

Natal origin of Pacific bluefin tuna *Thunnus orientalis* inferred from otolith oxygen isotope composition

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ABSTRACT: Pacific bluefin tuna *Thunnus orientalis* has 2 spawning grounds with different spawning seasons. One is in the northwest region of the Philippine Sea during May to June and the other is in the Japan Sea from late July to August. The oxygen isotope level in otoliths is affected by water oxygen isotope composition and negatively correlated to the ambient water temperature. We developed a technique using the temporal profiles of otolith $\delta^{18}\text{O}$ to discriminate the natal origin of Pacific bluefin tuna. For Age-0 tuna caught in the Japan Sea ($n = 35$), the $\delta^{18}\text{O}$ values varied from -2.5 to -2.2‰ between the otolith core and a distance of ca. $600\ \mu\text{m}$ from the core, and then the values increased (enriched) to about -0.8 to -1‰ at 700 to $1000\ \mu\text{m}$. For Age-0 tuna caught in the Pacific Ocean ($n = 41$), the otolith $\delta^{18}\text{O}$ values varied between -2.8 and -2.2‰ at 0 to $1000\ \mu\text{m}$ from the otolith core and then increased to ca. -1.5 to -1‰ at around 1200 to $1600\ \mu\text{m}$ from the core. The different profiles of otolith $\delta^{18}\text{O}$ values between tuna caught in the Pacific Ocean and the Japan Sea evidently reflect different water temperatures along the migratory route from their natal origin. Fish hatched in the Japan Sea experience water temperatures of $<15^\circ\text{C}$ in winter, about 4 mo after hatching, while fish hatched in the Philippine Sea experience winter water temperatures of 17 to 18°C about 6 mo after hatching. The temporal difference in experienced water temperatures produces an earlier increase of otolith $\delta^{18}\text{O}$ values for tuna caught in the Japan Sea than in the Pacific Ocean. The location of the first winter signal recorded as enriched $\delta^{18}\text{O}$ in the otolith is a valuable proxy for tracking the natal origin of Pacific bluefin tuna.

KEY WORDS: *Thunnus orientalis* · Pacific bluefin tuna · Otolith isotope

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INTRODUCTION

The Pacific bluefin tuna *Thunnus orientalis* is a highly migratory species that is widely distributed in the northern Pacific Ocean (Bayliff 2001), with occasional catches in the southern Pacific Ocean around New Zealand and Australia (Ward et al. 1995, Smith et al. 2001). Adult bluefin tuna spawn in the northwest region of the Philippine Sea, between eastern Taiwan and the Ryukyu Islands, from late April to June (Chen et al. 2006, Tanaka et al. 2006) and in the

southern Japan Sea in late July to August (Tanaka et al. 2007, Itoh 2009). Tuna hatched in the Philippine Sea are transported northward to Japan by the Kuroshio and Tsushima Currents and feed around coastal waters off Japan in the Pacific Ocean and the Japan Sea during the first year (Inagake et al. 2001, Itoh et al. 2003). On the other hand, Age-0 tuna hatched in the Japan Sea migrate mainly around the Japan Sea and the East China Sea (Itoh et al. 2003). As the tuna grow, they may leave the Japan Sea and migrate into the Pacific.

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The relative contributions of fish hatched in the 2 spawning grounds to the total Age-0 catch were tentatively estimated by Itoh (2009) from fertilization dates estimated using otolith daily increments. The sub-cohort fertilized up to early July was considered to originate from the spawning ground in the Philippine Sea and those fertilized after that period to come from the Japan Sea. However, the fertilization dates of tuna older than 1 to 2 yr cannot be reliably estimated from otolith daily increments because of accumulated errors with age (Radtke 1984). More refined methods are needed for determining the natal origin of older mature Pacific bluefin tuna in order to clarify their migratory life history.

In addition to age, fish otoliths record environmental information throughout the life of the fish (Campana 1999). Rooker et al. (2001) analyzed the otolith chemistry of juvenile Pacific bluefin tuna by solution-based inductively coupled plasma mass spectrometry. Otolith elemental composition clearly differed between samples collected in the Pacific Ocean off Shikoku and from the marginal seas, i.e. the Japan Sea and East China Sea, which suggests that elemental otolith composition could be an indicator to distinguish the nursery grounds. However, as mentioned above, juvenile tuna migrate from the 2 different spawning grounds to the nursery grounds in the coastal regions of Japan, so the nursery grounds do not necessarily represent the natal origin of fish, especially for those caught in the East China Sea and the Japan Sea, which support a mixture of fish from both spawning grounds (Itoh 2009).

Isotope levels in otoliths are also used as an indicator of the ambient environment (Campana 1999). Oxygen isotope levels in otoliths are affected by water oxygen isotope composition and ambient water temperature (e.g. Patterson et al. 1993, Thorrold et al. 1997, Høie et al. 2003). The otolith carbon isotope level is also affected by the carbon isotope ratios of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$), diet, and metabolism of the fish (Solomon et al. 2006). Otolith oxygen and carbon isotopes have been used to discriminate populations of Atlantic bluefin tuna *Thunnus thynnus* hatched and feeding in the Mediterranean Sea or western Atlantic Ocean (Rooker et al. 2008a,b, Schloesser et al. 2010).

Different isotope compositions may be recorded in otoliths of Pacific bluefin tuna hatched in the Philippine Sea and the Japan Sea because of differential hydrological characteristics, such as temperature and salinity, prevailing at their spawning grounds and along their migration routes to the nursery grounds. Instead of analyzing the whole otolith, we applied a topographical sampling technique to sequentially collect otolith materials from the core to the edge for isotope analysis (Shiao et al. 2009). This technique pro-

vides temporal resolution of otolith stable isotope composition along the core to the edge, which records the history of ambient hydrological characteristics through the larval, juvenile, and young life stages of the fish.

In this study, we determined the natal origin of Pacific bluefin tuna based on temporal and spatial profiles of otolith isotope ratios from the core to the edge via a topographical subsampling technique. The otoliths were collected from young-of-the-year fish caught in the Pacific Ocean and the Japan Sea by Japanese troll vessels and were considered as reference samples from the 2 spawning grounds (Philippine Sea, Japan Sea). Otolith oxygen and carbon isotope profiles from the core to the edge were compared for samples from the Pacific Ocean and the Japan Sea. The results indicated that the oxygen isotope composition could effectively determine the natal origin of Pacific bluefin tuna. Environmental factors which might possibly affect the isotope composition through their life history were compared with sea surface temperatures derived from satellite images.

MATERIALS AND METHODS

Fish collection. Pacific bluefin tuna ($n = 41$) were collected by port samplings in the main landing ports of coastal troll fisheries in Tosa Bay, which faces the Pacific Ocean, and 35 were captured in the Japan Sea (Fig. 1, Table 1). We concurrently sampled 'lots' of 5 to 10 fish at the same fishing port and the same fishing day. Samplings in the Japan Sea were conducted at Hagi-port in Yamaguchi Prefecture and Himi-port in Toyama Prefecture (Fig. 1). In addition, 5 fish were collected with a mid-water trawl in the southern Japan Sea (KY73-81) by RV 'Shunyo Maru' of the National Research Institute of Far Seas Fisheries (Tanaka et al. 2007). For clarity, only 'Pacific Ocean' and 'Japan Sea' will be used subsequently when referring to the regions sampled.

Otolith preparation and analysis. Sagittal otoliths were dissected from the tuna after the measurement of fork length and weight. Otoliths were embedded in epofix resin (Struers), and a transverse section approximately 400 μm thick was cut from the resin block by a slow speed saw (Isomet, Buehler) fitted with a diamond-edged blade. For the 5 smallest samples of lot J1, a section along the postrostrum and the core was cut for the oxygen and carbon stable isotope analyses. The section was embedded in epofix resin again, ground and polished repeatedly on 1 side to reveal the incremental pattern on a grinder-polisher machine (Metaserv 2000, Buehler). An image of the otolith section was taken by a compound microscope (Olympus BX-51) equipped with a digital camera

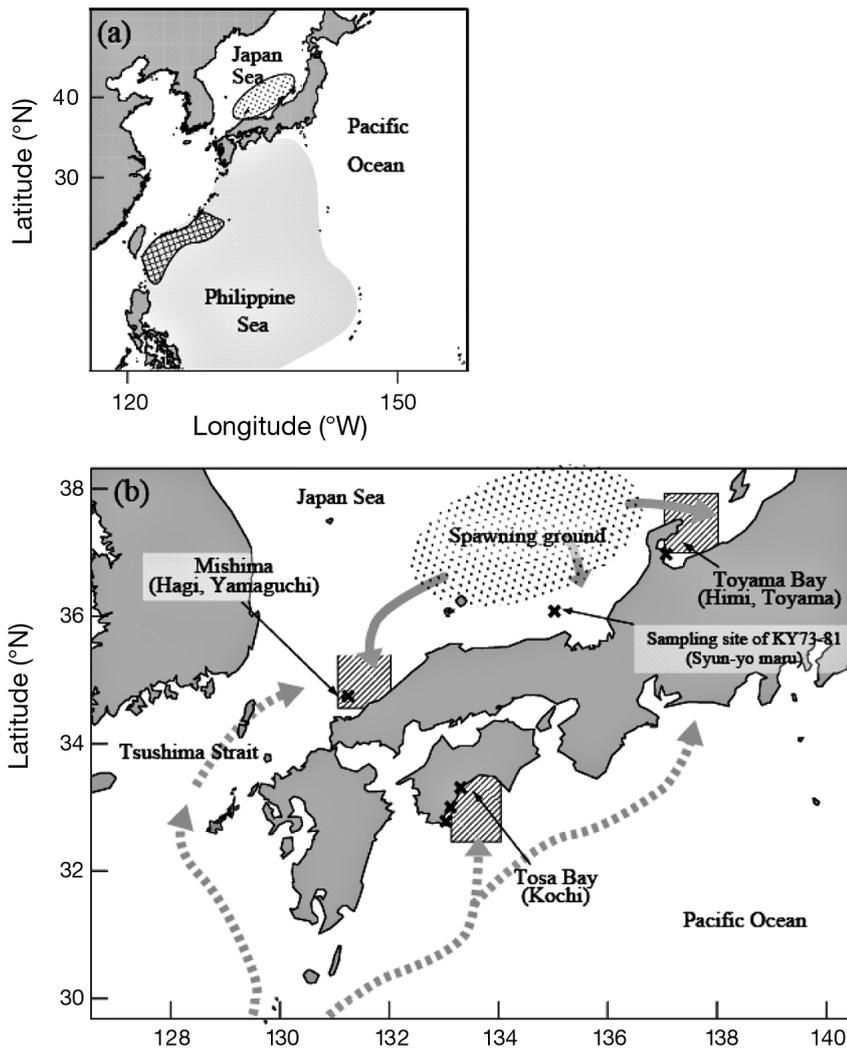


Fig. 1. *Thunnus orientalis*. (a) Assumed spawning grounds in the Japan Sea (dotted region) and the Philippine Sea (cross-hatched region) and (b) port sampling locations indicated by 'x'. The assumed routes of recruitment from the 2 spawning grounds are shown by arrows, and the fishing grounds near the sampling sites are shown by hatched squares

(Olympus DP-71) using transmitted light (Fig. 2). Otolith length was measured from the distal end to the core along the ventral-medial arm. The relationship between fish and otolith size was examined with the otolith lengths and fork lengths of corresponding fish.

Otolith powders were collected by a computerized micromill (Merchantek) along several segmented lines that followed the otolith growth zones marked on the real-time computer image from the camera on the top of the micromill. The micromill software interpolated new lines between 2 adjoining segmented lines according to the number of samples wanted (Fig. 2). Otolith powders, weighting approximately 25 to 35 μg , were collected from the distal end to the core along the ventral-medial arm as described by Shiao et al. (2009). Milled samples were then collected sequentially between each of the lines. Milling depth was set to approximately 200 μm . After each milling, the otolith image was recorded. The temporal resolution represented by each sample varied from <1 mo during the early life stage to 1 to 2 mo at the otolith edge.

The general trends of otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ profiles were defined by arbitrary categories of distance from the core to the edge, i.e. 0–250, 250–500 μm , and then every 100 μm to the otolith edge. Each isotopic

Table 1. *Thunnus orientalis*. Sampling data. Otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (‰) represent the mean values of all isotope data of lot J1. For other samples, otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ represent the mean values beyond 800 to 1000 μm from the otolith core, which are used for discriminant function analysis. N: sample size, FL: fork length. Data are means \pm SD

Lot ID	N	Sampling ports	Fishing area	Catch date	FL (mm)	Mass (g)	Otolith $\delta^{18}\text{O}$	Otolith $\delta^{13}\text{C}$
P1	5	Kamino-Kae	Tosa Bay, Pacific	12 Aug 2003	242 \pm 14	263 \pm 50	-2.56 \pm 0.19	-9.86 \pm 0.34
P2	5	Kamino-Kae	Tosa Bay, Pacific	27 Aug 2002	291 \pm 46	448 \pm 133	-2.56 \pm 0.04	-9.75 \pm 0.54
P3	5	Saga	Tosa Bay, Pacific	18 Oct 2000	412 \pm 31	1685 \pm 195	-2.57 \pm 0.2	-9.02 \pm 0.66
P4	5	Kanno-ura	Tosa Bay, Pacific	27 Nov 2003	471 \pm 14	2386 \pm 369	-2.63 \pm 0.07	-9.22 \pm 0.45
P5	10	Saga	Tosa Bay, Pacific	17 Dec 2003	468 \pm 10	2232 \pm 153	-2.28 \pm 0.27	-8.17 \pm 1.09
P6	5	Simono-Kae	Tosa Bay, Pacific	19 Feb 2007	532 \pm 10	3420 \pm 298	-2.71 \pm 0.26	-9.21 \pm 1.03
P7	6	Simono-Kae	Tosa Bay, Pacific	7 Mar 2007	540 \pm 44	3593 \pm 915	-2.37 \pm 0.34	-9.27 \pm 0.50
J1	5	Shoyo-maru	36° N 135° E, Japan Sea	18 Sep 2004	201 \pm 4	154 \pm 9	-2.30 \pm 0.12	-10.77 \pm 0.31
J2	10	Hagi	Near Mishima, Japan Sea	19 Dec 2002	363 \pm 15	932 \pm 108	-1.37 \pm 0.37	-8.39 \pm 0.74
J3	10	Himi	Toyama Bay, Japan Sea	24 Jan 2003	313 \pm 11	606 \pm 66	-1.54 \pm 0.36	-8.38 \pm 0.54
J4	10	Himi	Toyama Bay, Japan Sea	5 Feb 2004	338 \pm 29	771 \pm 255	-1.3 \pm 0.14	-7.84 \pm 0.67

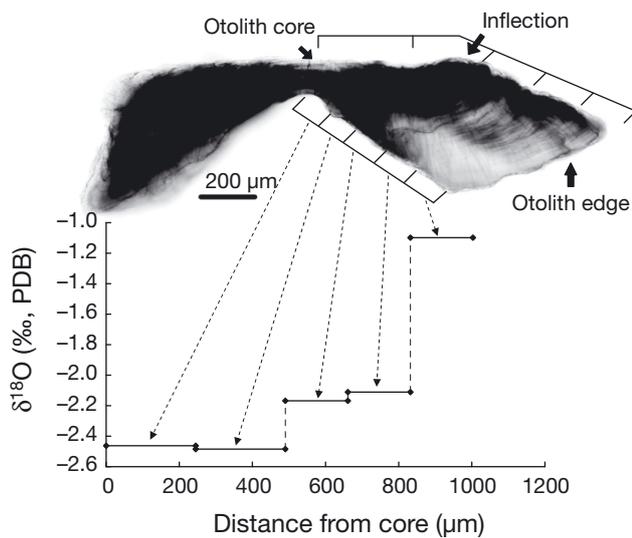


Fig. 2. *Thunnus orientalis*. Transverse section of the sagittal otolith of tuna No. 1914. The solid lines above and below the ventral-medial arm define the subsamples of the otolith collected by the micromill for isotopic analysis. The correspondence of otolith $\delta^{18}\text{O}$ values with each subsample is represented by the horizontal lines. Scale = 200 μm

data was assigned to a category according to the distance from the otolith core to the middle point of the subsample milled from the otolith. The mean and standard deviation of the data within a given category were calculated for individual tuna collected in the Japan Sea and the Pacific Ocean. This rule was not applied to the 5 smallest samples of lot J1 since their otoliths were not prepared for transverse section (see above). Annual variations of isotopic profiles were evaluated using tunas of similar age but collected in different years. Mean otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in each category of lots P1 to P2, P3 to P4, and J3 to J4 were analyzed by paired *t*-tests.

The oxygen and carbon isotope compositions were analyzed using a Finnigan MAT 253 mass spectrometer (Thermo Electron Corporation). The CO_2 gas for isotope measurements was produced by reaction with pure orthophosphoric acid at 70°C in an automated on-line system (Kiel carbonate device) with acid added to the sample in individual reaction vials. The long-term reproducibility of the Finnigan MAT 253 is better than $\pm 0.08\text{‰}$ for $\delta^{18}\text{O}$, and better than $\pm 0.05\text{‰}$ for $\delta^{13}\text{C}$ (1 SD) for an internal carbonate standard (25 to 35 μg) based on replicate measurements during this experimental period.

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

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000(\text{‰}) \quad (1)$$

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

Information on seawater $\delta^{18}\text{O}_{\text{SMOW}}$ in each sampling region was collected from published papers and database at NASA (<http://data.giss.nasa.gov/o18data/>). Water $\delta^{18}\text{O}_{\text{water}}$ values are reported on the Standard Mean Ocean Water (SMOW) scale that can be converted to the PDB scale by the equation: $\delta^{18}\text{O}_{\text{water}}$ (on the PDB scale) = 0.99978 ($\delta^{18}\text{O}_{\text{water}}$ on the SMOW scale) – 0.22 (Friedman & O'Neil 1977).

Otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ at different distance categories from the otolith core were used for discriminant function analysis (DFA) to determine the natal origin of the tuna. Lots P5 and J3, which were of similar age and were collected in the same year (2003), were used as training samples and the other lots as test samples. A jack-knife cross-validation was used to determine the classification accuracy for tuna collected from different localities, viz. the Pacific Ocean or the Japan Sea. DFA was performed with STATISTICA version 6.0 (StatSoft).

Sea surface temperature in the Pacific Ocean and the Japan Sea. Sea surface temperatures in the fishing grounds were derived from satellite images provided by the National Oceanic and Atmospheric Administration's (NOAA) Advanced Very High-Resolution Radiometer (AVHRR, <http://podaac.jpl.nasa.gov/>). The fishing grounds for the samples from Tosa Bay, near Mishima, and Toyama Bay were assumed to range from 133 to 134° E and 32.5 to 33.5° N, 131 to 132° E and 34.5 to 35.5° N, and 137 to 138° E and 37 to 38° N, respectively (squares in Fig. 1). This assumption of fishing grounds is based on the fact that Japanese troll fisheries are usually conducted near the coast within a 1 d trip range and land their catch at the nearest port from the fishing grounds of the day. Because temporal and spatial resolutions of the data were 5 d and 4 km, respectively, the sea surface temperatures within the assumed fishing area were simply averaged. The mean temperatures in the fishing grounds were compared with $\delta^{18}\text{O}$ values at the otolith edge, assuming the subsample milled from the otolith edge was deposited during the last 0.5 mo before the fish was caught for the 5 smallest samples in lot J1 (KY73-81) and 1.5 mo for the other samples. Since otolith $\delta^{18}\text{O}$ values were affected by water temperature and water oxygen isotope composition (Thorrold et al. 1997), values of $\delta^{18}\text{O}_{\text{otolith}}$ minus $\delta^{18}\text{O}_{\text{water}}$ (on the PDB scale) were linearly regressed against water temperature. Based on published data (Ki 1999, Postlethwaite et al. 2005), water $\delta^{18}\text{O}_{\text{SMOW}} = 0.2\text{‰}$ was used for lots P1 to P7, J1, and J2, and water $\delta^{18}\text{O}_{\text{SMOW}} = -0.4\text{‰}$ was used for lots J3 and J4 in the calculation (see 'Discussion').

RESULTS

Verification of tuna origin by size at catch

The average fork length of the samples was plotted with the predicted growth curves estimated by Itoh (2009) (Fig. 3). Itoh (2009) estimated fertilization days of young-of-the-year Pacific bluefin tuna by otolith daily increments, demonstrating that most of the samples caught in the Pacific Ocean were fertilized before early July while those caught in the Japan Sea presented 2 modes in the dates of fertilization: before and after early July. Itoh (2009) also found that fish caught in the Japan Sea originated from 2 spawning grounds in different spawning seasons. Itoh (2009) estimated 2 von Bertalanffy growth curves for the fish fertilized earlier (sub-cohort 1, SC1) and later (sub-cohort 2, SC2) than July 10, assuming dates of fertilization for SC1 and SC2 as the average of the estimated dates, i.e. 14 June for SC1 and 2 August for SC2 (Itoh 2009) (Fig. 3). Comparison of the predicted growth curves and the mean sample fork lengths in this study indicate that the tuna collected from the Pacific Ocean were hatched before early July, probably in the northwest region in the Philippine Sea. The tuna collected in the Japan Sea were probably hatched after early July, probably in the southern Japan Sea, considering that their size was smaller than that for the predicted growth curves of SC2.

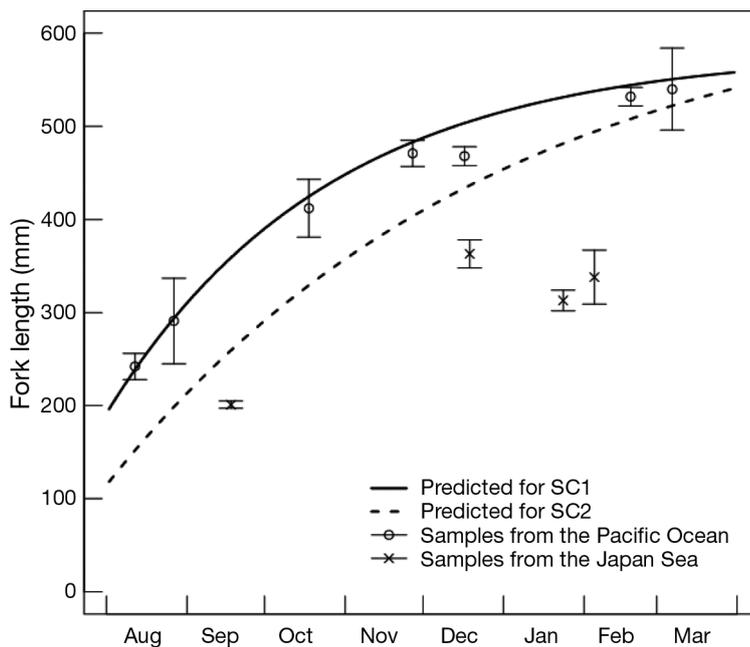


Fig. 3. *Thunnus orientalis*. Growth curves of young-of-the-year tuna derived from Itoh (2009) by assuming ova fertilization on 14 June in the Philippine Sea for sub-cohort 1 (SC1, solid line) and 2 August for SC2 (broken line) in the Japan Sea. Error bars show \pm SD of the fork length

Lot J2 caught in December had a relatively larger body size and otolith length compared to lots J3 and J4 collected in January and February, respectively (Fig. 3). Assuming similar hatching dates, these results suggest that tuna in lots J3 and J4 had a slower growth rate. A significant relationship was found between otolith length and fork length for Pacific bluefin tuna ($r^2 = 0.81$, $p < 0.01$, Fig. 4).

Variation in sea surface temperature

The mean daily sea surface temperature over several years in the sampled areas of the Pacific Ocean increased from 20 to 22°C in May to a peak at 28 to 29°C in August and then gradually decreased to 18–19°C in February (Fig. 5). For the sampled areas in the Japan Sea, mean daily temperatures gradually increased from 12 to 13°C in May to 23–25°C in August and then decreased to 9–10°C in February (Fig. 5). The mean temperature was always higher in the sampled areas of the Pacific Ocean than in the Japan Sea at the same period.

Oxygen stable isotope composition

Otolith $\delta^{18}\text{O}$ values of lots P1 to P4 only varied between -2.8 and -2.3‰ with 1 SD variations of $<0.4\text{‰}$ during their short life span of <6 mo (Fig. 6a–d). Samples of lot P5 collected in December also showed low (depleted) $\delta^{18}\text{O}$ between -2.8 and -2.2‰ from the otolith core to about 700 μm from the core. After the initial depleted stage, otolith $\delta^{18}\text{O}$ increased to -2.0 or -1.4‰ at the otolith edge (Fig. 6e). Otolith $\delta^{18}\text{O}$ values for lots P6 and P7, collected in February and March 2007, were also lower (depleted) $<-2.2\text{‰}$ before 800 to 1000 μm from the core, and then the values increased to about -1.5 or -1‰ at the otolith edge around 1400 to 1600 μm (Fig. 6f,g).

Lot J1 only showed depleted otolith $\delta^{18}\text{O}$ between -2.4 and -2.1‰ during their short life of <2 mo (Fig. 6h). Otolith $\delta^{18}\text{O}$ of lots J2 to J4, collected in winter, expressed consistent profiles among the fish, with depleted values <-2.2 to -2.8‰ before 600 μm from the core, and then the values increased to about -1.4 or -0.8‰ at the otolith edge (around 700 to 1000 μm from the core, Fig. 6i–k).

The initial depleted values of otolith $\delta^{18}\text{O}$, which might indicate warm water tempera-

ture, remained for a longer time in the samples from the Pacific Ocean (>1000 μm) than in those from the Japan Sea (<700 μm). Otolith $\delta^{18}\text{O}$ increased at an earlier stage in the samples from the Japan Sea than in those from the Pacific Ocean. Consequently, tuna from the Japan Sea might experience warm water temperatures for a shorter period in the summer and encounter decreasing water temperatures at a younger age. Mean otolith $\delta^{18}\text{O}$ did not vary significantly among otolith distance groups for the lots P1 to P2, P3 to P4, and J3 to J4 (paired *t*-test, all $p > 0.29$, Table 2).

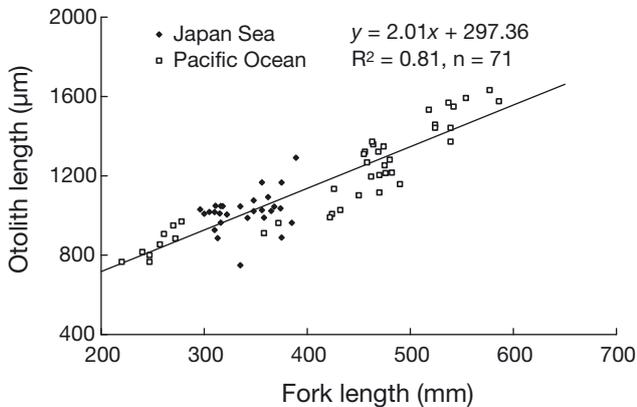


Fig. 4. *Thunnus orientalis*. Relationship between otolith length and fork length. Results from lot J1 are not included because their thin sections were cut from the postrostrum through the core, and were not a transverse section

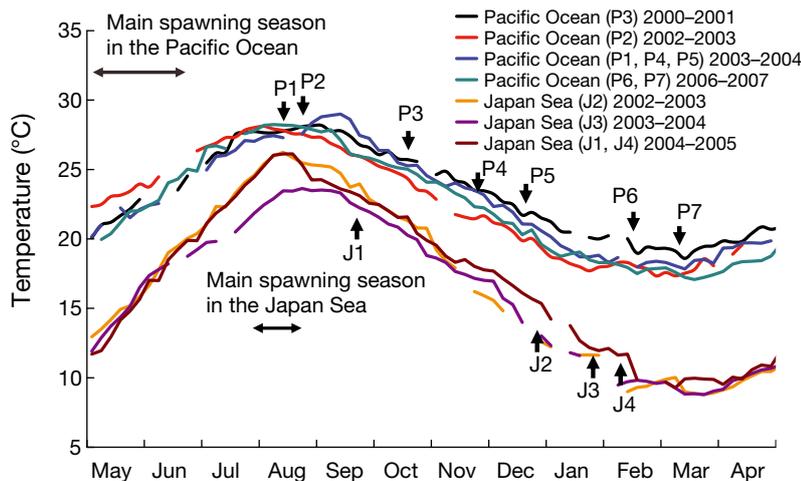


Fig. 5. Daily seawater temperatures for each sampling year at the fishing ground near each sampling port. Lines in different colors represent daily seawater temperature of each year from 2000 to 2007, except for 2005 in the Pacific Ocean, and from 2002 to 2005 in the Japan Sea. The water temperature at capture for each bluefin tuna lot (P1 to P7, J1 to J4) is indicated by a vertical arrow. The major spawning seasons in the Philippine Sea and the Japan Sea are indicated by horizontal lines (Chen et al. 2006, Tanaka et al. 2007). Missing data: no satellite images were available in the database for those periods, so temperatures could not be estimated

Carbon stable isotope composition

The values of otolith $\delta^{13}\text{C}$ ranged between -11 and -10‰ between the otolith core and 600 to 800 μm distance and then gradually increased to -9 or -6‰ at the otolith edge, depending on the fish age (Fig. 7a–k). Otolith $\delta^{13}\text{C}$ values of lot J1 varied around -11.5 to -10.5‰ due to younger fish ages (1 to 2 mo, Fig. 7h). Mean otolith $\delta^{13}\text{C}$ did not vary significantly among otolith distance groups for the lots P1 to P2, P3 to P4, and J3 to J4 (paired *t*-test, all $p > 0.05$, Table 2).

Isotopic trends in tuna of different origins

Average otolith $\delta^{18}\text{O}$ values within the defined otolith distance categories (Fig. 8a) clearly differed between tuna from the Japan Sea and from the Pacific Ocean. Although the average $\delta^{18}\text{O}$ values did not differ significantly between the core and about 400 μm , sample values from the Japan Sea were significantly higher after 500 μm than were the samples collected in the Pacific Ocean (Kruskal-Wallis 1-way analysis of variance, ANOVA, test on ranks, $p < 0.01$). In summary, the mean otolith $\delta^{18}\text{O}$ values of the tuna collected in the Japan Sea gradually increased from about -2.5‰ near 500 μm from the core to -1.0‰ at the otolith edge about 1000 μm distant. The mean otolith $\delta^{18}\text{O}$ values of tuna collected in the Pacific Ocean remained at a low level of about -2.5‰ from the otolith core to around 1000 μm and then slowly increased to about -0.8‰ at 1500 μm (Fig. 8a).

The mean otolith $\delta^{13}\text{C}$ profiles of Pacific Ocean samples declined over the first 3 distance groups, while the Japan Sea samples remained constant (Fig. 8b). Tuna from the Pacific Ocean and Japan Sea showed depleted otolith $\delta^{13}\text{C}$ isotope ratios up to 700 μm and then increased towards the edge. The isotope ratios between 250 and 1000 μm for tuna from the Japan Sea were significantly higher than for tuna from the Pacific Ocean (Kruskal-Wallis 1-way ANOVA test on ranks, $p < 0.01$).

Discriminant function analysis

A DFA of otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values at different distances indicated that the values between 800 and 1000 μm provided 100% classification success for most year classes, except for lot P7 in

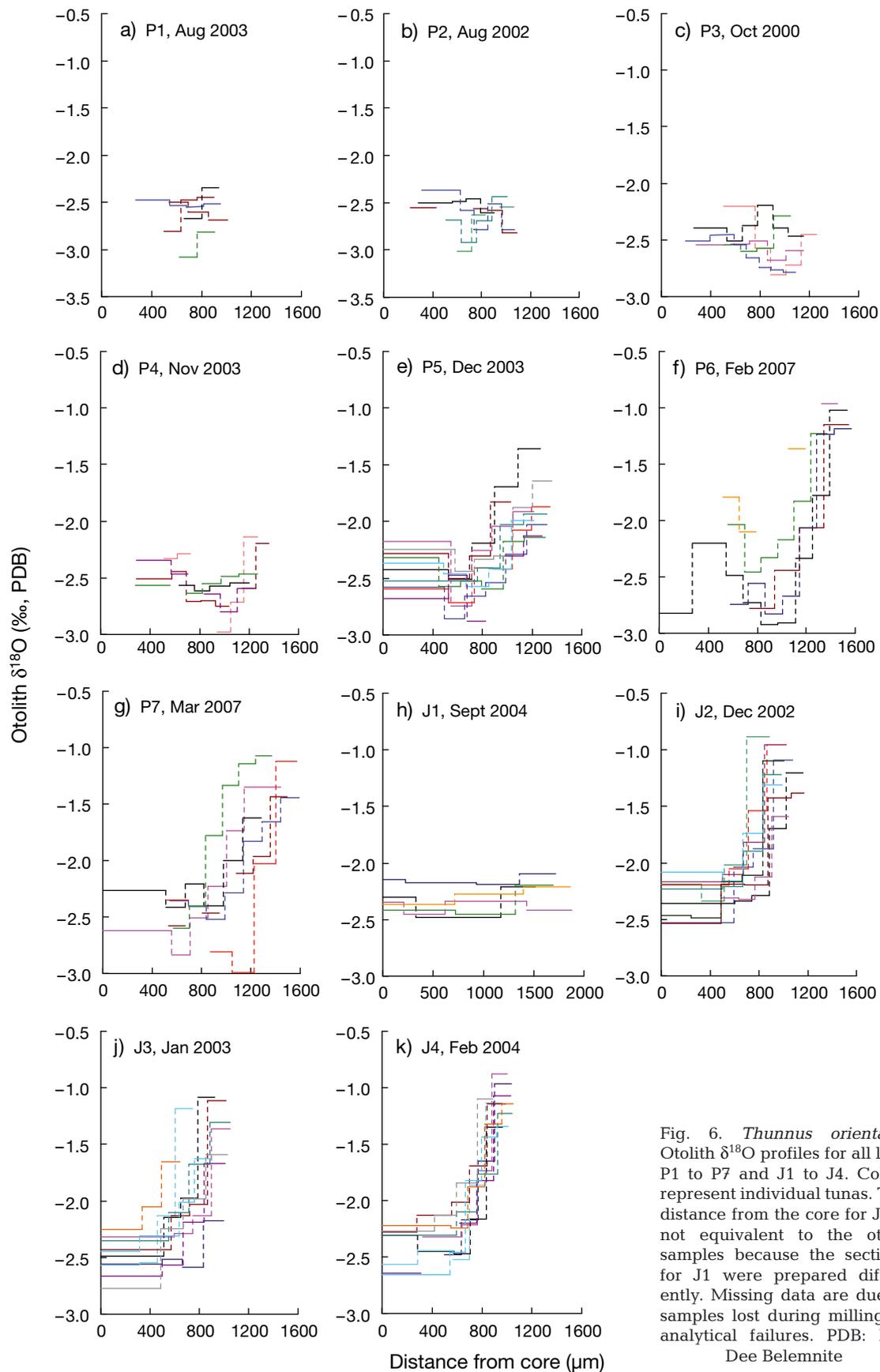


Fig. 6. *Thunnus orientalis*. Otolith $\delta^{18}\text{O}$ profiles for all lots, P1 to P7 and J1 to J4. Colors represent individual tunas. The distance from the core for J1 is not equivalent to the other samples because the sections for J1 were prepared differently. Missing data are due to samples lost during milling or analytical failures. PDB: Pee Dee Belemnite

Table 2. *Thunnus orientalis*. Paired *t*-test analysis of mean (\pm SD) $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (‰) in the defined distance from the otolith core between lots P1 to P2, P3 to P4, and J3 to J4. In some categories, only 1 value was available for the analysis. Numbers in parentheses show sample size

Lots	Otolith $\delta^{18}\text{O}$										p
	0–250 μm	250–500 μm	500–600 μm	600–700 μm	700–800 μm	800–900 μm	900–1000 μm	1000–1100 μm	1100–1200 μm		
P1	-2.47 (1)	-2.48 \pm 0.10 (3)	-2.81 (1)	-2.70 \pm 0.33 (3)	-2.58 \pm 0.08 (4)	-2.53 \pm 0.20 (4)	-2.689 (1)				0.70
P2			-2.68 (1)	-2.75 \pm 0.26 (4)	-2.61 \pm 0.13 (4)	-2.65 \pm 0.06 (2)	-2.51 \pm 0.07 (3)				
P3		-2.49 \pm 0.07 (3)	-2.50 \pm 0.04 (3)	-2.43 \pm 0.20 (3)	-2.54 \pm 0.12 (4)	-2.52 \pm 0.23 (4)	-2.59 \pm 0.23 (5)	-2.64 \pm 0.14 (4)	-2.45 (1)		0.29
P4		-2.47 \pm 0.12 (3)	-2.33 (1)	-2.44 \pm 0.12 (4)	-2.77 \pm 0.18 (3)	-2.62 \pm 0.08 (3)	-2.66 \pm 0.09 (3)	-2.65 \pm 0.22 (2)	-2.58 \pm 0.09 (5)		
J3	-2.51 \pm 0.22 (4)	-2.41 \pm 0.18 (9)	-2.21 \pm 0.33 (6)	-1.94 \pm 0.44 (5)	-2.07 \pm 0.30 (6)	-1.62 \pm 0.52 (3)	-1.54 \pm 0.37 (6)				0.48
J4	-2.42 \pm 0.18 (5)	-2.36 \pm 0.17 (7)	-2.30 \pm 0.25 (2)	-2.23 \pm 0.24 (8)	-1.97 \pm 0.21 (7)	-1.48 \pm 0.27 (9)	-1.10 \pm 0.17 (6)				
Lots	Otolith $\delta^{13}\text{C}$										p
P1	-11.08 (1)	-10.70 \pm 0.22 (3)	-11.17 (1)	-10.72 \pm 0.56 (3)	-10.85 \pm 0.27 (4)	-9.90 \pm 0.38 (4)	-9.73 (1)				0.89
P2			-10.82 (1)	-11.33 \pm 0.36 (4)	-10.78 \pm 0.55 (4)	-9.94 \pm 1.28 (2)	-9.75 \pm 0.38 (3)				
P3		-10.54 \pm 0.26 (3)	-10.58 \pm 0.47 (3)	-10.05 \pm 0.43 (3)	-9.44 \pm 0.52 (4)	-9.22 \pm 0.88 (4)	-8.82 \pm 0.61 (5)	-8.35 \pm 0.23 (4)	-7.99 (1)		0.32
P4		-10.52 \pm 0.50 (3)	-10.24 (1)	-10.56 \pm 0.53 (4)	-10.55 \pm 0.30 (2)	-9.57 \pm 0.67 (3)	-8.87 \pm 0.43 (4)	-8.21 \pm 0.38 (2)	-7.87 \pm 0.50 (5)		
J3	-10.16 \pm 0.10 (4)	-9.99 \pm 0.41 (9)	-9.98 \pm 0.16 (6)	-9.75 \pm 0.45 (5)	-9.47 \pm 0.40 (6)	-8.86 \pm 0.70 (3)	-8.25 \pm 0.53 (6)				0.052
J4	-10.00 \pm 0.30 (5)	-9.86 \pm 0.26 (7)	-9.95 \pm 0.21 (2)	-9.70 \pm 0.29 (8)	-9.18 \pm 0.45 (7)	-8.29 \pm 0.70 (9)	-7.28 \pm 0.74 (6)				

2007 (83%; Fig. 9, Table 3). The classification success of DFA models based entirely on $\delta^{18}\text{O}$ was also high (83 to 100 %). However, classification success based entirely on $\delta^{13}\text{C}$ was very low in almost all cases (0% for lots P1, P2, P4; 10 to 40% for lots P3, P6, P7, J2 to J4, and 66% for lot P5).

Relationship between otolith $\delta^{18}\text{O}$ and water temperature

Otolith $\delta^{18}\text{O}$ values nearest to the otolith edge were negatively related to water temperature between 16 and 28°C (Fig. 10a). Otolith $\delta^{18}\text{O}$ values of lots J3 and J4 were lower than the expected level extrapolated by the linear regression equation between water temperature and otolith $\delta^{18}\text{O}$ of other samples. After correcting the effects of water $\delta^{18}\text{O}_{\text{SMOW}}$, otolith $\delta^{18}\text{O}$ of lots J3 and J4 and other samples showed a strong ($R^2 = 0.93$) negative relationship with surface water temperature (Fig. 10b).

DISCUSSION

Otolith $\delta^{18}\text{O}$ values showed different profiles from the core to the edge for Pacific bluefin tuna from the Pacific Ocean and the Japan Sea, which indicated that oxygen isotope composition could be an effective indicator to determine natal origin. Tuna hatched in the Japan Sea in August experience their first winter (February) at 7 mo old, while tuna hatched in the Pacific Ocean in May to June experience their first winter (February) at 9 to 10 mo old. Otolith $\delta^{18}\text{O}$ increased by about 1.5‰ at around 700 to 1000 μm in tuna collected from the Japan Sea and at around 1200 to 1600 μm in tuna collected from the Pacific Ocean. The enrichment of otolith $\delta^{18}\text{O}$ reflects the decreasing winter water temperatures because otolith $\delta^{18}\text{O}$ is negatively related to water temperature (e.g. Campana 1999, Høie et al. 2003, present study). The first winter signal recorded in otolith $\delta^{18}\text{O}$ and its distance from the otolith core appears to be a useful natural marker to identify tuna natal origin.

Sea surface temperature in the possible spawning ground of the Japan Sea during the spawning season of late July to August (~25°C, Tanaka et al. 2007) is approximately 3°C colder than that in the spawning ground in the Philippine Sea during the spawning season of May to early July (~28°C, Tanaka et al. 2006), and also 2 to 3°C colder than that in the Tosa Bay sampling region of the Pacific Ocean in late July to August (Fig. 4). The colder water temperatures in the Japan Sea in summer to autumn would produce relatively enriched otolith $\delta^{18}\text{O}$ during the larval and juvenile

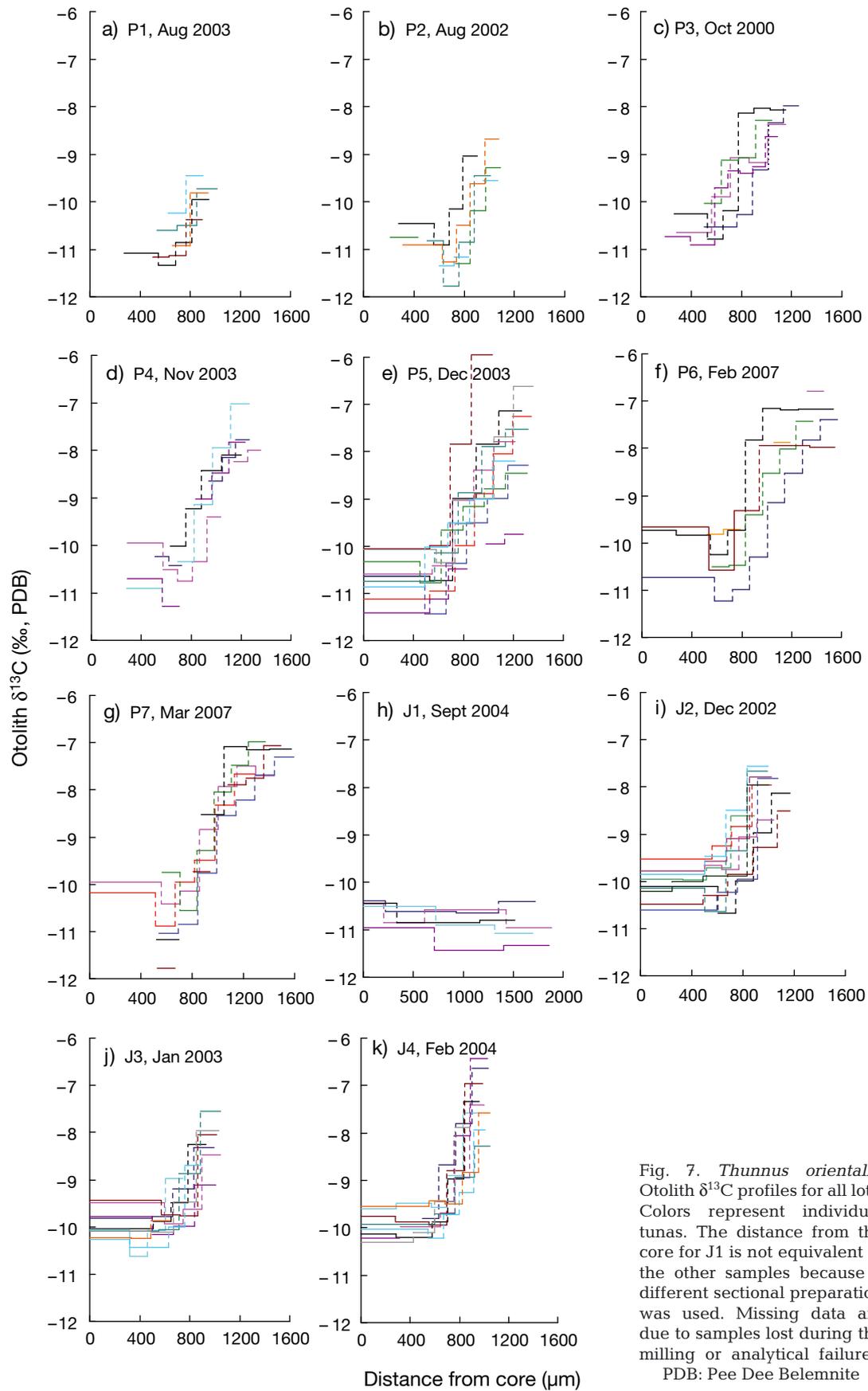


Fig. 7. *Thunnus orientalis*. Otolith $\delta^{13}\text{C}$ profiles for all lots. Colors represent individual tunas. The distance from the core for J1 is not equivalent to the other samples because a different sectional preparation was used. Missing data are due to samples lost during the milling or analytical failures. PDB: Pee Dee Belemnite

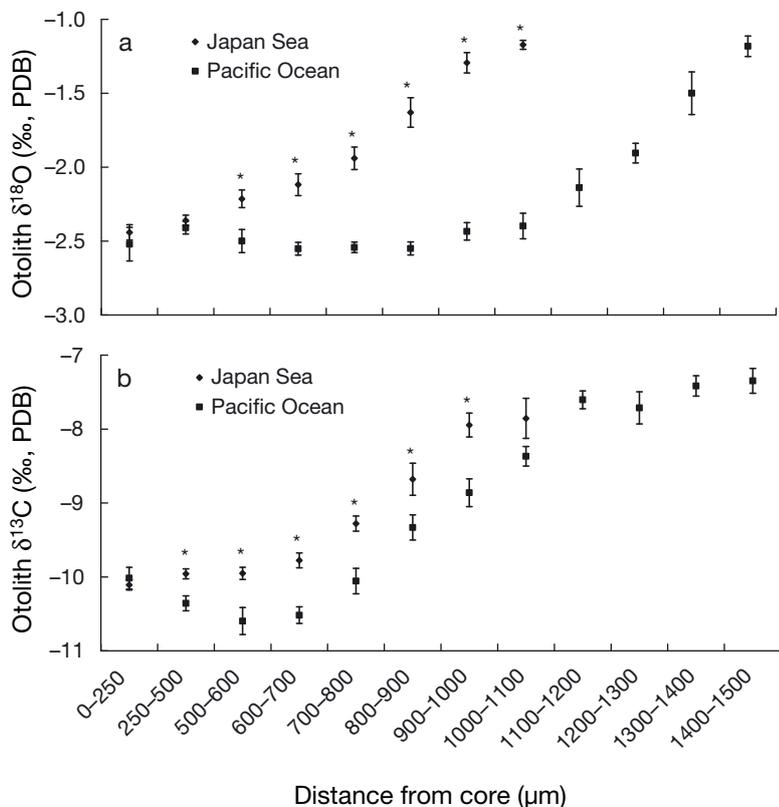


Fig. 8. *Thunnus orientalis*. Mean otolith (a) $\delta^{18}\text{O}$ and (b) $\delta^{13}\text{C}$ profiles of tuna collected in the Japan Sea and in the Pacific Ocean. Error bars are \pm SE. The asterisk indicates a statistically significant difference between 2 groups of the same distance category (Kruskal-Wallis 1-way ANOVA test on ranks, $p < 0.01$). PDB: Pee Dee Belemnite

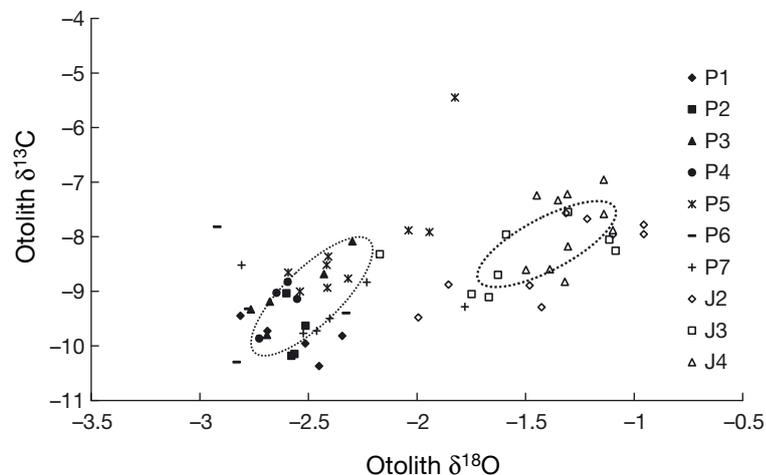


Fig. 9. *Thunnus orientalis*. Stable otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values from beyond 800 to 1000 μm from the core. Confidence ellipses (1 SD) are shown for tuna from the Pacific Ocean (lots P1 to P7) and the Japan Sea (lots J2 to J4). Refer to Table 1 for information on lots

stages compared to that of tuna feeding in the Pacific Ocean. However, this minor temperature difference between regions is not easily detected in tuna otoliths, especially those smaller than 500 μm (Fig. 8a). Otolith $\delta^{18}\text{O}$ profiles before the first winter overlapped for tuna collected in the Japan Sea and in the Pacific Ocean (Fig. 8a). Therefore, the stage from hatching to the first winter does not provide an effective signal to discriminate tuna originating from different spawning grounds.

This study shows a possible reflection of ambient water temperature in the isotopic ratio of tuna otoliths. The effect of the ambient water temperature on otolith $\delta^{18}\text{O}$ could be partly supported by Fig. 10. This result suggests that sea surface temperature could be an appropriate proxy of the ambient water temperature experienced by tuna, in conjunction with the fact that juveniles inhabit surface waters shallower than 100 m (Kitagawa et al. 2007).

Otolith $\delta^{18}\text{O}$ values are positively related to water $\delta^{18}\text{O}_{\text{SMOW}}$ composition as well as ambient water temperature (e.g. Campana 1999, Bastow et al. 2002). The seawater in the sampled region (i.e. Tosa Bay) in the Pacific Ocean is influenced by the Kuroshio Current, which is characterized by high salinity and enriched water $\delta^{18}\text{O}_{\text{SMOW}}$ values of about 0.4‰ (Shen et al. 2005). The water $\delta^{18}\text{O}_{\text{SMOW}}$ in the euphotic layer near the sampled regions of lots P1 to P7 in the Pacific Ocean were between 0 and 0.32‰, with a mean value of 0.2‰ ($n = 8$), derived from the Global Seawater Oxygen-18 Database (<http://data.giss.nasa.gov/o18data/>). In the Korea Strait and Tsushima Strait, the waters are characterized by the Tsushima Warm Current, which is composed primarily of waters of Kuroshio origin and has water $\delta^{18}\text{O}_{\text{SMOW}}$ values of 0 to 0.6‰ (Kim et al. 2005). Tuna of lot J2 were collected in this region. In the southern Japan Sea where tuna of lot J1 were collected, the sea surface water $\delta^{18}\text{O}_{\text{SMOW}}$ value varies between 0.02 and 0.22‰ (as shown in Fig. 2 of Postlethwaite et al. 2005). Based on the literature data, tuna of lots P1 to P7, J1, and J2 should experience a similar water $\delta^{18}\text{O}_{\text{SMOW}}$ of around 0.2‰ in each region sampled. This assumption was supported by the strong linear relationship ($R^2 = 0.91$,

Table 3. *Thunnus orientalis*. Classification of the natal origin for young-of-the-year tuna from the Pacific Ocean (lots P1 to P7) and the Japan Sea (lots J2 to J4). Lots P1 to P7 and J1 to J4 were assumed to have hatched in the Pacific Ocean and the Japan Sea, respectively. The natal origin of each tuna was determined by discriminant function analysis based on both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ or only $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values beyond 800 to 1000 μm from the otolith core. Small tuna ($n = 13$) that did not have isotopic data beyond 800 to 1000 μm from the otolith core were omitted from the discriminant function analysis

Lot ID	Assigned natal origin						Classification success (%)		
	Pacific Ocean			Japan Sea			Both $\delta^{18}\text{O}$		$\delta^{13}\text{C}$
	Both $\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$						
P1 (2003, Aug, n = 5)	5	5	0	0	0	5	100	100	0
P2 (2002, Aug, n = 4)	4	4	0	0	0	4	100	100	0
P3 (2000, Oct, n = 5)	5	5	2	0	0	3	100	100	40
P4 (2003, Nov, n = 4)	4	4	0	0	0	4	100	100	0
P5 (2003, Dec, n = 9)	9	8	6	0	1	3	100	88.9	66.7
P6 (2007, Feb, n = 4)	4	4	1	0	0	3	100	100	25
P7 (2007, Mar, n = 6)	5	5	1	1	1	5	83.3	83.3	16.7
J2 (2002, Dec, n = 9)	0	1	5	9	8	4	100	88.9	44.4
J3 (2003, Jan, n = 7)	0	0	5	7	7	2	100	100	28.6
J4 (2004, Feb, n = 10)	0	0	9	10	10	1	100	100	11.1

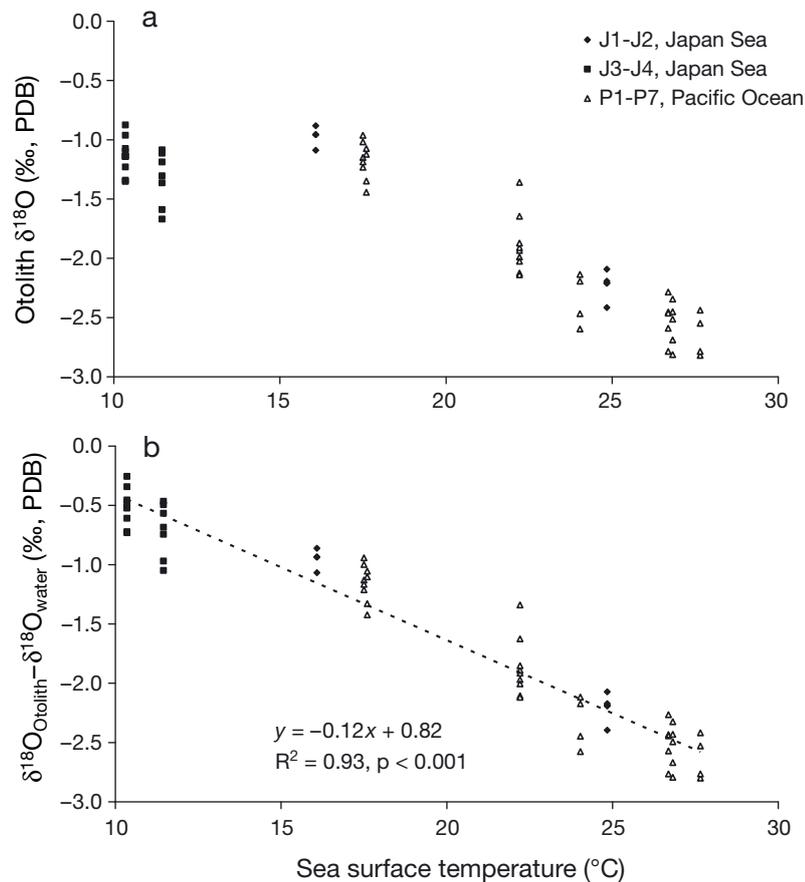


Fig. 10. *Thunnus orientalis*. Otolith $\delta^{18}\text{O}$ (a) at the edge and (b) the same, but corrected for the effects of water $\delta^{18}\text{O}_{\text{SMOW}}$, both relative to seawater temperature of the assumed fishing grounds. In (b), y-axis shows otolith $\delta^{18}\text{O}$ minus water $\delta^{18}\text{O}$ of Standard Mean Ocean Water (SMOW; 0.2‰ for lots P1 to P7 and J1 to J2, -0.4‰ for lots J3 and J4). PDB: Pee Dee Belemnite

$p < 0.001$) between otolith $\delta^{18}\text{O}$ and water temperature among these fish (Fig. 10a). For the samples of lots J3 and J4, their otolith $\delta^{18}\text{O}$ was lower than that expected from the fishing ground sea surface temperature. This suggests a possible nonlinear relationship of otolith $\delta^{18}\text{O}$ with ambient water temperature $< 16^\circ\text{C}$ or that factors other than water temperature may also affect otolith $\delta^{18}\text{O}$ of this tuna species. The depleted otolith $\delta^{18}\text{O}$ might be caused by relatively low (depleted) water $\delta^{18}\text{O}_{\text{SMOW}}$. Water $\delta^{18}\text{O}_{\text{SMOW}}$ in the Japan Sea is depleted in the deep waters ($\delta^{18}\text{O}_{\text{SMOW}} = -0.3$ to -0.8‰) that can upwell to the sea surface and reduce surface water $\delta^{18}\text{O}_{\text{SMOW}}$ to values of 0 to -0.4‰ (Ki 1999). The Japan Sea has a very weak vertical stability (Kim et al. 2002), and vertical mixing of surface and deep waters driven by the winter monsoon (Nakanishi & Minagawa 2003, Mooers et al. 2005) can extensively reduce water $\delta^{18}\text{O}_{\text{SMOW}}$ in the euphotic zone of the Japan Sea. Tuna of lots J3 and J4 were collected in late January and early February in the Japan Sea and thus may have experienced relatively depleted surface water $\delta^{18}\text{O}_{\text{SMOW}}$ that caused a depleted otolith $\delta^{18}\text{O}$ of around 0.5‰. This assumption is supported from the correction of otolith $\delta^{18}\text{O}$ by subtracting water $\delta^{18}\text{O}_{\text{SMOW}}$ of -0.4‰ , which improves the fit of lots J3 and J4 with other samples in the relationship with surface sea temperature (Fig. 10b).

Alternatively, Pacific bluefin tuna have a thermal conservation ability that can maintain a higher somatic temperature than that of the ambient water temperature (Kitagawa et al. 2007). It was possible that otolith $\delta^{18}\text{O}$ of lots J3 and J4 indicated temperatures 3 to 5°C higher than the actual ambient water temperatures if the thermal conservation ability functioned in these fish at water temperatures $< 16^\circ\text{C}$. Further studies are required to evaluate these 2 plausible explanations for the anomalous relation between otolith and ambient water $\delta^{18}\text{O}$ for tuna caught in the Japan Sea dur-

ing late January and February, i.e. depleted water $\delta^{18}\text{O}_{\text{SMOW}}$ or physiological effects on otolith $\delta^{18}\text{O}$ of bluefin tuna.

Otolith $\delta^{13}\text{C}$ represents a mixture of dietary carbon and $\delta^{13}\text{C}_{\text{DIC}}$ (Solomon et al. 2006, Weidel et al. 2007). $\delta^{13}\text{C}_{\text{DIC}}$ varied in a small range from 0.5 to 0.8‰ (April) and 0.4 to 1.2‰ (September) in the mixed layer in the Japan Sea off the western coast of Hokkaido (Itou et al. 2003). Near the Toyama Bay, Japan Sea, where tuna of lots J3 and J4 were collected, $\delta^{13}\text{C}_{\text{DIC}}$ varied between 0.4 and 1.04‰ during November to December 1995 (Kumamoto et al. 2008). The values of $\delta^{13}\text{C}_{\text{DIC}}$ off the Pacific Ocean coast of Japan varied from 0.7 to 1.3‰ (Itou et al. 2003, Bostock et al. 2010). $\delta^{13}\text{C}_{\text{DIC}}$ in the surface sea water was monitored along 137° E between 37° N to 1° S from 1981 to 1985, and $\delta^{13}\text{C}_{\text{DIC}}$ did not vary significantly from an average value of 1.3‰ (Inoue et al. 1987). Therefore, the natural variation of $\delta^{13}\text{C}_{\text{DIC}}$ within 1‰ cannot explain the large variation (~6‰) of tuna otolith $\delta^{13}\text{C}$ within several months. Ontogenetic effects such as metabolic rate (Høie et al. 2003) and diet (Nonogaki et al. 2007) may account for the consistent trend in otolith $\delta^{13}\text{C}$ profiles. Otolith $\delta^{13}\text{C}$ increased beyond about 750 μm from the core (Fig. 8b), which corresponds to a fork length of about 200 mm (Fig. 4). A fork length of 200 mm is the smallest size recruiting to the coastal area around Japan that is caught by troll fisheries. A rapid increase in otolith $\delta^{13}\text{C}$ was also found in the juvenile stage of southern bluefin tuna *Thunnus maccoyii*, and the authors speculated that the rapid increase of otolith $\delta^{13}\text{C}$ in *T. maccoyii* was due to the slowing down of fish metabolic rate (Shiao et al. 2009). Fish metabolic and growth rates are partly influenced by water temperature, which may indirectly affect otolith $\delta^{13}\text{C}$. Nevertheless, it is believed that otolith $\delta^{13}\text{C}$ is simultaneously controlled by $\delta^{13}\text{C}_{\text{DIC}}$, water temperature, and intrinsic factors such as physiology and ontogenetic development. The complicated nature makes otolith $\delta^{13}\text{C}$ an ineffective environmental proxy. Consequently, classification success of tuna natal origins by DFA was very low when entirely based on otolith $\delta^{13}\text{C}$.

CONCLUSIONS

The first winter signal recorded in otoliths of Pacific bluefin tuna as an enriched $\delta^{18}\text{O}$ signal and its distance from the otolith core can be used to discriminate their natal origin. Otolith $\delta^{18}\text{O}$ values increased over different time periods for tuna collected from the Japan Sea and Pacific Ocean. Values from the Pacific Ocean began increasing at around 1000 μm from the core of the otolith, while those from the Japan Sea began increasing at around 500 μm . DFA of tuna origin based

on $\delta^{18}\text{O}$ values beyond 800 to 1000 μm from the otolith core provided a high classification success rate. Additional studies utilizing this method will enable better understanding of the mixing of adolescent and adult tuna from different origins caught in all fishing grounds as well as their possible natal homing behavior.

Acknowledgements. We thank H.W. Chiang for assistance with isotope analysis, T.F. Yui for providing the carbonate standards, and B. Jessop, I. Thibault and 2 anonymous reviewers for useful suggestions. This study was financially supported by the Fishery Agency, Taiwan (98AS-10.1.1-FA-F4).

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Editorial responsibility: Stylianos Somarakis, Heraklion, Greece

Submitted: April 7, 2010; Accepted: October 8, 2010
Proofs received from author(s): November 29, 2010