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Identifying spatial structure of juvenile southern bluefin tuna using otolith microchemistry: initial results from a pilot project

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Abstract

A long standing question in SBT dynamics has been what proportion of the global population of juvenile SBT spend time in the Great Australian Bight (GAB) in summer. Tuna otoliths can provide such information about movements and residency because they act as a natural tag and contain a permanent record of the life history of fish. We ran an initial pilot project to determine if it is possible to identify otolith chemical fingerprints from different areas within the SBT range. The elements Ca, Mg, Sr, Li, Mn, Cu, Ba and Pb in twenty six SBT otoliths collected from juveniles and adults (45-166 cm FL) at 3 locations: the spawning grounds, the west coast of Australia and the Great Australian Bight; were measured by laser ablation inductively coupled spectrometry (LA-ICP-MS). Elements were measured continuously along the otolith growth axis from the earliest-formed primordial area to the margin, to provide a life history of elemental levels. Cyclical variation in all elements was observed. The variation in elemental levels was analysed to determine if site-specific signals can be identified, specifically if GAB-summer signals can be resolved from other "juvenile summer" otolith fingerprints.

Introduction

A critical question in SBT dynamics has been what proportion of the juvenile population spend summer in the Great Australian Bight (GAB), and whether this proportion is constant over time. This information is vital for interpreting the line-transect aerial-survey which provides an index of relative juvenile abundance in the GAB (Eveson et al., 2010). This index is now used in the CCSBT operating-model (OM), and may be part of the management procedure (MP) which the Commission will consider for adoption this year. The CCSBT-SC noted "the importance of additional research to determine what proportion of the juvenile SBT population enters the GAB as large variability or trends in the proportion would complicate the interpretation of these recruitment series." (Anon, 2007).

SBT otoliths have been collected and archived since the 1980's and stored in the CSIRO SBT hardparts archives and are used to monitor the age distribution in the commercial catch (Farley et al., 2010a). SBT otoliths from mature fish have been collected from the spawning grounds since 1994 and used to monitor changes in size and age of the spawning population (Farley et al., 2010b). The otoliths exist as pairs and only one otolith from each pair has been used for direct ageing in these projects so the remaining otoliths provide us with a valuable opportunity for further analysis.

Recent advances in otolith microchemistry have shown that trace element composition can be used to track tuna movements and can therefore be used as a stock identification tool (Rooker et al., 2003; Wang et al., 2009). Wang et al. (2009) identified a spawning site signal in the otoliths of SBT at the primordium, the earliest-deposited part of an otolith. At the otolith edge, which is formed later, they found significant differences between the otolith elemental composition in the sub-adults caught on the central Indian Ocean feeding grounds and adults on the spawning grounds.

This project aims to determine if it is possible to differentiate otolith chemical fingerprints of juvenile SBT; otoliths collected from different locations (the Great Australian Bight (GAB), west coast of Australia (west coast) and the spawning grounds) will be examined. If this proves successful, further work will aim to quantify the fraction of the stock that moves through southern Australia; the results will be relevant for the interpretation of the aerial survey index of abundance for juveniles, as well as the estimates of fishing mortality derived from the conventional tagging program, and the design of any future tagging programs.

Methods

Otolith removal and preparation

Sagittal otoliths from 35 southern bluefin tuna (SBT) collected from three regions off the Australian coast (GAB: n=15, spawning ground: n=10, west coast: n=10) were sourced from existing CSIRO collections (Fig 1. and Table 1.). Otoliths were examined whole under a dissecting microscope and the position of the primordium marked with a graphite pencil on the distal surface. Otoliths were mounted in epoxy resin blocks (Epofix, Struers) and sectioned transversely using a modified high-speed diamond cutting saw (GemastaTM) fitted with a 100 μm wide diamond impregnated blade. The resulting ~1 mm thick sections incorporated the primordium and otolith material laid down across the whole lifetime of each fish. Transverse sections were next mounted on a circular glass disc using wax and polished to expose daily growth increments near to the primordium using 1000× wet and dry sandpaper and 3μm lapping film. Sections were then turned over, mounted permanently on the glass disc using Araldite M, and the procedure repeated on the opposite side of the sample. The resulting thin sections were triple-rinsed in Milli-Q water and air dried overnight in a class 100 laminar flow cabinet, before being mounted on microscope slides with double-sided tape.

Trace element analysis

Otoliths were analysed using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). Elemental measurements were made using a Varian 810 quadrupole ICP-MS, coupled to a HelEx (Laurin Technic, Canberra, and the Australian National University) located at the School of Earth Sciences, The University of Melbourne. The HelEx system is constructed around a Compex 110 (Lambda Physik, Gottingen, Germany) ArF excimer laser (detailed descriptions of the system's performance can be found in Eggins et al. 1998, 2005 and Macdonald et al. 2008). Otolith mounts were placed in the sample cell and the primordium of each otolith was located visually with a 400× objective and a video imaging system. The intended ablation path on each sample was then digitally plotted in using GeoStar v6.14 software (Resonetics, USA).

Each otolith was ablated along a transect from the primordium to the terminal edge of the ventral arm using a 30 μm diameter laser spot. The laser tracked initially along the antisulcul margin to the first inflection, then continued along the ventral arm, distal to the ventral groove, towards the terminal edge (Fig.2). The laser was operated at 90 mJ, pulsed at 10 Hz and scanned at 3 μm.sec-1 across the sample. Dwell time for the analysis was set at 30 milliseconds. Ablation occurred inside a sealed chamber in an

atmosphere of pure He (flow rate, ~0.3 L/min) with the vaporised material transported to the ICP-MS in the Ar carrier gas (flow rate, ~1.23 L/min) via a signal smoothing manifold. Otoliths were analysed for a suite of elements including 7Li, 25Mg, 43Ca, 55Mn, 63Cu, 88Sr, 138Ba and 207Pb, and Ca was used as an internal standard to correct for variation in ablation yield among samples.

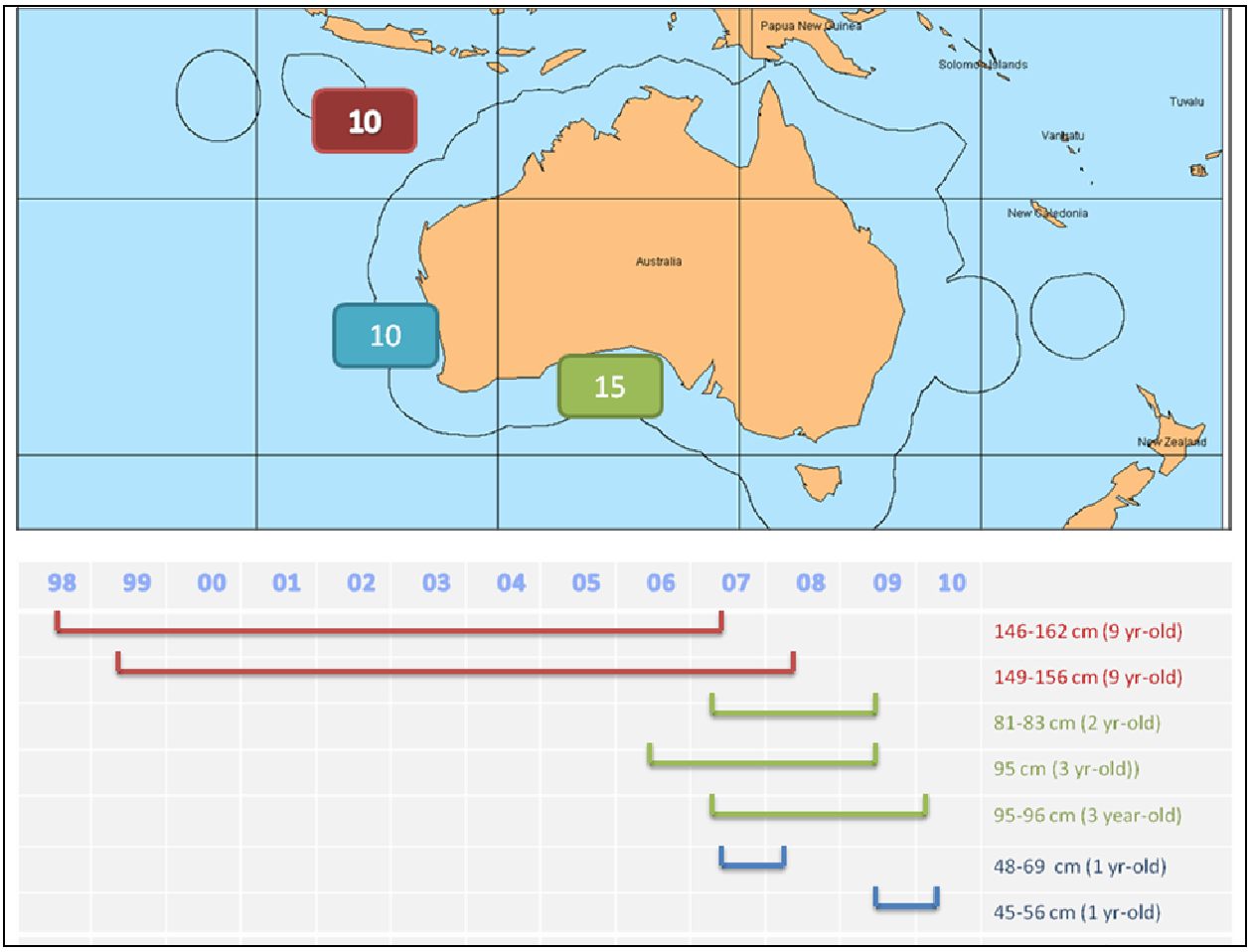

Figure 1. SBT otolith samples chosen for microchemistry analysis from 3 sites, 4 age classes and 5 spawning years. Coloured bars extend from the back-calculated spawning year to year-of-capture.

Data reduction and processing was done offline using the Iolite Version 2.13 (School of Earth Sciences, University of Melbourne) that operates within IGOR Pro Version 6.2.1.0 (WaveMetrics, Inc., Oregon, USA) (see also Woodhead et al. 2007). Subtraction of background ion counts from otolith counts was followed by the normalisation of each element to Ca using an external calibration standard (National Institute of Standards Technology, NIST 612) which was analysed after every 10 otolith samples. Finally, data from each otolith were expressed as element:Ca molar ratios (i.e. Mg:Ca, Sr:Ca). Measurement precision (% relative standard deviation - RSD) was determined based on analyses of MACS-3 (n = 4) reference standards run concurrently with the otolith samples. Mean %RSD across both analysis days for the MACS-3 was Li:Ca 0.41%, Mg:Ca 1.00%, Mn:Ca 0.15%, Cu:Ca 3.50%, Sr:Ca 2.31%, Ba:Ca 0.57%, Pb:Ca 0.30%.

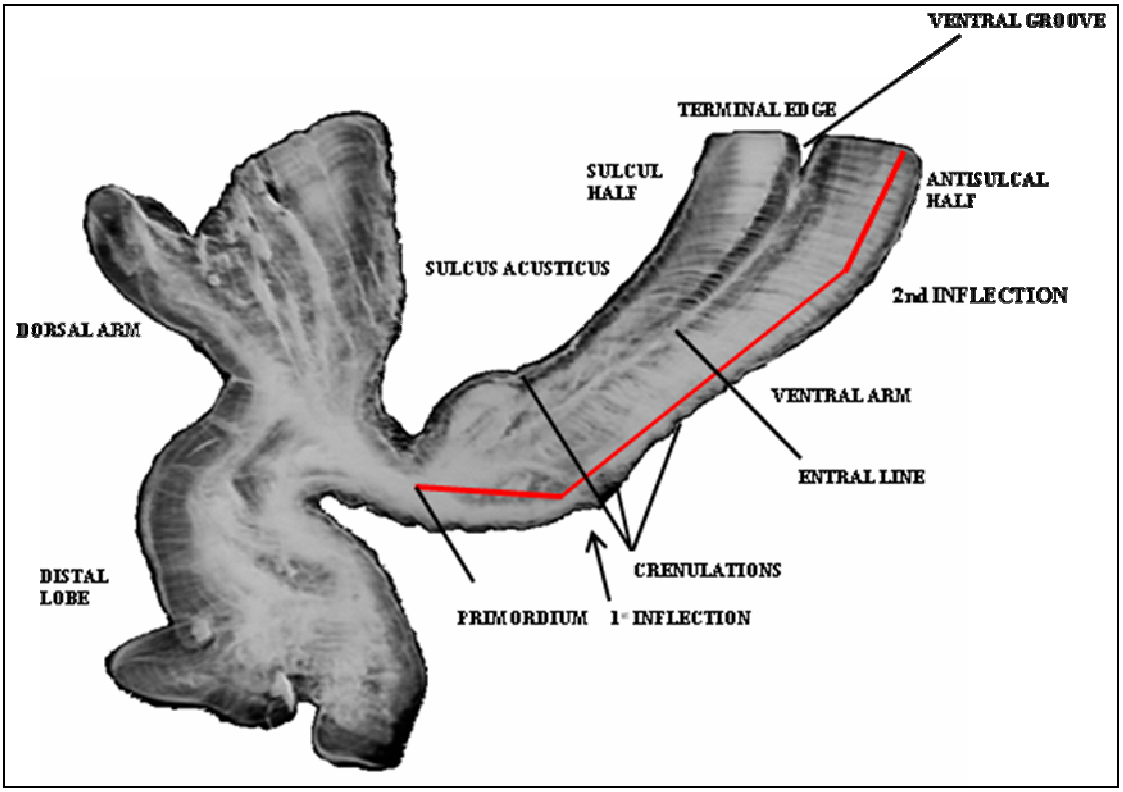

Figure 2. A transverse section of an SBT sagittal otolith showing the LA-ICP-MS path (red) along the ventral arm.

The Iolite program exported data for each analysis into an Excel spreadsheet, in a number of smoothed formats (i.e. unsmoothed data, smoothing over 0.5, 1, 2, 5 and 10 second integrations). The decision of whether to smooth the data or not is in essence driven by the temporal resolution required for a particular analysis. Depending on the question being asked, the ideal result is to expose the 'real' biologically driven trends in the data, while reducing the analytical noise generated by the experimental system. Data for each SBT sample was exported to Excel in unsmoothed and all smoothed forms.

The data was exported in ppm form for each element and converted into molar concentrations (mol) by dividing the ppm value for each element by its atomic weight. Molar concentration data for each element were then converted to molar ratios by expressing each element relative to Ca, and multiplying by 1000 for mmol.mol-1 (e.g. Sr:Ca) or 1000,000 for µmol.mol-1 (e.g. Ba:Ca). The distance that each data point represents along the transect was calculated by reference to the laser scan speed (3 µm.sec-1) and the time count. For each smoothing option, a single data point was integrated over a different time period, and this is reflected in the varying distances represented. The smoothing procedure in effect results in an average concentration for each element derived across several laser pulses. For example, for the 2 second smoothing option with the laser running at 10Hz (i.e. 10 pulses per second) and moved at 3 µm.sec-1, each data point represents otolith material analysed by 20 laser pulses and ~6 µm of distance across the transect.

Age Determination and Data Analysis

Using LA-ICP-MS, otolith composition was analysed along a continuous axis from near the primordium to a point at the otolith edge; the latter providing a site signal from the known area in which the fish were caught. Observations in between were related to age/season (Fig.3) using standard (well established and validated) ageing techniques (Gunn et al. 2008). SBT otoliths, as in most fish, grow incrementally and this is apparent in the sections as an annual pattern of opaque and translucent zones, which are formed due to seasonal changes in growth rate and the amount of organic material incorporated into the otolith matrix. These increments, along with particular surface features, allow a reader to count 'years' and hence assign an age to an individual fish.

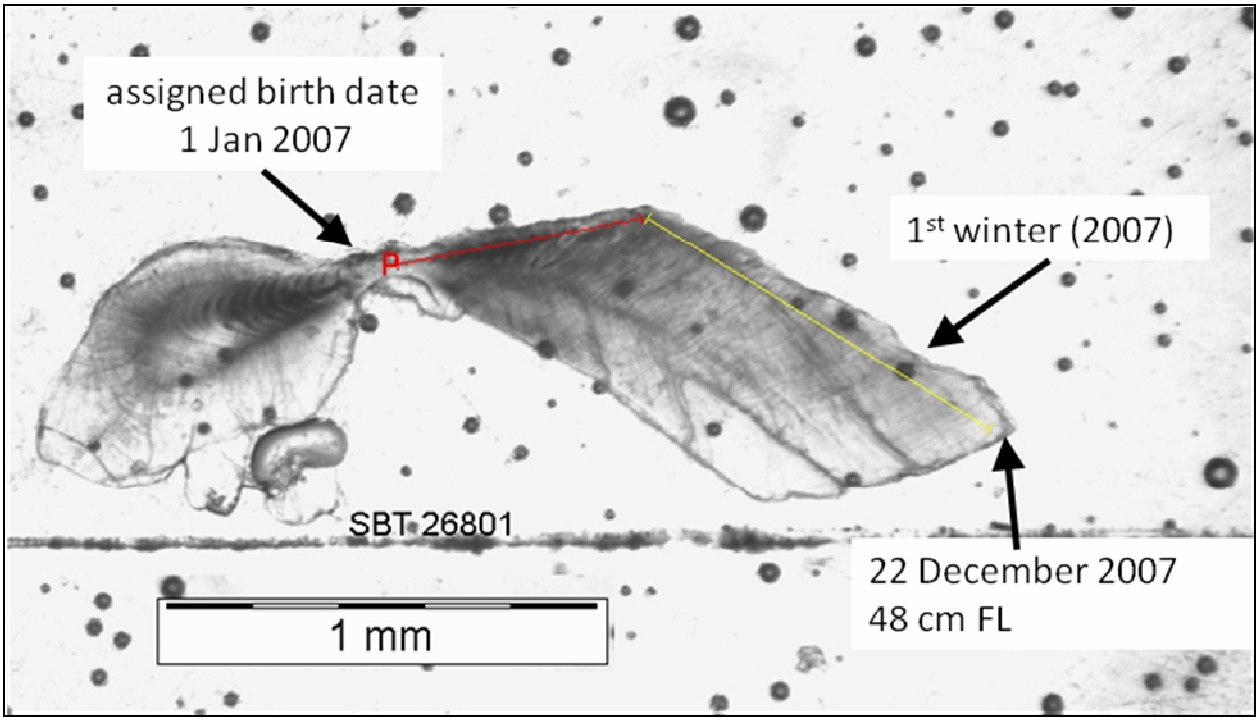

Figure 3. Transverse section of otolith from SBT #26801, caught off the west coast of Australia on 22 December 2007, estimated to be 1-year-old. Shown is the LA-ICP-MS path in red and yellow, and the area of the otolith where the first (winter) seasonal decrease in growth occurred.

Results and Discussion

Otolith removal and preparation

Of the 35 SBT chosen for analysis, 26 were prepared successfully and analysed using ICP-MS. The others were not used because either the primordium or margin was not included in the section, thus we were not able to analyse the complete growth axis. The loss of the primordium or margin in the section was due to either inaccurate sectioning or grinding the section too thin.

Trace element analysis

The elements 7Li, 25Mg, 43Ca, 55Mn, 63Cu, 88Sr, 138Ba and 207Pb were measured in the 26 otoliths (Fig. 4). It appears that Pb and Cu concentrations are very low and right on the limits of detection of the system therefore will be excluded from further analysis. Li was noisy, but above minimum detection limits and hence will not be excluded as it may be a useful as a discriminator among the three areas. Elements were expressed as a ratio to Ca, partly to account for ontogenetic changes as the fish grows.

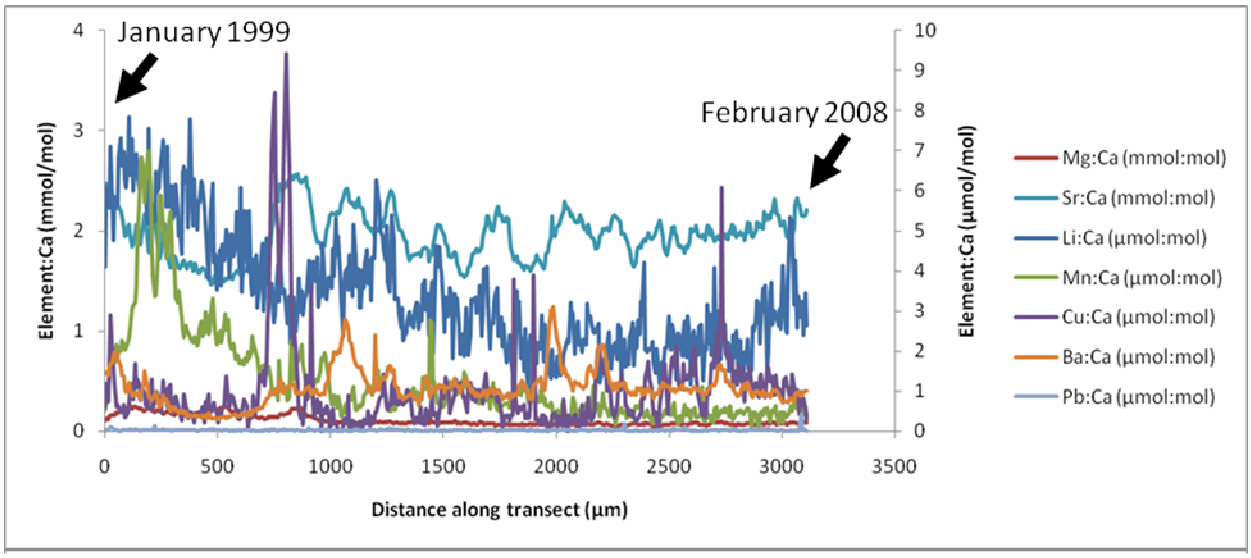

Figure 4. LA-ICP-MS data from SBT #26418, which was caught in February 2008 and estimated to be 9 years of age. Data have been smoothed over a 2 second period. The traces begin at the primordium (left hand side of graph) and cover the entire growth axis deposited during 9 years of life.

Age Determination and Data Analysis

After ICP-MS analysis the otolith sections were examined to relate the element:Ca ratios to periods during the life of the fish by identifying the position of annual increments in the otolith section and matching them with the corresponding position along the LA-ICP-MS transect. The opaque and translucent zones that comprise an annual increment appear as light and dark areas under a microscope and can be seen in Fig. 5. The growth axis from primordium to margin in Fig. 5 corresponds to the x-axis in Fig.4, which shows changes in elemental levels deposited during 9 years, before the was caught on the spawning grounds.

This pilot project has confirmed the feasibility of using LA-ICP-MS on a near-continuous scale on SBT as small as the pre-recruits. The project is still underway and, in the next phase, univariate and multivariate statistical analyses will be used to test for significant differences between areas and between years, in order to differentiate spatial and temporal otolith chemical fingerprints in SBT.

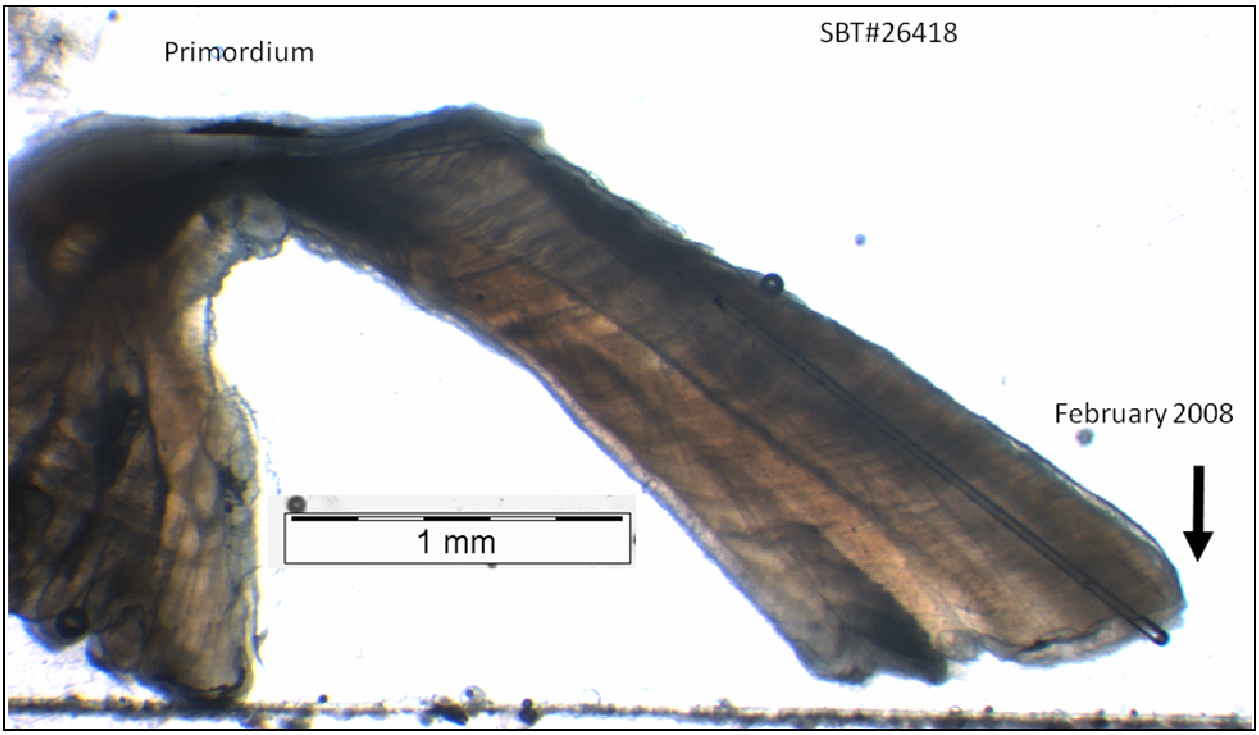

Figure 5. ICP-MS analysis of SBT#26418 was run in two directions, from primordium to 1st inflection, then from 1st inflection to the margin. Scars from the analysis can just be seen in this image.

If the results from this pilot project are successful we aim to use the techniques to provide estimates of the proportion of juveniles resident in the GAB over time, and estimate its variability, indicating whether the current assumption of a constant proportion (in the operating model and management procedure) is appropriate, and if not, provide alternative plausible assumptions. Secondly, results will be valuable for the design of future tagging programs and the interpretation of tagging (mark-recapture) data.

Both these outcomes will directly benefit the SBT assessment and management procedure through improved process understanding and provide an additional data source for estimating spatial distribution over time.

Acknowledgements

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Tables

Table 1. Collection data for all SBT otoliths chosen for LA-ICP-MS analysis.

	CSIRO SBT archive no.	FOP date	Catch Location	LCF (cm)	Age *	back calculated birth date
1	26801	22-Dec-07	west coast Australia	48	1	1-Jan-07
2	26803	12-Jan-08	west coast Australia	51	1	1-Jan-07
3	26806	12-Jan-08	west coast Australia	52	1	1-Jan-07
4	26807	12-Jan-08	west coast Australia	52	1	1-Jan-07
5	26816	12-Jan-08	west coast Australia	69	1 or 2	1-Jan-07
6	30766	09-Jan-10	west coast Australia	56	1	1-Jan-09
7	30770	11-Jan-10	west coast Australia	45	1	1-Jan-09
8	30785	14-Jan-10	west coast Australia	48	1	1-Jan-09
9	30786	14-Jan-10	west coast Australia	53	1	1-Jan-09
10	30789	14-Jan-10	west coast Australia	49	1	1-Jan-09
11	24489	03-Feb-07	spawning ground	150	9	1-Jan-98
12	24564	04-Feb-07	spawning ground	146	9	1-Jan-98
13	24718	11-Feb-07	spawning ground	162	9	1-Jan-98
14	24739	11-Feb-07	spawning ground	153	9	1-Jan-98
15	24763	11-Feb-07	spawning ground	153	9	1-Jan-98
16	26418	02-Feb-08	spawning ground	156	9	1-Jan-99
17	26443	02-Feb-08	spawning ground	152	9	1-Jan-99
18	26446	02-Feb-08	spawning ground	149	9	1-Jan-99
19	26470	02-Feb-08	spawning ground	153	9	1-Jan-99
20	26482	02-Feb-08	spawning ground	153	9	1-Jan-99
21	28908	27-Apr-09	Great Australian Bight	81	2	1-Jan-07
22	28920	27-Apr-09	Great Australian Bight	81	2	1-Jan-07
23	28923	27-Apr-09	Great Australian Bight	83	2	1-Jan-07
24	28924	27-Apr-09	Great Australian Bight	82	2	1-Jan-07
25	29234	12-May-09	Great Australian Bight	83	2	1-Jan-07
26	31318	09-Apr-10	Great Australian Bight	95	3	1-Jan-07
27	31220	29-Mar-10	Great Australian Bight	95	3	1-Jan-07
28	31237	29-Mar-10	Great Australian Bight	96	3	1-Jan-07
29	31251	06-Apr-10	Great Australian Bight	96	3	1-Jan-07
30	31308	09-Apr-10	Great Australian Bight	96	3	1-Jan-07
31	28868	15-Jan-09	Great Australian Bight	95	3	1-Jan-06
32	28951	28-Apr-09	Great Australian Bight	95	3	1-Jan-06
33	29185	06-May-09	Great Australian Bight	95	3	1-Jan-06
34	29188	06-May-09	Great Australian Bight	95	3	1-Jan-06
35	29331	03-Jun-09	Great Australian Bight	95	3	1-Jan-06

*Ages 1-3 years were assigned using age-length keys; the fish aged 9 years were chosen from a group assigned this age using direct ageing, independent from this study.