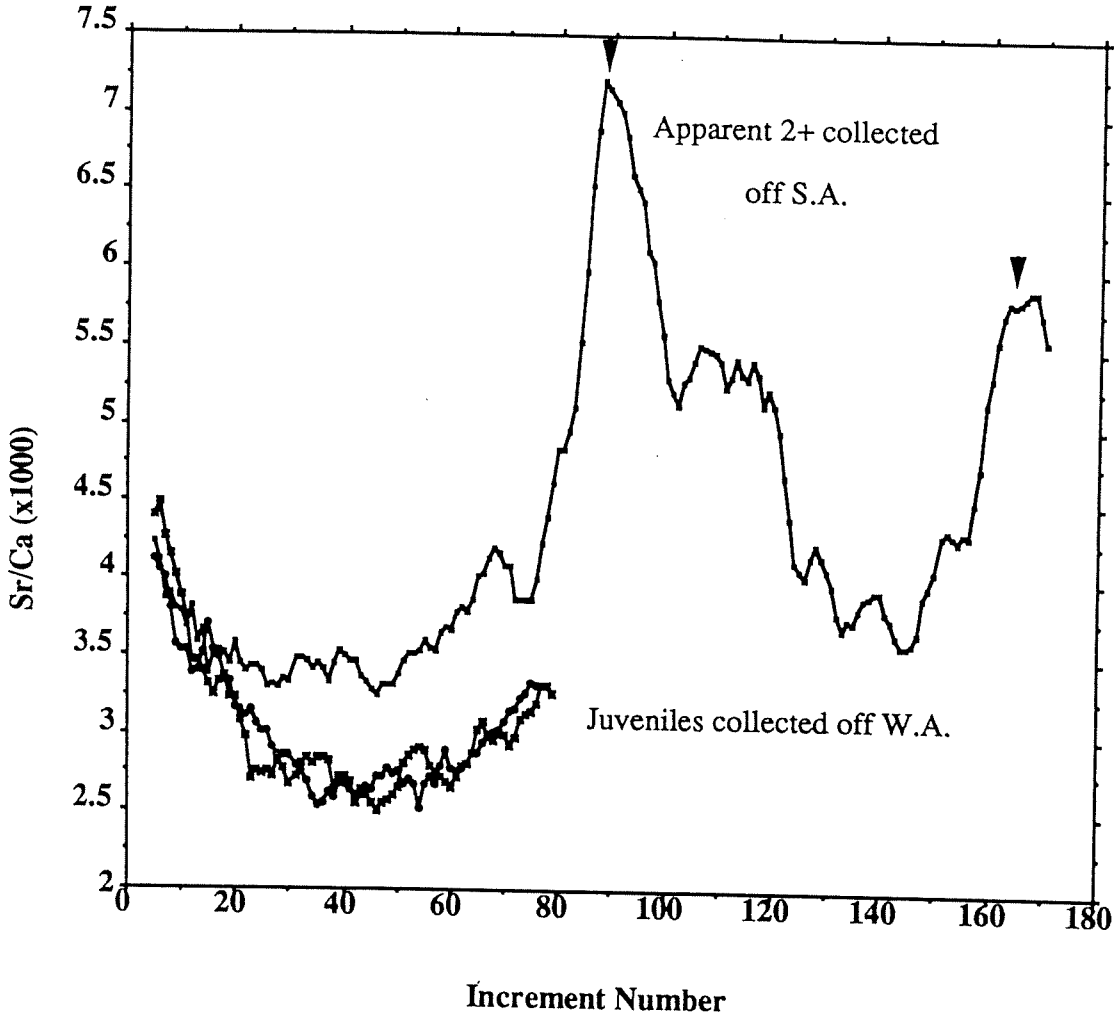

Fig.1 Stronitum-calcium ratio life-history scans across otoliths from a 61cm SBT collected off S.Aust. and two 25cm SBT collected off Dirk Hartog Is., W.Aust. The arrows indicate what we believe to be annual peaks in Sr/Ca.

Fig.2

(a) Strontium-calcium ratio life-history scan across an otolith from a 177cm SBT. The numbered arrows indicate what we believe to be annual peaks in Sr/Ca. The major peak at increment no.25 is not considered to be the first annual peak (and is presently unexplained), as all other SBT we have scanned in this manner show the first major peak at approximately increment 75 to 120. The position of this first numbered peak is consistent with the predicted rate of otolith growth during the first year. The first three or four peaks in these Sr/Ca scans often exhibit 'split' peaks, perhaps indicative of inshore-offshore migrations.

(b) An expanded view of the outer portion of the Sr/Ca life-histrory scan shown in Fig.2(a).

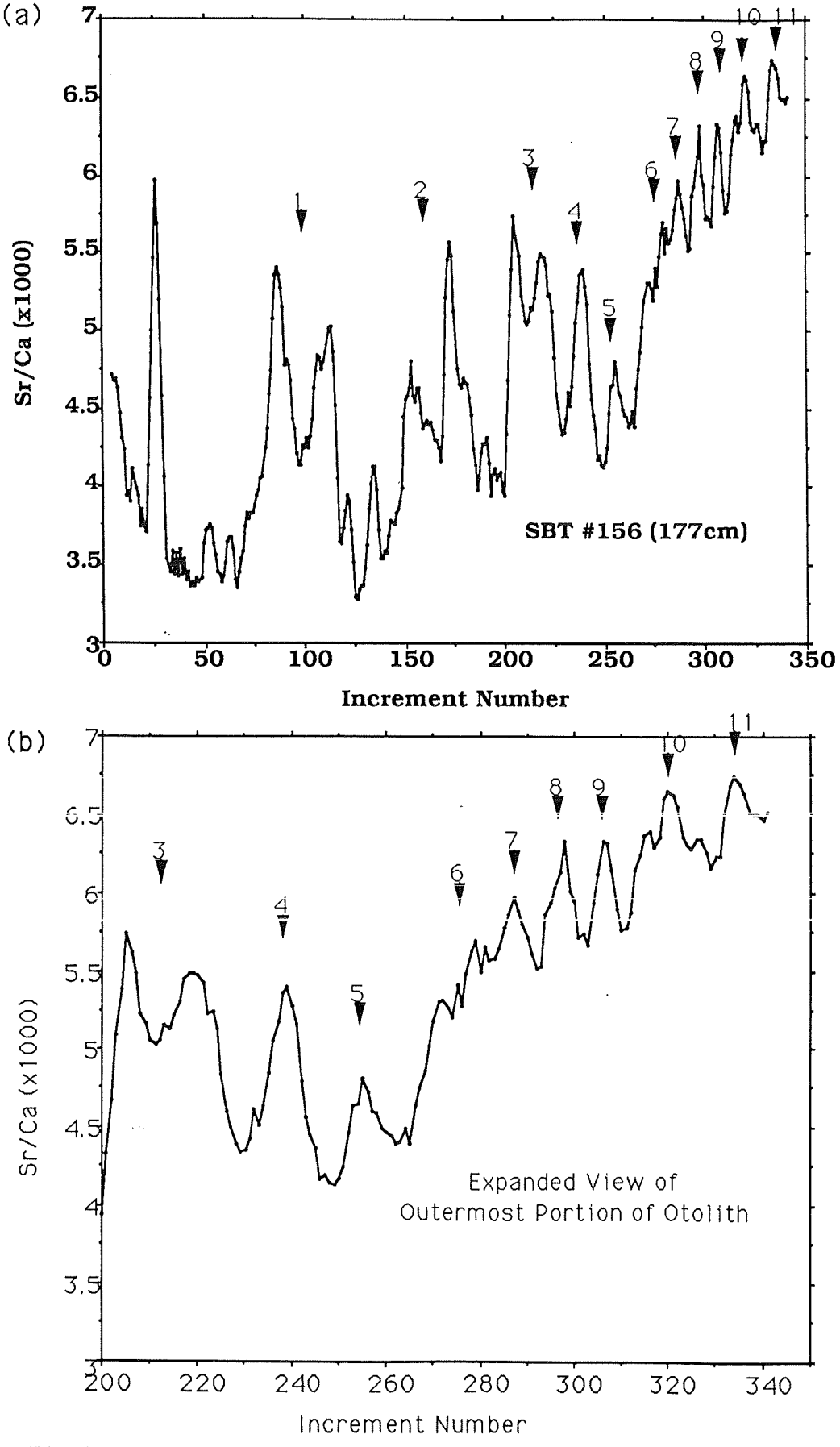
(a)
Sr/Ca (x1000)
Increment Number
SBT #156 (177cm)
(b)
Sr/Ca (x1000)
Increment Number
Expanded View of
Outermost Portion of Otolith

Fig.2