

BA/CA RATIOS IN OTOLITHS OF SOUTHERN BLUEFIN TUNA (*THUNNUS MACCOYII*) AS A BIOLOGICAL TRACER OF UPWELLING IN THE GREAT AUSTRALIAN BIGHT

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Key words: Southern bluefin tuna; otolith elemental composition; migratory environmental history; upwelling.

ABSTRACT

The southern bluefin tuna (*Thunnus maccoyii*) is a long-lived, large, and highly migratory marine fish in the Indian Ocean. They can live up to 40 years and migrate to the Great Australian Bight in the summer when 1-4 years old. The Great Australian Bight is characterized as the most productive coastal upwelling zone in southern Australia and is the largest area of cool-water carbonate sedimentation in the world. The barium (Ba) level is poor in the open ocean but rich in the upwelling area. This study used otolith Ba/Ca ratios as a natural tag to confirm that southern bluefin tuna seasonally occupy the upwelling area. Southern bluefin tuna were collected from the central Indian Ocean and the spawning ground between the island of Java, Indonesia and northwestern Australia. The temporal variation of trace elements in otoliths of the specimen was measured by laser ablation inductively coupled plasma mass spectrometry. Otolith Ba/Ca ratios were significantly elevated in the summer growth zone, which corresponds to the upwelling season when juvenile tuna enter the Great Australian Bight at the age of 1-4 years old. Although almost all of the mature southern bluefin tuna collected in the spawning ground had previously migrated to the Great Australian Bight upwelling area as juveniles, some fish collected from the Central Indian Ocean didn't migrate to the Great Australian Bight upwelling area, perhaps because they are a vagrant population and may contribute less to the spawning stock.

I. INTRODUCTION

The southern bluefin tuna (SBT, *Thunnus maccoyii*) is a long-lived, large, and highly migratory marine fish. They can live for up to 40 years and grow up to 2 m in length and over 200 kg in weight [18]. The tuna is mainly distributed between 30°-50°S latitude throughout three Oceans in the southern hemisphere [8]. Its only spawning ground is located around 7°-20°S latitude and 100-125°E longitude between the island of Java, Indonesia and northwestern Australia [8]. The SBT spawns during the austral summer between September and March [43]. After hatching, the post-larval fishes disperse southwards along the western coast of Australia with the tropical Leeuwin Current to the inshore waters between Perth and Esperance [8]. The SBT arrives in waters of southern Western Australia during their juvenile stage, and they migrate east along the southern coast towards nursery grounds in the Great Australian Bight (GAB). The migration of juvenile SBT in the GAB was confirmed by tracking with archival tags [21]. They migrate to the east or west of Australia within the latitudes of 30-50°S during autumn, and return to the GAB in the spring before 4 years old [19]. The GAB, the largest area of cool-water carbonate sedimentation in the world, is characterized as the most productive coastal upwelling zone in southern Australia [26]. In addition, the GAB plays an important role in primary production for Australian fisheries. After 5 years old, almost all SBT have recruited to the adult stock in the open ocean. They then circumglobally disperse to temperate feeding grounds between 30°S and 50°S [8]. After maturation at 8-12 years old, the tuna only migrates between their tropical spawning ground in the tropical zone and feeding grounds in the temperate zone [20].

Otoliths function for the hearing and balance of the fish. They are mainly composed of calcium carbonate with a minor organic matrix and a few trace elements. The otoliths are deposited in a daily and annual schedule that is synchronized with the photoperiod and seasonal temperature variation. As such, it yields recognized growth checks, which are daily growth increments or annuli in the otolith. This allows fish age determination at daily or annual cycles. At least 31 elements have been found to be deposited in the otolith during the growth of fish [7]. Otolith growth increments are metabolically inert once deposited and become a permanent record of fish life history. The otolith trace element such as barium (Ba) is taken up from

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the ambient water and is positively correlated to ambient water concentrations [3]. The barium in estuaries and bays mostly originates from freshwater but both coastal and oceanic upwelling areas are rich with barium. An increased barium concentration in fish otoliths might be a signature of oceanic upwelling [3, 9]. Thus, it should be possible to determine whether fish have encountered an upwelling area by examining the temporal change of the barium concentration in their otoliths.

The archival tag has been used to track the population structure and migration of juvenile SBT in the GAB [21] and northern bluefin tunas in the Atlantic and Pacific Oceans [4, 25]. The population structure, migratory environmental history and physiology of the SBT has also been studied by analyzing the otolith elemental composition by EPMA (electron probe microanalyzer) [35] and by laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) [44]. However, knowledge of the migratory behavior and habitat use of the SBT in association with its environment remains fragmented. And few studies have explored the otolith elemental signature of the tuna in relation to oceanic upwelling [39].

This study tested whether the Ba in otoliths is a reliable natural tag to explore the migration of the SBT to upwelling areas. Laboratory and field studies of otolith Ba/Ca ratios in marine fish in relation to fish migration to an upwelling area have shown a positive relationship between the Ba/Ca concentration in the ambient water and otolith. Thus, we analyzed the temporal change of Ba/Ca ratios in the otolith of SBT by LA-ICPMS, and examined the life history relationship between the migration behavior of SBT and the variation in otolith microchemistry.

II. MATERIAL AND METHODS

1. Specimen collection

The SBT was collected from two different habitats - their feeding ground in the central Indian Ocean (CIO) and their spawning ground in the waters between southern Java and northwestern Australia (SPAWN). Otolith trace elements were analyzed by ICPMS for 14 SBT from the CIO and for 15 SBT from the SPAWN (Fig. 1). The mean length, weight, and age of the specimens are shown in Table 1. Otoliths of SBT from the CIO were removed immediately after capture in the fishing boat by the observer with a battery powered hole-saw drill, while those from the SPAWN were removed in the fish market 1 to 7 days after capture. After collection, the otolith specimens were cleaned with de-ionized water, air-dried, and then stored in Eppendorf microcentrifuge tubes for age determination and otolith microchemistry analysis.

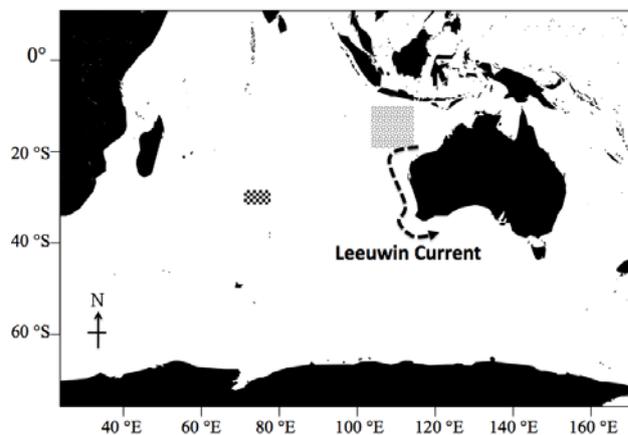


Fig. 1. Sampling sites of southern bluefin tuna in the central Indian Ocean (CIO, 31-33°S, 71-77°E, black area) and the spawning ground south of Java (SPAWN, 10-20°S 110-120°E, grey area) [8].

2. Otolith preparation for microchemistry analyses by LA-ICPMS

For the analysis of trace elements, each otolith was cleaned with 5% H₂O₂ to remove all remaining organic material from the otolith surface, ultrasonically cleaned with de-ionized water, oven-dried overnight at 60°C, and embedded in epofix resin and transversely sectioned with a low speed saw (Isomet, Buehler) into slices approximately 300 μm thick with the primordium in the middle. The sectioned otolith was ground with 2000 grit sandpaper followed by 2400 grit carborundum until the primordium appeared. Finally, the otolith sections were polished with 0.05 μm alumina paste to smooth the surface.

The elements in the otoliths were measured from the primordium to the edge of the long arm (Fig. 2) by a high resolution LA-ICPMS (Finnigan MAT ELEMENT 2, Thermo Electron corp., Bremen, Germany) connected with a Merchantek LUV 266™ Nd: YAG UV laser microprobe (New Wave Research, Inc). The laser was pulsed at a repetition rate of 20 Hz, at a scan speed of 15 μm·sec⁻¹ with an ablation transect diameter of approximately 150 μm. The elements, ²³Na, ²⁴Mg, ⁴⁴Ca, ⁵⁵Mn, ⁸⁸Sr, and ¹³⁸Ba, were measured because they always remain at least 10 times higher than the background levels. The setting took about 2.46 sec to produce one data point, which represented approximately 37 μm on the otolith transect. Standards (NIST 612) were collected before each series, with each series comprising 2-3 otoliths. At the start of each otolith analysis, background counts were collected for 30 seconds, and the average was then subtracted from the sample counts to correct for the background level. The ablation chamber was purged for 60 seconds after sampling each otolith. All measurement data were expressed as ratios of element to Ca concentrations (ppm·ppm⁻¹) by estimating the relative response

Table 1. Mean (± SD) and range of fork length, body weight, and age of southern bluefin tuna used for otolith trace element analysis, which were collected from the central Indian Ocean (CIO) and the spawning ground South of Java (SPAWN). (n: sample size).

Sampling sites	Sampling Date	n	Fork length (cm)	Body weight (kg)	Age (yrs)
CIO (31-33°S, 71-77°E)	14 July - 3 August 2004	14	122.7 (± 22.9) 91 - 170	34.1 (± 21.4) 12 - 79	10.1 (± 7.7) 4 - 27
SPAWN (10-20°S, 110-120°E)	1 Jan & 3 Feb 2005	15	172.9 (± 8.6) 160 - 189	98.9 (± 20.6) 75 - 137	16.3 (± 3.2) 13 - 25

factor of the instrument to the known concentration in the standard (NIST 612).

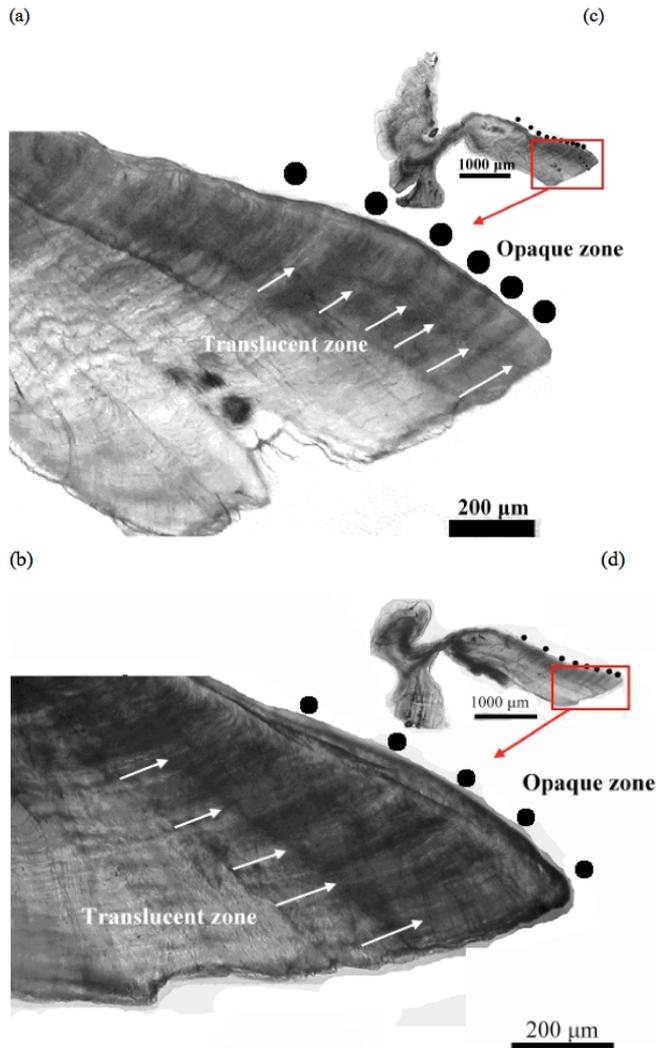


Fig. 3. Comparison between sampling seasons of the marginal increment from the last annulus (opaque zone) to the edge of southern bluefin tuna otoliths. (a) Collected in southern Java in February 2005 (austral summer), FL = 165 cm; (b) collected from the CIO in July 2004 (austral winter), FL = 151 cm. Solid circles: annuli (opaque zones). (a) and (b) are magnified from (c) and (d).

3. Otolith annulus and microstructure examination

The annulus (opaque zone) on the polished otolith sections of SBT was identified from a photograph taken under transmitted light with a compound microscope equipped with a digital camera. The SBT otolith annulus consists of a dark opaque zone and a light translucent zone under transmitted light (Fig. 2). The age of the tuna was determined by counting the number of annuli along the ventral arm, following the manual for age determination of southern bluefin tuna *Thunnus maccoyii* [1]. The terminology describing the otolith used in this study followed Rees et al. [38]. Respectively, the first and second inflection points in the otolith were identified to examine the temporal change in the otolith elemental signature

during the early life stage of the fish and during maturation.

4. Data analyses

The general spatio-temporal variation in otoliths of the six elements examined (Na, Mg, Ca, Mn, Sr, and Ba) was described by Wang et al. [44]. The spatial variation of barium in otoliths has now been demonstrated for a variety of species [12, 23]. A positive relationship exists for barium between the otolith and ambient water, where the water temperature and salinity do not vary greatly [31]. Ambient data for barium can be used to predict the spatial and temporal variation of barium in otoliths. Likewise, barium concentrations in the otolith can potentially be used to reconstruct histories of the ambient water mass [22]. In this study, we focused on the timing of the peak Ba/Ca ratios in the otolith in relation to fish age and the relative position of the annulus to understand the timing of SBT recruitment to the upwelling area of the GAB. The criterion in the judgement of the otolith Ba/Ca ratio as a signal of upwelling for the tuna was determined from the average otolith Ba/Ca ratio (3.7×10^{-6}) of 14 marine species reviewed by Campana [7] and that of other marine fish species (less than 4.0×10^{-6}) [16, 22, 41]. Otolith Ba/Ca ratios greater than 4.0×10^{-6} were identified as a signal of upwelling. The age and season when the tuna migrated to the upwelling area was determined from the position of the peak Ba/Ca ratio and the annulus of the otolith. The patterns of migration to the upwelling area were classified into 7 types according to the occurrence of peak Ba/Ca ratios, and the type frequency was also compared between CIO and SPAWN by life stage. Life stage I refers to the larval stage (0 to 55 days old), stage II refers to the juvenile stage (55 days to 1 year old), stage III refers to the juvenile to sub-adult stage (1 to 4 years old), and stage IV refers to the sub-adult to adult stage (more than 4 years old). [See Table 2]

III. RESULTS

1. Seasonal occurrence of otolith annuli

The first inflection point in the otoliths of SBT occurred between the primordium and first annulus and corresponds to the transition from larval to juvenile life stage (Fig. 2). Otolith annual growth increments were smaller after the second inflection point, which corresponds with sexual maturation at ages 8-12, indicating a reduction in growth rate after maturation (Fig. 2).

The marginal increment in SBT otoliths from the last annulus (opaque zone) to the otolith edge was different between CIO and SPAWN (Fig. 3). A translucent zone was present in SBT collected from the spawning ground in February of the austral summer (Fig. 3a), but an opaque zone appeared just at the otolith edge for SBT collected from the CIO in July of the austral winter (Fig. 3b). This indicated that an opaque zone was deposited in the austral winter and a translucent zone in the summer.

2. Migratory types of SBT and occurrence season of upwelling signal in otolith

The migratory patterns of SBT in the Great Australian Bight upwelling area as indicated by the upwelling signal of otolith peak Ba/Ca ratios were divided into 7 types (Table 2). Type A:

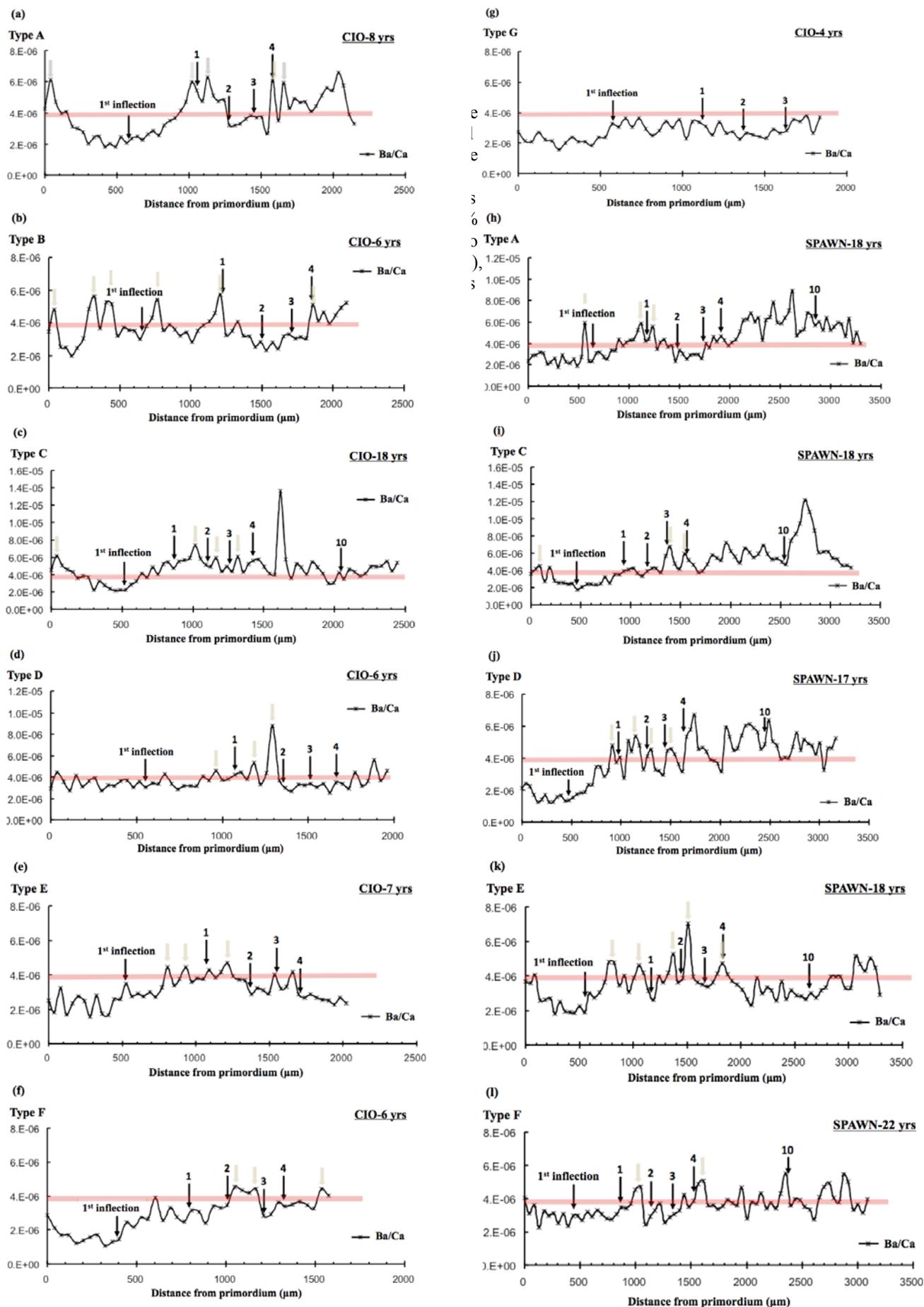


Fig. 4. Temporal changes in otolith Ba/Ca ratios of southern bluefin tunas collected from the central Indian Ocean (CIO, Types A-G) and from the spawning ground south of Java (SPAWN, Types A and C-F). The Types A-G are described in Table 2. The locations of annuli with numerals and of peak Ba/Ca ratios greater than 4.0×10^{-6} (the criterion as upwelling signal, red band) are indicated by black and grey arrows, respectively. Age is indicated with the sampling site of each fish. The 1st inflection is shown in Fig.2.

The peak otolith Ba/Ca ratios of all 29 SBT appeared in the translucent zone of the otolith, indicating that they encountered a barium-rich area in the summer (Fig. 4).

3. Comparison of migratory patterns between the CIO and the SPAWN by age group

Type D was dominant among the 7 types of migratory patterns, reaching 60.0% for the SPAWN and 28.6% for the CIO (Table 3). This implied that most SBT (90%) migrate to the upwelling area after their juvenile stage (ages 1-4). The frequency of the occurrence of peak SBT otolith Ba/Ca ratios was not consistent between CIO and SPAWN ($X^2=6.040$, $p < 0.05$) (Fig. 5), indicating the migratory pattern was different for the SBT between sampling location. Although only a low percentage of larval stage (before the first inflection) tuna migrated to the upwelling area for both CIO and SPAWN (20-40%), the percentage was higher in SPAWN (60%) than in CIO (30%) for age groups 2-3 and 3-4 ($X^2= 4.441$ and 4.209 , $p < 0.05$).

IV. DISCUSSION

1. Linkage between the peak of otolith Ba/Ca ratio and upwelling events

Juvenile SBT enter southern Australian waters and aggregate in the Great Australian Bight (GAB) during the austral summer, then disperse to the east or west of Australia within latitudes 30-50°S during the autumn and return to the GAB in the spring [19]. The GAB is characterized as the most productive coastal upwelling zone in southern Australia and is the largest area of cool-water carbonate sedimentation in the world [26]. The otolith Ba/Ca ratio of SBT peaked during the austral summer, mostly at the ages of 1-4 years old, which corresponds to the age and the season of SBT appearing in the GAB. This suggests

Table 3. Comparison of the occurrence frequency of the type of upwelling signature in otolith of southern bluefin tuna between Central Indian Ocean (CIO, n=14) and spawning area (SPAWN, n=15). Total sample size =29. Types A-G are described in Table 2

Occurrence frequency (%) of the type of upwelling signature in otolith			
Type	CIO	SPAWN	Total
A	21.4	6.7	13.8
B	7.1	0	3.4
C	7.1	13.3	10.3
D	28.6	60.0	44.8
E	14.3	6.7	10.3
F	14.3	13.3	13.8
G	7.1	0	3.4
Total	100	100	100

that the otolith Ba/Ca ratio is a reliable natural tag for recording

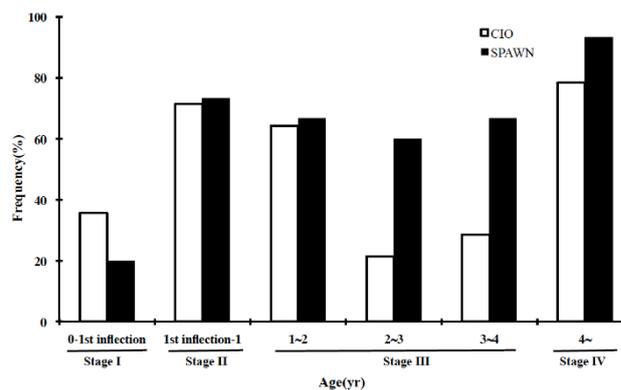


Fig. 5. Comparison of the frequency of occurrence of peak Ba/Ca ratios in southern bluefin tuna otoliths, by age group, for the central Indian Ocean (CIO) and the spawning ground south of Java (SPAWN).

the migratory environmental history of SBT to the upwelling area (GAB).

2. Occurrence season of the peak Ba/Ca ratios in otolith

Studies of the gut contents suggest that SBT migrated to the GAB to forage [29, 45]. The primary food items of juvenile SBT during the summer in the GAB are pilchards (*Sardinops sagax*) and anchovy (*Engraulis australis*). These forage fish spawn in the upwelling area during the austral summer and autumn [45], which might attract SBT to migrate to the GAB during the austral summer. This supports the idea that the peak SBT otolith Ba/Ca ratios were deposited during their foraging migration to the GAB during the summer season. The variation in the magnitude of peak otolith Ba between years might be due to the inter-annual change in the strength of the upwelling.

The otolith Ba/Ca ratios of other fish are weakly affected by temperature, which might influence the estimation of the upwelling effect on the peak otolith Ba/Ca ratios, and subsequently the migratory environmental history of the fish [3, 22, 30]. Salinity may also influence the incorporation of barium into the otolith, particularly in diadromous or freshwater fishes [11, 22]. The SBT lives in the open ocean which has a comparatively constant environment relative to that of diadromous or freshwater fishes in the estuary or freshwater. Thus, salinity might not greatly influence the otolith Ba/Ca ratios of tunas in the open ocean. Barium concentration varies positively with the environmental water chemistry [13, 30]. We have no data to verify whether the summer peak in SBT otolith Ba/Ca ratios was influenced by seasonal changes in water temperature. If the effect of the temperature on the barium uptake of the fish was significant, then another peak otolith Ba/Ca ratio would be expected when the SBT migrated between the tropical spawning area south of Java after maturation at the age of 8- 12 years [20]. However, no such seasonal peak Ba/Ca ratio was found for most SBT, suggesting a negligible temperature effect on otolith Ba/Ca ratios.

3. Individual variation in peak otolith Ba/Ca ratios

The peak of SBT otolith Ba/Ca ratios occurred mostly at the age of 1-4 years old, which corresponds to the age of the

SBT migration to the upwelling area of the GAB. However, the age of the occurrence of peak otolith Ba/Ca ratios varies among individuals, which might indicate that the timing at recruitment to the upwelling area of GAB differs among individuals. Fish of Types A-C had peak otolith Ba/Ca ratios before the first inflection at approximately 45–55 days old which corresponds to the life-stage transition from larva to juvenile and a habitat shift away from the inshore nursery area in northwestern Australia. Two types of upwelling occur in the Indian Ocean, wind-driven coastal upwelling and current-induced oceanic upwelling. Both types of upwelling may have a different influence on *T. maccoyii*. Larvae <45–55 days old (or before the first inflection point) are usually distributed between southern Java and north-western Australian but otolith Ba:Ca concentration ratios were low at this age, suggesting that no upwelling happened in this location as found by Wyrki (1962) [47]. This indicated that SBT might encounter Ba-rich waters in the spawning area at the larval-juvenile stage transition. The only known SBT spawning ground is located between southern Java and northwestern Australia (about 7°–20°S and 100–125°E) where several currents occur, including the Indonesian through flow (ITF), South Java current (SJC), South Equatorial current (SEC), Eastern Gyral current (EGC) and the Leeuwin current (LC). The mixing of these currents might produce an upwelling of the Ba-rich deep water to the surface and enable a high Ba uptake by some larval SBT. There is an upwelling along Java and Sumatra near the spawning area [5, 36]. However, the appearance of the upwelling signature was controlled by the annual monsoon and remotely by ENSO (El Niño-Southern Oscillation) [42]. This can explain the elevated Ba/Ca ratios in the otolith of SBT at the juvenile stage. The larval SBT is usually transported from the spawning ground by the southward-flowing Leeuwin Current to the Great Australian Bight along the west Australian coast where no upwelling occurs [47]. The productivity in the coastal waters of western Australian is low because of downwelling and the poleward-flowing Leeuwin Current [14, 24]. The southward Leeuwin Current is characterized by warm and low salinity waters that seasonally change with maximum flows in autumn and winter [34]. Sardine eggs and larvae are abundant along the southwestern coasts of Australia from June to August and from December to February, which is linked to the coastal upwelling [15, 17, 33]. The occurrence of some SBT with peak otolith Ba/Ca ratios before age-1 might be the result of coastal upwelling along the southwestern Australia coast. A higher barium upwelling signature helped distinguish the different nursery grounds of the northern bluefin tuna (*Thunnus orientalis*) [39].

In addition, stable isotopes of oxygen in the otolith have been validated for reconstructing the temperature histories of fish [40]. The $\delta^{18}\text{O}$ values in otoliths were negatively correlated with the ambient temperatures experienced by the fish. After spawning the larval SBT migrated to the southern temperate zone from the tropics. Shiao et al. (2009) used the composition of both O and C isotopes to reconstruct the migratory temperature history of SBT and show a correlation between otolith $\delta^{18}\text{O}$ values and the ambient temperature for SBT less than one-year-old [40]. The otolith $\delta^{18}\text{O}$ values increased gradually as the temperature decreased when fish migrated from the

spawning ground to the nursery ground. After age one, the thermal conservation ability of the fish developed gradually, however, the seasonal change of $\delta^{18}\text{O}$ values in otolith was still obvious (see Fig. 4 in Shiao et al. 2009) [40]. The $\delta^{18}\text{O}$ values between two annual otolith increments in the summer zone were higher than those in the winter zone at ages less than 4 years. This indicated the fish migrated to a low temperature upwelling area during the high temperature summer season. The GAB is the largest cool-water carbonate upwelling area in the world. The match between the temperature history of the SBT as revealed from both otolith oxygen isotopes and Ba/Ca ratios indicates that the otolith Ba/Ca of SBT can be used to examine fish habitat residency in areas of oceanic upwelling.

The Ba concentration in the surface waters of the Southern Ocean ranges between 4.2–5.5 $\mu\text{g}\cdot\text{L}^{-1}$, which is similar to that in the GAB upwelling area (5–6 $\mu\text{g}\cdot\text{L}^{-1}$) [22, 27]. Thus, the peak Ba/Ca ratios in otolith of SBT older than 5 years might be due to fish migrating to the southern ocean or polar front. Between age 5 and maturation at the age of 8–12 years, SBT migrate from the Great Australian Bight eastward and westward to the circumglobal feeding grounds between 30 and 50° S in the open ocean. The area 30–50° S is located at the boundary between subarctic and central Indian Ocean waters, which is a divergence zone where Ba-rich deep water may upwell to the surface layer of the ocean. Thus, a peak otolith Ba:Ca ratio may occur annually, but not regularly, in *T. maccoyii* after maturation. The difference in amplitude of the Ba:Ca ratios might indicate annual changes in the strength of the upwelling. The SBT from the CIO where no peak otolith Ba/Ca occurred between the ages of 1–4 years might indicate that some larvae entered the central Indian Ocean directly via the South Equatorial current without entering the GAB after hatching. The higher percentage of SBT with peak otolith Ba/Ca ratios from the SPAWN than from the CIO might indicate that inhabiting areas of upwelling during the juvenile stage might contribute to improved spawning stock recruitment.

V. CONCLUSION

We conclude that otolith Ba/Ca ratios and associated age determinations can be used as a biological tracer to identify the age and season of SBT migrating to an upwelling area. Seasonal movements in the GAB might be linked to food availability, which is influenced by the upwelling. The higher percentage of SBT on the spawning ground with peak otolith Ba/Ca ratios indicates that inhabiting areas of upwelling may improve spawning stock recruitment.

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