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# Otolith O and C Stable Isotope Compositions of Southern Bluefin Tuna *Thunnus maccoyii* (Pisces: Scombridae) as Possible Environmental and Physiological Indicators

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**Jen-Chieh Shiao, Tzen-Fu Yui, Hans Høie, Ulyssess Ninnemann, and Shui-Kai Chang (2009)** Otolith O and C stable isotope compositions of southern bluefin tuna *Thunnus maccoyii* (Pisces: Scombridae) as possible environmental and physiological indicators. *Zoological Studies* **48**(1): 71-82. This study evaluated the use of stable isotopic signatures stored in otoliths to study the migratory life history of the southern bluefin tuna (SBT, *Thunnus maccoyii*). Otolith powders were sequentially collected from the edge to the core for stable oxygen (O) and carbon (C) isotopic analysis. All specimens (*n* = 11) analyzed showed a 3-stage profile of stable O and C isotope compositions. Values of $\delta^{18}O$ decreased from approximately -1.5‰ to -3.0‰ in early life, followed by an increase to approximately -1‰ at an age of 1 yr, and the values remained at the $\delta^{18}O$-enriched level with moderate fluctuations for the remaining lifetime. Hatchling SBT appeared to stay in the spawning ground for approximately 1 mo and then migrate southward, experiencing a temperature decrease of 8-10°C to about 20-25°C until 1 yr old. Water temperatures varied between approximately 15 and 25°C with no evident trend over the remaining life. However, the ambient water temperature might have been overestimated during the subadult to adult stages of the SBT when their thermal conservation ability was fully developed. The otolith $\delta^{13}C$ profile followed broadly similar trends to those of $\delta^{18}O$, showing depletion from approximately -6.5‰ to -10.5‰ in the early life stages, followed by abrupt enrichment up to -6‰ to -8‰ at an approximately age of 1 yr. Otolith $\delta^{13}C$ reached a plateau earlier in the 1st year than did $\delta^{18}O$ and showed greater variation over the remaining life. Interpretation of otolith $\delta^{13}C$ values is complicated, but the results suggest progressive transitions of ontogeny, metabolism, and trophic levels for the SBT. Otolith stable O and C isotope analyses provide continuous information about the migratory life history and contribute to our presently limited understanding of the SBT migratory cycle. http://zoolstud.sinica.edu.tw/Journals/48.1/71.pdf

**Key words:** *Thunnus maccoyii*, Southern bluefin tuna, Otolith, Stable isotope, Migratory life history.

The southern bluefin tuna (SBT, *Thunnus maccoyii*) is a highly migratory species which spawns southeast of Java, Indonesia (Shingu 1981, Nishikawa et al. 1985). Most, if not all, juveniles migrate south along the west coast of Australia to the Great Australian Bight (GAB), which is an important area for fish 1-5 yr old. SBT can live for up to 40 yr (Kalish et al. 1996). Fishery data indicate that SBT are found throughout the southern hemisphere, mainly in waters at 30°-50°S, but are rarely found in the eastern Pacific (Caton 1991).

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Many events in the migratory life history of the long-lived SBT are still unclear, such as the dispersion of larvae and spawning biology of adults. Based on present knowledge, SBT larvae experience warm water in the tropical spawning ground, and young SBT (< 1 yr) experience a water temperature decline during migration to a temperate area, such as the GAB. A cool-water environment is also expected for subadult and adult SBT, which usually stay in a temperate area. To reproduce, mature SBT must return to the warmer waters of the tropical spawning grounds. SBT as young as 8 yr old occur at the spawning ground, but the age at spawning is usually greater and can exceed 30 yr old (Farley and Davis 1998, Davis et al. 2001, Farley et al. 2007).

Although electronic devices, such as archival tags, can accurately log ambient water temperatures (Block et al. 1998a b, Gunn and Block 2001), recording such data for a fish's entire life is not possible because very small fish cannot carry these devices. Fish otoliths provide an alternate means to discern time-specific environmental conditions throughout the life of fish. Stable isotopes of oxygen in otoliths are deposited close to an isotopic equilibrium, with a well-defined fractionation effect of the ambient temperature (Patterson et al. 1993, Thorrold et al. 1997), and have been used to reconstruct temperature histories in both ancient and modern water masses (Patterson 1998, Weidman and Millner 2000).

Stable carbon isotopes in otoliths can reflect the metabolism, diet, and trophic level of fish (Kalish 1991, Schwarcz et al. 1998, Sherwood and Rose 2003). Atlantic bluefin tuna (*T. thynnus*, ABT) forage for different food items at different life stages and in different habitats (Sarà and Sarà 2007). Like the ABT, the SBT is an opportunistic feeder, preying on a wide variety of fish, cephalopods, and crustacea (Young et al. 1997). Tissue stable $^{13}C$ and $^{15}N$ isotope signatures suggest that adult ABT feed at a trophic level similar to pelagic sharks (Estrada et al. 2005). Combining otolith chronology and its stable C and O isotopic signatures may provide insights into the behavior, physiology, and environmental conditions over the life of SBT.

By applying a high-resolution sampling technique, SBT otoliths were sequentially examined from the edge to the core for stable C and O isotope analyses. The objectives of this study were to (1) evaluate whether chronological variations in otolith stable O isotopes can represent changes in ambient water temperatures experienced by the SBT, (2) determine whether water temperatures reconstructed by otolith stable O isotopes can disclose the sequence of migratory events throughout an SBT's life, and (3) infer the autecology of SBT by chronological variations of otolith stable C and O isotopes.

## MATERIALS AND METHODS

### Sample collection

Sagittal otoliths were collected from spawning SBT landed at Benoa Port, Bali, Indonesia in Jan. and Feb. 2005. From these collections 10 pairs of otoliths were randomly selected for analysis (fork length: 140-200 cm, nos. 2-11 in Table 1). Otoliths were also collected from fish caught by longline in the feeding grounds of the central Indian Ocean. Most individuals caught in this area were young fish (Shiao et al. 2008) but a larger fish (fork length: 177 cm, no. 1 in Table 1) that may have been older was chosen for the analysis. Otoliths were extracted from the head with a battery-powered hole-saw drill after measuring the fork length (to the nearest 1 cm) and dressed weight (to the nearest 1 kg). Otoliths were cleaned with fresh water and air-dried before transfer to the laboratory for subsequent analyses.

### Otolith preparation and analysis

One otolith from each fish was prepared to estimate the daily age during the early life stages by counting the daily growth increments (Jenkins and Davis 1990, Itoh and Tsuji 1996), and the other otolith was prepared to estimate the annual age and to run the isotope analyses. For the age estimation and isotope analyses, otoliths were embedded in epofix resin, and a transverse section approximately 400 μm thick was cut from the resin block by a slow-speed saw (Isomet, Buehler, Evanston, IL, USA) fitted with a diamond-edged blade. The section was embedded in epofix resin again, ground and polished repeatedly on 1 side to expose the core on a grinder-polisher machine (Buehler, Metaserv 2000, Evanston, IL, USA), then etched with 0.05 M HCl to reveal the daily growth increments by observation with a compound light microscope (Olympus BX51, Tokyo, Japan) and a scanning electron microscope (FEI Quanta 200 SEM, FEI, Hillsboro, OR, USA) after being coated with a layer of gold (Cressington 108 Sputter Coaters, Watford, UK). Increments assumed to

represent daily growth were counted from the core to approximately the first inflection point, and incremental widths were measured. The 2nd sagittal otolith from the same fish was prepared by a similar procedure, but the otolith section was only slightly ground and polished until the annuli were clearly visible under a stereomicroscope. The prepared otolith section was approximately 300 μm thick. An image of the otolith section was taken with a digital camera (Olympus DP 71, Tokyo, Japan) mounted on a compound microscope (Olympus BX51, Tokyo, Japan) using transmitted light (Fig. 1). Annual growth increments were identified and counted following Anonymous (2002), which is based on validation studies of Kalish et al. (1996) and Clear et al. (2000).

To collect otolith powder, an otolith was placed on a computerized micromill (Merchantek, Fremont CA, USA), and several segmented lines that followed the growth zones of the otolith were marked on the real-time computer image taken by a camera mounted on the micromill (Fig. 1). The micromill software interpolated new lines between 2 adjoining segmented lines according to the number of samples to be obtained. Otolith powder, approximately 15-20 μg for sample no. 1 and 25-30 μg for sample nos. 2-11, was collected from the distal end to the core along the ventral-medial arm by the micromill as described by Høie et al. (2004a b). Milled samples were then sequentially collected between each of lines. Milling depth was set to approximately 200 μm. After each milling, an otolith image was taken. Therefore, the corresponding age of each milled sample could be determined by comparing the images taken for the age estimation and the images after milling. The otolith growth rate decreased as the fish grew in length (Fig. 1). Therefore the number of samples per year decreased with fish age, from approximately 7-12 during yr 1 to 1 or 2 after yr 10 (Fig 1). Hence the temporal resolution represented by each sample varied from approximately l-2 wk during the early life stage, approximately 1-2 mo before age 1, and to 0.5-1 yr during the adult stage (> 10 yr).

SBT no. 1 was analyzed at the Department of Earth Science, University of Bergen, Bergen, Norway while the other 10 samples were analyzed at the Institute of Earth Sciences, Academia Sinica, Taipei, Taiwan. The stable-isotope composition was analyzed using a Finnigan MAT 253 mass spectrometer at the University of Bergen and a Finnigan MAT 252 mass spectrometer at Academia Sinica. In both laboratories, gas for isotope measurements was produced by a reaction with orthophosphoric acid at 70°C in an automated on-line system (i.e., the Kiel carbonate device) with acid added to the sample in individual reaction vials. The long-term reproducibilities of the MAT 253 were ± 0.1‰ for $\delta^{18}O$ and ± 0.04‰ for $\delta^{13}C$

**Table 1.** Biological information on the southern bluefin tuna (SBT) used in this study. SBT nos. 2-11 were breeding individuals caught at the spawning ground and sampled at Benoa Port, Bali, Indonesia. SBT no. 1 was an adult fish in a nonbreeding condition when it was caught. The daily growth increments (DGIs) in the initial growth zone were estimated by counting otolith increments from the core to the first inflection. Numbers in parentheses are the corrected ages after adding 13 d to the count, as per Itoh and Tsuji (1996)

| SBT no. | Dressed weight (kg) | Fork length (cm) | Sex | DGI in initial growth zone | Age when caught (yr) | Date | Sampling location |
|---|---|---|---|---|---|---|---|
| 1 | 76 | 177 | - | - | 14 | Aug. 2003 | Central Indian Ocean 31°S, 89°E |
| 2 | 62 | 154 | F | - | 15 | Feb. 2005 | Bali, Indonesia |
| 3 | 97 | 172 | F | 24 (37) | 19 | Jan. 2005 | Bali, Indonesia |
| 4 | 101 | 170 | F | 20 (33) | 17 | Jan. 2005 | Bali, Indonesia |
| 5 | 166 | 200 | M | - | 19 | Jan. 2005 | Bali, Indonesia |
| 6 | 112 | 180 | F | 24 (37) | 16 | Jan. 2005 | Bali, Indonesia |
| 7 | 80 | 165 | F | 22 (35) | 16 | Jan. 2005 | Bali, Indonesia |
| 8 | 73 | 161 | F | 25 (38) | 15 | Jan. 2005 | Bali, Indonesia |
| 9 | 93 | 176 | F | 23 (36) | 14 | Jan. 2005 | Bali, Indonesia |
| 10 | 65 | 140 | M | 20 (33) | 10 | Jan. 2005 | Bali, Indonesia |
| 11 | 73 | 165 | M | 16 (29) | 12 | Jan. 2005 | Bali, Indonesia |

(standard deviation) for sample masses of 6-90 μg based on replicate measurements of an internal carbonate standard over a period of months. Precisions of the measurements performed on the MAT 252 were ± 0.1‰ for $\delta^{18}O$ and ± 0.09‰ for $\delta^{13}C$ (standard deviation) for small sample masses (25-30 μg). Each mass spectrometer was tuned to handle small samples after calibration with a series of standards of different weights.

All isotope values are reported according to standards of the International Atomic Energy Agency, Vienna. $\delta^{18}O$ and $\delta^{13}C$ values of the otoliths are reported in standard $\delta$ notation relative to Vienna Pee Dee belemnite (VPDB) through calibration against the NBS19 standard:

$$\delta = (\frac{R_{sample} - R_{standard}}{R_{standard}}) \times 1000 (‰);$$

where $R$ is the $^{18}O{:}^{16}O$ or $^{13}C{:}^{12}C$ ratio in the sample or standard.

Otolith $\delta^{18}O$ levels can be used as a proxy for the ambient water temperature based on the equilibrium deposition of stable oxygen isotopes in the otoliths. Several equations suggest that aragonite $\delta^{18}O$ is inversely related to the ambient water temperature with a slope of approximately -0.20 to -0.23 (Grossman and Ku 1986, Radtke et al. 1996 1998, Høie et al. 2004a b), although the intercepts significantly differ between species (e.g., Patterson et al. 1993, Thorrold et al. 1997, Høie et al. 2004a b). The various published equations produce different estimated temperatures but the relative trends within an individual are almost the same. No $\delta^{18}O$ fractionation relationship specific to tuna has been published. The equation for inorganic aragonite fractionation: $\delta^{18}O_{oto} - \delta^{18}O_{water}$ = 3.71 - 0.206 T (°C) (Kim and O'Neil 1997, Campana 1999) was used in this study since the estimated water temperatures are mostly close to the actual seawater temperatures in each corresponding area. Kalish (1991) reported the water $\delta^{18}O$ value for Tasman Sea surface water to be approximately 0‰ on the SMOW scale (Kalish 1991). According to the global seawater $\delta^{18}O$ database at NASA (http://data.giss.nasa.gov/o18data/), the water $\delta^{18}O$ in temperate areas of the Indian Ocean is close to 0‰ (SMOW), but the water $\delta^{18}O$ is more enriched in subtropical areas, up to approximately 0.4‰ (SMOW). Since the SBT spend more time in temperate areas, we used 0‰ (SMOW) as the water $\delta^{18}O$ value,
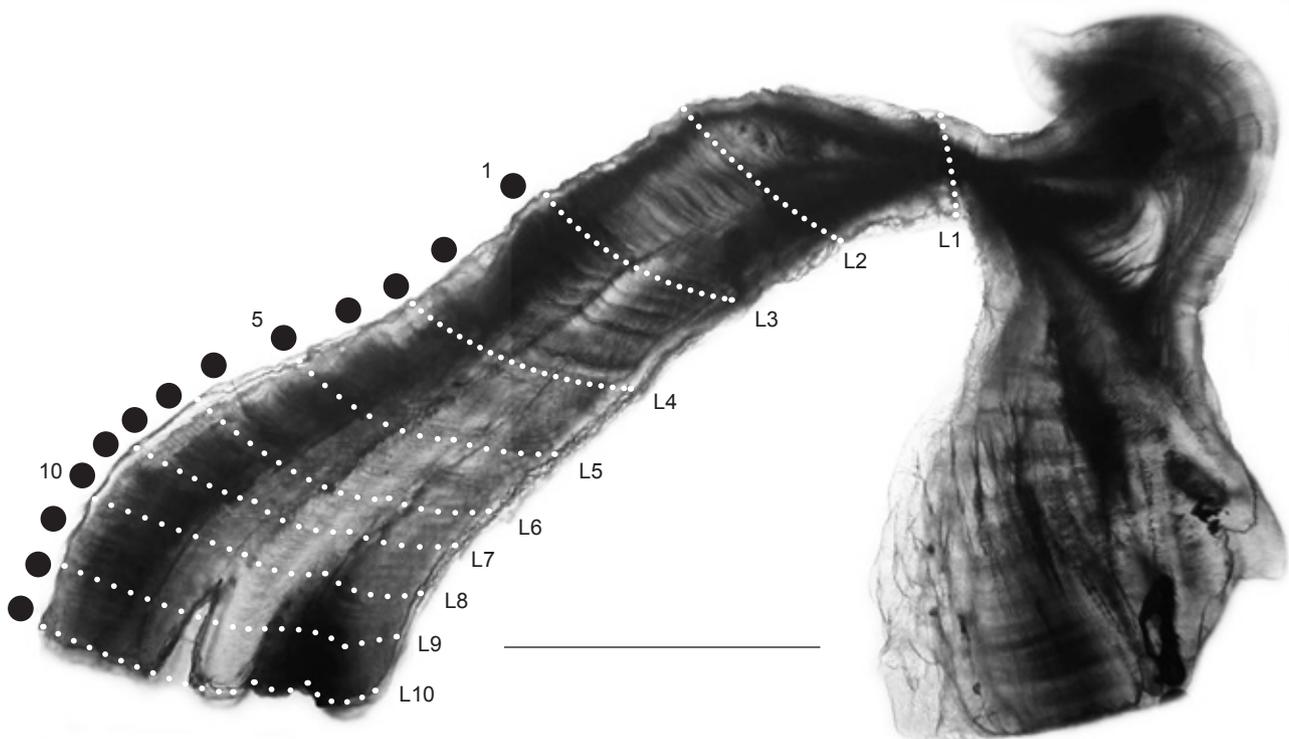


**Fig. 1.** Transverse section of a sagittal otolith of southern bluefin tuna no. 6. Ten dashed lines (L1-10) are marked following the growth zone of the otolith. Three or 4 samples were milled in the sampling paths interpolated between 2 adjoining dashed lines. Numbers and solid dots indicate annuli. Scale bar = 1 mm.

and this value was converted to the VPDB scale by the equation: $\delta^{18}O_{water}$ (on the VPDB scale) = $0.99978(\delta^{18}O_{water}$ on the SMOW scale) - 0.22 (Friedmann and O'Neill 1977) when calculating the water temperature. The results may underestimate the temperature by 2°C if the value of 0.4‰ is used as the water $\delta^{18}O$ value in the calculation. This bias is relatively small compared to temperature changes experienced by the SBT. Furthermore, the bias may be trivial since SBT are seldom found in subtropical areas during the subadult to adult stages.

## RESULTS

### Morphology and microstructure of otolith sections

A transverse section through the core of an SBT sagittal otolith showed a short dorsal-medial growth arm and a long ventral-medial growth arm with a very deep sulcus in between. The core of the otolith section is usually dark when observed under a compound light microscope and viewed with transmitted light. Jenkins and Davis (1990) and Itoh and Tsuji (1996) observed an average of 13 narrow daily growth increments (1-12 μm) near the core, which were not observed in this study either by the light microscope or SEM. This result suggests that the primordium of our samples was lost. However, wide daily growth increments outside the core were discernible. The daily incremental widths gradually decreased from 40 μm to approximately 20 μm from the core to the first inflection (Fig. 2). The daily growth increments were counted from the first discernible increment outside the core to approximately the first inflection on the ventral-medial arm. The counted daily ages were corrected by adding 13 d, since the narrow daily increments near the primordium were lost. The first inflection appeared at around 1 mo, ranging 29-38 d after hatching (Table 1). We noted a distinct layer of calcium carbonate covering the distal and proximal surfaces of the otolith from the core to around the first inflection (Fig. 2A). This layer does not exist in otoliths of young tuna species and is probably deposited during the subadult to adult stages.

The otolith growth direction changed twice over the life of the SBT, resulting in 2 inflections along the ventral arm. Annual growth zones were broad and diffuse, and contained multiple translucent and opaque sub-annual bands during the young and subadult stages. The growth zones became less diffuse and closer as the fish grew. Around age 10 yr and older, the annuli were usually regular in width and appearance. During this stage, the yearly growth zone spacing was narrower than that of the middle and inner zones. A typical otolith section is shown in figure 1, and all estimated ages range 10-19 yr (Table 1). Sample no. 5 was estimated to be only 19 yr old after multiple readings by 2 persons. However, SBT of this size (200 cm in fork length) can be 40 yr old. We have no explanation for this disagreement, but the length and weight of no. 5 as measured by the scientific observer on the market might have been incorrect.

### Oxygen-isotope composition

The analyzed otoliths (*n* = 11) showed a consistent pattern in $\delta^{18}O$ profiles (Fig. 3). Otolith $\delta^{18}O$ decreased in all fish at the initial few points, but the level of $\delta^{18}O$ varied among individuals. For example, $\delta^{18}O$ values of nos. 1, 3, and 8 decreased from approximately -1.5‰ to -2.2‰, and those of nos. 2, 5, and 6 decreased from approximately -2.0‰ to -3.0‰ in the early stage (Fig. 3). This $\delta^{18}O$-depleted stage corresponded to the otolith growth from the core to the first inflection of the ventral-medial arm. The next stage showed a dramatic increase in $\delta^{18}O$ that reached a plateau of around -1‰ at approximately 1 yr old. Results for this phase indicate that the SBT might experience a period of environmental shifts during the first year of life. The 3rd phase was a $\delta^{18}O$-enriched period lasting from about 1 yr old to the adult stage. For most individuals, $\delta^{18}O$ varied from -1.6‰ to -1‰ with minor fluctuations, indicating a relatively stable environment over the remaining life of the SBT. However, $\delta^{18}O$ values of nos. 9 and 10 showed relatively large fluctuations during the subadult to adult stage. This may reflect the nature of the highly migratory SBT with seasonal or yearly movements between temperate and tropical areas.

Ambient water temperatures estimated by otolith $\delta^{18}O$ showed a 3-stage life history in SBT (Figs. 4, 5). Stage I included larval and juvenile stages with ambient water temperatures increasing 3-5°C to the highest level in the initial few points. In stage II, the young SBT experienced water temperature decreases of 8-10°C from the highest level at approximately 1 mo to around 18-20°C at approximately age 1 yr. Stage III included the subadult to adult stages which showed relatively

cool water temperature of 22-24°C from an approximate age of 1 yr to the end of the life, such as in nos. 1, 2, 4, and 6. However, sample no. 8 showed a water temperature increase from approximately 20 to 26°C, and no. 9 showed large fluctuations between 16 and 26°C from the subadult to adult stages. Water temperature evidently decreased during the last few years in nos. 2, 5, and 9, which may indicate that the fish fed further south as they grew.
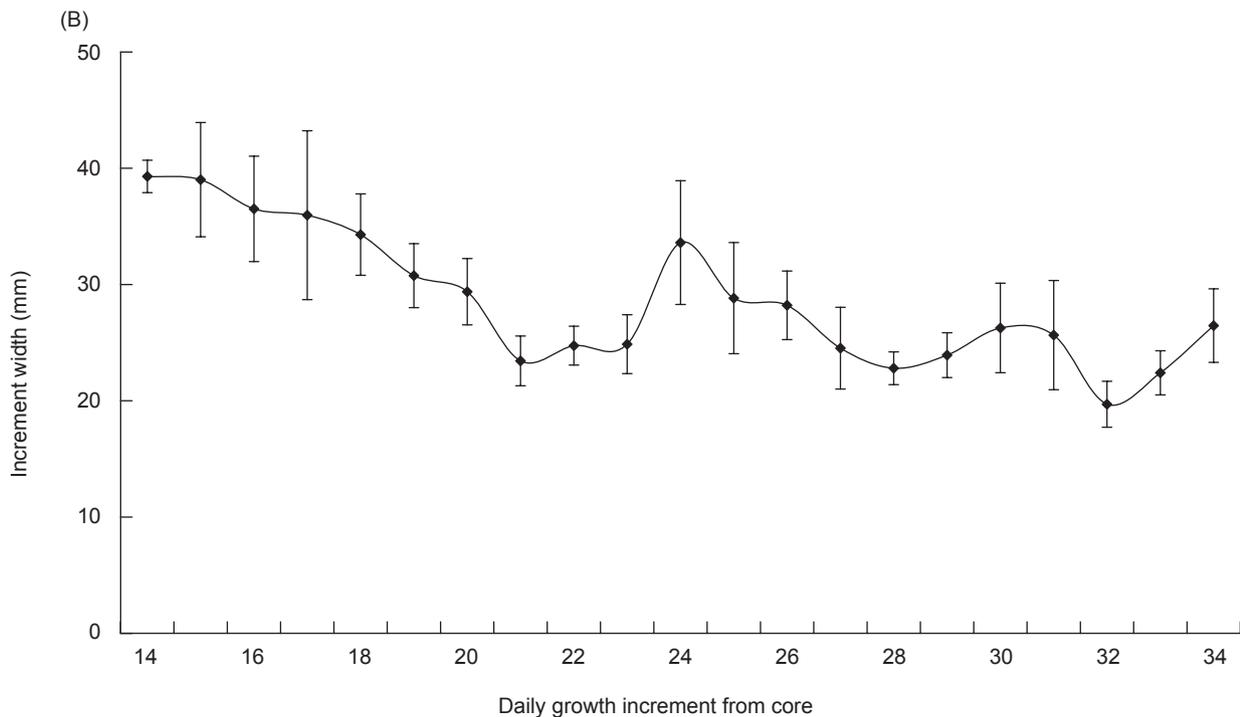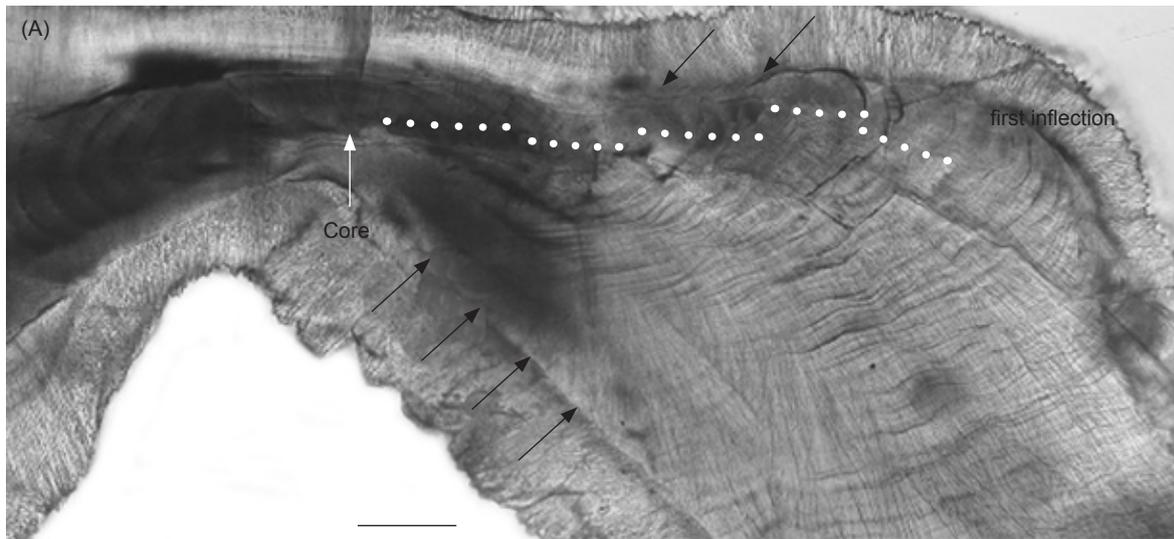
**Carbon-isotope composition**



**Fig. 2.** (A) Otolith microstructure of southern bluefin tuna no. 9. Daily growth increments are indicated by white dots between the core and the first inflection on the ventral-medial growth arm. A distinct structure is visible along the distal and proximal surfaces of the otolith, which can be distinguished by a boundary (indicated by the black arrows). This special layer of calcium carbonate was probably deposited at the subadult to adult stage and has no daily growth increments. Scale bar = 100 μm. (B) Temporal changes in the mean daily growth increment width counted from the core to approximately the first inflection of the 8 samples. The first increment measured is assumed to be the 14th d after hatching due to the presumed loss of 13 narrow rings near the core as shown by Itoh and Tsuji (1996). Data are shown as the mean ± SEM.

The otolith $\delta^{13}C$ profile was generally similar to the $\delta^{18}O$ profile. A decline in $\delta^{13}C$ in the early stage was found in all fish, but the level of $\delta^{13}C$ found varied among individuals. For example, those of nos. 3 and 9 decreased from approximately -7‰ to -8.5‰, while those nos. 2, 5, and 6 decreased from approximately -8‰ to -9.5‰ in the early stage (Fig. 3). However, nos. 3 and 8 showed a depletion of otolith $\delta^{13}C$ from approximately -6.5‰ to -8.3‰. This $\delta^{13}C$-depleted stage corresponded to otolith growth from the core to the first inflection of the ventral-medial arm. The duration of the $\delta^{13}C$-depleted stage was similar to the $\delta^{18}O$-depleted stage (approximately 1 mo) (Table 1). The 2nd stage was followed by a much-enriched otolith $\delta^{13}C$ that leveled off at about -6‰ to -8‰. Levels of $\delta^{13}C$ generally increased faster than for $\delta^{18}O$ and also leveled off earlier than did $\delta^{18}O$. For most fish, otolith $\delta^{13}C$ reached a plateau before an age of 1 yr, but continued at high levels throughout life, even for fish older than 10 yr, such as nos. 4, 5,

and 6. However, some fish showed more variation in the $\delta^{13}C$ profile after 1 yr. Nos. 4 and 5 showed continued increases in the $\delta^{13}C$ profile while nos. 2 and 3 showed large fluctuations.

## DISCUSSION

A short-term rearing experiment for 4 mo suggested that otolith $\delta^{18}O$ levels of adult ABT (aged 19-24 yr) may reflect the brain or body temperature rather than ambient water temperature (Radtke et al. 1987). Bluefin tuna maintain their brain and body temperature above the water temperature through their thermal conservation ability. By archival tagging, Block et al. (2001) found that ABT maintained a relatively constant internal peritoneal temperature (25-30°C) when the fish experienced water temperature of 2.8-30.6°C at different depths. Otolith $\delta^{18}O$ levels in adult SBT may indicate temperatures 3-5°C higher than the actual ambient water temperatures, based on data
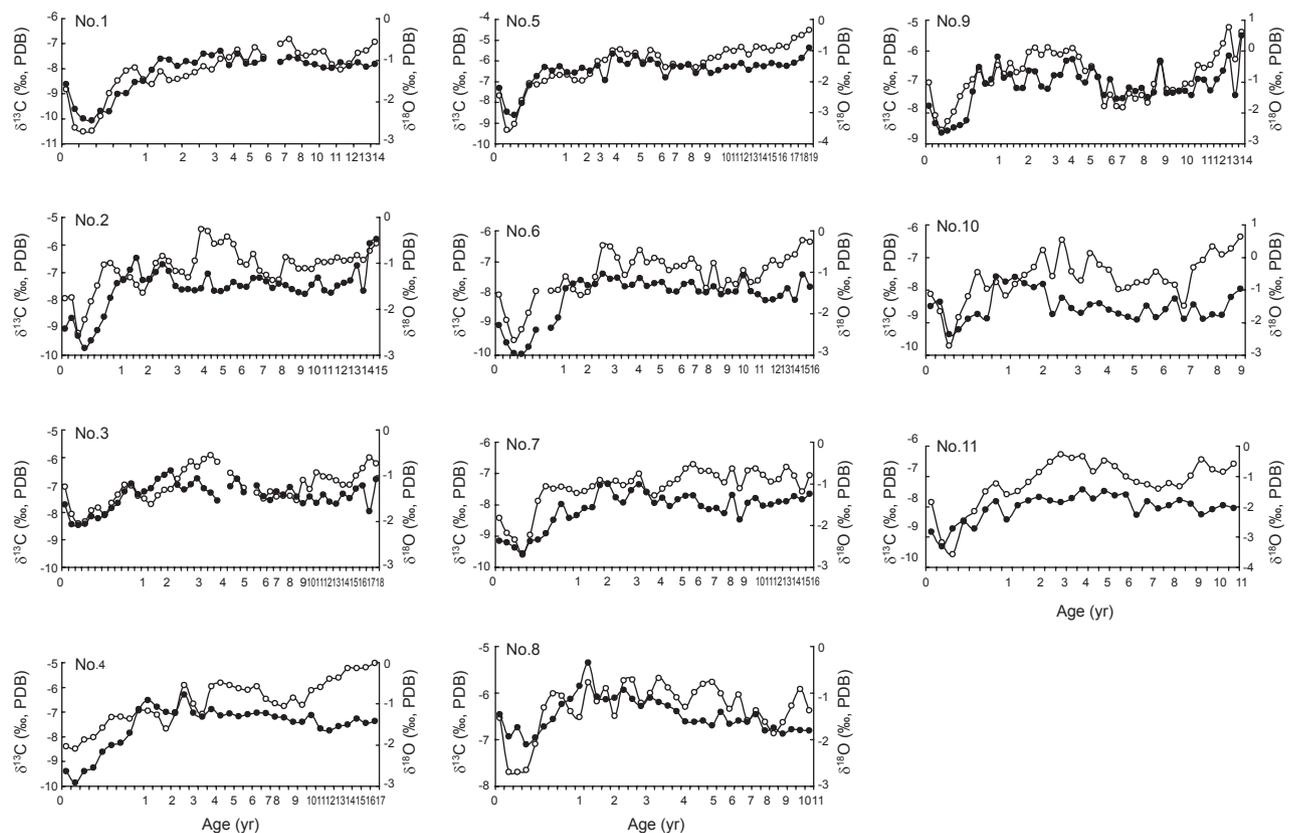


**Fig. 3.** Southern bluefin tuna otolith $\delta^{13}C$ (open dots) and $\delta^{18}O$ (solid dots) profiles, collected from the edge (to the right of the X-axis) to the core (to the left of the X-axis). The X-axis represents the annulus of each otolith. The temporal resolution of a single data point can be roughly estimated from the numbers of isotopic data between 2 annuli. Some data were lost in fish nos. 1, 3, 6, and 8.

of Radtke et al. (1987). Sample no. 8 showed a water temperature increase from 18°C at an age of 1.5 yr to 25-26°C after the age of 5 yr. Sample nos. 2, 3, 4, 6, and 9 showed an increase of 2-4°C in water temperature higher during the subadult to adult stages compared to the young stage of 1-2 yr. This phenomenon might be caused by endothermy by the SBT. However, it is unknown when the thermal conservation ability of SBT and other tuna species is fully developed (Dickson et al. 2000). The water temperature estimated by otolith δ18O levels reasonably matched the previously measured or reported water temperature before the age of 1 yr (see below). These results suggest that endothermy by the SBT is not fully functional before an age of 1 yr, and estimation of water

temperature by otolith δ18O is not affected by the thermal conservation ability in very young SBT.

The daily age from the otolith core to the first inflection was likely underestimated in our examined otolith sections. This underestimation was likely due to technical difficulties of revealing the primordium of adult otoliths by transverse sectioning and grinding. Itoh and Tsuji (1996) observed the entire otolith etched by diluted HCl without cutting or grinding/polishing. They identified 3 stages of otolith growth in SBT juveniles. The daily growth increments in the 1st stage were narrow for tuna otoliths, approximately 1-12 μm near the otolith primordium for a mean duration of 13 (range, 9-16) d. This stage corresponded to the larval stage of SBT
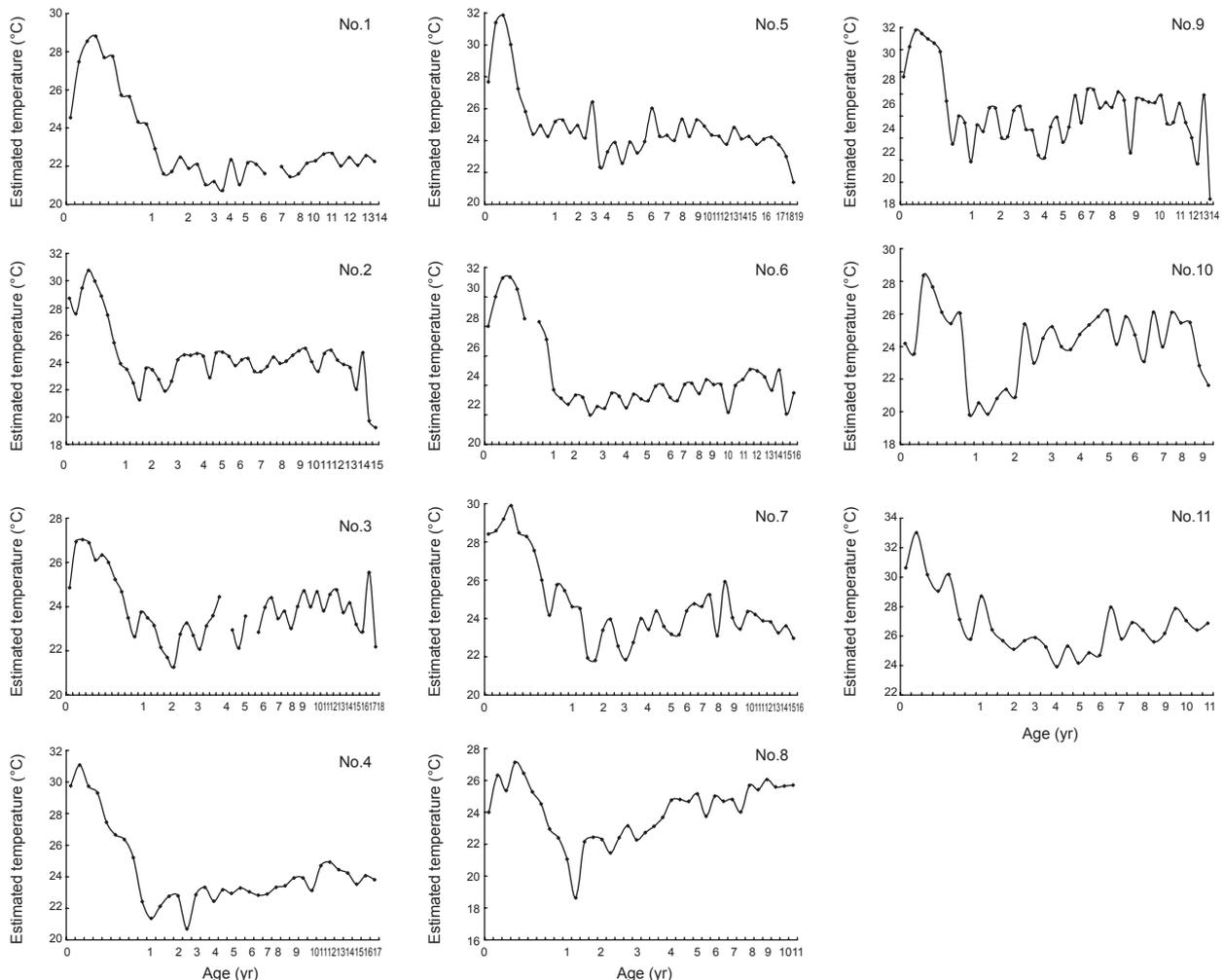


**Fig. 4.** Ambient water temperature inferred from otolith δ18O of the southern bluefin tuna (SBT), showing 3 stages of the migratory life history. Stage I: larval and juvenile stage (from the beginning to the highest temperature), stage II: young SBT (from the highest temperature to an approximate age of 1 yr), stage III: subadult to adult stage (from an approximate age of 1 yr to the end of life). The X-axis represents the annuli of each otolith. The number of isotopic data between 2 annuli roughly reflects their temporal resolution.

as observed by Jenkins and Davis (1990). The increment width dramatically increased and reached a maximum of 55-95 μm at 40-50 d in the 2nd stage (Itoh and Tsuji 1996), which was very similar to the profile of increment width observed herein. Consequently, the 1st stage that represents the larval stage of the SBT as described by Itoh and Tsuji (1996) was not observed in our otolith samples, but the later profile of increment widths was similar to our observations as shown in figure 2b. We corrected the daily age from the otolith core to the first inflection by adding 13 d. The corrected age may be still be underestimated by several days since the larval stage could last approximately 20 d (Jenkins and Davis 1990). Therefore, the first inflection of the ventral-medial arm was formed approximately 30-40 d after hatching.

The patterns of isotopic enrichment and depletion were similar across the 11 otoliths examined. The water temperatures (27.2-27.7 °C) measured at the sampling locations of SBT larvae (7-18 d old) by Jenkins and Davis (1990) were in the ranges of water temperatures estimated from otolith $\delta^{18}O$ values (Figs. 4, 5). Otolith $\delta^{18}O$

profiles suggested that SBT larvae and juveniles (approximately 1 mo old) experienced water temperatures that increased by approximately 3-5°C (Figs. 4, 5). The reason for the increased water temperatures during the early larval stage is unknown, given that water temperatures on the spawning grounds southeast of Java do not increase by 3-5°C over a 30-40 d period during the peak spawning season (Nov.-Feb.). The increased water temperatures are not caused by fish moving large distances or changing depths because larval collection data and satellite-tracked drogues suggest that most larvae likely complete development in the mixed layer at depths of < 35 m (Davis et al. 1990) near the spawning site (Jenkins and Davis 1990). Water temperatures may be underestimated by the analysis of otolith $\delta^{18}O$ levels near the core due to the larger portions of later deposited calcium carbonate from the distal and proximal surface in the composition of milled powder obtained from the core to the first inflection (Fig. 2A). The measured otolith $\delta^{18}O$ values were higher than the actual value due to the mixing of enriched $\delta^{18}O$ calcium carbonate deposited in the subadult to adult stages.
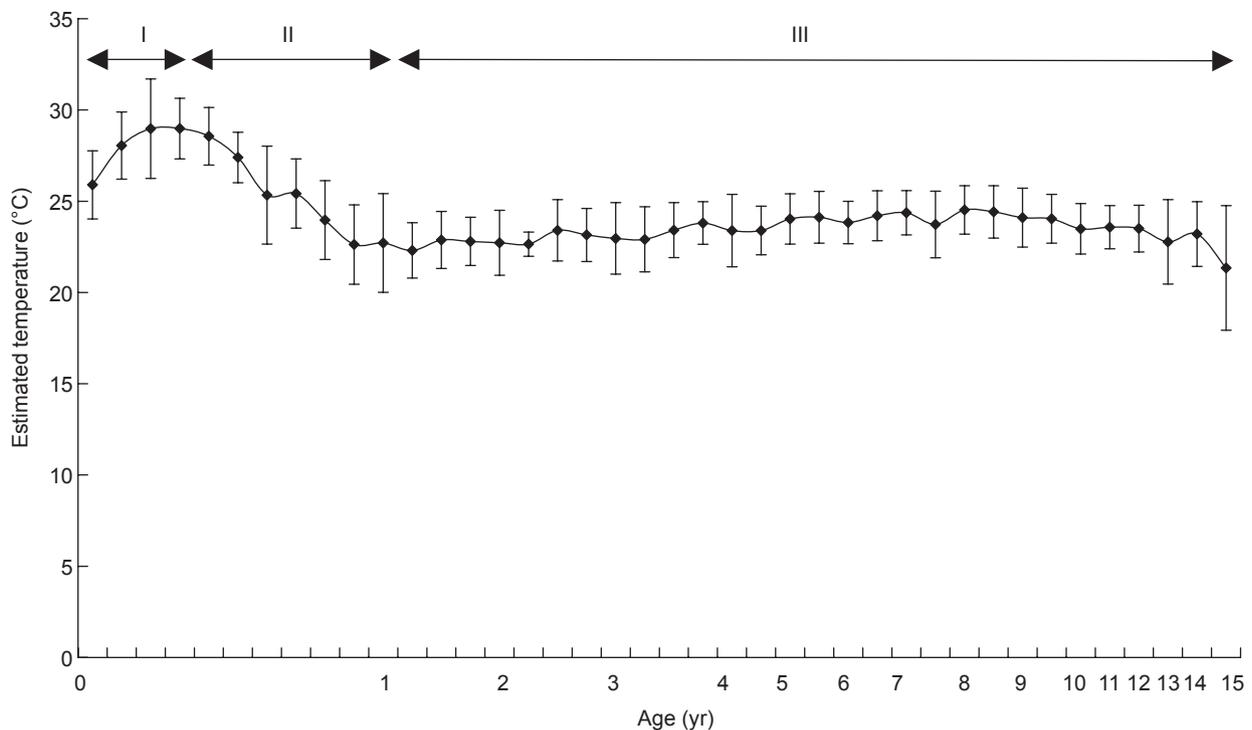


**Fig. 5.** Mean (± SD) ambient water temperatures inferred from otolith $\delta^{18}O$ values of 11 southern bluefin tuna (SBT) showing 3 stages of the migratory life history. Stage I: larval and juvenile stage (from the beginning to the highest temperature), stage II: young SBT (from the highest temperature to an approximate age of 1 yr), stage III: subadult to adult stage (from an approximate age of 1 yr to the end of life).

Water temperatures inferred from otolith $\delta^{18}O$ profiles decreased rapidly by 8-10°C during the 2nd stage corresponding to ages from about 1-12 mo in all examined samples. Fishery data suggest that most, if not all, juvenile SBT emigrate from the tropical ocean to Western Australia near the western edge of the GAB as 1 yr olds and become available to the Australian surface fishery as 2-4 yr old. The southward migration of juvenile SBT may be aided by the Leeuwin Current that flows strongly southwards along the Western Australian coast from North West Cape (~22°S) to Cape Leeuwin (34°S) and continues into the GAB (Cresswell 1991). During the summer, young SBT aggregate near the surface in the coastal waters off southern Australia according to fishery and ultrasonic telemetric survey data (Davis and Stanley 2002). Therefore, the continuous water temperature decrease after the first inflection of the otolith, at about 1 mo of age, may represent the beginning of an active southerly migration of the SBT from the spawning ground to the nursing grounds. The mean Feb. surface water temperature difference of 8-10°C (Tchernia 1980) between the SBT spawning grounds (approximately 28°C) and the GAB (approximately 19-20°C) corresponds to changes in ambient water temperature estimated from otolith $\delta^{18}O$ values.

The 3rd stage showed relatively cool water temperatures varying approximately 15-25°C. Estimated water temperatures were generally in the range of SSTs where the SBT of these ages are found (30-50°S) (Tchernia 1980), and they also agree with values measured by ultrasonic telemetry (Davis and Stanley 2002). However, otolith edges of samples collected from the central Indian Ocean and tropical spawning grounds did not show the expected high water temperature signature, nor was a cyclic hot and cold pattern found in otoliths after the age-at-maturity (12-13 yr), which would be expected as mature SBT migrate to and from the spawning ground.

The temporal resolution of the estimated water temperature history based on the present milling and analytical approaches may be unable to define the spawning period of SBT in the tropical ocean. Each subsample milled from the otolith section deposited at the adult stage (>10 yr) may represent a time span of 6-12 mo. Block et al. (2001) found, from archival tags results, that the ABT can cross the Atlantic in 40 d. Teo et al. (2007) suggested that mature ABT on average spend 39 ± 11 d at the breeding ground during the spawning season. The duration of the SBT at the spawning ground may be similar to that of the ABT. Farley and Davis (1998) estimated 19-39 d for an adult SBT to migrate from the spawning ground to Tasmania. Therefore, SBT may be able to move to the spawning grounds in a comparably brief time span. Both the ABT and SBT are believed to breed near the sea surface (Block et al. 2001), but the SBT spends most of the time in deeper zones of the tropical spawning grounds (Davis and Farley 2001) to regulate body temperature or avoid overheating (Kitagawa et al. 2001). Therefore, the SBT's experience of high water temperatures in the tropical spawning grounds might be too short to be recorded in otolith $\delta^{18}O$.

Otolith $\delta^{13}C$ represents a mixture of dietary carbon and dissolved inorganic carbon (DIC). Variations in otolith $\delta^{13}C$ are negatively related to a fish's metabolic rate, which is influenced by such factors as temperature, activity level, and age. About 17%-30% of otolith C is metabolically derived, while the rest is derived from DIC in the ambient water (Høie et al. 2003, Solomon et al. 2006). Since DIC shows little variability with water temperature and latitude in the open ocean (~1‰, Kroopnick 1980), metabolism, diet, and trophic level may have more-prominent effects on otolith $\delta^{13}C$ than does DIC (Schwarcz et al. 1998, Jamieson et al. 2004).

SBT otolith $\delta^{13}C$ levels generally followed the same pattern as otolith $\delta^{18}O$, but the values were very negative, probably due to the highly active metabolism of the tuna. The lowest otolith $\delta^{13}C$ of -9‰ to -10‰ in the early life stage corresponded to the highest metabolic rate and warmer water temperatures. Fish metabolic rate is negatively related to its size but positively related to temperature (Gillooly et al. 2001). The mean value of 11 otoliths analyzed herein was -7.03‰ ± 0.94‰, which is close to the reported value of -7.9‰ for the same species using a whole otolith by Kalish (1991). Mean otolith $\delta^{13}C$ values of the SBT and ABT (Radtke et al. 1987, Rooker and Secor 2004) are the most negative values in a review of 60 marine species (Sherwood and Rose 2003). The rapidly increasing (enriched) period of otolith $\delta^{13}C$ corresponds to the southward migration of the juveniles to the nursery grounds before an age of 1 yr. During the southerly migration, SBT grow to 50 cm in fork length in approximately 1 yr (Itoh and Tsuji 1996), but the water temperature continuously decreases by approximately 8-10°C. A larger size and lower water temperatures may slow the fish's metabolism and enrich otolith $\delta^{13}C$, as may a shift in the diet from low to high trophic levels. Sarà

and Sarà (2007) observed a dietary shift in ABT from zooplankton and small pelagic fish at age 0+ to cephalopods and medium-sized pelagic fish at an age of > 2 yr. Older SBT (> 10 yr) tend to have more-enriched otolith $\delta^{13}C$ values. This result is consistent with the observation of an increasing trophic level as the ABT grows (Sarà and Sarà 2007). A high diversity of prey species (Young et al. 1997), wide water temperature tolerance, and different environmental characteristics of the geographic habitats may account for variable otolith $\delta^{13}C$ levels during the adult stage of the SBT. Otolith $\delta^{13}C$ is influenced by multiple factors, including, DIC, diet, and water temperature, which makes its interpretation difficult. However, this study for the first time reveals chronological variations in SBT otolith $\delta^{13}C$ values, which is useful and additive to the understanding of tuna autecology.

This study demonstrates the value of otolith stable isotope analyses for studying the migratory life history of the SBT, especially at the young stage before the thermal conservation ability is fully developed. Further studies that reveal the timing to acquire endothermy of tuna and collect information on water $\delta^{18}O$ values will be useful to improve our ability to interpret a tuna's life history from otolith $\delta^{18}O$ levels. Data on chronological variations in stable isotope concentrations add to our present understanding of the SBT and may be useful in studying other migratory marine species that experience environmental changes, particularly in water temperature, during their life history.

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