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Spatio-temporal variation in the elemental compositions of otoliths of southern bluefin tuna *Thunnus maccoyii* in the Indian Ocean and its ecological implication

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The elements Na, Mg, Mn, Ca, Sr and Ba in otoliths of southern bluefin tuna *Thunnus maccoyii*, collected from their feeding ground in the central Indian Ocean and spawning ground between southern Java and north-western Australia were measured by laser-ablation inductively coupled plasma mass spectrometry (LA-ICPMS) and compared among sampling locations and developmental stages. The Na, Mg and Mn to Ca concentration ratios were significantly higher at the larval stage than at the adult stage, and the ratio reached a peak at the first inflection point of the otolith, mean ± s.d. 43·3 ± 4·9 days after hatching and decreased sharply to a low level thereafter. The temporal change of the elements:Ca ratios in the first inflection point corresponded to the life stage transition from larva to juvenile, indicating that the uptake rate of elements from ambient waters was significantly influenced by the ontogenetic change in the fish. The elemental composition at the otolith edge differed significantly in sub-adults on the feeding grounds and adults on the spawning grounds. Thus, the otolith elemental composition can be used as a biological tracer to study the time of the ontogenetic shift and to reconstruct the past migratory environmental history of *T. maccoyii*. In addition, the elemental composition of the otolith core of the adult was similar between feeding and spawning grounds, indicating that the fish in the Indian Ocean had the same larval origin, which is consistent with the single spawning population hypothesis.

Key words: migratory environmental history; ontogenetic change; otolith elemental composition.

INTRODUCTION

The southern bluefin tuna *Thunnus maccoyii* (Castelnau) is a long-lived and highly migratory pelagic fish in the Indian Ocean. Their only known spawning ground is located between southern Java and north-western Australia (*c*. 7–20° S; 100–125° E), but their feeding grounds are circumglobally distributed between 30 and 50° S (Caton, 1991). The fish spawns from September to March of the austral summer (Ueyanagi, 1969; Shingu, 1970; Farley & Davis, 1998). After spawning,

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larval and juvenile *T. maccoyii* disperse southward with the tropical Leeuwin Current to the inshore waters between Perth and Esperance, Western Australia (Caton, 1991). Most juveniles aged from 1 to 4 years appear to aggregate in the Great Australian Bight during the austral summer, then migrate to the east or west within latitudes 30–50° S during autumn and return to the Great Australian Bight in spring (Stanley, 2002). By age 5 years, almost all fish have recruited to the adult stock in the open ocean. After maturation at the age of 8–12 years, they migrate between temperate feeding grounds and the tropical spawning ground (T. L. O. Davis, J. H. Farley & J. S. Gunn, unpubl. data; J. S. Gunn, J. H. Farley & N. P. Clear, unpubl. data). *Thunnus maccoyii* can reach 40 years in age, >200 kg mass and up to 200 cm fork length (L_F) (Kalish *et al*., 1996; Glencross *et al*., 2002; J. S. Gunn, N. P. Clear, T. I. Carter, A. J. Rees, C. A. Stanley, J. M. Kalish & J. M. Johnston, unpubl. data). Knowledge of ontogenetic movements and migratory environmental history is essential for fishery management and sustainable fish exploitation. The migratory environmental history of northern bluefin tunas *Thunnus thynnus* (L.) in the Atlantic and Pacific Oceans have been studied with advanced archival tags (Kitagawa *et al*., 2002; Itoh *et al*., 2003; Stokesbury *et al*., 2004; Block *et al*., 2005). An archival tag trial was also applied to *T. maccoyii* aged 2–4 years collected in the Great Australian Bight to understand the migration orientation of juveniles (Polacheck *et al*., 2006). The archival tag is, however, too large to apply to larval *T. maccoyii*; therefore, knowledge of the migratory environmental history of larvae is still fragmentary.

Fish otoliths are used in hearing and balance and are mainly composed of calcium carbonate and an organic matrix, both of which are deposited in daily and annual increments as the fish grows. This allows the ages of fishes to be determined on a daily and annual basis (Pannella, 1980). Depositions of elements in the growth increments of the otolith represent a permanent record of the environmental conditions experienced by the fish at a particular time (Campana *et al*., 2000). Jenkins & Davis (1990) verified daily formation of increments using marginal increment analysis and the daily progression of increment numbers in otoliths sampled from a single cohort. Itoh & Tsuji (1996) examined daily growth increments in otoliths of juvenile *T. maccoyii* and estimated their mean L_F to be 50·8 cm at age 1 year and 78·6 cm at age 2 years. Clear *et al*. (2000) validated the deposition of annual marks in the otoliths using strontium-marking experiments. For chemical analyses, Proctor *et al*. (1995) used two types of probe microanalysers to examine otolith elemental composition for studying the population structure of *T. maccoyii* from different locations and found that the environmentally correlated variation of elements was too low to provide a robust test of the diversity of migratory routes. The non-significance might be due to relative homogeneity of the pelagic environment and a weak effect of environmental factors on the concentration of elements present in otoliths at level ≥1 ppm. The use of higher resolution techniques such as inductively coupled plasma mass spectrometry (ICPMS) has enabled the measurement of otolith elemental composition <1 ppm. This form of analysis has been used to explore the small differences in otolith elemental composition for tunas (Scombridae) of different populations in the Pacific Ocean, Atlantic Ocean and Mediterranean Sea (Secor & Zdanowicz, 1998; Rooker *et al*., 2001, 2003; Secor *et al*., 2002).

This study attempted to measure the temporal change in otolith elemental composition by more advanced laser-ablation ICPMS (LA-ICPMS) and used daily and annual otolith increments to reconstruct the past migratory environmental history

of *T. maccoyii*. The otolith elemental composition was compared among life stages and between sampling locations in order to understand the effects of ontogeny and environment on the elemental composition of the otolith. The feasibility of using elemental composition of the otolith as a natural tag was also examined.

MATERIALS AND METHODS

SAMPLE COLLECTION

A total of 18 sagittal otoliths was collected from *T. maccoyii* on their feeding ground in the central Indian Ocean and 15 from their spawning ground between southern Java and north-western Australia (Fig. 1 and Table I). Otoliths of *T. maccoyii* from the feeding ground were collected from freshly caught fish by observers on long-line fishing vessels by a battery powered hole-saw drill. The otoliths of fish from the spawning ground were collected by hole-saw drill from landings at Benoa Port, Bali, Indonesia, from fish kept in ice immediately after capture and transported to the port 1–7 day later. After collection in the field, the otoliths were cleaned with de-ionized water, air-dried and stored in Eppendorf microcentrifuge tubes before preparation for age determination and otolith elemental composition analyses.

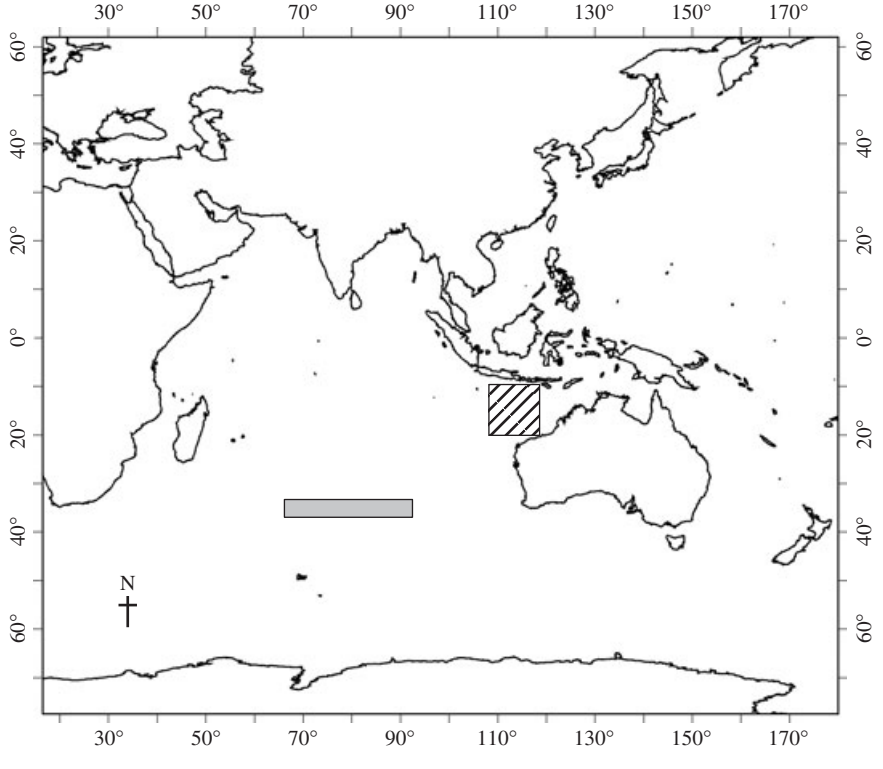


FIG. 1. The sampling area for *Thunnus maccoyii* in their feeding ground in the central Indian Ocean around 30–33° S and 67–89° E (▬) and in the spawning ground between southern Java and north-western Australia around 10–20° S and 110–120° E (▨).

TABLE I. *Thunnus maccoyii* mean ± S.D. and (range) of fork length (L_F), body mass (M) and age collected from the central Indian Ocean and south of Java which were used for otolith trace-element analysis by laser-ablation inductively coupled plasma mass spectrometry

Sampling location	Sampling date	Sample size	L_F (cm)	M (kg)	Age (years)
Central Indian Ocean (31° S; 89° E)	13–14 July 2003 and 14 July–3 August 2004	18	127·8 ± 20·5 (91–170)	30·3 ± 12·8 (12–79)	4·5 ± 1·9 (3–21)
South of Java (10–20° S; 110–120° E)	1 January and 3 February 2005	15	172·9 ± 8·6 (160–189)	98·9 ± 20·6 (75–137)	16·3 ± 3·2 (13–25)

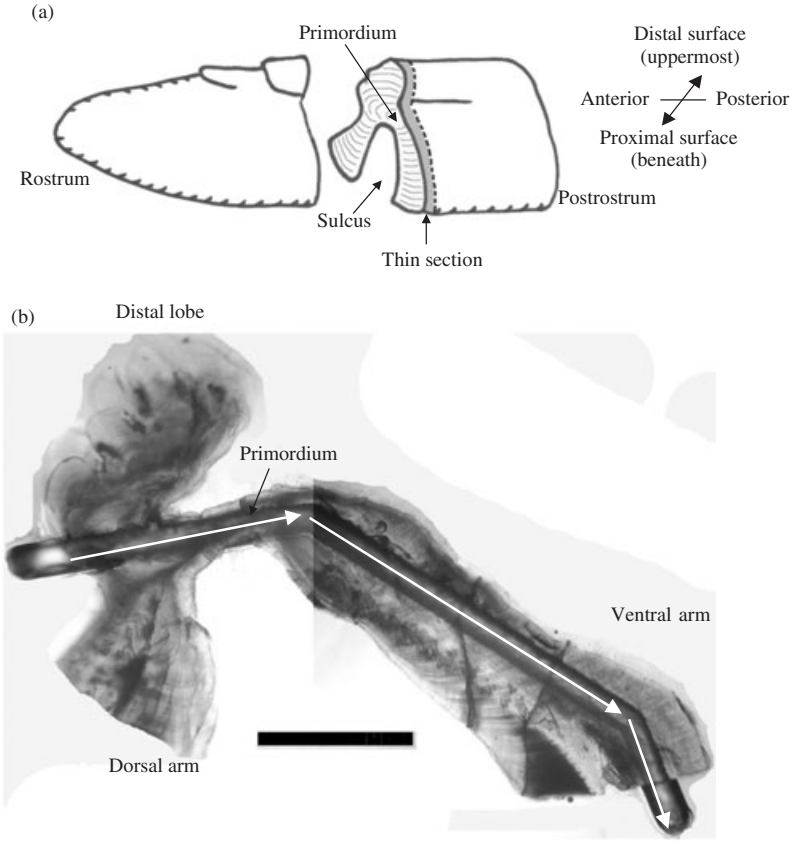


FIG. 2. (a) Drawing showing where the transverse section was taken of the left sagittal otolith of and (b) the laser-ablation inductively coupled plasma mass spectrometry measurement axis along the ventral arm of the otolith from the primordium to the otolith edge.

AGE DETERMINATION AND OTOLITH MICROCHEMISTRY ANALYSES BY LA-ICPMS

Otoliths were immersed in 5% ultrapure H_2O_2 for a few minutes to oxidize all remaining organic material from the otolith surface, ultrasonically cleaned with de-ionized water, air-dried and finally preserved in acid-washed Eppendorf microcentrifuge tubes. The otolith was embedded in epofix resin and transversely sectioned with the primordium in the middle at *c*. 300 μm in thickness by the diamond-coated blade of a slow speed saw (Isomet, Buehler; www.buehler.com) [Fig. 2(a)]. The otolith sections were further polished with a micro cloth and 0·05 μm alumina paste until the primordium was revealed. The annuli of the polished otolith sections were examined with a transmitted light microscope, and the ages of the fish were determined by counting the number of annuli along the ventral arm (longer arm), following the manual for age determination of *T. maccoyii* (Anon., 2002). A part of the otolith section was etched with ethylenediaminetetraacetic acid (EDTA) to examine the microstructure of the otolith before age 1 year with a scanning electron microscope. The daily age of the fish at an early stage was determined by the number of otolith daily growth increments plus 5, because *T. maccoyii* otolith daily growth increment is assumed to be deposited daily and the first increment is deposited *c*. 5 days after hatching (Itoh *et al*., 2000).

The elements in the ventral arm of the otolith sections were measured along the growth axis from the primordium to the otolith edge by using a Merchantek LUV 266 Nd:YAG UV laser microprobe (New Wave Research, Inc.; www.new-wave.com) connected to a Finnigan MAT ELEMENT 2 high resolution inductively coupled plasma mass spectrometry (LA-ICPMS) (Thermo Electron Corp.; www.thermo.com). The otolith sections were placed in a sealed chamber and viewed through a microscope connected to a computer monitor to programme the analysed transects [Fig. 2(b)]. The laser was pulsed at a repetition rate of 20 Hz, at a scan speed of 15 μm s^{-1} with an ablation transect diameter of *c*. 150 μm. The elements including ^{23}Na, ^{24}Mg, ^{44}Ca, ^{55}Mn, ^{88}Sr and ^{138}Ba were measured because these elements remain constantly at least 10 times higher than background levels. The setting took *c*. 2·46 s to produce one data point, which represented *c*. 37 μm on the otolith transect. Standards (NIST 612) were collected before each series, with each series comprising two to three otoliths. At the start of each otolith analyses, background counts were collected for 30 s, and the average was subtracted from sample counts to correct for background levels. The ablation chamber was purged for 60 s after sampling each otolith. All counting data were expressed as ratios of element:Ca concentrations (ppm ppm^{-1}) by estimating relative response factor of the instrument to the known concentration in standard (NIST 612).

DATA ANALYSES

The temporal variation for each of the element:Ca concentration ratios in the otolith was plotted by distance from the primordium to otolith edge. The first inflection point of the growth axis of the otolith and age was marked to examine how the temporal variation of the otolith elemental composition changed with developmental stage and growth of the fish. Six individuals were randomly selected from the 33 samples to examine the temporal variation of elements:Ca ratios in the otolith. In addition, to examine the differences in otolith elemental composition among different life stages and habitats (feeding and spawning grounds), the temporal change of elements:Ca concentration ratios in the otolith of the adult fish was divided into three sections corresponding to the larval and juvenile stages and the adult stage at capture. The elemental composition of the otoliths at the larval and juvenile stages was retrieved from the otolith core regions of sub-adult and adult fish collected in the feeding and spawning grounds because the larval and juvenile specimens were not available. The elemental composition of the first three points from the otolith primordium of sub-adult and adult fish from the feeding and spawning grounds were regarded as deposited in the early larval stage, and the subsequent five points as deposited in the late larval stage. The last point at the edge of the sub-adult and adult otoliths represented deposition in the central Indian Ocean and spawning (Fig. 1) areas. The ventral arm otolith radius from the primordium at the early larval stage in the sub-adult and adult otoliths was *c*. 0–111 μm and at the late larval stage was 112–297 μm, representing ages of *c*. 0–27 and 28–43 days after hatching, respectively.

Significant differences in elements:Ca concentration ratios among life stages (early larval, late larval and adult or sub-adult) and between habitats (central Indian Ocean and spawning areas) were tested by multiple ANOVA. The elements:Ca ratios were logarithmically transformed to meet the assumptions of normal distribution and homogeneity of variance. Four observations of spawning areas-edge and two of central Indian Ocean-edge were identified as outliers and excluded from further statistic analyses because these element:Ca ratios were more than 3 S.D. from the mean. A sequential Bonferroni procedure (Holm, 1979) was conducted to avoid possible type I errors in the testing of differences in otolith elemental composition among groups (Quinn & Keough, 2002). A canonical discriminate function analysis (DFA) was conducted to examine similarity and differences in the otolith elemental composition among groups of *T. maccoyii* of different life stages and habitats. The classification success of the fish for each group was determined by the jack-knife method (leave-one-out approach) of DFA. In addition, the eigenvectors of the individual elements:Ca ratios that contributed to the affinity among groups were also calculated.

RESULTS

DAILY AND ANNUAL GROWTH INCREMENTS AND OTOLITH GROWTH RATE

The daily growth increment width in the core region was narrow, but it dramatically increased before the first inflection point in the ventral arm of the otolith of a 3 year-old *T. maccoyii* [Fig. 3(a)]. In addition, the width before the first inflection point was approximately twice as wide as that after the first inflection point [Fig. 3(a) I–III]. The direction of the growth axis also changed at the inflection. The annulus in the otolith of a 17 year-old adult is shown in Fig. 3(b). The first annulus in the ventral arm of the otolith was located at approximately twice the distance of that from the primordium to the first inflection point. The first inflection point was located at mean ± S.D. $548{\cdot}6 \pm 88{\cdot}7$ μm ($n = 33$) from the primordium and deposited $43{\cdot}3 \pm 4{\cdot}9$ days (mean ± S.D.) ($n = 6$) after hatching. The mean age at the second inflection point was *c*. 8–12 years old, which corresponds to the age of *T. maccoyii* at first maturity. The width of annual increments before the second inflection point was greater than that after the second inflection point [Fig. 3(b)]. The otolith growth rate dramatically changed with fish developmental stage, particularly at the early and mature stages as indicated by the daily and annual increments.

TEMPORAL VARIATION IN OTOLITH ELEMENTS:CA CONCENTRATION RATIOS

Compared to the four other elements:Ca ratios, the Sr:Ca ratios were relatively homogeneous and stable for the six individuals randomly selected from the 33 specimens collected from the feeding ground [Fig. 4(a)–(c)] and spawning ground [Fig. 4(d)–(e)]. The Sr:Ca ratios slightly decreased in the early stage at an otolith radius *c*. 300 μm from the primordium and then gradually increased. The Sr:Ca ratios fluctuated between 3 and 6‰ through entire life history irrespective of whether the otoliths were collected from the feeding ground or spawning ground. In contrast, otolith Na:Ca, Mg:Ca and Mn:Ca ratios all changed markedly in the early life stage, reaching a peak ratio at the ages around 45–55 days at the ventral arm of the otolith approximately corresponding to the first inflection point and then sharply decreased

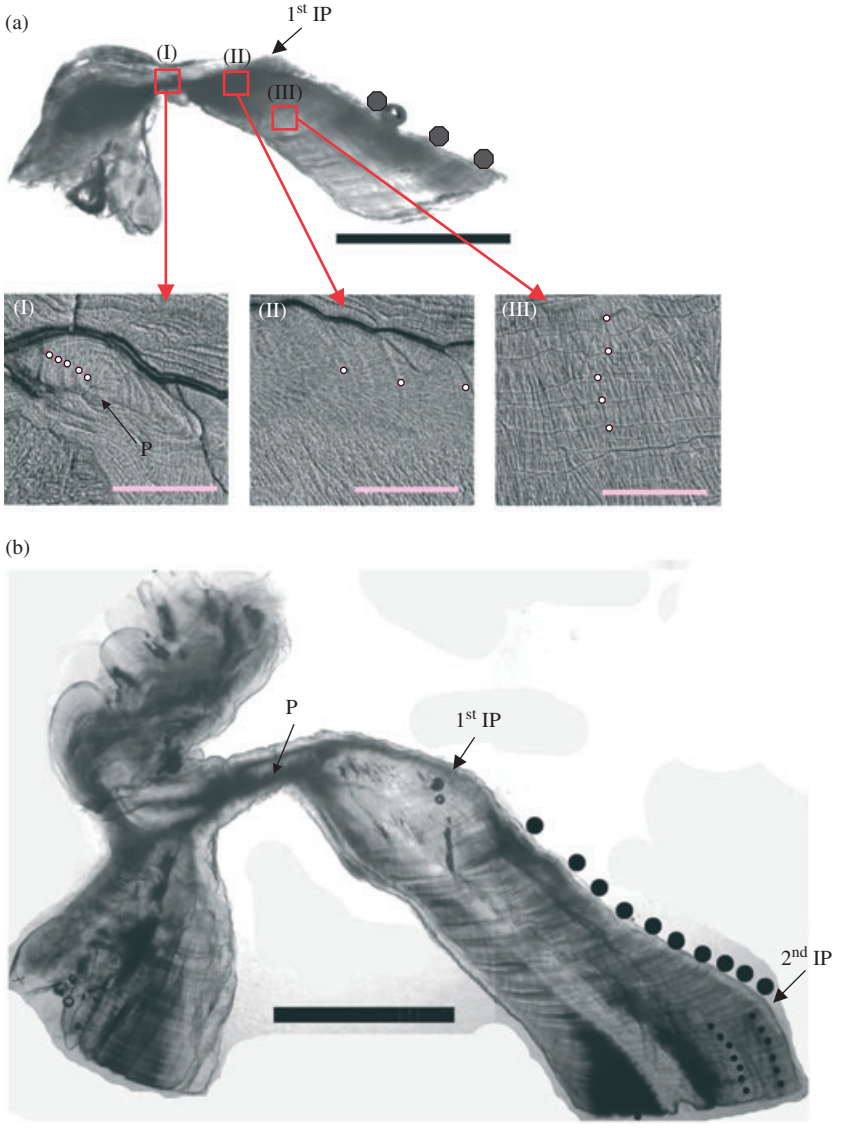


FIG. 3. (a) The daily growth increment in the ventral arm of the otolith of a 3 year-old *Thunnus maccoyii* (96 cm fork length, L_F), and (b) the annuli in the otolith of a 17 year-old fish (172 cm L_F). The daily growth increments in the core region (I) and in the section of the otolith before (II) and after (III) the first inflection point are marked (○) to show difference in daily growth increment width among sections. P, primordium, 1st and 2nd IPs, 1st and 2nd inflection points; ●, annuli. Scale bar = 1000 μm (a) and (b), 50 μm (I), (II) and (III).

to a low level. Otolith Ba:Ca ratios, however, were all extremely low in the early stage, then markedly increased at ages after the first inflection point. The changing pattern of the timing and level for the Ba:Ca ratios in otolith were not consistent among individuals.

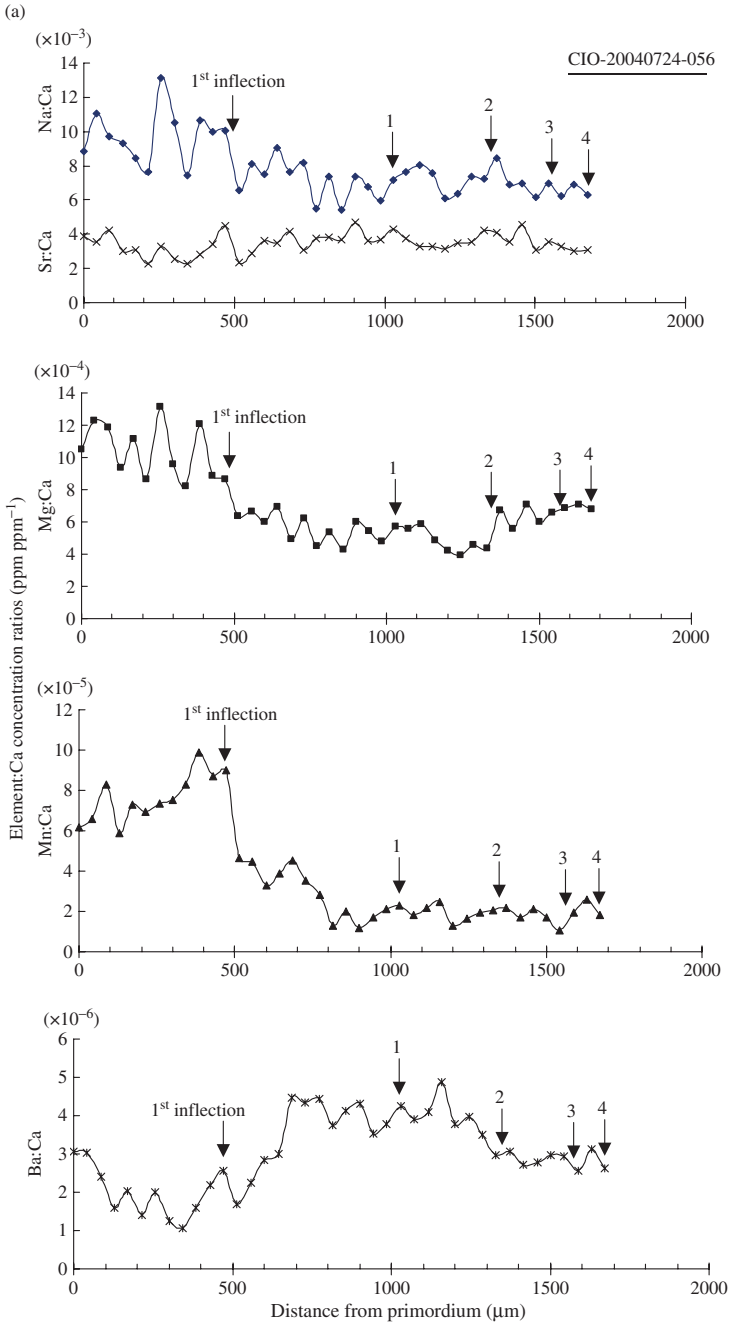
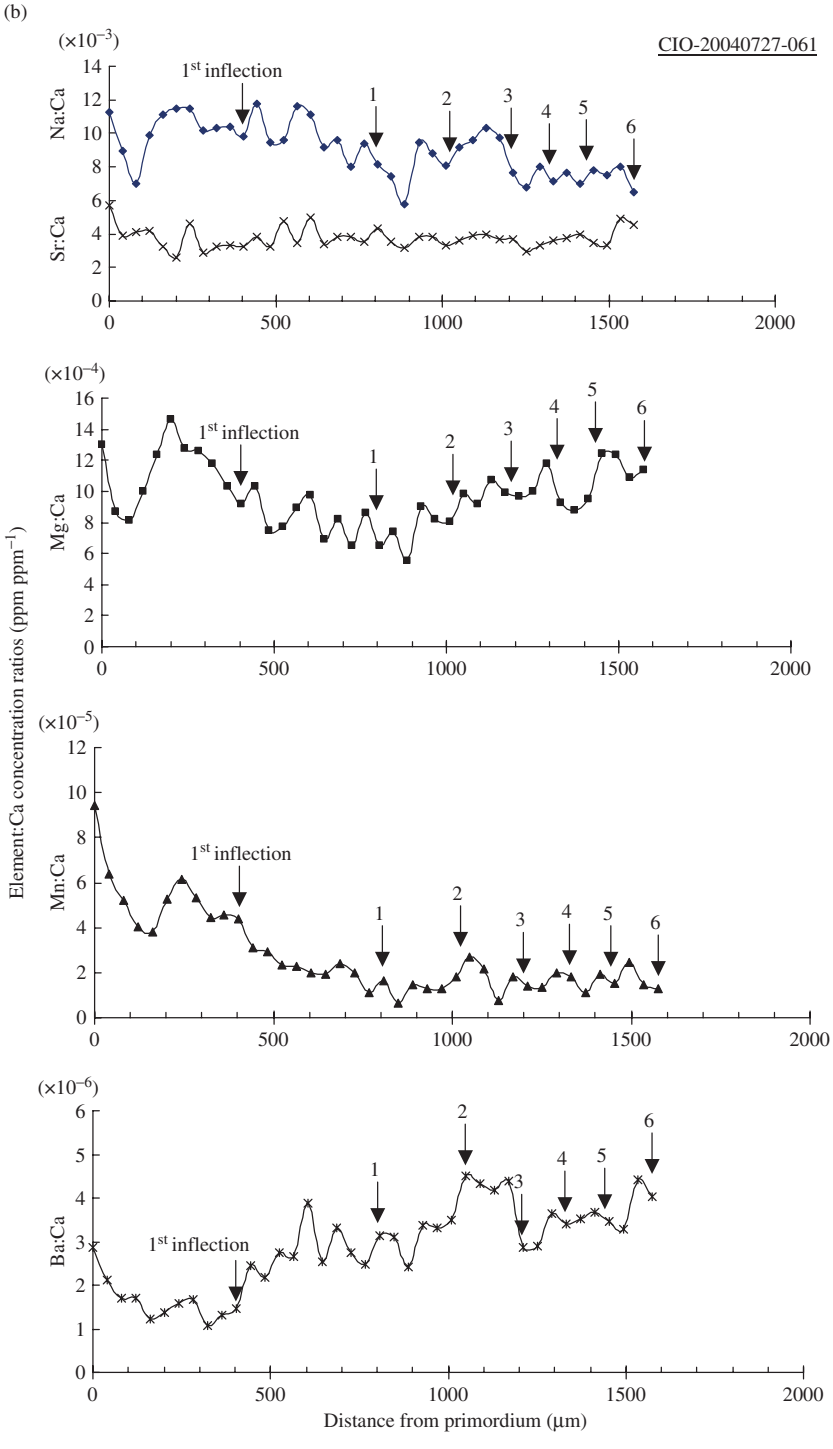


FIG. 4. Chronological changes in the five elements:Ca concentration ratios in the otoliths of six representative *Thunnus maccoyii* collected from (a)–(c) the feeding ground in the winter of 2004 and from (d)–(f) the spawning ground in the summer of 2005. Arrows with numerals indicate age of the fish in years. The first inflection point in the ventral arm of the otolith is indicated. Fish fork length and age were: (a) 96 cm and 4 years, (b) 91 cm and 6 years, (c) 158 cm and 25 years, (d) 165 cm and 22 years, (e) 176 cm and 17 years and (f) 182 cm and 20 years.



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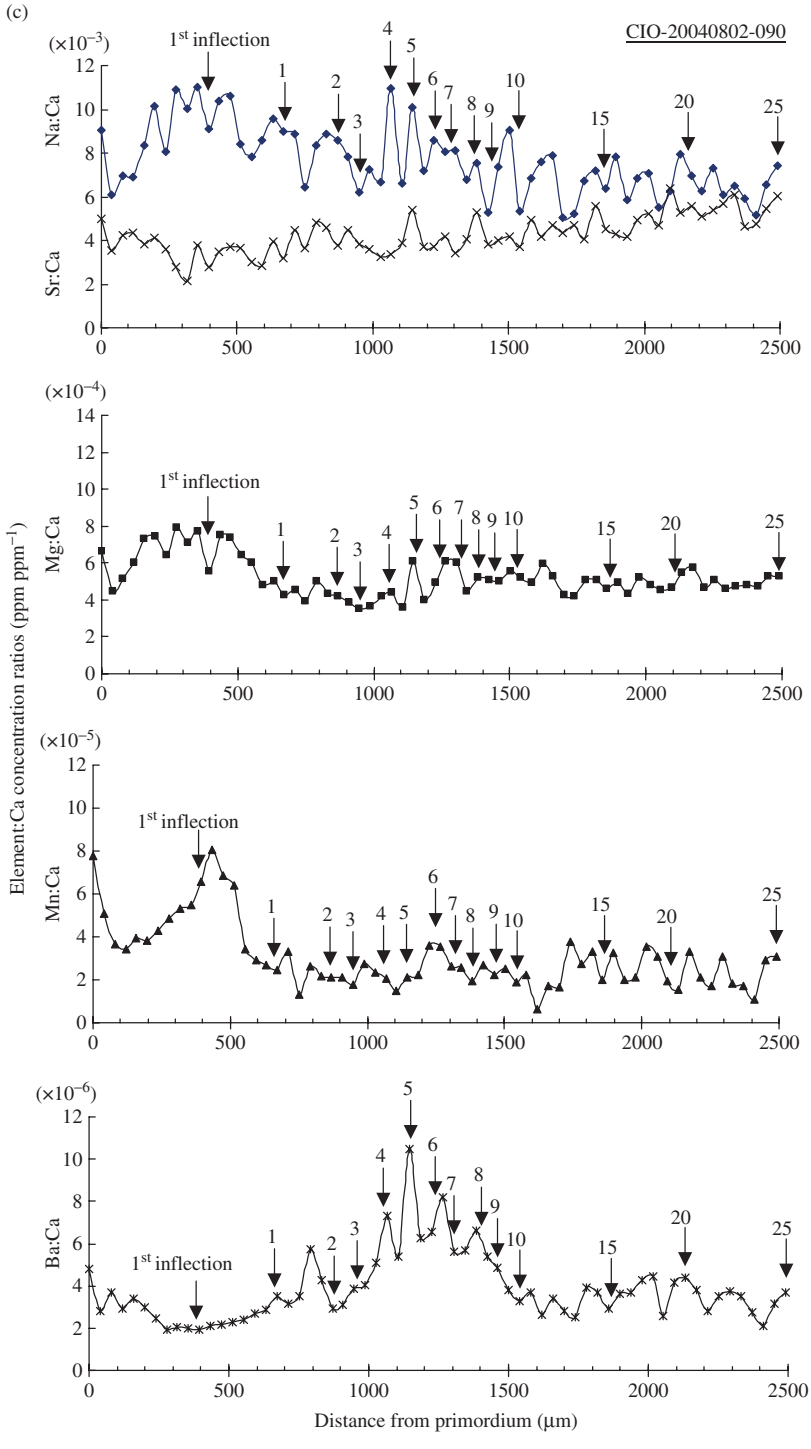

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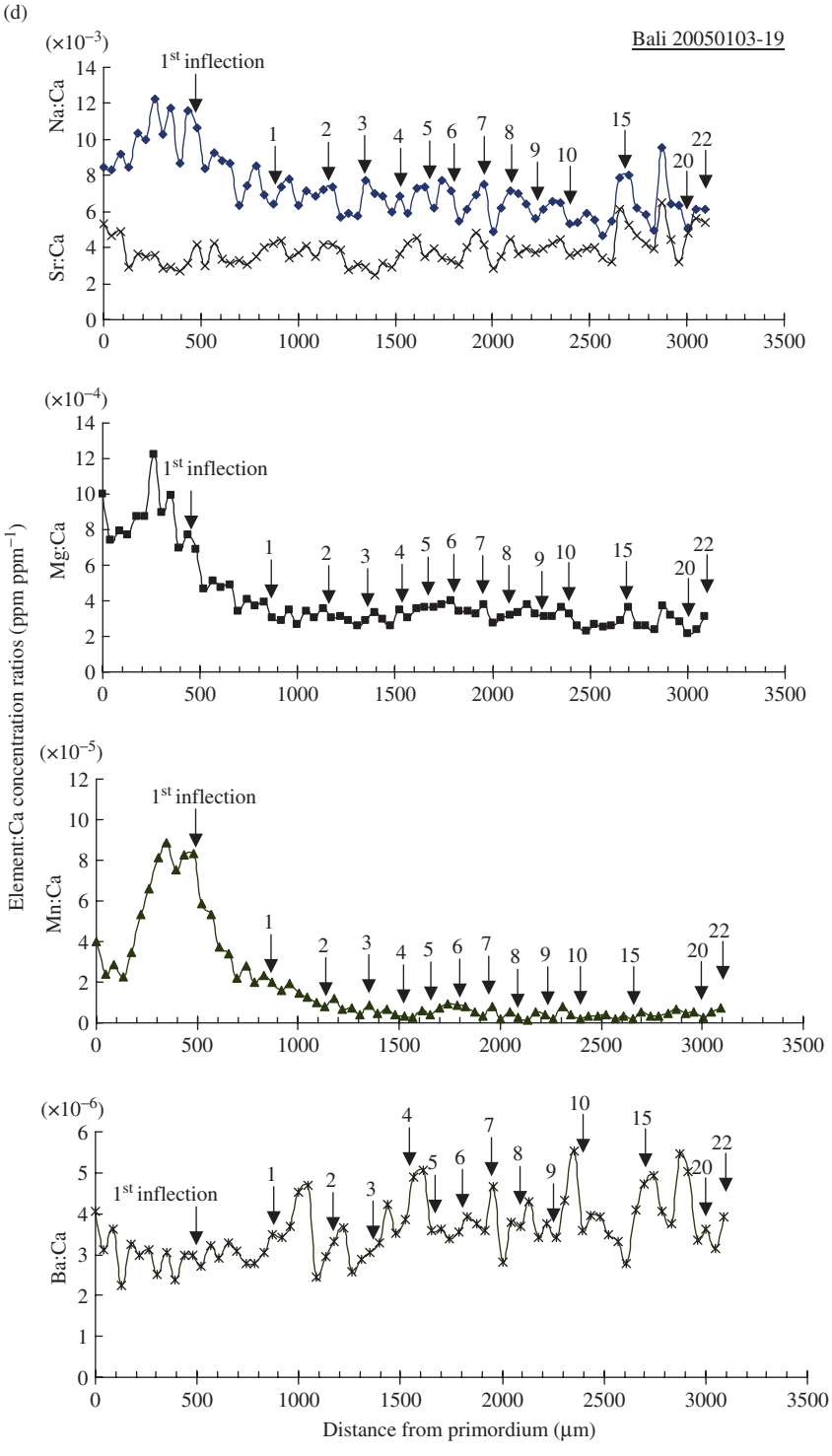

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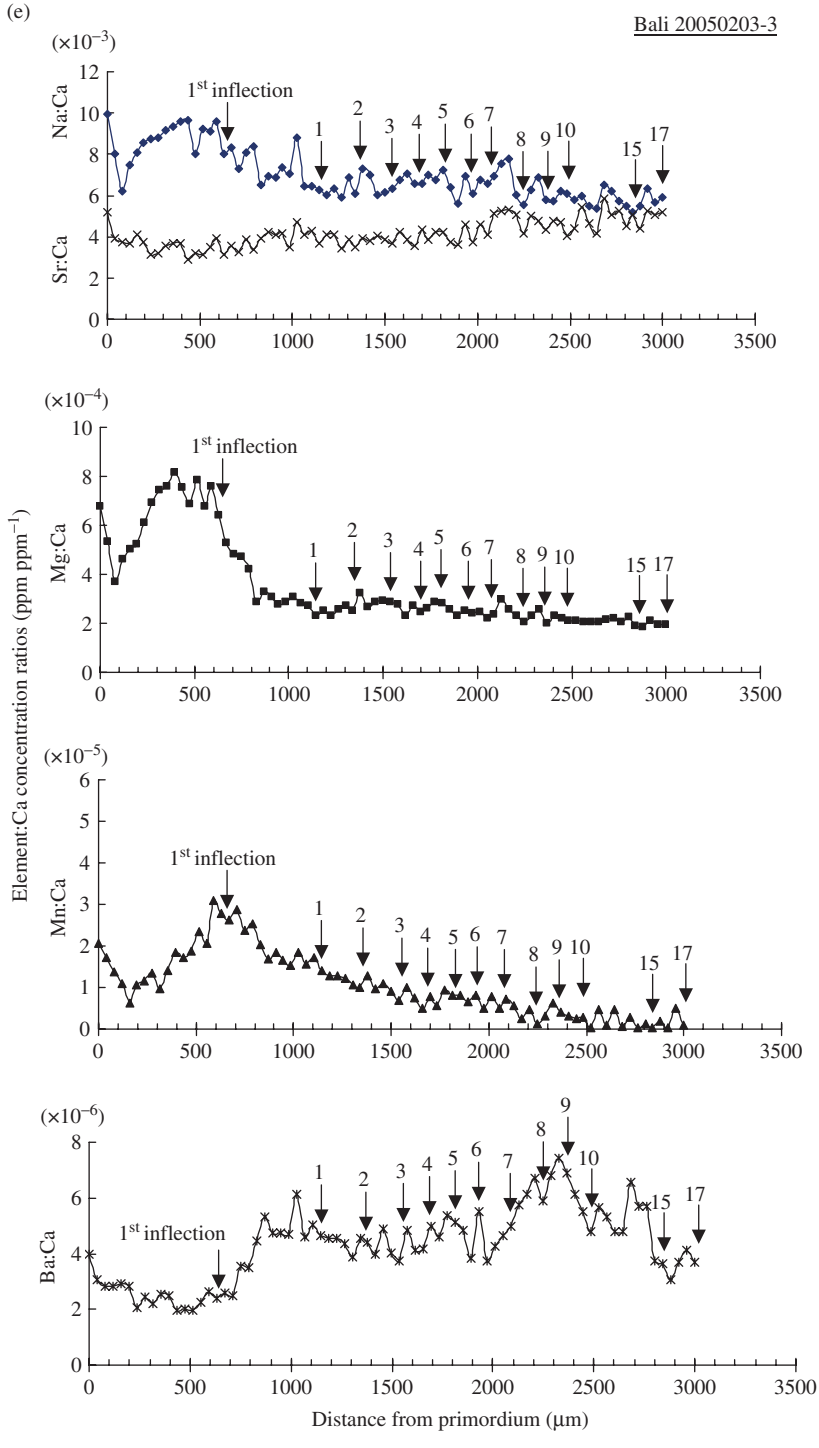

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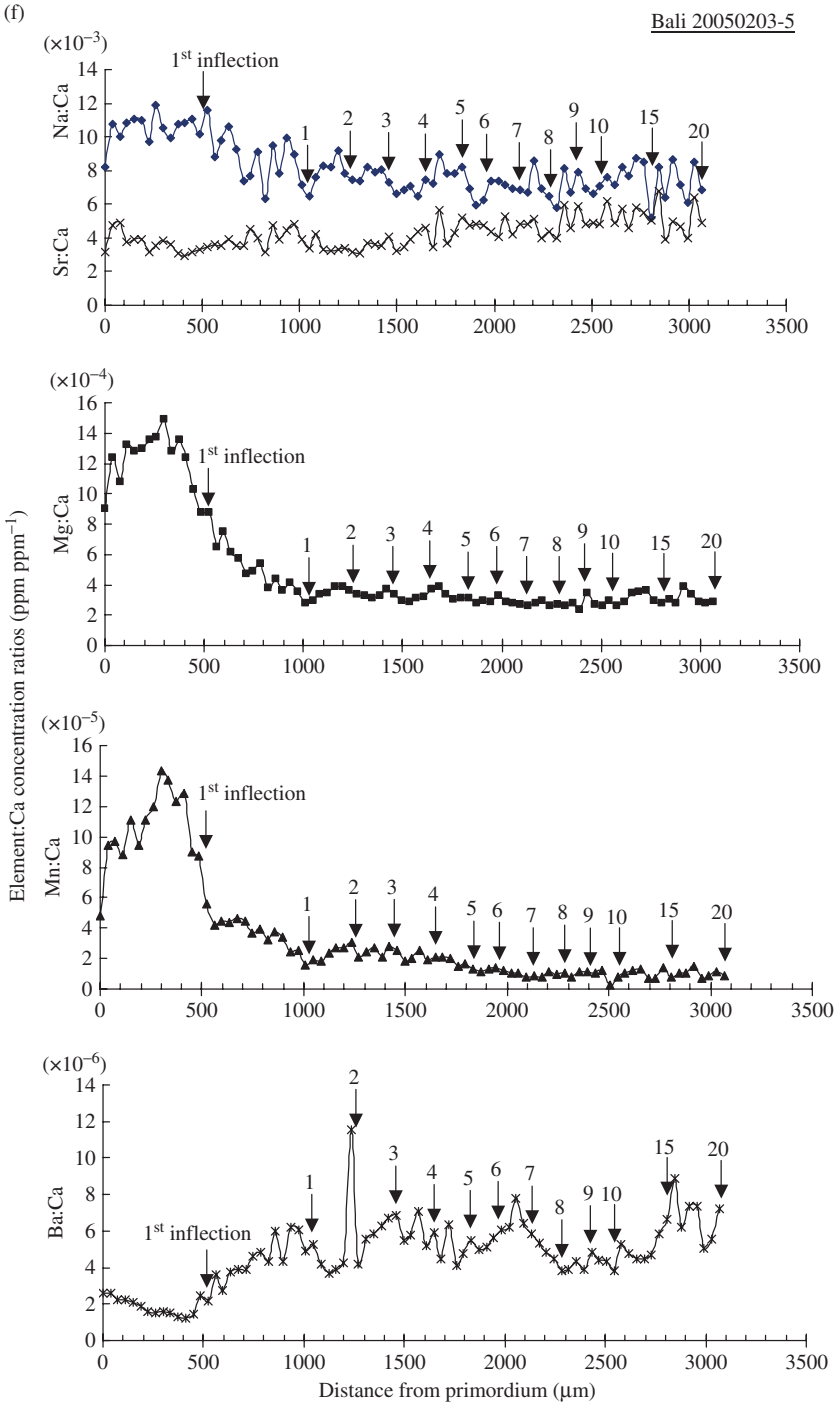

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TABLE II. Between-group comparison of *Thunnus maccoyii* otolith elemental composition for otolith sections divided into early (L), and late (J) larval stages and the adult stage at the otolith edge (E), by location [spawning ground (spawn) and central Indian Ocean (CIO)]. A sequential Bonferroni procedure was used to adjust *P* values

Comparison between groups	Pillai trace	d.f.	*F*	*P*	Adjusted *P*
Spawn-J × spawn-E	0·966	5,19	108·98	<0·001	<0·001
Spawn-J × CIO-E	0·921	5,24	55·94	<0·001	<0·001
CIO-J × spawn-E	0·928	5,23	58·96	<0·001	<0·001
CIO-L × CIO-E	0·901	5,25	45·40	<0·001	<0·001
CIO-L × spawn-E	0·940	5,20	62·67	<0·001	<0·001
CIO-J × CIO-E	0·824	5,28	26·21	<0·001	<0·001
Spawn-L × CIO-E	0·845	5,24	26·14	<0·001	<0·001
Spawn-L × spawn-E	0·863	5,19	23·91	<0·001	<0·001
Spawn-L × CIO-J	0·716	5,26	13·08	<0·001	<0·001
Spawn-E × CIO-E	0·733	5,21	11·52	<0·001	0·001
Spawn-L × spawn-J	0·692	5,22	9·87	<0·001	0·002
CIO-L × spawn-J	0·660	5,23	8·93	0·001	0·003
Spawn-L × CIO-L	0·533	5,23	5·25	<0·01	<0·01
CIO-L × CIO-J	0·439	5,27	4·23	<0·01	<0·05
Spawn-J × CIO-J	0·366	5,26	3·01	<0·05	<0·05

COMPARISON OF OTOLITH ELEMENTAL COMPOSITION AMONG LIFE STAGES AND SAMPLING SITES

MANOVA indicated that the elemental compositions in otoliths all differed significantly between the 15 pair-wise comparisons derived from the six groups of three life stages (early larval, late larval and adult at capture) from the feeding and spawning grounds (MANOVA, $F_{25,410}$, $P < 0{\cdot}001$; Table II). The first 10 between-groups comparisons of the otolith elemental composition at the otolith edge were highly significantly different (Table II). The Pillai trace for the remaining five between-groups of within early larval and late larval stages decreased to 0·660 in feeding–early larval × spawning–late larval (F, adjusted $P < 0{\cdot}001$), 0·439 in feeding–early larval × feeding–late juvenile (F, adjusted $P < 0{\cdot}05$) and 0·366 in spawning–late larval × feeding–late larval (F, adjusted $P < 0{\cdot}05$). In other words, the degree of the differences in the elemental composition was lower in the core region in early life stages than at the otolith edge in the adult fish between feeding and spawning grounds.

SIMILARITY AND DISSIMILARITY IN OTOLITH ELEMENTAL COMPOSITION AMONG LIFE STAGES AND FISHING AREAS

The DFA indicated that the elemental compositions of the otoliths were similar in the early larval stage between feeding and spawning areas. The degree of similarity, however, gradually decreased in the late larval stage and finally became completely separated at the adult stage (Fig. 5). The first two functions in the canonical discriminant analysis of the five elements:Ca ratios explained 93% of the total variance. Function 1 scores received positive weightings from Mn, Sr and Ba and negative weightings from Na and Mg. Function 2 was positively associated with Mg, Mn and Ba and negatively associated with Na and Sr (Fig. 5). In other words, function 1

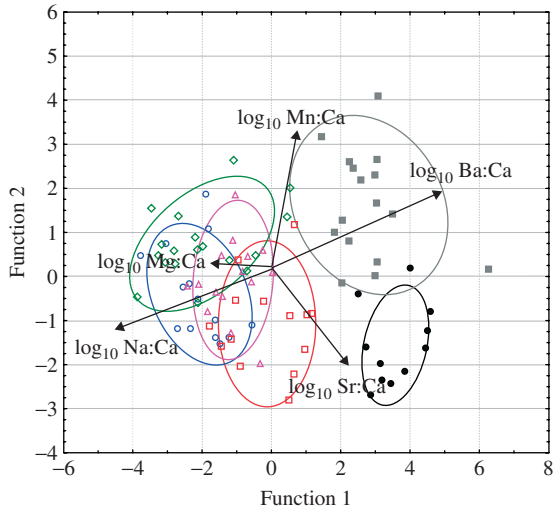

FIG. 5. Canonical variable plot from the discriminant analysis of the five elements:Ca ratios in the otoliths of 33 *Thunnus maccoyii* and the factor loading plot of the first two eigenvectors illustrating the relative contribution of the elements in the grouping. The otolith elemental composition at the early and late larval stages and the otolith edge of the fish was measured from different adult otolith sections collected from both spawning ground and the central Indian Ocean [○, spawning–late larval; □, spawning–early larval; ◇, feeding–late larval; △, feeding–early larval; ●, spawning–edge; ■, feeding–edge]. The elliptical circles indicate 95% CI for each group.

TABLE III. Classification per cent accuracy of *Thunnus maccoyii* collected from the central Indian Ocean (CIO) and spawning ground (spawn) based on the elemental composition in otolith sections from adults at the early (L) and late (J) larval stages as well as the otolith edge (edge)

	Correct (%)	Spawn-J	Spawn-L	CIO-J	CIO-L	Spawn-edge	CIO-edge
Spawn-J	79	79	0	21	0	0	0
Spawn-L	71	7	72	7	14	0	0
CIO-J	56	22	0	56	11	0	11
CIO-L	67	0	13	20	67	0	0
Spawn-edge	91	0	0	0	0	91	9
CIO-edge	88	0	0	0	0	12	88
Total	74						

scores contributed to the discrimination of the larval (feeding–early and feeding–late larval and spawning–early and spawning–late larval) and adult stages (feeding–edge and spawning–edge). Function 2 contributed to the classification of the adult stage with respect to the feeding ground and spawning ground. The jack-knife classification matrix assigned group membership correctly for 88 and 91% for the adults in the feeding ground (feeding-edge) and spawning ground (spawning-edge), respectively, but the percentage of success classification decreased to 56–79% for the fish in the early and late larval stages. The overall classification success was 74% (Table III).

DISCUSSION

In contrast to the earlier electron and proton probe microanalyses (Proctor *et al*., 1995), LA-ICPMS was able to detect significant changes in elemental composition of *T. maccoyii* otoliths. The resolution of the probe microanalyser was limited to detecting the most physiologically related major and minor otolith elements as well as Sr (Campana, 1999), which are generally little changed in the open ocean and thus failed to discriminate and predict *T. maccoyii* migration between different environments. In this study, LA-ICPMS measured the minor and trace elements <1 ppm. The elements detected in the otolith differed significantly between life stages and between feeding and spawning areas, thus discriminating among the timing of ontogenetic shifts and the environmental differences experienced by *T. maccoyii*. The elemental compositions in the otoliths were similar in the early larval stages but significantly different at the adult stage between the feeding and spawning grounds. *Thunnus maccoyii* spawned in the area between southern Java and north-western Australia during September to March. After hatching, the larvae dispersed from the spawning ground southward with the Leeuwin Current through a similar environment to the inshore waters of Western Australia (Shingu, 1967; Maxwell & Cresswell, 1981; Farley & Davis, 1998). There is currently only one known spawning ground for *T. maccoyii* (Proctor *et al*., 1995; Grewe *et al*., 1997). These results confirmed that the fish collected in both the feeding and spawning grounds were coming from a single spawning ground. After the juvenile stage, the fish migrated from the nursery to the open ocean for feeding and then to the spawning grounds where they experienced different water masses with different elemental compositions. The ambient water chemistry plays an important role in influencing the elemental composition in otolith when fishes migrate through different water masses in the ocean (Radtke & Shafer, 1992; Campana, 1999; Rooker *et al*., 2001, Ashford *et al*., 2007). Accordingly, the otolith elemental composition can be used as a tool for population discrimination and delineation of the migratory history of *T. maccoyii*.

The concentration of the minor element Na and the trace elements Mg and Mn were extremely elevated in the early life stage but dramatically decreased after first inflection point in the ventral arm of the otolith at an approximate age of 43 days after hatching. This age was very close to the metamorphosis period of *T. maccoyii* from larvae to juveniles. Thus, the elemental signature in the otolith can be used as a natural tag to trace the timing of ontogenetic shift of *T. maccoyii*. The deposition of elements in the otolith is a complex biogeochemical process influenced by the chemistry of the ambient water (Campana, 1999), the physiology and ontogeny of the fish (Kalish, 1989; Tzeng, 1996) and the otolith crystal structure (Brown & Severin, 1999; Melancon *et al*., 2005; Tzeng *et al*., 2007). An elevated Mn concentration at the otolith core has also been found in the Atlantic herring *Clupea harengus* L. by Brophy *et al*. (2004), and they speculated that the elevated Mn concentration in the core did not reflect the external environment and may be associated with embryological development. Michibata & Hori (1979) also reported Mn concentration in the eggs and embryos of the freshwater fish *Oryzias latipes* (Temminck & Schlegel) that were several orders of magnitude greater than concentrations in the surrounding water. This is attributed to the active uptake of Mn, which is involved in enzyme activation during the embryological development of the respiratory system. Similarly, the peak Mg:Ca concentration ratio in the early stage of *T. maccoyii* should

also reflect the fish physiological processes, particularly at the life stage transition as for fishes reported elsewhere (Bath Martin & Thorrold, 2005).

Accordingly, the elevated Na:Ca, Mg:Ca and Mn:Ca concentration ratios around the age of 43 days after hatching might be a signature of physiological and ontogenetic effect rather than an environmental effect. Although there is no experimental study on *T. maccoyii* at *c*. 45–55 days old to prove this hypothesis directly, the drastic decrease in the elements:Ca concentration ratios around this age corresponds to the life-stage transition from larva to juvenile and a habitat shift from the inshore nursery area in north-western Australia to the Great Australian Bight. The presence of an otolith daily growth increment width approximately twice as great in larval stage II than in stage III might indicate that the stage II development of *T. maccoyii* was very active and required rapid uptake of elements such as Na, Mg and Mn from the ambient water with the consequent elevation of Na, Mg and Mn before the first otolith inflection point. The otolith structural and chemical change signals the ontogenetic change when *T. maccoyii* migrate to the western coast of Australia and metamorphose from larva to juvenile. After the juvenile stage (1–4 years old), they emigrate from Western Australia to the Great Australian Bight. By age 5 years, they migrate eastward and westward for feeding within the latitudes of 30–50° S (Caton, 1991). On maturation, they migrate from the feeding ground (30–50° S) to the spawning ground (7–20° S and 100–125° E). During the feeding and spawning migration, they pass across different water masses, including sub-Antarctic water, Indian Ocean central water and Indian Ocean equatorial water. The difference in elemental composition at the otolith edge of *T. maccoyii* at capture between feeding and spawning grounds should reflect their migratory environmental change. Alternatively, the elemental composition at the otolith edge of adults collected in the spawning ground was not consistent with that of larval-stage fish although both larvae and adults should have experienced the same ambient water mass in the spawning ground and thus their otolith element composition should be similar. This was not the case, however, because of the significant ontogenetic effect on the uptake of the elements of otolith from the ambient water masks the environmental effect on element uptake. Thus, the otolith elemental composition of different developmental stages cannot be used to identify population units because of the significant ontogenetic effect on the uptake rate of elements. The effect of this fine-scale variation of ontogenetic effect on population identification, however, has not been considered in studies of other tuna species (Secor & Zdanowicz, 1998; Rooker *et al*., 2001, 2003). It is not clear why composition of the otolith core and otolith edge is different even though these parts of the otolith were deposited when the fish were in the same area. An ontogenetic change in endothermy was found in juvenile black skipjack tuna *Euthynnus lineatus* Kishinouye (Dickson *et al*., 2000). The ability of *T. maccoyii* to thermoregulate develops when the fish are still juveniles (Shiao *et al*., 2009). Whether the differential uptake of elements varied with the ability of SBT to thermoregulate and whether this can be detected in the elemental signals along an otolith section would make an interesting future study.

On the other hand, the Ba:Ca concentration ratios were independent of the change of Na, Mg and Mn and increased after the first inflection point possibly indicating that the fish migrated to a Ba-rich area. In the marine environment, fluvial sediments within an estuary are rich in Ba, as are the upwelling areas in coastal waters and the open ocean (Li & Chan, 1979; Coffey *et al*., 1997). The Ba content of the

ocean is more abundant in upwelling than in non-upwelling zones (Wolgemuth & Broecker, 1970), and the Ba concentration is rich and the productivity is higher in the coastal upwelling and equatorial divergence zones in the Pacific and Indian Oceans (Breymann *et al*., 1992). Ocean upwelling returns essential nutrients to the euphotic zone where they can be utilized by phytoplankton to produce organic materials. *Thunnus maccoyii* is an oceanic migratory fish and does not move to estuaries, thus the peak Ba:Ca ratio in the otolith may imply that the feeding fish migrated to a highly productive upwelling area rather than to an estuary. The elevation of Ba, as indicated in Fig. 4, happened at different ages. Accordingly, the age and Ba content of the otolith can be used to track the frequency and duration of individual fish in the upwelling area. 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 Wyrtki (1962). Juveniles at ages 1–4 years migrate to the Great Australian Bight and, at this stage, are found to have elevated Ba in the otolith, perhaps reflecting the coastal upwelling that the fish experienced in the Great Australian Bight. An archival tagging experiment of a 3 year-old *T. maccoyii* indicated that it had migrated to the highly productive upwelling area to the west of the Bass Strait (Bestley *et al*., 2008; Patterson *et al*., 2008). By age 5 years, they migrate from the Great Australian Bight eastward and westward to the circumglobal feeding ground between 30 and 50° S in the open ocean before maturation at the age of 8–12 years. The area 30–50° S is located at the boundary between subarctic and Indian Ocean central oceanic waters, which is a divergence zone where Ba-rich deep water may upwell to the surface layer of the ocean. Thus, the peak Ba:Ca concentration ratio in *T. maccoyii* otoliths after maturation will occur annually. The peak Ba:Ca ratios in otoliths after maturation seemed to occur annually but not regularly. The difference in amplitude of the Ba:Ca ratios might indicate that the strength of the upwelling changed annually. These hypotheses, however, need long-term historic environmental data and archival tag data that can confirm the migratory route to prove the relationship between upwelling and *T. maccoyii* otolith Ba:Ca ratios.

In conclusion, the five elements:Ca concentration ratios in otoliths of *T. maccoyii* measured by LA-ICPMS can be used as a natural tag to trace the timing of ontogenetic shift and habitat changes and to reconstruct their migratory environmental history in the Indian Ocean. Among the five elements, Na:Ca, Mg:Ca and Mn:Ca ratios seemed related to an ontogenetic shift, while Sr:Ca and Ba:Ca ratios may reflect environmental change such as upwelling in the ocean. This study suggests that the otolith elements reflect both the content of the elements in the ambient water and also physiological functions in early development.

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