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Identifying residency signals of SBT using trace elements in otoliths: insights from a pilot study

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Important disclaimer

Acknowledgments

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Contents

Abstract

A long-standing question in SBT spatial dynamics has been the proportion of the population of juvenile SBT that spend summer in the Great Australian Bight (GAB) foraging grounds. Otoliths may be able to provide such information about movements and residency because they act as a natural archive of the environmental conditions experienced over the life history of fish. Here we report on an initial pilot study to determine if it is possible to identify chemical fingerprints from different areas within the SBT range using archived otoliths of SBT. The concentrations of elements (i.e. Ca, Mg, Sr, Li, Mn, Cu, Ba and Pb) were measured by laser ablation inductively coupled spectrometry (LA-ICP-MS) for 25 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. Elements were measured continuously along the growth axis from the earliest-formed primordial area to the margin of the otolith to provide a chronology of elemental ratios. 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 spends summer in the Great Australian Bight (GAB), and whether this proportion is constant over time. This information is central for interpreting the index of relative juvenile abundance in the GAB from the line-transect aerial-survey as an index for the juvenile population of SBT as a whole (Eveson et al., 2010). This index is one of two input series to the CCSBT management procedure (MP) and is also the only fishery-independent relative abundance index in the operating model (OM). The CCSBT-SC (Anon., 2007) 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." The potential to address this question via otolith microchemistry has been identified as a priority activity in the CCSBT Scientific Research Program 2014-18 (Anon., 2013).

SBT otoliths have been collected and archived by CSIRO and others 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; Farley et al., 2014). 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.

Advances in otolith microchemistry have shown that trace element composition can be used to infer tuna movements and 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 pilot project aims to determine if it is possible to differentiate otolith chemical fingerprints of SBT caught from three different locations: the Great Australian Bight (GAB), west coast of Australia and the spawning grounds. If this initial examination indicates there is potential for the technique to address the question of juvenile dynamics, analysis of a larger number of otoliths from a more comprehensive spatial and temporal design will aim to quantify the average fraction of the juvenile population that spends the summer in the GAB and the extent to which this varies systematically over time. If feasible, the results of such a study would be relevant for the interpretation of the aerial survey index as well as the validity of the assumption about the extent of mixing related to the conventional tagging program, and the design of any future tagging programs (Anon., 2013).

Methods

Otolith removal and preparation

Sagittal otoliths from 25 southern bluefin tuna (SBT) collected from three regions off the Australian coast (GAB: n=10, spawning ground: n=7, west coast: n=8) 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 polished on one side 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 polishing procedure repeated on the opposite side of the sample. The resulting thin sections were triple-rinsed in Milli-Q water and air dried overnight before being mounted on microscope slides with double-sided tape.

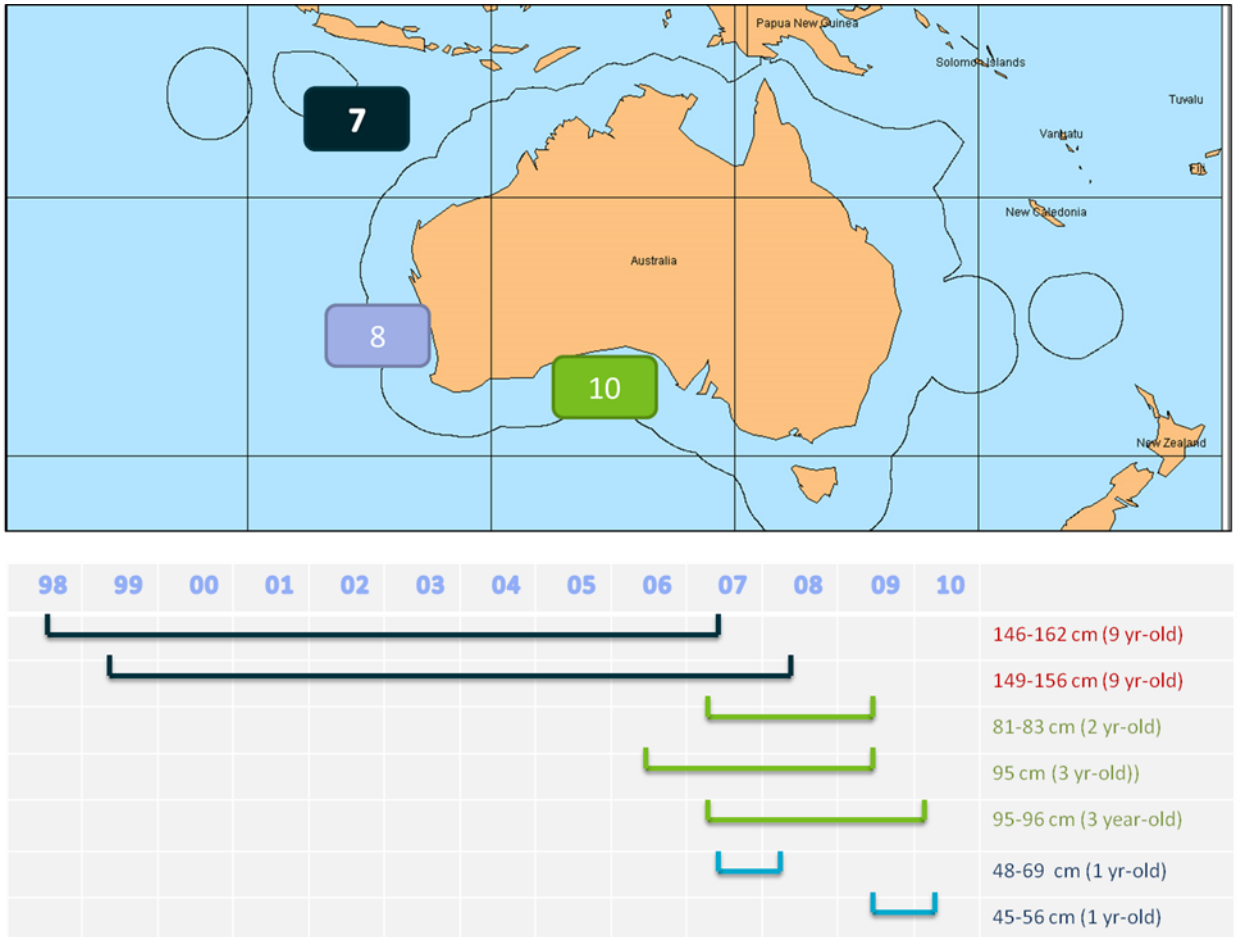

Figure 1. SBT otolith samples chosen for microchemistry analysis from 3 sites, 4 age classes and 5 spawning years.

LA- ICP-MS 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).

Table 1. SBT otoliths analysed by LA-ICP-MS

	CSIRO fish number	catch date	LCF (cm)	catch location	estimated age (yrs)
1	26801	22-Dec-07	48	west coast WA	1
2	26803	12-Jan-08	51	west coast WA	1
3	26806	12-Jan-08	52	west coast WA	1
4	26816	12-Jan-08	69	west coast WA	1
5	30770	11-Jan-10	45	west coast WA	1
6	30785	14-Jan-10	48	west coast WA	1
7	30786	14-Jan-10	53	west coast WA	1
8	30789	14-Jan-10	49	west coast WA	1
9	28908	27-Apr-09	81	GAB	2
10	28923	27-Apr-09	83	GAB	2
11	29234	27-Apr-09	82	GAB	2
12	28934	12-May-09	83	GAB	3
13	29185	6-May-09	95	GAB	3
14	29188	6-May-09	95	GAB	3
15	31237	29-Mar-10	96	GAB	3
16	31251	6-Apr-10	96	GAB	3
17	31308	9-Apr-10	96	GAB	3
18	31318	9-Apr-10	95	GAB	3
19	24489	3-Feb-07	150	spawning ground	9
20	24564	4-Feb-07	146	spawning ground	9
21	24718	11-Feb-07	162	spawning ground	9
22	24763	11-Feb-07	153	spawning ground	9
23	26418	2-Feb-08	156	spawning ground	9
24	26446	2-Feb-08	149	spawning ground	9
25	26470	2-Feb-08	153	spawning ground	9

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 initially tracked along the anti-sulcul 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: 7Li, 25Mg, 43Ca, 55Mn, 63Cu, 88Sr, 138Ba and 207Pb. Ca was used as an internal standard to correct for variation in ablation yield among samples.

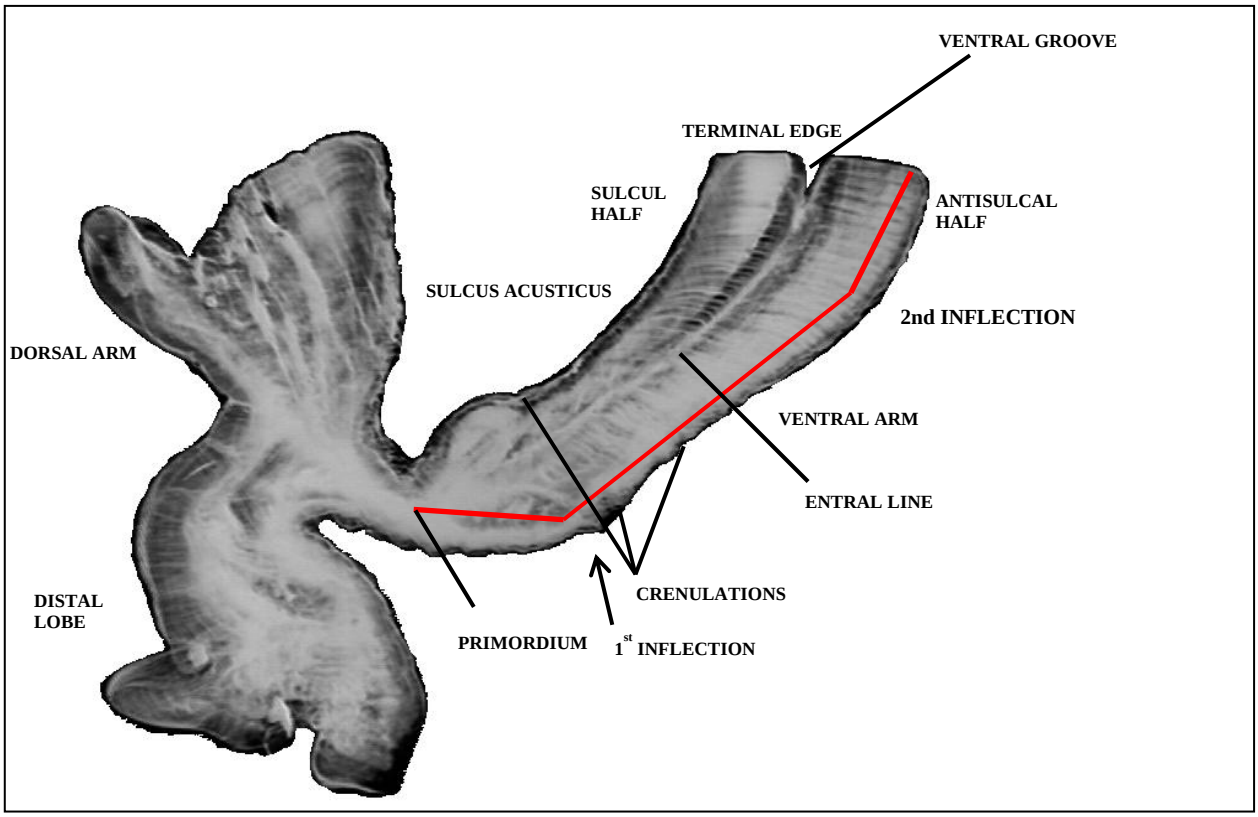

Figure 2. A transverse section of an SBT sagittal otolith showing the LA-ICP-MS path (red) along the ventral arm

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. The 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 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%.

Data for each SBT sample were exported to Excel in a smoothed form. The smoothing integrated elemental concentrations across the number of laser pulses achieved in 2 seconds. With the laser running at 10Hz (i.e. 10 pulses per second) and moving at 3 µm.sec-1, each data point in the 2 second smoothed output represents otolith material analysed by 20 laser pulses and ~6 µm of distance across the transect.

Age Determination

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 of the otolith 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. The position of the increments was identified along the section from primordium to margin.

A back-calculated birth date was assigned to each fish as the 1st of January, assumed to be a mid-point in the spawning season. All fish were caught in the summer/early autumn and the year of the spawning season was calculated by:

Spawning year = catch year - estimated age (years), if catch date was from January to May;

= catch year - estimated age (years) + 1, if catch date was in December.

For example, SBT #26801 was caught in December 2007 and SBT #26803 was caught in January 2008; both were aged 1-year-old and both had an assigned birth of 1st January 2007.

The events that occur in the first year of life of SBT and the corresponding growth of the otolith during the first year of life are of particular interest in this study. To assign time-stamps to these life events, we estimated the number of days from primordium to 1st inflection and 1st increment using results from studies that have attempted to age young SBT using daily increments (Itoh and Tsiji, 1996; Rees et al., 1996; Wang et al., 2009; Shiao et al., 2009).

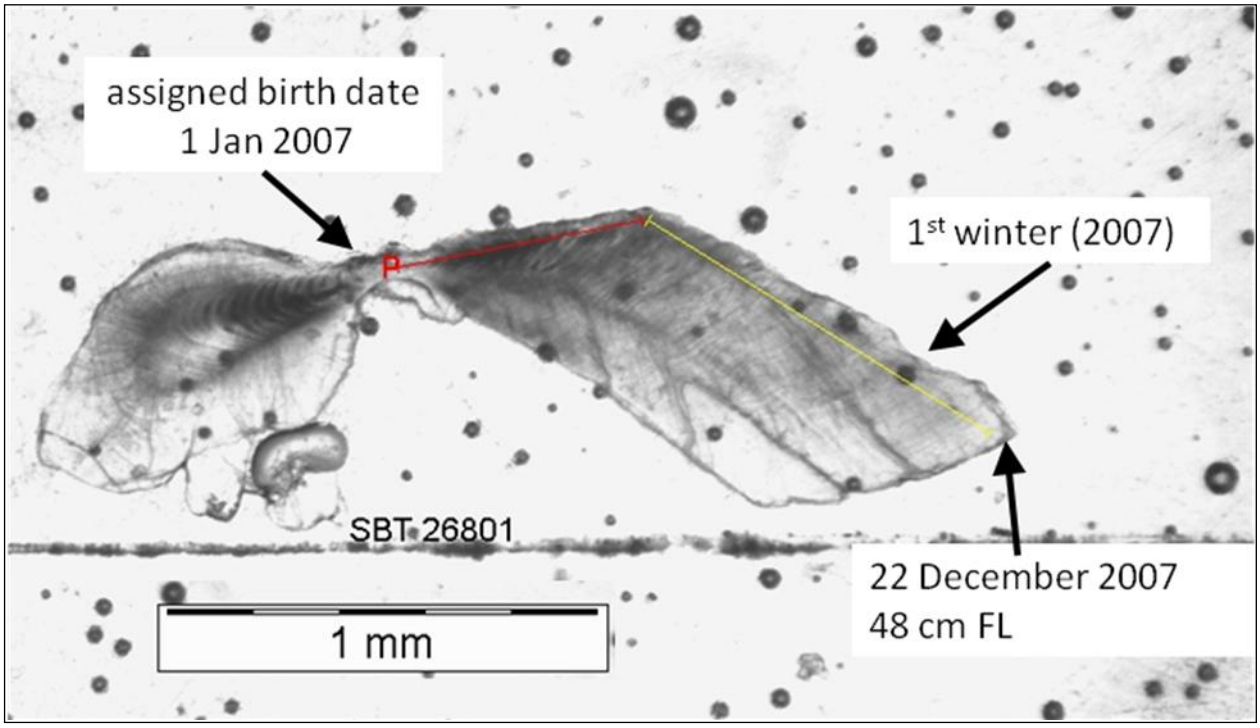

Figure 3. Transverse section of otolith from SBT #26801, caught off the west coast of Australia on 22 December 2007, estimated to be 1-year-old. Shown is the LA-ICP-MS path in red and yellow, and the area of the otolith where the first (winter) seasonal decrease in growth occurred.

Data Analysis

We explored variation among capture locations and years in trace element concentrations at the otolith margin and core. For the margin, we averaged the data for each element over the last 20 microns of the transect, which we estimated to correspond to about the last month of life. We did not use a greater distance because we wanted to ensure that the data corresponded to the location of capture. For the core, we averaged the data between ~10 and 200 microns from the primordium, which we estimate to correspond to about the first 5 weeks of life post-hatch (see below).

To investigate differences among capture locations, we initially used one-way analysis of variance (ANOVA) to consider each element separately. We then considered combinations of elements using multivariate analysis of variance (MANOVA) and linear discriminant function analysis (LDFA). Data for all elements were natural log transformed to better meet the assumptions of normality and homoscedasticity (i.e., equality of variances). We determined which combination of elements optimised discrimination success for the LDFA using an iterative procedure that considered all combinations of up to 3 elements (because the sample size for the spawning ground capture location was only 4, the maximum number of elements that could be considered in the LDFA was 3). Classification success was calculated using jackknife classification (also referred to as leave-one-out cross-validation), and randomisation tests were used to determine if the jackknife classification estimates were significantly different from random (i.e., the jackknife classification success was calculated using a random permutation of the catch locations, and this was repeated 1000 times to determine the null distribution of success) (see White and Ruttenberg, 2007).

Results and Discussion

Trace element analysis

The elements 7Li, 25Mg, 43Ca, 55Mn, 63Cu, 88Sr, 138Ba and 207Pb were measured in the 25 otoliths (Fig. 4) but a full data set is currently available for only 21 of the 25 otoliths due to a complication with the production of the smoothing data sets for those 4 otoliths. The outstanding data will be available soon and will be included in further reporting of this work.

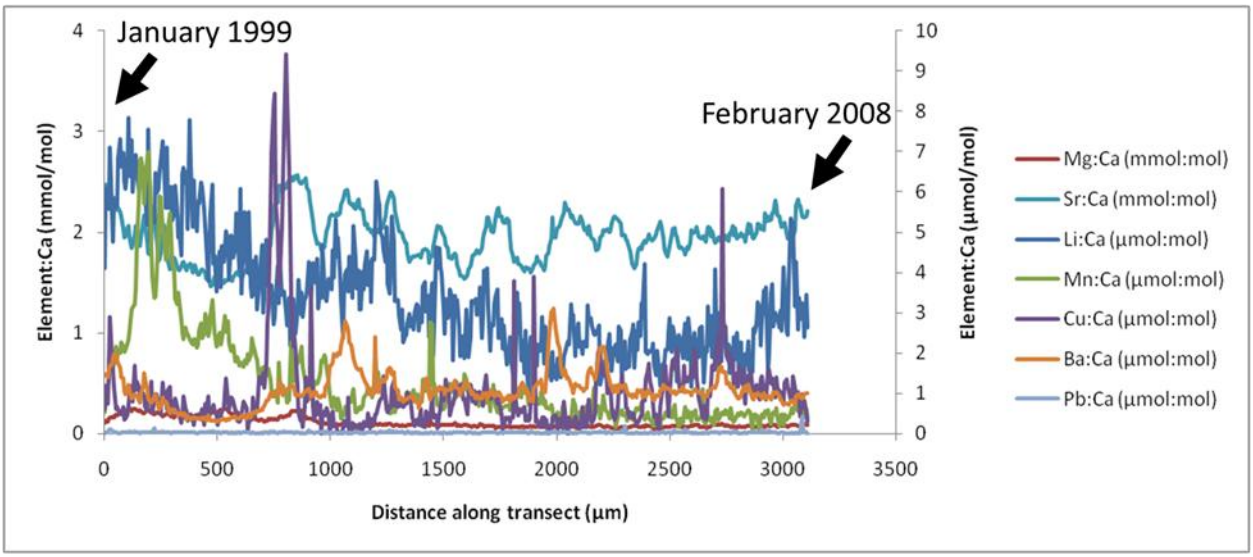

Figure 4. LA-ICP-MS data from SBT #26418, which was caught in February 2008 and estimated to be 9 years of age. Data have been smoothed over a 2 second period. The traces begin at the primordium (left hand side of graph) and cover the entire growth axis deposited during 9 years of life.

Age Determination

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. In the part of the otolith deposited before the first increment, the timing of the larval stage and first inflection was estimated as follows:

0-111 microns = 0-27 days

112-297 microns = 28-43 days

500 microns = 40-50 days, 1st inflection (end of larval stage)

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 fish was caught on the spawning grounds.

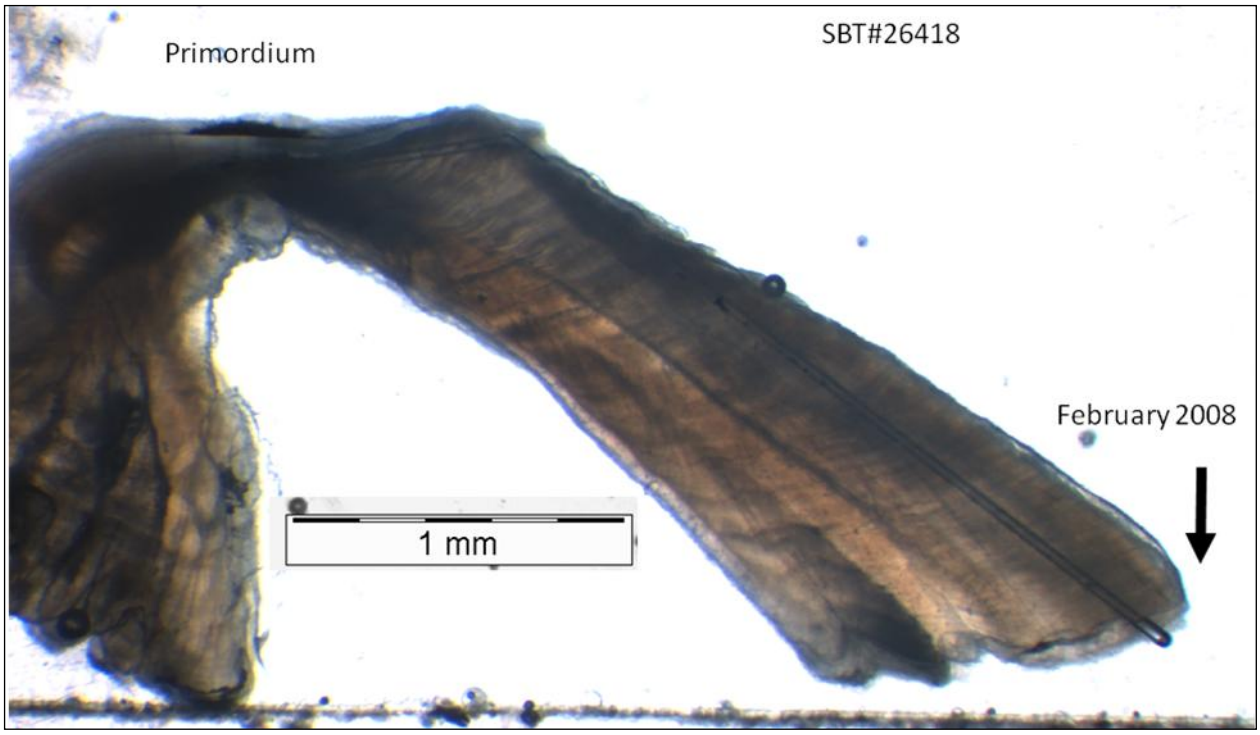

Figure 5. ICP-MS analysis of SBT#26418 was run in two directions, from primordium to 1st inflection, then from 1st inflection to the margin. Scars from the analysis can just be seen in this image.

Data Analysis

Examination of individual elements

Plots of the element:Ca ratios versus distance along the otolith transect (from core to margin) show a lot of variability between otoliths, but also reveal some common patterns (Fig. 6). For example, for all elements except Pb and Cu, the element:Ca ratios show similar trends between 0 and 1000 microns (estimated to correspond to the first 4-5 months of life). In particular, Li:Ca, Mg:Ca and Mn:Ca are dome-shaped (increase to a peak then decline), whereas Sr:Ca and Ba:Ca are U-shaped (decline then increase). Wang et al. (2009), who investigated elemental composition of otoliths for SBT caught in the central Indian Ocean and on the spawning grounds, found similar patterns for Mg:Ca, Mn:Ca and Ba:Ca in this early life stage, but did not observe the same U-shaped pattern for Sr:Ca.

A number of otoliths have large peaks in their Ba:Ca concentrations between ~1000-1500 microns along the transect (Fig. 6). These otoliths all come from fish spawned in the 2006/2007 spawning season (i.e., the 2007 cohort), which we estimate would be experiencing their first late winter/early spring during this time period. Otoliths from other cohorts do not show these same peaks. If we assume all SBT migrate from the spawning ground down the west coast of Australia during their first year of life, then this suggests there can be significant differences in element concentrations between years in roughly the same region of the ocean.

Note that our estimates of age are approximate because we assign the same birth date (January 1st) to all fish regardless of their true birth date and individual growth rates (since these quantities are unknown). This could account in part for the offset in the elemental peaks, e.g. the peaks in the Ba:Ca ratios that correspond to first winter/spring do not all line up at exactly the same distance along the section transect.

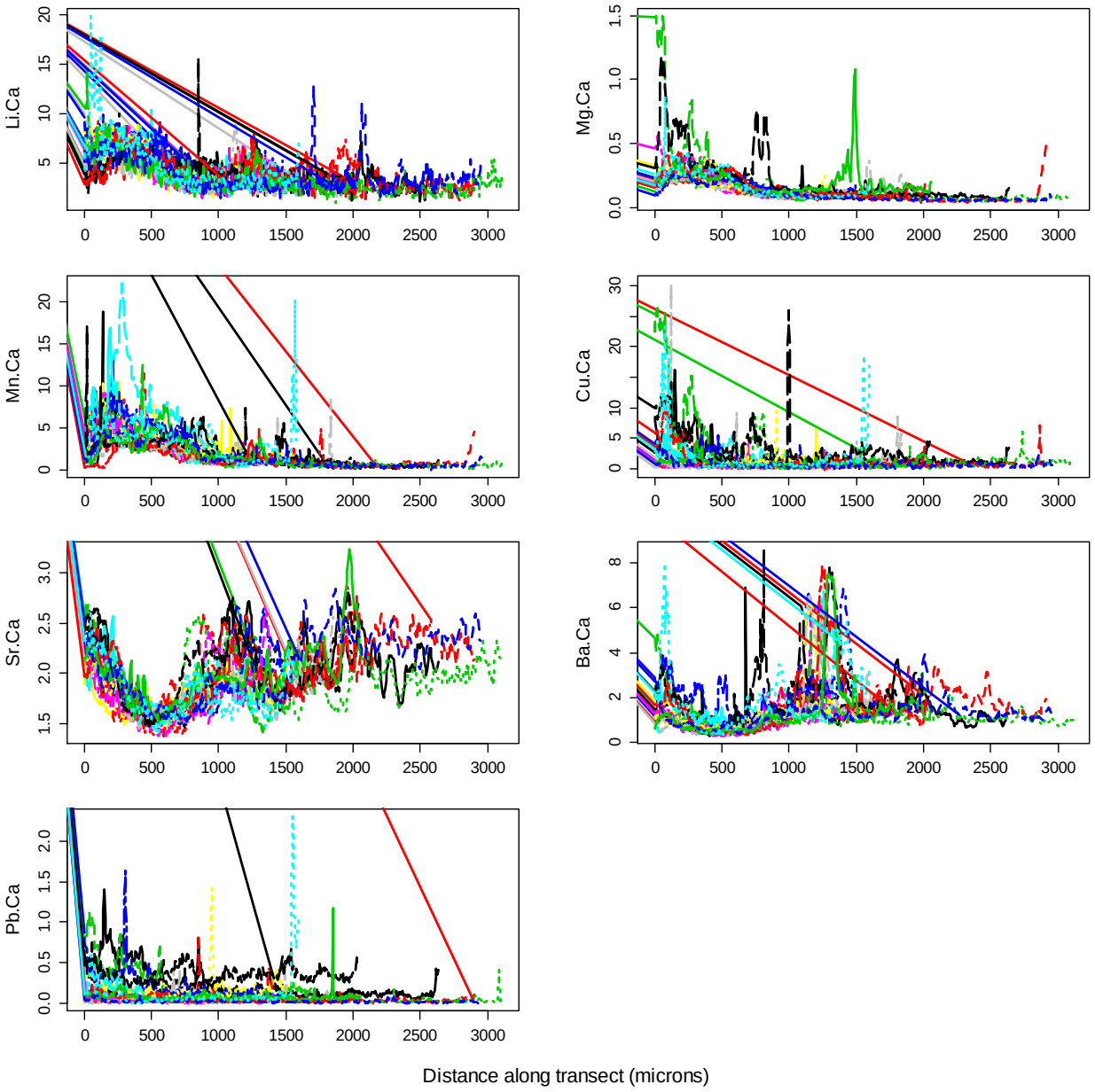

Figure 6. Trace element to calcium ratios measured along transects ablated from the otolith core to the terminal edge along the ventral arm for all otolith samples (each sample represented by a different colour and line type). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

Smooths of the element:Ca transects for fish caught on the spawning grounds at age 9 suggest there are some cyclic patterns over time in several of the elements (Fig. 7), which may correspond to cyclic migration patterns. The data also show that, for many elements, the spawning ground signal is different at the core than at capture (e.g., Mn:Ca concentration is significantly higher at the core than the margin for all spawning ground caught fish). This is investigated further below in the section "Effects of ontogeny, physiology and environment on elemental chemistry".

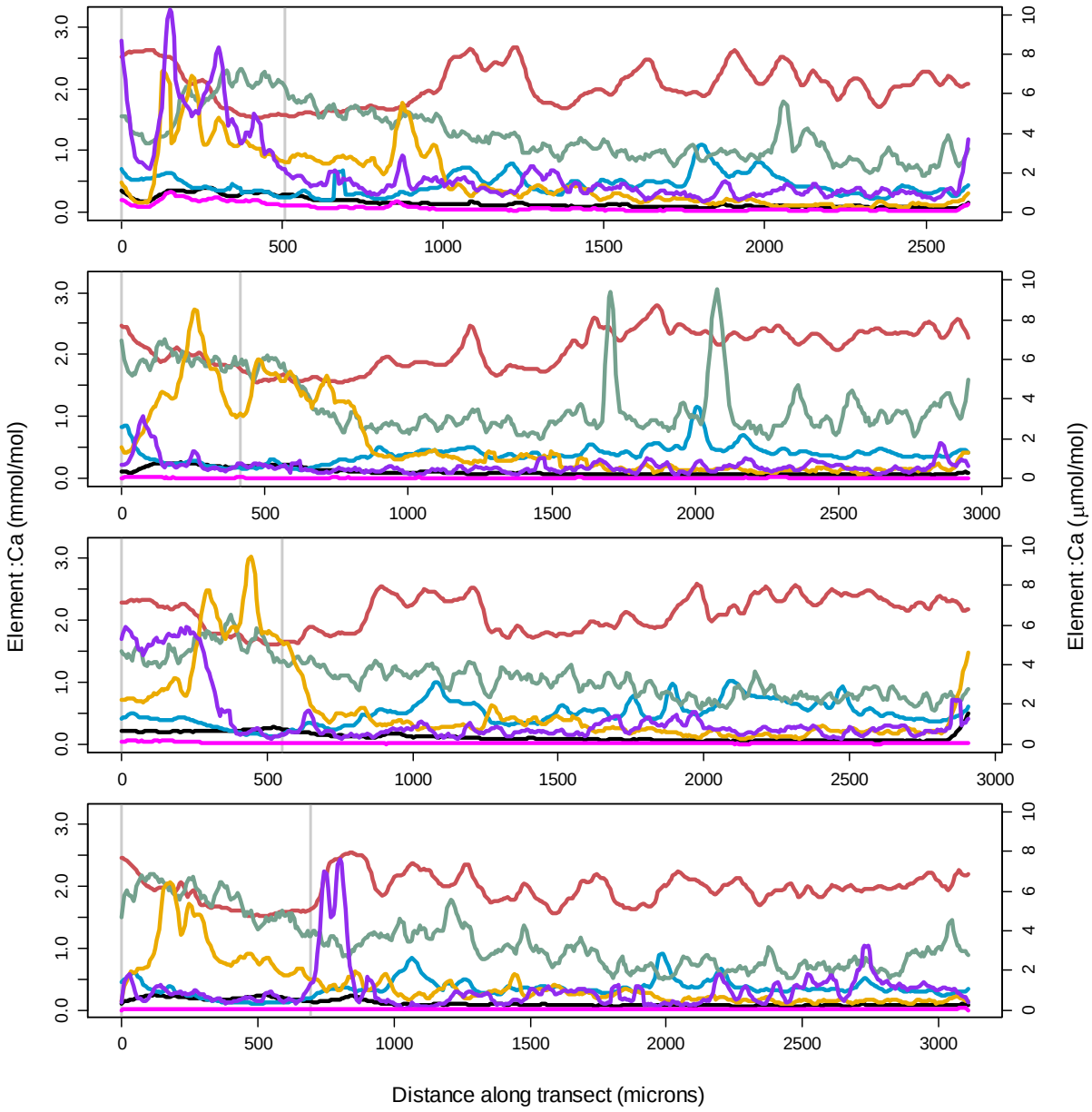

Figure 7. Trace element to calcium ratios measured in otoliths from four fish caught on the spawning ground at age 9 (each panel represents a different fish). Concentrations of Sr:Ca (red) and Mg:Ca (black) are shown in mmol/mol and relate to the left axis, whereas concentrations of Ba:Ca (blue), Li:Ca (green), Mn:Ca (yellow), Cu:Ca (purple), and Pb:Ca (pink) are shown in µmol/mol and relate to the right axis. The grey vertical lines indicate the position of the otolith primordium (left) and first inflection (right).

Results for the otolith margin

Significant differences among capture locations were observed only for Sr:Ca measured at the otolith margin based on univariate ANOVAs (Table 2), with the west coast of Australia (WA) having a lower level than the GAB or spawning grounds (SG) (Fig. 8). Even when considering multiple elements simultaneously, results from the MANOVAs confirmed that using only Sr:Ca discriminated most successfully between capture locations (Pillai's trace of 7.04, approx. F of 4.9 with 2, 18 df; p=0.003). For the LDFA with only Sr:Ca as an explanatory variable, the jackknife classification success rate was 76% (8 correctly classified as GAB and 8 as WA; 4 SG fish incorrectly classified as GAB, and 1 GAB fish incorrectly classified as WA). This success rate is significantly different than the randomized tests, which had a mean success rate over the 1000 iterations of 41%.

It is not surprising that Sr differentiates the three widely-dispersed and varying locations because otolith Sr concentrations are known to closely reflect ambient salinity and temperature (Campana et al., 2000). Otolith chemistry is thought to be determined in part by the environmental conditions experienced by the fish, and in part by physiological processes. Some elements in the otolith, such as Sr and Ba, have strong linear correlations with ambient concentrations whereas others, such as Na, K and S are known to be strongly physiologically regulated. However Campana et al., (2000) noted that if physiologically controlled elements do differ significantly among groups there is no reason to exclude them from the chemical 'fingerprint'.

Table 2. Univariate analyses (ANOVAs) among capture locations in each trace element concentration at the otolith margin. Analyses were performed on log transformed data. Significant differences at the 0.05 significance level are shown in bold.

		df	SS	MS	*F*	*p*
Li:Ca	Location	2	0.22	0.11	2.66	0.10
	Residual	18	0.74	0.04		
Mg:Ca	Location	2	0.38	0.19	1.08	0.36
	Residual	18	3.16	0.18		
Mn:Ca	Location	2	2.30	1.15	2.79	0.09
	Residual	18	7.42	0.41		
Cu:Ca	Location	2	0.03	0.01	0.02	0.98
	Residual	18	11.19	0.62		
Sr:Ca	Location	2	0.15	0.07	9.41	**0.00**
	Residual	18	0.14	0.01		
Ba:Ca	Location	2	0.57	0.28	2.08	0.15
	Residual	18	2.47	0.14		
Pb:Ca	Location	2	2.15	1.08	1.18	0.33
	Residual	18	16.45	0.91		

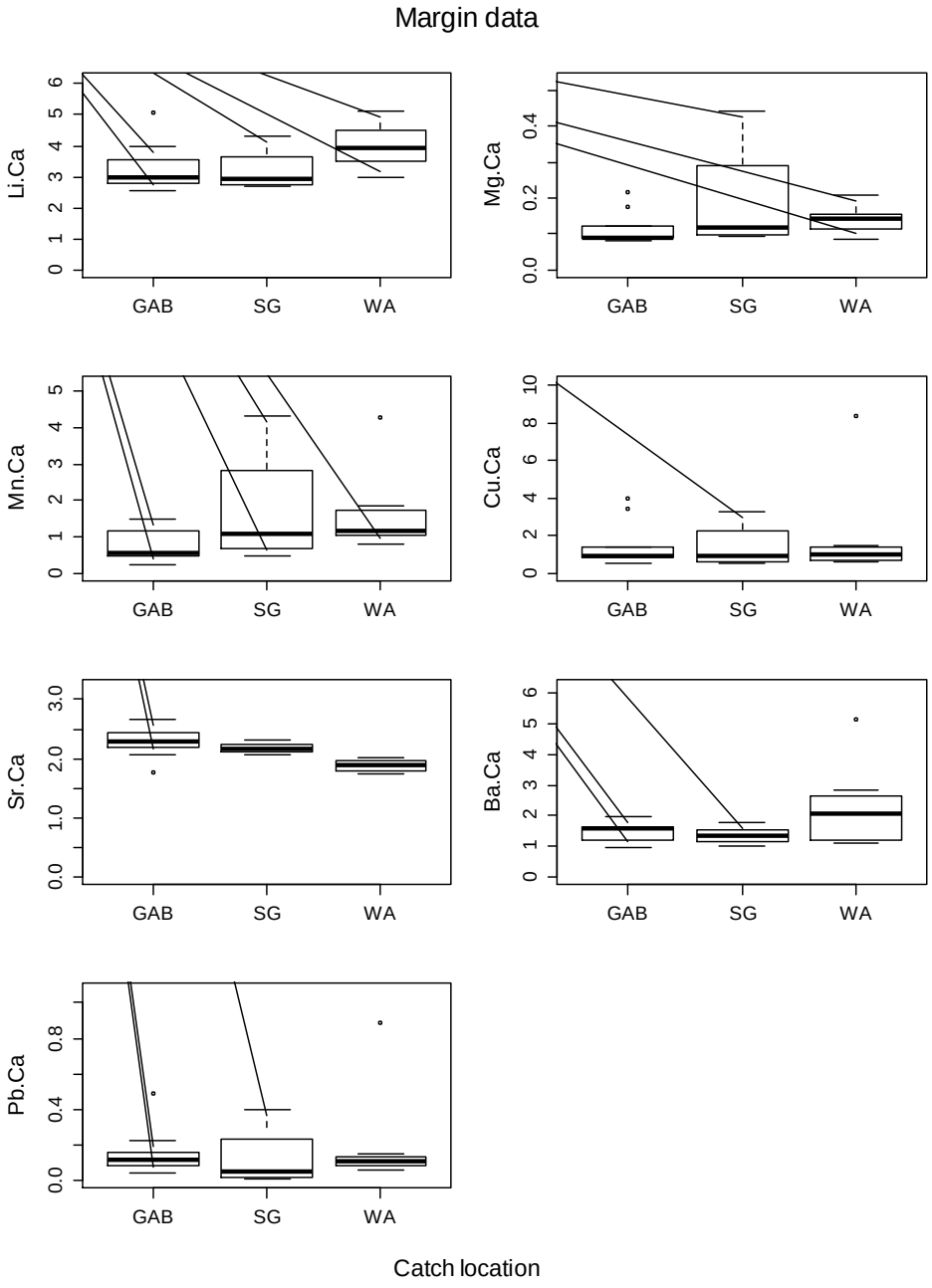

Figure 8. Boxplots comparing trace element to calcium ratios measured at the otolith margin for fish captured from the Great Australian Bight (GAB), spawning grounds (SG) and west coast of Western Australia (WA). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

Differences in elemental concentrations at the margin between years for the same location are difficult to evaluate because of low sample sizes. Nevertheless, as an initial investigation, for the GAB, we compared margin data from the 5 fish captured in 2009 with the 4 fish captured in 2010 (Fig. 9), and for the west coast of Australia (WA), we compared the 4 fish captured in 2008 with 4 fish captured in 2010 (Fig. 10). For the GAB, a significant difference between years exists for Mn:Ca; whereas for WA, a significant difference between years exists only for Ba:Ca. This is consistent with our earlier observation that Ba:Ca differed

between cohorts during their first year of life when we expect them to be moving down the west coast of Australia.

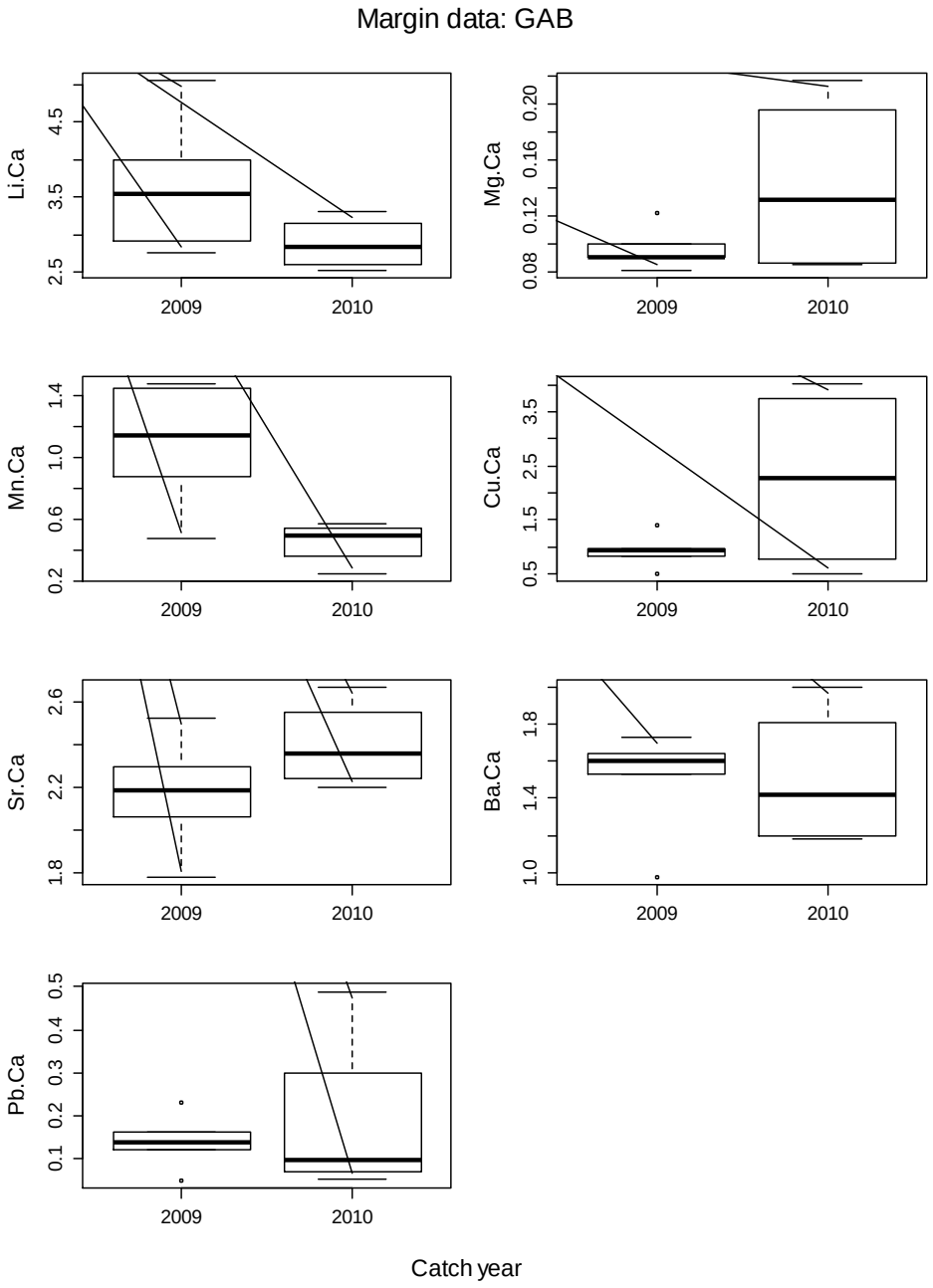

Figure 9. Boxplots comparing trace element to calcium ratios measured at the otolith margin for fish captured from different years from the Great Australian Bight (GAB) (n=5 for 2009, n=4 for 2010). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

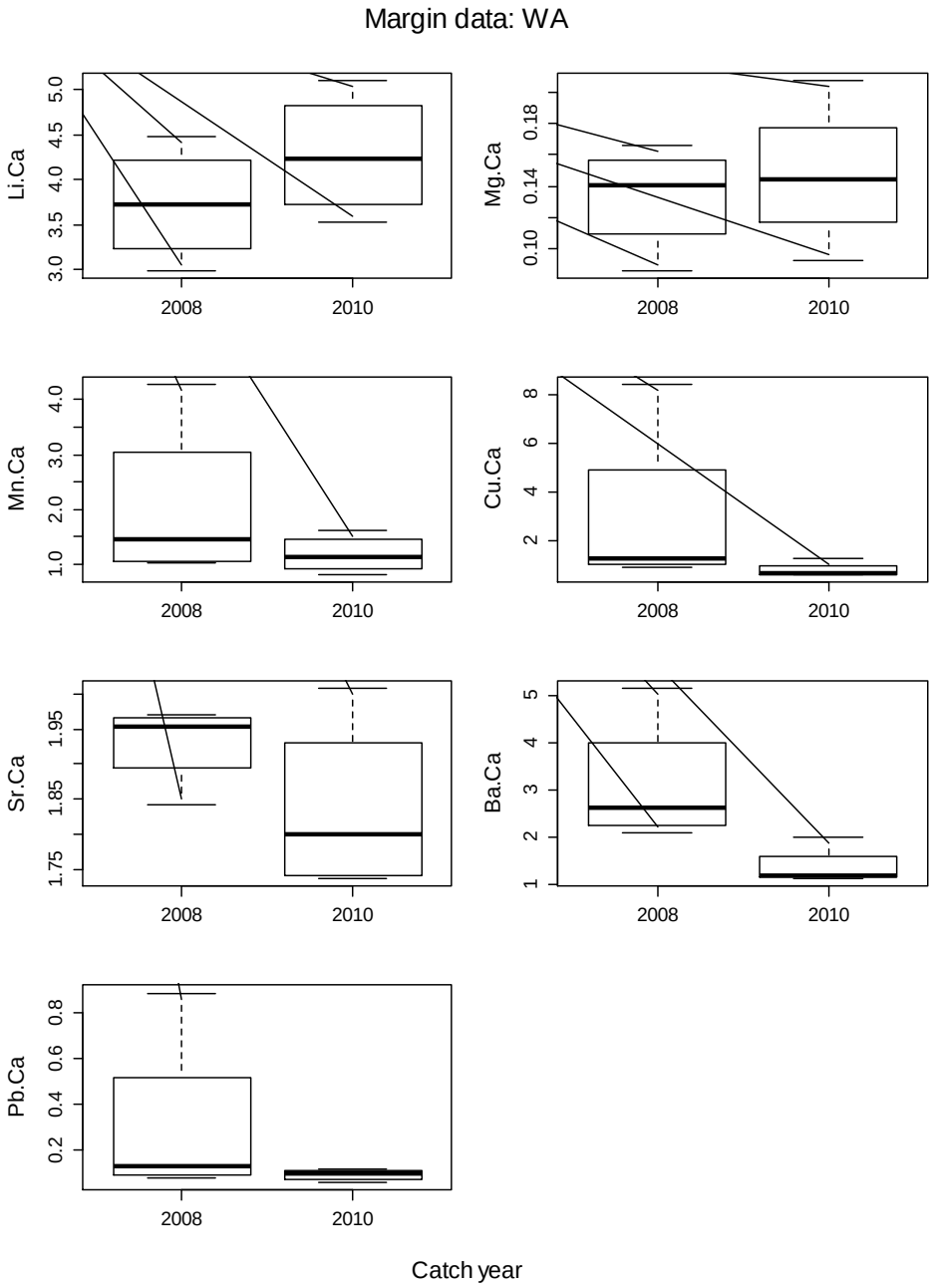

Figure 10. Boxplots comparing trace element to calcium ratios measured at the otolith margin for fish captured in different years off the west coast of Western Australia (WA) (n=4 for 2008, n=4 for 2010). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

Results for the otolith core

Significant differences among capture locations were observed for Li:Ca and Mn:Ca measured at the otolith core based on ANOVAs for each element separately (Table 3), with the west coast of Australia (WA) having a higher level of both elements than the GAB or spawning grounds (SG) (Fig. 11). In terms of the multivariate analyses, results from the MANOVAs suggest that using a combination of Li:Ca, Mn:Ca and Mg:Ca discriminated most successfully between capture locations (Pillai's trace of 0.89, approx. F of 4.3 with 6, 32 df; p=0.003). For the LDFA with these 3 elements as an explanatory variables, the jackknife classification success rate was 70% (4 correctly classified as GAB, 2 as SG, and 8 as WA; 1 GAB fish incorrectly classified as SG, 3 GAB fish incorrectly classified as WA, and 2 SG fish incorrectly classified as

GAB). This success rate is significantly different than the randomized tests, which had a mean success rate over the 1000 iterations of 38%.

We assume that all fish would have been on the spawning grounds during the time their otolith cores developed but we found significant differences in the elemental make-up of their cores based on where they were recaptured several (1-9) years later. Possible explanations are that fish from the different capture locations were spawned in different years, so differences in their otolith cores could be due to differences between years (i.e., spawning year and capture location are confounded). Interannual differences in the oceanography of the spawning grounds occur and these may produce temporal differences in the trace element patterns in the otolith cores of fish caught in different years. However, we also found differences in the cores of some fish estimated to be spawned in the same year, suggesting that smaller-scale spatial and temporal changes on the spawning grounds affect otolith composition. Unfortunately, the sample sizes we currently have are too small once broken down by age, year and location to investigate this further.

Table 3. Univariate analyses (ANOVAs) of differences among capture locations in each trace element concentration at the otolith core. Analyses were performed on log transformed data. Significant differences at the 0.05 significance level are shown in bold.

		df	SS	MS	*F*	*p*
Li:Ca	Location	2	0.22	0.11	2.66	0.10
	Residual	18	0.74	0.04		
Mg:Ca	Location	2	0.38	0.19	1.08	0.36
	Residual	18	3.16	0.18		
Mn:Ca	Location	2	2.30	1.15	2.79	0.09
	Residual	18	7.42	0.41		
Cu:Ca	Location	2	0.03	0.01	0.02	0.98
	Residual	18	11.19	0.62		
Sr:Ca	Location	2	0.15	0.07	9.41	**0.00**
	Residual	18	0.14	0.01		
Ba:Ca	Location	2	0.57	0.28	2.08	0.15
	Residual	18	2.47	0.14		
Pb:Ca	Location	2	2.15	1.08	1.18	0.33
	Residual	18	16.45	0.91		

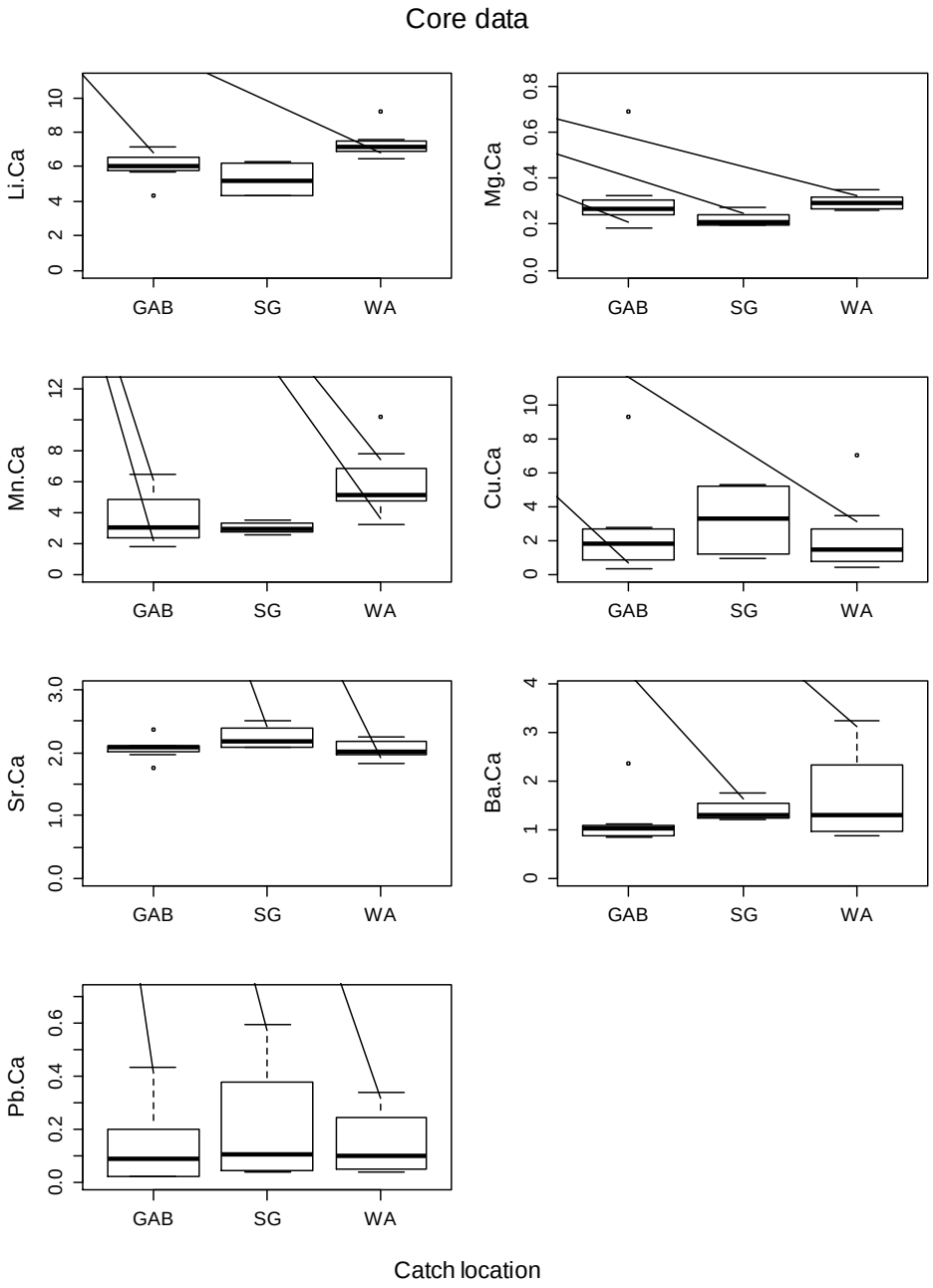

Figure 11. Boxplots comparing trace element to calcium ratios measured at the otolith core for fish captured from the Great Australian Bight (GAB), spawning grounds (SG) and west coast of Western Australia (WA). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

Effects of ontogeny and environment on elemental chemistry

Location is one factor that can potentially affect the otolith chemistry of fish if the concentration of elements differs between locations. However, ontogeny (age) and may also affect otolith chemistry. We see evidence of this when we compare the element:Ca concentrations at the core from all fish, which corresponds to when they were larval fish on the spawning grounds, with the element:Ca concentrations at the margin from fish caught on the spawning grounds at age 9 (Fig. 12). Even though both the core and margin data come from the spawning grounds, there are significant differences in Li:Ca and Mn:Ca concentrations. These differences are too large and consistent to be attributed to year effects. Some caution is needed in interpreting the results of elements such as Mn that are under strong physiological

regulation, where physiology may outweigh environmental influences (Miller, 2009; Sturrock et al., 2014). Despite the fact that they reflect the ambient water chemistry less than other elements, they may still contribute to a unique chemical fingerprint for different groups of fish. A comparison of element:Ca ratios in otoliths from adult fish of the same age caught in different areas, such as the central Indian Ocean and New Zealand, could address such questions .

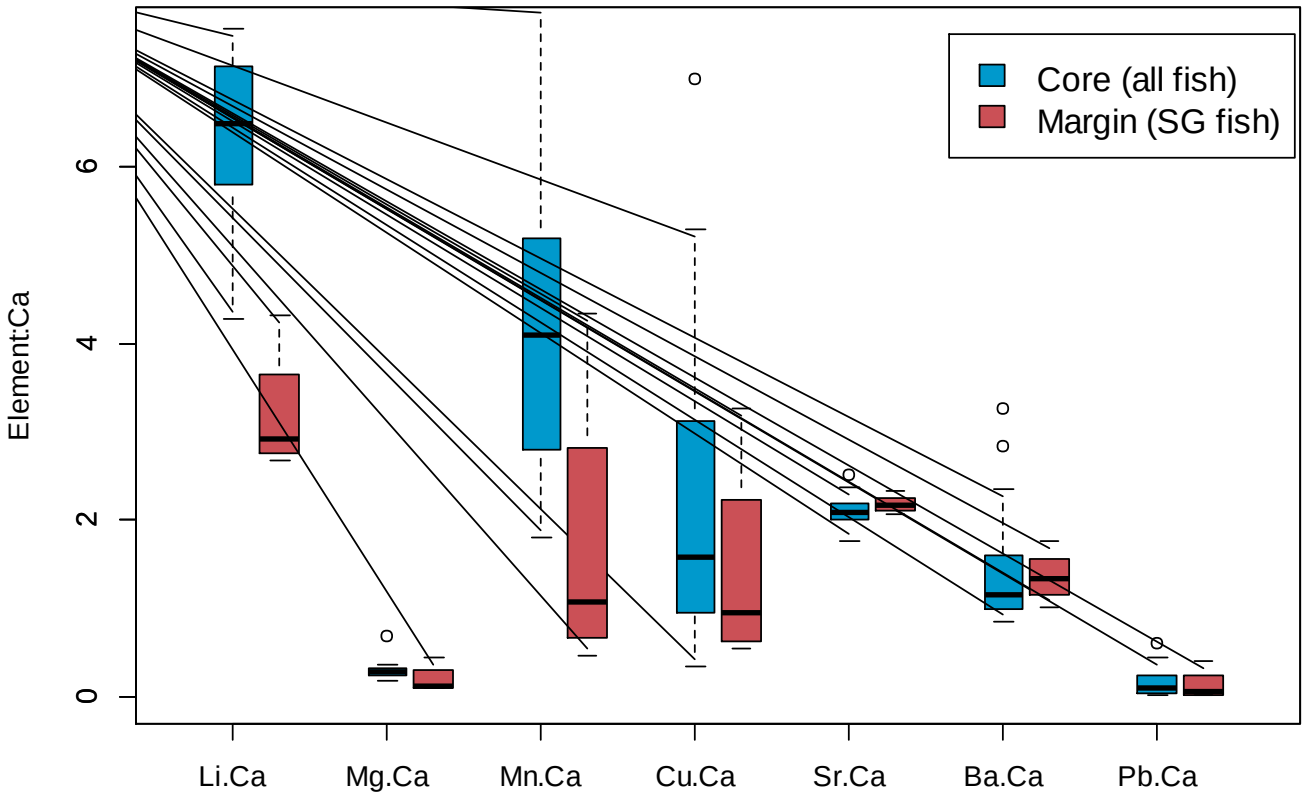

Figure 12. Boxplots comparing trace element to calcium ratios measured at the otolith core for all fish (assumed to be formed on the spawning grounds) versus the otolith margin for fish captured on the spawning grounds (SG). Concentrations of Sr:Ca and Mg:Ca are shown in mmol/mol, whereas concentrations of Li:Ca, Mn:Ca, Cu:Ca, Ba:Ca and Pb:Ca are shown in µmol/mol.

Conclusions / Summary

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. Although the element to calcium ratios along the otolith transect (from core to margin) showed a lot of variability between otoliths, they also revealed some common patterns, especially during the first 4-5 months of life. Moreover, for fish caught on the spawning grounds at age 9, cyclic patterns were apparent in several of the element:Ca ratios over time, which may correspond to cyclic migration patterns.

Analyses of element:Ca ratios at the otolith margin showed significant differences in Sr:Ca concentration among capture locations, with fish caught off the west coast of Australia having a lower level than the GAB or spawning ground caught fish. Using linear discriminant function analysis, we were able to classify the capture location of fish based on Sr:Ca with a 76% success rate. Sr concentrations are known to closely reflect ambient salinity and temperature and so indicate that the three locations: GAB, west coast of Australia and spawning grounds have different physical attributes that is reflected in otolith chemistry.

We also found significant differences among capture locations in Li:Ca and Mn:Ca concentrations at the otolith core, with fish caught off the west coast of Australia having higher levels of both elements than the GAB or spawning ground caught fish. Presumably all fish would have been on the spawning grounds during the time their otolith cores were developed; therefore significant differences in the elemental make-up of their otolith cores based on where they were recaptured several years later were not expected. Differences between spawning years and ages of fish may be contributing to these differences, but current sample sizes are too small once broken down by age, year and location to address this issue.

Lastly, a comparison between element:Ca concentrations at the core from all fish, which corresponds to larval stage on the spawning grounds, with the element:Ca concentrations at the margin from fish caught on the spawning grounds showed significant differences in Li:Ca and Mn:Ca concentrations, which suggests ontogenetic effects.

In summary, the results from this pilot study suggest it is possible to differentiate otolith chemical fingerprints of juvenile SBT caught in the Great Australian Bight (GAB) from those caught on the west coast of Australia. However, there is also evidence of age and year effects that may be confounding difference between locations. These issues could be addressed with otolith chemistry traces from a larger sample of fish specifically selected for this purpose (e.g., with a sufficient overlap between years, ages and locations); for more details refer to Davies et al. (2014) [CCSBT-ESC/1409/27].

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