

Detecting the difference of life history in a fish group by using micro-scale oxygen isotopic analysis of otoliths

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Japanese sardine (*Sardinops melanostictus*) has been reported its abundance periodically fluctuates during last 3000 years (Kuwaie *et al.*, 2017). More recently the catch of Japanese sardine declined to about 1/150 in 20 years (Fisheries Agency, 2011), but the mechanism of this phenomena is not well understood. In order to investigate the causes of such fluctuations and enable sustainable fishery, it is necessary to elucidate basic ecological information of sardine, such as their spawning place and migratory route. In that situation, recent progress of analytical technique have allowed us utilize chemical compositions in fish otolith, which is formed in inner ear, to detect the life history of individual fishes. Otolith is a hard tissue composed of calcium carbonate (CaCO_3 : aragonite), and once it crystallizes will not be resorbed. Therefore, the environmental history experienced by individuals is preserved in otolith as chemical composition of CaCO_3 , especially as stable isotopic compositions. Furthermore, in the juvenile period, concentric growth ring are formed daily from the center of otolith. On the other hand, stable oxygen isotope ratio ($\delta^{18}\text{O}$) of CaCO_3 depend on $\delta^{18}\text{O}$ of sea water and surrounding water temperature when inorganic aragonite is formed (Kim *et al.*, 2007). Recent study have also reported that many species of fish show temperature dependency of otoliths $\delta^{18}\text{O}$ almost same as inorganic aragonite. Accordingly, the history of ambient water temperature through the life history of individual fish can be estimated by $\delta^{18}\text{O}$ record in otoliths.

In this study, we analyzed the otoliths of Japanese sardine caught in three area in the Sea of Japan, off Tottori (around Oki, 25/Feb./2015), off Nagasaki (17/Mar. /2015) and Toyama bay (23/Apr. /2015). The otoliths were continuously milled along the growth ring by high-precision micro-milling system (GEOMILL326, Izumo-web Ltd., Japan). After the milling, the stable carbon and oxygen isotopic compositions of otoliths powder were determined by microvolume isotope ratio mass spectrometry system (MICAL3c with IsoPrime100), then the migration pathway of each individuals were estimated based on high-resolution $\delta^{18}\text{O}$ analysis of otoliths. The purpose of analysis are (1) to clarify the difference of migration routes of sardine among three sampling area, and (2) to examine individual differences in $\delta^{18}\text{O}$ history of sardine otoliths in the same areas.

As a result, $\delta^{18}\text{O}$ of otoliths in three area indicate different migration history clearly. As for the sardine caught in off Tottori, comparing the $\delta^{18}\text{O}$ histories of 6 individuals, we recognized that the those are clearly divided into two groups. One group showed ^{18}O -depleted $\delta^{18}\text{O}$ values ($-0.49 \pm 0.18 \text{ ‰}$) and the other group showed more ^{18}O -enriched $\delta^{18}\text{O}$ values ($-0.05 \pm 0.20 \text{ ‰}$) after about 600 μm from the center of otolith which is calculated to be about 80 days after birth (Ohshimo *et al.*, 1997). We concluded that these two groups experienced totally different water temperature history after around 80 days. In this study, we identified the difference of migratory route among each sampling area. Besides, we realized the extraction of ecological information by estimating the formation time of the sardine groups by using high resolution isotopic analysis of otolith.

Keywords: otolith, Stable oxygen isotope, Japanese sardine