論 文 の 内 容 の 要 旨

論文題目：
Studies on the estimation of water temperature experienced during the larval stage of Pacific bluefin tuna *Thunnus orientalis* using SIMS oxygen isotope analysis of otoliths

（SIMSによる耳石酸素安定同位体比分析を用いたクロマグロ仔魚期の経験水温の推定に関する研究）

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1. General introduction

Ocean warming has significant impacts on marine species and ecosystems. Species like Pacific bluefin tuna *Thunnus orientalis* that spawn seasonally in relatively limited areas, namely in the Sea of Japan and the western North Pacific, are particularly vulnerable to increasing water temperature. In fact, numerical simulation shows that their spawning grounds will likely shrink and move northward in response to water temperature rise in the coming decades. However, the effect of ongoing climate change on the spawning ecology and the early life history survival and growth of fish remain largely unexplored due to difficulties in monitoring long-term responses to such climatic stressors. This study aimed to reconstruct water temperature experienced during the larval stage of Pacific bluefin tuna using oxygen isotope analysis of otoliths measured by secondary ion mass spectrometry (SIMS). As estimated temperature experienced during the larval stage likely reflect the natal environment near the spawning grounds, we also evaluated the feasibility of using water temperature estimates based on otolith core $\delta^{18}O$ values to identify the natal origin of an individual fish.

2. Development of sample preparation protocol for SIMS analysis

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. In this chapter, we developed sample preparation methods that minimizes the effect of sample surface irregularities with an appropriate polishing procedure for calcium carbonate like otoliths. The sample preparation method for a single otolith thin section

embedded in epoxy 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. The sample preparation method for multiple otolith thin sections embedded in indium mounts greatly increases the sample throughput per session and allows for more efficient analysis.

3. Reconstruction on temperature experienced during larval stage

In this chapter, we established a method for estimating water temperature experienced during the larvae stage of Pacific bluefin tuna by combining SIMS and microvolume Isotope Ratio Mass Spectrometry (IRMS) stable oxygen isotope ratio ($\delta^{18}O$) analyses of otoliths. Since SIMS is a method that is affected by matrix effects resulting from the difference in composition between the analytical sample and the standard materials, the measured values obtained from SIMS analysis are biased to a certain extent mainly due to the biological organic matter contained in the otolith. Therefore, the $\delta^{18}O$ value of the same samples that were measured by SIMS were measured using IRMS, a method that is not affected by organic materials, to determine the correction coefficient. As a result of SIMS analysis, the $\delta^{18}O$ profiles of all individuals showed an increasing trend toward the first annual increment that is thought to be formed in winter. Given the negative relationship between $\delta^{18}O$ and water temperature, this increase in $\delta^{18}O$ was considered to reflect the actual water temperature decrease experienced by the individuals. In addition, the ambient water temperature experienced during the larval stage estimated using the oxygen isotope fractionation equation between the otolith $\delta^{18}O$ of bluefin tuna larvae and the water temperature overlapped with temperatures associated with the larval occurrence reported in the past larval collection surveys. However, since some individuals appeared to have experienced higher water temperature of about 30°C, it is necessary to further study the estimation accuracy of the water temperature, which is discussed in Chapter 4.

4. Determination of natal origin

Since ambient water temperature experienced immediately after hatching is considered to reflect the surface water temperature in the natal habitat and Pacific bluefin tuna is known to spawn in different water temperatures in the Sea of Japan (cooler temperature) and the western North Pacific (warmer temperature), this chapter examined whether such water temperature estimate is a useful environmental indicator for determining the place of birth of the fish. As a result of otolith $\delta^{18}O$

measurements made by SIMS for 51 Pacific bluefin tuna individuals, the otolith core $\delta^{18}O$ values representing the larval stage significantly differed among the individuals. The results of cluster analysis showed that the 51 individuals could be divided into three distinct groups: cluster 1 consisting of fish that experienced lower temperatures (the Sea of Japan cluster) and clusters 2 and 3 consisting of fish that experienced higher temperatures (the western North Pacific cluster). The estimated water temperatures experienced by the Sea of Japan cluster were approximately 1°C higher than the surface water temperature of the corresponding spawning season, and those experienced by the western North Pacific appeared to have experienced 1 to 2°C higher temperatures than the actual temperatures. The estimation error in water temperature of about 1 to 2°C is most likely due to a combination of the precision error of the SIMS measurements and the SIMS–IRMS offset correction coefficient. Although there is a certain degree of estimation error in water temperature, the clustering results should not be affected significantly since the effects of organic materials deposited on the same growth stage of otoliths on the SIMS $\delta^{18}O$ measurements can be considered relatively similar. Therefore, SIMS $\delta^{18}O$ values of the otolith core is a useful proxy for determining the natal origin of an individual fish. Also, as most fish in the western North Pacific cluster experienced temperatures around 30°C even with possible error considered, Pacific bluefin tuna larvae may be capable of surviving such extreme warm temperatures in the wild. The estimated water temperatures based on the otolith core $\delta^{18}O$ value provides an important evidence on thermal physiological traits of fish in the wild, that cannot be obtained from the laboratory environment.

5. General conclusion

The method to estimate the ambient water temperature experienced during the larval stage of an individual fish using SIMS $\delta^{18}O$ analysis of otoliths provided new opportunities for investigating the physiological thermal traits. In addition, the SIMS $\delta^{18}O$ values measured on the core region representing the larval stage was proven to be an effective environmental indicator for determining the place of birth of an individual fish and showed a potential in the use of assessing the impacts of ocean warming on the spawning and stock dynamics of Pacific bluefin tuna. In order to reveal the long-term effects of ocean warming on the spawning ecology, stock dynamics, and early life history of this species, SIMS $\delta^{18}O$ analysis needs to be conducted with individuals of different age groups with a larger sample size and estimate the recruitment contribution in each spawning ground. The methods developed in this study are expected to be applied to various fish species to investigate the effects of ocean warming on their physiological and ecological traits as well as stock fluctuations.