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Short communication

# Temperature dependence of $\delta^{18}O$ in otolith of juvenile Japanese sardine: Laboratory rearing experiment with micro-scale analysis

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ABSTRACT

We evaluated the use of the stable oxygen isotope ($\delta^{18}O$) in the otolith as a proxy for the temperature history of Japanese sardine *Sardinops melanostictus* individuals. Japanese sardine juveniles were reared in three different water temperatures over the course of a month. Otolith $\delta^{18}O$ ($\delta_{otolith}$) was analyzed by extracting the portions formed during the rearing period using a micromill. $\delta^{18}O$ of the rearing water ($\delta_{water}$) was also analyzed. A linear relationship between otolith $\delta^{18}O$ and ambient water temperature was identified as follows: $\delta_{otolith} - \delta_{water} = -0.18 * T + 2.69$ ($r^2 = 0.91$, $p < 0.01$). This equation is different from that proposed for inorganic aragonite or other *Sardinops* spp., with resulting application to wild Japanese sardine captured in the Pacific Ocean showing that it estimates a more realistic *in situ* temperature. Our findings suggest that the Japanese sardine-specific isotopic fractionation equation should be used when interpreting otolith $\delta^{18}O$ of the Japanese sardine, and the methods presented here could also be useful to understand the temperature history of other fish species.

## 1. Introduction

The overall abundance of the Pacific stock of the Japanese sardine *Sardinops melanostictus* has fluctuated considerably in amplitude at inter-decadal time scales (e.g. Ito 1961; Yasuda et al., 1999). The annual variation of recruitment abundance, which depends on the number of eggs produced and survival rates during the egg, larval, and juvenile stages, has been suggested to drive this fluctuation (Watanabe et al., 1995). Although the relationships between the annual variation of recruitment and environmental variability in the western North Pacific are well studied (e.g. Noto and Yasuda, 1999; Yatsu et al., 2005; Watanabe, 2009; Nishikawa et al., 2013), the mechanisms connecting the environment to sardine survival rate remain unclear.

The age-0 Japanese sardine migrate a long distance from the temperate Kuroshio region to the subarctic Oyashio region during spring to autumn. Environmental factors such as temperature and food density along the migration route have been suggested to affect survival rate and ultimately control recruitment abundance (Takasuka et al., 2007, 2008; Takahashi et al., 2008, 2009; Nishikawa et al., 2013). These hypotheses have yet to be verified since it is difficult to accurately define the environment that the sardine individuals experience during migration. For large bodied species such as the bluefin tuna, archival tagging has been a useful tool for tracking the migration routes of individuals (e.g. Block et al., 2005). Due to their small size and fragility, it is impossible to tag a larval or juvenile sardine. Due to this limitation, estimates have been made using numerical models (Okunishi et al., 2009, 2012). Modeling approaches are inherently based on many assumptions, often difficult to validate (Humston et al., 2000) and the accuracy of the results must be verified by comparison with *in situ* data. A new method to determine the environmental factors that sardines experience during early life stages is therefore necessary.

The stable oxygen isotope ratio ($\delta^{18}O$) in biogenic carbonates has often been used as a temperature proxy of ambient water. The otolith of fish is mainly composed of aragonite (Campana, 1999) and the temperature dependency of otolith $\delta^{18}O$ has been quantified in various species (Kalish et al., 1991; Thorrold et al., 1997; Høie et al., 2004; Storm-Suke et al., 2007; Godiksen et al., 2010; Geffen, 2012; Kitagawa et al., 2013). This temperature dependency is often expressed using the

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following simplified formula, referred to as the fractionation equation:

$$\delta_{otolith} - \delta_{water} = a*T + b \quad (1)$$

where $\delta_{otolith}$ is the $\delta^{18}O$ of the otolith, $\delta_{water}$ is the $\delta^{18}O$ of ambient water, and $T$ is the temperature of ambient water, while $a$ and $b$ are constants. Generally, the fractionation equation constants of otoliths are not significantly different from those reported for inorganic aragonite (Kim et al., 2007), indicating that the otolith is formed in or near isotopic equilibrium with ambient water (e.g. Høie et al., 2004). Several authors have noted that the intercepts in Eq. (1) differ significantly among species, although the slopes are similar (Høie et al., 2004; Storm-Suke et al., 2007; Godiksen et al., 2010). Hence, to obtain an accurate estimation of the temperatures an individual has experienced, species- or genus-specific fractionation equations have been recommended instead of the equation for inorganic aragonite (Storm-Suke et al., 2007; Godiksen et al., 2010).

In the Japanese sardine, temperature dependency of otolith $\delta^{18}O$ has not been quantitated. Regarding species in the *Sardinops* genus, Dorval et al. (2011) determined the following formula for the Pacific sardine, *Sardinops sagax*, from rearing experiments using three different temperatures:

$$\delta_{otolith} - \delta_{water} = -0.132*T + 2.455 \quad (2)$$

It should be noted that the slope of Eq. (2) is exceptionally gradual compared to those of inorganic aragonite and other species examined previously. One limitation of this equation is that Dorval et al. (2011) estimated the $\delta^{18}O$ of the otolith deposited during the rearing period indirectly by calculating a mass-balance relationship. This calculation is based on the trend across the whole otolith $\delta^{18}O$ composition with an increase of otolith weight expected with growth (Kalish, 1991; Høie et al., 2004). The use of this estimation was inevitable since the newly formed region of the otolith was too small to directly analyze the isotopic composition by conventionally used mass spectrometry. Due to indirect estimation being inherently uncertain, Eq. (2) must be tested for the ability to precisely estimate temperatures experienced by the Japanese sardine.

In the present study, we aimed to accurately determine the relationship between temperature and otolith $\delta^{18}O$ of the Japanese sardine. To accomplish this, juvenile Japanese sardines were reared at three different temperatures. Using a micro-volume analyzer and micro-scale sampling techniques, we directly analyzed the oxygen isotope composition of the otolith regions deposited during the rearing period, which minimized potential errors that could be caused by indirect estimation.

## 2. Materials and methods

### 2.1. Laboratory experiments

Juvenile Japanese sardines were caught in Sukumo Bay, in the Kochi Prefecture of western Japan, in April 2015, and were transported to the Hakatajima station (Ehime Prefecture) of the National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency. A total of approximately 200 specimens were maintained in a 2-ton circular tank under a photoperiod cycle of 14 h light and 10 h dark. A total of 59 specimens randomly selected were available for otolith analysis. Fish were fed daily with approximately 3% of their body weight (g) of commercial dry pellets (Marubeni Nisshin Feed Co., Ltd., Tokyo, Japan, New Arteck: protein 52%, oil 11%, ash 18%, fiber 3%).

Specimens were kept at three different temperatures maintained using a hot water circular pump system or cooler (IWAKI Co., Ltd, Tokyo, Japan, REI-SEA FZ-602AY). The water temperature in the tank was recorded every hour using a data logger (Onset, MA, USA, Tidbit V2). First, specimens were kept in the 2-ton rearing tank at temperatures ranging from 18 °C to 19 °C for 35 days and then were reared in water at 22 °C for 35 days. Finally, a total of 20 randomly selected specimens were kept in the 1-ton rearing tank at temperatures ranging from 14 °C to 15 °C for 28 days, after two days for assimilation prior to the start of the experiment. The filtered seawater exchange rate was maintained at 360% (300 L $h^{-1}$) of tank volume/day in the 2-ton rearing tank, while it was maintained at 180% (75 L $h^{-1}$) of tank volume/day in the 1-ton rearing tank. The ranges of dissolved oxygen and pH over the experiments were 5.8–6.3 mg/L and 6.9–7.5, respectively. At the end of each period characterized by a constant temperature, 19–20 juveniles were randomly removed from the tank. Standard lengths of the individuals were measured before freezing. Rearing water samples for isotopic analysis were taken from the tank at least twice during each fixed temperature period. Water samples were placed in 2 mL glass screw vials and preserved in a refrigerated storage area until isotopic analysis to prevent evaporation.

### 2.2. $\delta^{18}O$ analysis of otoliths and rearing water

Sagittal otoliths were dissected out and adherent bits of tissue were removed using a needle and a thin paintbrush under 10–20 magnification. Otoliths were rinsed with Milli-Q water and air-dried for 2–3 h. After these cleaning procedures, otoliths were embedded in epoxy resin Petropoxy 154 (Burnham Petrographics LLC), ground with sandpaper (no. 2000), polished with a lapping film (no. 4000), and smoothed with alumina polishing suspension (BAIKOWSKI International Corporation).

Otolith microstructures were observed using an otolith measurement system (Ratoc System Engineering Co. Ltd., Tokyo, Japan). To distinguish the portion of the otolith recently deposited during the rearing period from that deposited when individuals were maintained in a tank at a constant temperature, 28 daily rings were counted from the edge of otoliths with clearly visible rings. Otoliths with indistinctive increments were excluded from further analysis. Photos were taken of each area distinguished for micro-scale sampling (Fig. 1a).

Newly deposited areas were extracted using a high-precision micromilling system (GEOMILL326, Izumo-web, Japan). This system consisted of a micromill, a CMOS camera, a video monitor, and an image analyzer controlled by a computer, and allowed for accurate sampling at 1/1000 mm scales, which enabled us to avoid contamination of pre-experiment otolith material (Sakai, 2009). After milling, otolith powder was collected into an aluminum micro-Petri dish for isotope analysis. We confirmed that the drill pass did not invade the inner area by checking each remaining portion of the otolith using the otolith measurement system (Fig. 1b).

Stable oxygen isotope ratios of the otolith powder were determined using a continuous-flow isotope ratio mass spectrometry system (MICAL3c with IsoPrime 100) at the National Institute of Technology, Ibaraki College. Using this system, we analyzed the $\delta^{18}O$ of ultra-microvolume carbonate samples (as low as 0.2 μg) with high precision and accuracy (Ishimura et al., 2004, 2008; Kitagawa et al., 2013), which allowed us to directly analyze the portion deposited during the rearing experiment. Collected powder was reacted with phosphoric acid at 25 °C, and the resulting liberated $CO_2$ gas was introduced into the mass spectrometer via vacuum purification line. The $\delta^{18}O$ values were reported in δ-notation against the VPDB (Vienna Pee Dee Belemnite) reference standard, and given as a ‰ value. Reproducibility was better than ± 0.10‰ throughout the entire analysis. In order to facilitate the comparison with isotopic values reported in previous studies, we used the acid fractionation factor of calcite.

Oxygen isotope composition of rearing water samples was determined using the Picarro L2120-i Analyzer at the Atmosphere and Ocean Research Institute, University of Tokyo. Before introduction into the analyzer, samples were filtered using membrane filter (pore size: 0.45 μm, Toyo Roshi Kaisha, Ltd.) to reduce suspended particles in order to avoid blocking the sampling line. Data were reported in δ-notation against the VSMOW (Vienna Standard Mean Ocean Water) reference standard and long-term instrument reproducibility was ±