Reconstruction of temperature experienced by Pacific bluefin tuna *Thunnus orientalis* larvae using SIMS and microvolume CF-IRMS otolith oxygen isotope analyses

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ABSTRACT: This study aimed to reconstruct temperatures experienced during the larval period by adult Pacific bluefin tuna *Thunnus orientalis* using high-resolution otolith stable oxygen isotope (δ¹⁸O) analysis. A novel otolith sample preparation protocol for secondary ion mass spectrometry (SIMS) analysis developed in this study reduced the background noise of SIMS measurements, enabling analyses of >10 times higher resolution around the otolith core compared to previous studies using conventional isotope ratio mass spectrometry (IRMS). The values obtained from SIMS were compared to those obtained by microvolume δ¹⁸Ootolith analysis using micromilling and conventional continuous-flow IRMS (CF-IRMS). There was a systematic offset (average 0.41‰ with SIMS resulting in lower values) most likely caused by matrix effects on SIMS δ¹⁸Ootolith values that can be calibrated using a strong linear relationship between SIMS and CF-IRMS measurements (r² = 0.78, p < 0.001). The core-to-edge δ¹⁸Ootolith of 5 Pacific bluefin tuna revealed fine-scale seasonal variations in water temperature agreeing with known migration patterns. In addition, the ambient water temperature experienced during larval stages (about 10–20 d post hatch) estimated from otolith core δ¹⁸O ranged from 26.7 to 30.7°C, overlapping with temperatures associated with the occurrence of larval Pacific bluefin tuna. Combining SIMS and microvolume CF-IRMS δ¹⁸Ootolith analyses offers a microscale examination of fish ecology that is not possible with conventional IRMS techniques. This novel method is particularly useful for understanding the early life history of fish that may be affected by climate change and reconstructing a well-resolved migration history for fish species that have small otoliths and/or narrow growth increments.

KEY WORDS: Temperature reconstruction · Otolith · Oxygen isotope analysis · Secondary ion mass spectrometry · SIMS · continuous-flow isotope ratio mass spectrometry · CF-IRMS · Pacific bluefin tuna · Sample preparation protocol
1. INTRODUCTION

Ocean warming has significant impacts on marine species and ecosystems, including high mortality, distribution shifts, and loss of spawning and nursery habitats (Perry et al. 2005, Kimura et al. 2010, Muhling et al. 2011, 2015). Species that spawn seasonally in relatively limited areas are particularly vulnerable to increasing water temperature, as their optimum range in spawning temperatures tends to be restricted. Pacific bluefin tuna Thunnus orientalis (PBT) is a highly migratory species that spawns in waters near the Nansei Islands in the western North Pacific from May to June and in the Sea of Japan from July to August (Yonemori 1989, Ohshimo et al. 2017). Adult fish in the western North Pacific spawn at temperatures between 26 and 29°C, whereas those in the Sea of Japan initiate spawning at temperatures greater than 20°C (Chen et al. 2006, Tanaka 2011, Suzuki et al. 2014, Okochi et al. 2016). In the laboratory, the growth rate and survival of PBT larvae significantly decrease when temperatures exceed 29°C (Kimura et al. 2007). In fact, projected temperature in the current spawning sites is expected to increase by more than 3°C by 2100 under the most extreme Intergovernmental Panel on Climate Change (IPCC) climate-warming scenario (IPCC 2007) and become unsuitable for PBT to spawn (Kimura et al. 2010). As PBT larvae are particularly vulnerable to thermal stress, warming sea temperatures are likely to have significant impacts on their early growth and survival. However, the effects of ongoing climate change on the early life stages of PBT are poorly understood due to a lack of empirical evidence and methods to study such effects.

Oxygen isotope ratios (δ18O) in otoliths, biogenic calcium carbonate (aragonite) found in the inner ear of teleost fish (ray-finned bony fish), has been widely used as a natural tag to reconstruct water temperatures and salinity conditions experienced by fish (Thorrold et al. 1997, Campagna 1999, Jones & Campagna 2009). Such reconstructions of past environment are possible because otoliths generally develop at or close to the isotope equilibrium with ambient water, and many studies have demonstrated the temperature dependency of otolith δ18O for various fish species under laboratory conditions (Kalish 1991, Thorrold et al. 1997, Heie et al. 2004, Kitagawa et al. 2013). Existing methods of temperature reconstruction for fish mostly rely on δ18Ootolith measurements by conventional isotope ratio mass spectrometry (IRMS), which often involves a milling process to obtain a relatively large amount of otolith powder for analysis (usually more than a few tens of micrograms with a minimum weight requirement of 15 μg). Ambient water temperatures have previously been reconstructed using IRMS for sockeye salmon Oncorhynchus nerka (Zazzo et al. 2006), alewife Alosa pseudoharengus (Dufour et al. 2008), Atlantic cod Gadus morhua (Jones & Campagna 2009, von Leesen et al. 2020), turbot Scophthalmus maximus (Inlsand et al. 2014), and chub mackerel Scomber japonicus (Higuchi et al. 2019), most of which have a monthly to annual resolution depending on the otolith size. The limited temporal resolution due to the sample mass requirement of the IRMS is inevitable and makes it particularly difficult when analyzing the otolith core and edge.

Recent developments in secondary ion mass spectrometry (SIMS) δ18O analysis of otoliths have enabled a high-resolution reconstruction of migration and life history characteristics of marine species (Hanson et al. 2010, Matta et al. 2013, Shiao et al. 2014, Helser et al. 2018a, Shirai et al. 2018, Willmes et al. 2019). Unlike conventional IRMS, SIMS is capable of determining isotopic composition within a spatial resolution of 5 to 15 μm, which allows subannual, seasonal, and even weekly or much shorter timescale analyses with high accuracy and precision (Kita et al. 2009, Valley & Kita 2009). While a recent study (Sakamoto et al. 2019) reconstructed migration histories of an individual Japanese sardine Sardinops melanostictus with 10 to 30 d resolution (20 to 30 d around the core regions and 10 to 15 d toward the edge) using microvolume isotope analysis measured by continuous-flow IRMS (CF-IRMS), SIMS provides even finer temporal resolution, particularly for the otolith core and edge. High-resolution reconstruction of experienced temperatures using SIMS δ18Ootolith is an effective method for investigating the early life history of fish in response to increasing water temperature associated with climate change.

In this study, we developed a method to reconstruct ambient water temperatures experienced during the larval period of an individual fish using SIMS δ18Ootolith analysis. δ18Ootolith values of 5 adult PBT otolith samples were measured from the otolith core to edge by SIMS, and the measured SIMS δ18Ootolith values were compared to those measured by CF-IRMS. Water temperatures were then estimated using a temperature-dependent oxygen isotope fractionation equation for PBT larvae that had already been established in a previous study (Kitagawa et al. 2013). The temperature reconstruction technique presented here allows high-resolution investigation of the early life history of fish and provides a more
thorough understanding of the characteristics of survivors and the thermal environment that may constrain their early growth and survival.

2. MATERIALS AND METHODS

2.1. Sample collection and sample preparation protocol

Sagittal otoliths, the largest of 3 pairs of otoliths, were collected from the heads of PBT caught in waters south of Japan in the Pacific by local longline fishing vessels and small ships from 2017 to 2018 (Fig. 1, Table 1). Catch location, date of catch, and biological information of PBT samples are shown in Table 1. Otoliths were cleaned and rinsed with double deionized water (Milli-Q water) to remove any remaining muscle tissues, air-dried in a clean environment, and stored in microtubes for later analysis. Five adult fish samples were randomly selected and 1 of the paired otoliths from each individual was used for SIMS $\delta^{18}O$ analysis. For CF-IRMS $\delta^{18}O$ analysis, the 3 otolith sections that had been analyzed by SIMS were used.

Since SIMS is a surface analytical method, any irregularities of the sample surface, such as the existence of cracks, sample relief, and inclination of the surface, can cause degraded accuracy and precision of SIMS measurements (Kita et al. 2009, Valley & Kita 2009). It is also difficult to prepare samples with otolith cores and standard materials exposed on the same flat mirror-finished surface because otolith cores are usually very small (<5 μm). Thus, we first developed a sample preparation protocol (Fig. 2) that minimizes the effect of sample surface irregularities with an appropriate polishing procedure for calcium carbonates. This protocol is appropriate for preparing a thin otolith section with a transverse (or sagittal) cross-sectional plane. First, an otolith was mounted on a microscope slide with thermoplastic cement perpendicular to its longest axis, with the sulcus side facing down. The otolith core was then observed under an inverted microscope (IX-71, Olympus) equipped with a high-resolution color CMOS camera (DP-74, Olympus). Next, straight lines were drawn on the glass slide at 270 to 300 μm on each side of the otolith core using waterproof ink and a comic pen (Zebra

Table 1. Biological information, sampling data, and $\delta^{18}O$ analyses of Pacific bluefin tuna

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Catch location</th>
<th>Date of catch</th>
<th>Weight (kg)</th>
<th>Estimated fork length (cm)</th>
<th>Number of SIMS spots</th>
<th>Number of CF-IRMS measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>T64R</td>
<td>30.0°N, 134.0°E</td>
<td>26 April 2017</td>
<td>146.0</td>
<td>191.1</td>
<td>78 (35 and 43)</td>
<td>5 (2)*</td>
</tr>
<tr>
<td>T75R</td>
<td>30.0−32.0°N, 136.0°E</td>
<td>30 April 2017</td>
<td>52.2</td>
<td>136.2</td>
<td>42</td>
<td>−</td>
</tr>
<tr>
<td>T104L</td>
<td>27.0−28.0°N, 132.0−134.0°E</td>
<td>18 May 2017</td>
<td>99.2</td>
<td>168.3</td>
<td>53</td>
<td>8 (3)*</td>
</tr>
<tr>
<td>T118R</td>
<td>Nearshore off Kii Peninsula</td>
<td>22 May 2017</td>
<td>138.0</td>
<td>187.6</td>
<td>44</td>
<td>9 (4)*</td>
</tr>
<tr>
<td>T131R</td>
<td>Nearshore off Kii Peninsula</td>
<td>28 May 2017</td>
<td>118.0</td>
<td>178.2</td>
<td>42</td>
<td>−</td>
</tr>
</tbody>
</table>

Fig. 1. Catch locations of Pacific bluefin tuna *Thunnus orientalis* (red circles). Two samples (T118R and T131R) were caught in nearshore waters off Kii Peninsula by a small local boat and thus the locations are not shown on the map.
comic pen nib holder with Tachikawa T-99 round pen nib), and a thin otolith section was cut out together with the slide glass along the drawn lines with an automatic low-speed precision cutter (IsoMet 5000, Buehler) equipped with a 0.3 mm thick diamond blade (IsoMet 15LC, Buehler). The sectioned otolith was then removed from the strip of glass by carefully rinsing it with acetone, and was allowed to air-dry in a laminar flow hood. The appropriate thickness of a sectioned otolith is subject to change depending on the species, and it is recommended that the thickness which provides the clearest view of the otolith growth increments should be determined for each species.

The sectioned otolith was fixed in the center of a 2.54 cm silicon mold and embedded in mounting resin (EpoxyCure 2 Resin, Buehler) along with a calcite standard, UWC-3 ($\delta^{18}O = 12.49\%$o, Vienna Standard Mean Ocean Water [VSMOW], Kozdon et al. 2009), that was placed just above the otolith. It was then kept at room temperature for 24 h to cure the resin. The resulting epoxy disk containing the otolith thin section and standard material was ground with a grinding machine equipped with 70 and 13 μm diamond cup wheels (Discoplan-TS, Struers) until the distance from the otolith surface to the core was 15 to 20 μm. It was then successively polished using 6, 3, and 1 μm diamond pastes on a fine grinding disc (MD-Largo, Struers) to expose the core on a mirror-finished surface. Before analysis, the samples were cleaned in an ultrasonic cleaner and dried in a vacuum oven at 40°C for 2 h. They were then sputter-coated with approximately 60 nm gold. This protocol reduces sample preparation time by eliminating the need to embed otoliths in epoxy resin twice (once for sectioning in the transverse or sagittal plane and once for embedding with standard materials and polishing). It also provides flexibility in deciding desired section thickness and minimizes the portion of the sample that needs to be polished.
2.2. SIMS $\delta^{18}$O analysis

Otolith oxygen isotope ratios were measured in situ using a CAMECA IMS 1280-HR large radius, multi-collector ion microprobe (SIMS) at the Kochi Institute for Core Sample Research, JAMSTEC. Five otolith thin sections were prepared for SIMS $\delta^{18}$O_{otolith} analyses using the sample preparation protocol developed in the present study (Fig. 2). The $\delta^{18}$O_{otolith} values were measured from otolith core to edge along the growth axis for each otolith sample (Fig. 3a).

The SIMS analytical conditions that were used for $\delta^{18}$O_{otolith} measurements in this study have been described in detail by Kita et al. (2009). The sample surface was sputtered by a 20 kV accelerated $^{133}$Cs$^+$ primary ion beam at 1.5 to 1.8 nA focused to a diameter of 10 to 15 μm, resulting in a pit of approximately 1 μm depth (Fig. 3b). The secondary ions ($^{16}$O$^-$, $^{18}$O$^-$, and $^{16}$OH$^-$) were accelerated at 10 kV and detected simultaneously by 3 Faraday cup detectors. Since hydrogen is present in the SIMS chamber even under ultra-high vacuum conditions, measured $^{16}$OH$^-$/1$^{16}$O$^-$ ratios were background-corrected by subtracting the average $^{18}$OH$^-$/1$^{16}$O$^-$ ratio of the UWC-3 standard bracketing analyses (nominally anhydrous minerals) from the $^{18}$OH$^-$/1$^{16}$O$^-$ ratio of $\delta^{18}$O_{otolith} measurements. The background-corrected $^{16}$OH$^-$/1$^{16}$O$^-$ ratios served as a proxy for the relative hydrogen content contained in otolith samples. Each analysis took approximately 3 min, consisting of pre-sputtering (10 s), automatic centering of the secondary ion beam (90 s), and the isotopic measurements with 20 analytical cycles (40 s). The count rates for $^{16}$O$^-$ and $^{18}$O$^-$ were 1.7 to 2.5 × 10$^9$ and 3.5 to 5.1 × 10$^6$ counts per second (cps), respectively.

For accurate calibration of SIMS $\delta^{18}$O measurements in biogenic carbonate samples, a homogeneous biocarbonate standard with a matched-matrix is needed. The UWC-3 standard is a chemically and isotopically homogeneous calcite standard which has a similar chemical composition to otoliths (aragonite), and thus all $\delta^{18}$O_{otolith} measurements were normal-

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Fig. 3. (a) Cross-sectional electron probe micro analyzer (EPMA) image of bluefin tuna otolith with beam spot locations. (b) SIMS beam spot sputtered with a $^{133}$Cs$^+$ primary ion beam focused to a diameter of 10 to 15 μm. (c) Otolith thin section from Pacific bluefin tuna *Thunnus orientalis* embedded in epoxy resin with a calcite UWC-3 standard. The sample surface was mirror-finished and coated with gold.
ized with this standard in our study (Kozdon et al. 2009). Every 10 to 15 unknown sample measurements were bracketed by 10 analyses of a UWC-3 calcite standard (5 analyses before and after each group of unknown samples) to calculate the spot-to-spot precision of sample analyses and to correct for instrumental mass fractionation. The precision of sample analyses for all 5 otolith thin sections was ±0.3 to ±0.6‰ (2 standard deviations).

After analysis, each spot was observed on scanning electron microscope (SEM) images taken with an electron probe micro analyzer (JXA-8230, JEOL) to check for any cracks and inclusions that might bias the resulting δ¹⁸O values (e.g. Weidel et al. 2007). No spots had such surface irregularities. In addition, the secondary ion yield (¹⁸O⁻, cps nA⁻¹) relative to the mean of the UWC-3 standard bracketing analyses were used to assess the quality of each spot measurement and check for any extreme outliers. Raw SIMS δ¹⁸O measurements are presented in Table S1 in the Supplement at www.int-res.com/articles/suppl/m649_p175_supp.xlsx.

For comparison purposes, all δ¹⁸O_{otolith} values were converted from VSMOW to Vienna Pee Dee Belemnite (VPDB) by using the latest published conversion equation ($δ^{18}O_{VSMOW} = 1.03092 \times δ^{18}O_{VPDB} + 30.92$, Brand et al. 2014, Kim et al. 2015).

### 2.3. Microvolume δ¹⁸O analysis by CF-IRMS

Microvolume CF-IRMS δ¹⁸O analysis was conducted to compare δ¹⁸O_{otolith} values measured by SIMS and CF-IRMS. Three otolith thin sections that had been analyzed by SIMS were used for microvolume CF-IRMS δ¹⁸O analysis. δ¹⁸O analyses were performed with an IsoPrime100 isotope ratio mass spectrometer (Isoprime) equipped with a customized continuous-flow gas preparation system (MICAL3c) at the National Institute of Technology, Ibaraki College, Japan. This system can measure isotope ratios of calcium carbonate samples with a minimum sample mass of 0.2 μg (about 1/100 of the sample mass required for commercially available IRMS systems) with high precision and accuracy (Ishimura et al. 2004, 2008). By micromilling the otolith material deposited during the same growth period as that analyzed by SIMS, it is possible to compare average SIMS and CF-IRMS δ¹⁸O_{otolith} values.

A high precision micromilling system (Geomill-326) was used for milling the specific regions of otolith samples. This system comprised a carbide bur fixed over an XYZ sample stage, a high-resolution camera, and a computerized image analyzer. An otolith image with marks indicating the target milling areas was imported into the system and milling paths were configured on the computer. The target milling areas were set in the otolith region where the measured SIMS δ¹⁸O_{otolith} values were stable, and they covered roughly 3 to 4 SIMS beam spots (Fig. 4). First, an unwanted area right next to the target milling path was milled and removed from the otolith to avoid cross-contamination. The removed otolith powders were also collected and used for analysis as supplementary samples to increase the dataset. For each otolith sample, 2 to 3 target paths were milled along the growth rings to obtain powder samples. The resulting milled paths were 15 to 80 μm wide, 250 to 350 μm long, and 60 to 90 μm deep.

![Fig. 4. Otolith thin section from Pacific bluefin tuna Thunnus orientalis (T64R) used for micromilling. (a) Before milling, (b) after milling unwanted areas to avoid cross-contamination, and (c) after milling the target milling path (the area inside the red line). (d) Beam spot locations where SIMS δ¹⁸O_{otolith} values are stable (~ −3.01, ~ −2.93, ~ −3.04‰, respectively, from top to bottom [VPDB]). The images were taken under an optical microscope for (a), and a stereo microscope for (b) and (c).](image-url)
The life stages of the corresponding milled paths were estimated based on the distance from the otolith core and the location of the annual growth increments. Each path corresponded to either the juvenile (a few months old), immature (about 5 mo old to age 1+), or sub-adult stage (age 2 to 3). The non-parametric Kruskal-Wallis test was used to determine the statistical difference in resulting offset values between SIMS and CF-IRMS among different life stages.

The amount of powder produced from each path was 0.9 to 3.5 μg. The milled powder was carefully collected and placed on to a small piece of glass using a needle under a microscope, and then put into the bottom of a reaction tube. The aragonite powder was then reacted with 104% phosphoric acid at 25°C, and the evolved CO₂ was purified in a stainless steel vacuum line. After further purification using a helium-purged purification line, the purified CO₂ was introduced into the mass spectrometer. Samples that weighed more than 2.0 μg were analyzed twice. The results are reported in standard δ notation (‰) relative to VPDB. We measured a laboratory standard CO₂ gas for the determination of the analytical precision of pure CO₂ gas (δ¹³CVPDB = −1.56‰ and δ¹⁸OVpdb = −4.42‰, Nishida & Ishimura 2017) 3 or more times every day. The analytical precision was better than ±0.1‰ (±1 SD) for the entire analysis.

To compare the difference between SIMS and CF-IRMS values, the average SIMS δ¹⁸O_{otolith} values were calculated by averaging the SIMS spot measurements adjacent to or within each milling path and then compared to the CF-IRMS δ¹⁸O_{otolith} value. A linear regression analysis was performed to determine the correlation between values measured by the 2 methods. In addition, the Wilcoxon signed rank test was applied to test the statistical difference between the average SIMS and CF-IRMS δ¹⁸O_{otolith} values, and the Tukey’s test was used to detect outliers. All statistical analyses were performed using R software (version 3.3.2).

2.4. Temperature reconstruction for larval stages of adult PBT

Ambient water temperatures experienced during larval stages of adult PBT were estimated using SIMS δ¹⁸O_{otolith} values of otolith core regions. The daily growth increments were counted on the SEM images and the SIMS spot measurements that were made within 20 days post hatch (DPH) were used for the temperature estimation. For an accurate estimation of ambient water temperatures, a species-specific fractionation equation and δ¹⁸O_{seawater} are needed. To reconstruct ambient temperatures, we used the oxygen isotope fractionation equation for PBT larvae proposed by Kitagawa et al. (2013):

\[ \delta^{18}O_{otolith} (VPDB) = 5.193 - 0.270 \times T \quad (1) \]

where \( \delta^{18}O_{water} \) is the δ¹⁸O value of ambient water, and \( T \) is the water temperature in °C. For \( \delta^{18}O_{water} \) in the equation, we applied the \( \delta^{18}O \) value of +0.22‰ (VSMOW) which is the average \( \delta^{18}O_{water} \) of the main spawning ground around the Nansei Islands (24.04 to 26.09° N, 123.56 to 131.00° E) in May to June from 2008 to 2010 (Uozato 2011). The \( \delta^{18}O_{water} \) (VSMOW) value was corrected on the VPDB scale by subtracting 0.22‰ (Friedman & O’Neil 1977), following the same protocol as Kitagawa et al. (2013). The mean temperatures were calculated for the samples that had multiple \( \delta^{18}O_{otolith} \) measurements made around the core regions. Although the focus was on the reconstruction of ambient water temperatures experienced during the larval period of the fish, the lifetime temperature history was estimated to evaluate how well \( \delta^{18}O_{otolith} \) records ambient water temperatures in the older stages of PBT.

3. RESULTS

3.1. Comparison of SIMS and CF-IRMS δ¹⁸O_{otolith} Measurements

In total, 22 paths were milled by a micromilling system and the \( \delta^{18}O_{otolith} \) of collected powders from each milled path was measured by CF-IRMS (Table S2 in the Supplement). After carefully examining the accuracy of how well each milled path captured the same growth zone as the SIMS spots, 18 samples, including 9 main samples and 9 supplementary samples, were selected to assess the difference between SIMS and CF-IRMS \( \delta^{18}O_{otolith} \) values. The average values of multiple CF-IRMS measurements were used when the milling accuracy of a single milling path was low (see Table S3 in the Supplement for a complete list of data). The precision of \( \delta^{18}O_{otolith} \) values measured by CF-IRMS was better than that of SIMS. The \( \delta^{18}O_{otolith} \) values measured by CF-IRMS were significantly higher than those measured by SIMS (Fig. 5a, Wilcoxon signed-rank test, p < 0.001) except for 2 measurements in which the CF-IRMS value was 0.12 and 0.47‰ lower than the average SIMS value, respectively. We considered the CF-IRMS \( \delta^{18}O_{otolith} \) measurement that was 0.47‰ lower than the average SIMS \( \delta^{18}O_{otolith} \) value to be an outlier, based on the Tukey’s outlier detection method. This measurement was taken from a relatively large area of the otolith.
deposited during the second to third years of life, where interannual variation in water temperature is expected. It is likely that this temperature variation was not well reflected in the average SIMS value since only 3 spot measurements were averaged over more than a half-year period. Therefore, this measurement was excluded for calculating a linear regression curve and the average SIMS–CF-IRMS difference.

There was a significant positive correlation between SIMS and CF-IRMS $\delta^{18}$O otolith values ($r^2 = 0.79$, $p < 0.001$) with a slope of 1.1408 and an intercept of 0.0704. The slope is 1 within the 95% confidence interval (lower limit = 0.82, upper limit = 1.46) with a high correlation coefficient ($r^2 = 0.78$), and thus a linear regression with a slope of 1 was fitted to the data to calculate the $y$-intercept, which is the average difference (Fig. 5b). The average offset between SIMS and CF-IRMS $\delta^{18}$O otolith values was 0.41‰, with SIMS yielding lower values. The SIMS–CF-IRMS $\delta^{18}$O otolith correction equation for PBT can be expressed as:

$$\text{SIMS} \delta^{18}\text{O}_{\text{otolith}}(\text{VPDB}) = \text{CF-IRMS} \delta^{18}\text{O}_{\text{otolith}}(\text{VPDB}) - 0.41$$

No significant difference was observed between $\delta^{18}$O otolith measurements at different life stages (Kruskal-Wallis test, $p = 0.29$), and thus the consistent application of this offset correction equation to all SIMS measurements was considered appropriate.

### 3.2. Seasonal variations in SIMS $\delta^{18}$O otolith profiles

In total, 259 $\delta^{18}$O otolith measurements were made for 5 PBT otoliths by SIMS (Table 1). The total number of spots measured per otolith was 42 to 78 (2 life-history transect lines were analyzed for T64R). The length of each transect ranged between 2.5 and 2.8 mm with a spot-to-spot distance ranging from 16 to 153 μm around the core region, and 26 to 337 μm toward the edge. The spatial resolution around the core region in one of the samples was more than 10 times higher compared to the conventional IRMS method previously used for PBT otoliths (Shiao et al. 2010). The temporal resolution of SIMS spots was 3 to 5 d near the core region and roughly several weeks to a month on the outer edge depending on the age of fish.

The offset-corrected SIMS $\delta^{18}$O otolith values for 5 PBT samples are plotted in Fig. 6 (see left $y$-axis). High-resolution $\delta^{18}$O otolith profiles of all otolith samples showed distinct seasonal variations with an increasing trend from the otolith core to about 1250 μm. The average $\delta^{18}$O otolith values from the core to about 750 μm ranged between $-3.1$ and $-2.5$‰ (VPDB), and sharply increased toward the first annual increment (opaque zone), peaking at $-1.3$ to $-0.4$‰. After the increase, the $\delta^{18}$O otolith values decreased, showing a cyclical pattern toward the
edge fluctuating mostly between $-2.5$ and $-1.5\%$ (VPDB). The $\delta^{18}O_{\text{otolith}}$ of the core regions corresponding to PBT larval stages (10 to 20 DPH) ranged between $-3.1$ and $-1.9\%$ (VPDB).

The background-corrected $^{16}\text{OH}^+/^{16}\text{O}^-$ ratios measured for the 5 otoliths ranged between 0.017 and 0.031, and had a general inverse relationship with the SIMS $\delta^{18}O_{\text{otolith}}$ values (there is no impact of individual differences on this relationship) (Fig. 7a). This indicates that the relative hydrogen content in the otolith increases with lower SIMS $\delta^{18}O_{\text{otolith}}$ values. Overall, higher $^{16}\text{OH}^+/^{16}\text{O}^-$ ratios resulted in larger SIMS–CF-IRMS $\delta^{18}O_{\text{otolith}}$ differences (Fig. 7b), which is consistent with the inverse trend seen in Fig. 7a.

### 3.3. Estimation of temperature experienced during larval period

Core-to-edge water temperature profiles of all samples are shown in Fig. 6 (see right $y$-axis). The estimated temperatures experienced during the larval stages ranged between 26.7 and 30.7°C among the individuals (T64R: $30.7 \pm 1.3^\circ$C [~20 DPH], T75R:...
27.9 ± 1.0°C (~10 DPH), T104L: 26.7 ± 1.0°C (~12 DPH), T118R: 28.9 ± 0.9°C (~12 DPH), T131R: 28.4 ± 1.4°C (~12 DPH). After Year 0, temperature ranged mostly between 24 and 30°C, and never reached 35°C.

4. DISCUSSION

An increasing number of experimental and modeling studies have shown significant impacts of projected climate change on the early growth and survival of various fish species (Kimura et al. 2007, Pankhurst & Munday 2011, Moyano et al. 2017). Generally, fish larvae are more sensitive to temperature variations than juveniles and adults as they have narrower thermal tolerance ranges (Pörtner & Peck 2010, Moyano et al. 2017), making them particularly vulnerable to climate change. However, the effects of ongoing climate change-driven ocean warming on the early life history of fish remain largely unexplored for many species, mostly due to difficulties in monitoring long-term responses to climatic stressors. Thus, the advancement of techniques that can quantitatively estimate past environments actually experienced by fish during key phases of the life cycle is essential to fill this knowledge gap. In this study, a high-resolution temperature reconstruction technique using SIMS δ¹⁸O_otolith analysis was developed and applied to PBT, a species of great economic importance, whose early larval growth and survival may be constrained by climate change.

The 0.41‰ offset observed between SIMS and CF-IRMS δ¹⁸O_otolith values for PBT otoliths is most likely due to incomplete correction for ‘matrix effects’ by SIMS methods. Matrix effects refer to an instrumental mass fractionation caused by different chemical compositions and structures between given samples and standard materials, which shifts measured values (Eiler et al. 1997, Riciputi et al. 1998, Śliwiński et al. 2016, 2018, Wycech et al. 2018). Otoliths contain a small amount of organic proteins, namely otolith matrix protein-1 and Otolin-1 (Murayama et al. 2000, 2002). The presence of these proteins in the otolith would be responsible for a subtle change in instrumental mass fractionation, which results in lower SIMS δ¹⁸O_otolith values relative to those measured by CF-IRMS, and higher ¹⁶OH⁻ ion yields compared to that of the calcite standard. The general inverse relationship between ¹⁶OH⁻/¹⁸O⁻ ratios and SIMS δ¹⁸O_otolith values (Fig. 7a) may be the result of the incorporation of more proteins into the otolith matrix, which is thought to relate to fast growth in summer (or less proteins in winter due to slow growth). The water content (OH⁻) of the otoliths, if any, is also responsible for the lower δ¹⁸O_otolith values. These organic proteins and water content bias SIMS δ¹⁸O_otolith values because they are measured together with calcium carbonates, whereas they do not affect CF-IRMS δ¹⁸O_otolith values since these proteins do not react at 25°C with the phosphoric acid that is used in a digestion process to generate CO₂ gas. Furthermore, the systematic difference in isotopic fractionations caused by sputtering different crystalline structures (the biogenic aragonite samples and calcite standard) may contribute to the observed offset (Linzmeier et al. 2016). Although matrix effects are likely the primary cause of the SIMS–CF-IRMS difference, other potential factors (e.g. milling and roasting effects) may influence the measurement results of SIMS and CF-IRMS. The effects of roasting and other factors have been discussed in detail by Wycech et al. (2018), who investigated the δ¹⁸O difference between SIMS and IRMS using foraminiferal shells.
The offset of 0.41‰ found in this study is within the range of offset values previously reported for otoliths of other fish species and biocarbonate samples. Orland et al. (2015) reported that there is a consistent sample-dependent offset in δ¹⁸O of typically less than 1.0‰, but this can go up to 1.8‰ with different sample matrices including biocarbonates and speleothems. Matta et al. (2013) used roasting to remove organic materials and observed an offset of about 1‰ in SIMS δ¹⁸O values between roasted and unroasted otoliths of yellowfin sole Limanda aspera. Helser et al. (2018b) also observed a 0.5‰ offset between SIMS and CF-IRMS δ¹⁸O values measured in otoliths of Pacific cod Gadus macrocephalus. The temperature estimation without the correction of the 0.41‰ offset using SIMS δ¹⁸O values of PBT would cause a bias of 1.5°C in the estimates, resulting in some unrealistically high temperature estimates (i.e. >34°C). While the extent to which organic proteins and water content contribute to an overall offset is unknown, it is necessary to determine these offsets between SIMS versus IRMS when estimating ambient water temperatures from SIMS δ¹⁸O values for different species.

The SIMS analyses performed on 5 otolith samples from PBT revealed fine-scale δ¹⁸O profiles from the core to the edge with clear seasonal variations (Fig. 6). As water temperature and δ¹⁸O values are negatively correlated (Devereux 1967, Thorrold et al. 1997, Høie et al. 2004), the initial increase in the δ¹⁸O values observed toward the first annual increment (opaque zone) for all otolith samples indicates that the PBT experienced a decreasing water temperature. Age 0 juveniles predominantly inhabit the surface mixed layer (Kitagawa et al. 2000), and they are known to migrate northward in summer along the coastal regions of Japan and southward for overwintering in the East China Sea and nearshore waters on the Pacific side of Japan (Itoh et al. 2003, Fujioka et al. 2018). The increase in the δ¹⁸O values thus reflects the actual water temperature change experienced by the individuals from autumn to winter. The increasing patterns of the δ¹⁸O in the first year of life observed in this study are consistent with the results previously reported by Shiao et al. (2010), with much greater temporal resolutions (d to wk) with high precision and accuracy achieved by SIMS techniques. The δ¹⁸O profiles after age 0 also showed similar seasonal fluctuations, but with much less variation. This narrow δ¹⁸O range observed after age 0 is likely due to the effect of the development of thermoregulatory ability by the PBT. For endothermic fish (warm-bodied) such as PBT and other tuna species, the δ¹⁸O of immature and adult stages do not merely reflect ambient (in situ) water temperatures but, rather, the elevated, internal body temperatures. Using counter current heat exchangers known as retia mirabilia (Dickson & Graham 2004), PBT have the capacity to elevate the temperature of their viscera, red (slow-twitch, oxidative) myotomal muscle fibers, eyes, and brain (Linthicum & Carey 1972, Carey & Lawson 1973). When juvenile PBT reach about 20.0 cm in fork length at age 0 (about 2 mo after hatching) (Kubo et al. 2008), thermoregulatory ability begins to develop and they are able to maintain their body temperature <1°C above that of the surrounding water (Furukawa et al. 2017). The thermoregulatory ability of PBT increases as the fish grow and develop. For example, water temperatures of the peritoneal cavity of adult fish (a 250 kg PBT) could be 10°C higher than ambient temperatures constructed from acoustic telemetry data (Kitagawa et al. 2006). Since temperatures >35°C are lethal for PBT, the observed upper temperature range (30°C) in immature and adult stages is likely a result of physiological thermoregulation to avoid overheating. Understanding how thermoregulatory ability changes with body size and its physiological effects on δ¹⁸O will facilitate better interpretation of the temperature data obtained from this technique.

The estimated temperatures experienced during the larval stages ranged between 26.7 and 30.7°C among the individuals, with a mean temperature of 28.5 ± 0.1°C (±2 SD). Field surveys have collected PBT larvae in sea surface temperatures (SSTs) between 23.5 and 29.5°C in the 2 main spawning grounds (Yonemori 1989, Abe et al. 2014, Suzuki et al. 2014), with higher concentrations of larvae found around 27°C. The estimated temperatures overlap with the range of temperatures observed for larval occurrence of PBT. As newly hatched PBT larvae tend to stay in warm waters within or near the spawning grounds for their optimal growth and survival, temperatures estimated from the core δ¹⁸O corresponding to the early larval period may also serve as a useful indicator of spawning temperatures or the location of spawning grounds. One of the specimens (T64R) appeared to have experienced relatively warm temperatures (>30°C) associated with decreased growth rates and survival of larvae in the laboratory (Kimura et al. 2007). Although there is evidence that the eggs of PBT hatched as normal larvae at 31.5°C in rearing experiments (Miyashita et al. 2000), the higher temperature estimated for this specimen could be a result of the high-resolution sampling of SIMS which captured several warmer
days. Despite the need to increase sample size to accurately judge whether the estimated temperatures are realistic, particularly for the specimens with higher temperatures, our results suggest that SIMS δ¹⁸O<sub>otolith</sub> analysis coupled with a microvolume CF-IRMS δ¹⁸O<sub>otolith</sub> analysis and a species-specific temperature-dependent fractionation equation is an effective method for reconstructing ambient water temperatures experienced by fish and inferring their early life characteristics, which are difficult to obtain with the limited resolution of conventional methods.

5. CONCLUSIONS

We have developed a novel method to estimate ambient temperatures experienced during the larval stage of fish species using SIMS and microvolume CF-IRMS δ¹⁸O<sub>otolith</sub> analyses. Microvolume δ¹⁸O<sub>otolith</sub> analysis revealed that the SIMS δ¹⁸O<sub>otolith</sub> values were 0.41‰ lower on average than CF-IRMS δ¹⁸O values. High-resolution SIMS δ¹⁸O<sub>otolith</sub> analysis of PBT otoliths achieved greater spatial and temporal resolution with high precision and accuracy compared to the conventional IRMS methods. The δ¹⁸O<sub>otolith</sub> profiles of all samples showed distinct seasonal variations, reflecting ambient water temperatures experienced by an individual fish. The developed protocol is useful especially for smaller otoliths with narrow growth increments. SIMS δ¹⁸O<sub>otolith</sub> analysis coupled with micromilling and microvolume δ¹⁸O<sub>otolith</sub> analysis allows microscale examinations of otoliths, and more detailed information on the thermal life history of fish can be obtained compared to conventional IRMS methods. This novel method is a powerful tool for the reconstruction of environmental histories of various fish species and has important implications for understanding how ocean warming is potentially affecting the early life history of fish.

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