Estimation of migratory routes of Japanese sardine in the Tsushima Warm Current area by combining the microscale stable isotopic analysis of otoliths and a data assimilation mode

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Japanese sardine (*Sardinops melanostictus*) has been reported its abundance periodically fluctuates during last 3000 years (Kuwae *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 phenomenon is still undetermined. In order to understand the mechanism of those fluctuations and hence enable future sustainable fishery, it is necessary to elucidate basic ecological information of sardine, such as their spawning place and migration pathway. In that situation, recent progress of analytical technique has 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), the concentric growth rings are formed from the center of otolith. The stable oxygen isotope ratio ($\delta^{18}O$) of $CaCO_3$ depend on (1) $\delta^{18}O$ of sea water and (2) ambient water temperature. Accordingly, the history of ambient water temperature through the life history of individual fish can be estimated by $\delta^{18}O$ record in the otoliths. Besides, by combining the otoliths $\delta^{18}O$ analysis and an individual-based model, recent study successfully clarified the migration pathway of Japanese sardine in the western North Pacific (Sakamoto *et al.*, 2019).

The purpose of this study is (1) to estimate the migration pathway of Japanese sardine in the Tsushima Warm Current area by combining methods of the high-resolution $\delta^{18}O$ analysis and the data assimilation model, and (2) to extract the biological information of Japanese sardine in the Tsushima Warm Current area.

In this study, we collected the otoliths of Japanese sardine caught in three sampling areas in the Sea of Japan: off Toyama (25/Aug./2016), Tsushima Strait (5/Sep./2016) and off Goto Islands (13/Sep./2016), and analyzed the otoliths in three individuals from each sampling area. The otoliths were continuously milled along the growth ring at every 10days (except near the core) by high-precision micro-milling system (GEOMILL326). 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 $\delta^{18}O$ analysis of otoliths and the data assimilation model.

As a result, there was no significant difference in the otolith $\delta^{18}O$ histories between the three individuals collected in each sampling area, and also we found similar trend in isotopic shift (experienced temperature) among sampling areas. The estimated location at the time of the hatching and sampling corresponded to the actual observed spawning grounds and the actual sampling area, respectively, which confirmed the consistency of the estimated migration pathways. In addition, it was suggested that all individuals grew in the same place (offshore west of Kyushu) after hatching and migrated to their caught area by different migration pattern from late June to early July. Our experimental study has successfully

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drawn the variations of migration pathways of Japanese sardine in the Tsushima Warm Current area.

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