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Oxygen isotopic distribution along the otolith growth axis by secondary ion mass spectrometry: Applications for studying ontogenetic change in the depth inhabited by deep-sea fishes

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ABSTRACT

This study using tuna otoliths as working standards established a high lateral resolution and precision analysis to measure $\delta^{18}O_{otolith}$ by secondary ion mass spectrometry. This analytical approach of the ion probe was applied to deep-sea fishes to reconstruct the likely depths inhabited by the fishes at different life history stages based on the measured $\delta^{18}O_{otolith}$ values as a proxy of water temperature. Dramatic increases up to 5–6‰ in $\delta^{18}O_{otolith}$, representing a temperature decrease of approximately 20 °C, were detected in a blind cusk eel (*Barathronus maculatus*) otolith and in the otoliths of *Synaphobranchus kaupii* during leptocephalus metamorphosis to glass eel, inferred from the drop of otolith Sr/Ca ratios and increase of otolith growth increment width. $\delta^{18}O_{otolith}$ profiles clearly divided the fish's life history into a planktonic stage in the mixed layer of the ocean and a benthic stage on the deep-sea ocean bottom. The habitat shift signal was recorded within a 150 μm width of otolith growth zone, which was too narrow to be clearly detected by mechanical drilling and conventional isotopic ratio mass spectrometry. However, variations down to −7‰ were found in $\delta^{18}O_{otolith}$ profiles as the result of Cs^{2+} beam sputter in the core and larval portions of the otoliths. Carbon mapping by electron probe microanalyzer and staining by toluidine blue suggested abundant proteins existed in the areas with anomaly negative $\delta^{18}O_{otolith}$ values, which cannot be interpreted as a habitat change but due to the isotopic fractionation by O emission from the proteins. These results implied that careful design and understanding of the chemical composition of the analytical areas or tracks on the heterogeneous otolith was essential for highly accurate and precise analysis.

1. Introduction

Otoliths are calcium carbonate structures in the inner ears of teleostean fish that function as balance and auditory organs (Popper and Coombs, 1982; Cruz et al., 2009). The acellular and metabolically inert otoliths grow continuously throughout the life of a fish and record the environmental characteristics e.g., water temperature experienced by the fish (Campana and Neilson, 1985). Examination of otolith microstructure and chemical compositions has enabled scientists to reveal life history events such as hatching, metamorphosis, settlement, and habitat shifts of the fishes (Hislop et al., 2001; Lin et al., 2012).

$\delta^{18}O_{otolith}$ is deposited in equilibrium with ambient water $\delta^{18}O_{water}$ and $\delta^{18}O_{otolith}$ is influenced by salinity and temperature. The fractionation factor of $\delta^{18}O_{otolith}$ is temperature-dependent (Thorrold et al., 1997) and the negative linear relationship between water temperature and $\delta^{18}O_{otolith}$ has been validated for several species (e.g., Høie et al., 2004). $\delta^{18}O_{otolith}$ as a proxy of water temperature has wide applications in such diverse fields as migration studies (Northcote et al., 1992), stock discrimination for fishery management (Shiao et al., 2010), and for the reconstruction of paleoclimate (Patterson et al., 1993).

In past decades, mechanical milling and bulk analysis by conventional isotopic ratio mass spectrometer (IRMS) have been the major methodologies used to measure variations in $\delta^{18}O_{otolith}$ values throughout the life of the fish (Wurster et al., 1999). Conventional IRMS provides high precise and accurate data, which are able to detect small isotopic variations (<1‰) in fish otoliths. However, these methods are limited in temporal resolution due to the need for sample masses of ca. 10–80 μg, depending on machine types and instrument settings. The requirement for relatively large sample masses means that these methods can track seasonal isotopic

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variations in larger otoliths, but cannot detect isotopic changes at scales of days or weeks.

In contrast, the ion probe technology has greatly advanced in capability in recent decades and is now able to measure isotopic ratios from samples within μm-scale spots with a precision and accuracy comparable to conventional IRMS (Hanson et al., 2010). The in situ analysis on small areas (~10 μm) by secondary ion mass spectrometry (SIMS) is the only current methodology that can measure isotopic distribution in small otoliths or detect isotopic variations at a daily scale (Weidel et al., 2007). However, the principal challenge for high-precision SIMS analysis is the strong and variable instrumental mass fractionation (IMF) of isotopic compositions that may occur during analysis. Fractionation mediated by sputtering and ionization is one of the complex factors, which may mainly depend on the surface properties of the samples (i.e., chemical composition). This phenomenon is referred to as "matrix effects" or compositionally dependent fractionation (Riciputi et al., 1998). To minimize matrix effects in high precision SIMS analysis, correction by a mineral specific standard is usually essential. Otoliths are not pure minerals; rather, they are biomineralized structures containing minor organic materials e.g., proteins (Degens et al., 1969; Söllner et al., 2003) and various elements intercalated within the calcified daily increments (Campana, 1999). The chemical composition and textures can vary within the same otolith at different life stages. Therefore, correction for IMF with a pure calcium carbonate standard such as NBS-19 may not be the best choice.

This study aims to test the feasibility of using tuna otoliths instead of pure $CaCO_3$ as a working standard for $\delta^{18}O$ analysis by SIMS. Otoliths are impure $CaCO_3$ structure whose organic component may constitute up to 10% by weight (Sasagawa and Mugiya, 1996; Murayama et al., 2002). The heterogeneity of otolith composition may cause fractionation mediated by matrix effects, and was also investigated. Otoliths of Pacific bluefin tuna (*Thunnus orientalis*), deep-sea eels (*Synaphobranchus kaupii*) and blind cusk eels (*Barathronus maculatus*) were chosen for this work. The $\delta^{18}O_{otolith}$ values from one sagittal otolith of Pacific bluefin tuna has been determined by mechanical drilling and conventional IRMS in our previous study (Shiao et al., 2010) and the other sagittal otolith from the same individual was used here as the working standard. The blind cusk eel is viviparous, giving birth to live young. Very little is known about the life history of the blind cusk eel. The newly-born larvae of the blind cusk eel were believed to live near the sea bottom after birth (Nielsen et al., 1999). However, this assumption was contradictory to the finding of pelagic larvae of *B. pacificus* in shallower waters (Okiyama and Kato, 1997). We using SIMS technology to clarify the mysterious life history of the blind cusk eel (*B. maculatus*) by measuring the $\delta^{18}O_{otolith}$ profile. The synaphobranchid eels undergo distinct life stages including a planktonic leptocephalus in the warm, shallow oceanic layer (Minagawa et al., 2007) and benthic life on the cold deep-sea floor during juvenile and adult stages (Trenkel and Lorance, 2011). Metamophosis of synaphobranchid eels from leptocephalus to glass eel stage shall be accompanied with decrease of otolith Sr/Ca ratios and increase of otolith growth increment widths, which were regarded as the common traits that had been extensively found in many other eel species e.g., anguilid eels, conger eels, moray eels (Otake et al., 1994, Correia et al., 2003; Lin et al., 2005). We hypothesize that $\delta^{18}O_{otolith}$ varies as eels transition between habitats of different depths from pelagic larval stage to benthic juvenile and adult. Such kind of ontogenetic habitat shift to the seafloor accompanied with the increase of $\delta^{18}O_{otolith}$ values has been found in the deep-sea grenadiers (Lin et al., 2012). However, the leptocephalus stage of synaphobranchid eel may have a slow otolith growth rate similar to that for anguillid eel and conger eel e.g., <1 μm per day (Shiao et al., 2002; Correia et al., 2003) that records transient isotopic variations in narrow otolith growth zones. The signal change within a small growth zone is not easily studied by mechanical drilling and conventional IRMS (Hanson et al., 2010; Lin et al., 2012). The SIMS methodology established in this study provides further insight into the understanding of the mysterious life history of deep-sea fishes and will have wide applications for other species.

2. Materials and methods

2.1. Fish collection

The Pacific bluefin tuna (*T. orientalis*, No. PBT3607) is collected in the landing port of Saga, Kochi Prefecture, Japan, which faces the Pacific Ocean on the 17th December 2003. Another Pacific bluefin tuna (No. PBT1910) is collected in the landing port of Hagi, Yamaguchi Prefecture, Japan, which faces the Sea of Japan on the 19th December 2002 (Shiao et al., 2010). A deep-sea blind cusk eel (*B. maculatus*) and Kaup's cutthroat eels (*S. kaupii*) are collected from the sea-floor at the depth of 2356 m for the former and 1242 m for the later off the east coast of Taiwan by the beam trawl during the cruises of Ocean Researcher I in June 2005 and July 2009, respectively (Table 1). Hydrological data, including salinity and temperature, are measured in situ with a SeaBird CTD (Conductivity–Temperature–Depth) recorder (SBE 9/11 plus, SeaBird Inc., USA).

2.2. Otolith preparation

Sagittal otoliths were dissected from the fish after the measurement of total or fork length and weight. One otolith of each tuna was analysed for $\delta^{18}O$ values by conventional isotope mass spectrometry (Shiao et al., 2010) and the other otolith from the same tuna was prepared for secondary ion mass spectrometry in this study. Tuna otoliths were embedded in epofix resin (Struers, Demark) 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 one side with a grinder-polisher machine (Buehler, Metaserv 2000, Evanston, IL, USA) to reveal the incremental pattern. Images of otolith sections were taken by a compound microscope (Olympus BX-51, Japan) equipped with a digital camera (DP-71, Olympus, Japan) using transmitted light.

Otolith powders was collected by a computerized micromill (Merchantek, USA) 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 two adjoining segmented lines according to the desired number of samples. Otolith powder samples, weighing approximately 25–35 μg, were collected from the distal end to the core along the ventral-medial arm. 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.

Otoliths of the deep-sea fish were also embedded in epofix resin (Struers, Demark) and the sagittal planes were ground and polished until the core was near the surface. The otolith thin sections of tuna and polished otoliths of deep-sea fish were cast within the central 1.5 cm of a 2.54 cm diameter round epoxy (Epxi Cure®, Buehler) block. These otoliths were slightly ground and polished by hand using 30 μm, 12 μm, 3 μm polishing sheets (3 M) until the cores of the deep-sea fish otolith were exposed on the surface. The samples were finally polished by a vibrating polisher (VibroMet® 2 Vibratory Polisher, Buehler) until the surface was