論文の内容の要旨

水圏生物科学専攻

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論文題目　Studies on sardine (*Sardinops* spp.) stocks using oxygen stable isotope ratios in otoliths

（耳石の酸素安定同位体比を用いたマイワシ属資源の研究）

Sardines, *Sardinops* spp., are short-lived, small pelagic fish that occur widely in temperate areas of global oceans, especially abundant in highly productive areas such as the Kuroshio-Oyashio system or coastal upwelling regions. They efficiently feed on planktons and play crucial roles in energy transfer from low to high trophic levels in marine food webs. Sardines are also economically important because they are a major target of coastal pelagic fisheries. Caught sardines are mainly processed to fishmeal, of which demand is increasing year by year due to the expansion of aquaculture and stock breeding industries or canned for human consumption. However, their abundances are known to fluctuate intensely in multi-decadal scale. The fluctuations are presumably driven by environmental variabilities, although the actual mechanisms have not been revealed. In addition, sardines are highly mobile, and their habitat areas shrink and expand following the variabilities of abundance, which often makes population structures unclear. These cause large difficulties in fisheries managements aiming for sustainable and efficient use of sardine stocks.

Introduction of high-resolution analysis of oxygen stable isotope ($\delta^{18}O$) in otoliths can cut off new aspects of sardine ecology, and thus contribute to solve the problems. Otoliths are calcium carbonate crystals formed in the inner ear of fish. Oxygen stable isotope ratios ($\delta^{18}O$) in otoliths are known to reflect ambient water temperature. Although the application of the isotope analysis has been limited to large otolith species due to the analytical limitation of conventional mass spectrometry, recent remarkable developments in microscale sampling techniques and microvolume analysing systems have opened the door for applications to small otolith species such as sardines. Otolith $\delta^{18}O$ analysis would allow direct and quantitative estimations of ambient water temperature that the fish experienced, which has never been possible in other methods used in researches of sardine stocks.

Here in this thesis, high-resolution otolith $\delta^{18}O$ analysis was introduced to sardines for the first time in the world, to describe movements and the effect of temperature on growth rates during early life history stages of sardines. By extending the target of the analysis to the sardines off South Africa, Japan and California coasts, I also tried to clarify general differences in ecology of sardines in western and eastern boundary current systems.

A first, the temperature dependence of $\delta^{18}O$ in sardine otolith was calibrated through rearing experiment. Japanese sardine juveniles were reared in three different water temperatures over the course of a month. Otolith $\delta^{18}O$ ($\delta_{otolith}$) was then analysed by extracting the portions formed during the rearing period using a micromill. $\delta^{18}O$ of the rearing water (δ_{water}) was also analysed. 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 slightly different from that proposed for inorganic aragonite, with resulting application to wild Japanese sardine captured in the Pacific Ocean showing that it estimates a more realistic *in situ* temperature than equations previously used. Therefore, it was concluded that the sardine-specific isotopic fractionation equation should be used when interpreting otolith $\delta^{18}O$ of sardines.

As a first application, geographical differences in nursery environments of the South African sardine were examined to test the multiple stock hypothesis. The sardine is found off entire coast of South Africa, which includes the western cool region dominated by coastal upwelling and the south-eastern warm region dominated by the western boundary current Agulhas Current, and has recently been hypothesised to be comprised of two or three discrete subpopulations. Sardine otoliths were collected from sardines captured in west, central, south and east coast in summer and winter during 2015–2017, both adults and juveniles, and $\delta^{18}O$ and growth rates during the first 2 months from hatch were examined. From west to south coast, adults and summer captured juveniles showed clear longitudinal gradients in both ambient water temperature and growth rates, while the gradient was not evident in winter captured juveniles. The difference between seasons was attributed to the seasonality of upwelling, which are often intensified even in the south coast during summer. It was concluded that sardines in the west and south coast have significant differences in their nursery environments and resulting larval growths, although the extent may vary seasonally, and provided a new evidence that supports the existence of multiple subpopulations in the region.

Next, I tried to figure out how temperature variations contributed to the recent increase of stock abundances of two sardines in the North Pacific, the Japanese and the Pacific sardine. Although many studies concluded that sardine abundance in the western North Pacific increases in cooler period while in the eastern North Pacific the abundance increases in warmer period, the mechanism connecting the environmental change and sardine production has not been revealed. On otoliths of age-0 Japanese sardines collected during 2006–2010 and 2014–15 and age-1 Pacific sardines collected during 1987, 1991–1998 and 2005–2007, δ^{18}O analysis in 15–30 days resolution and microstructure analysis were performed to estimate temperature and growth histories during early life history stages. The mean temperature histories showed the difference in basic thermal environment between the two regions, warmer in the western North Pacific especially in larval stage. The comparison between temperature histories and growth trajectories showed that in both sides of North Pacific, larval growths are enhanced in relatively warmer waters while juvenile growths showed no or weak negative correlation with temperature, suggesting the general feature of *Sardinops* species. The positive correlations were stronger in the Japanese sardine in larval stages and were stronger in the Pacific sardine in the early juvenile stage, suggesting that the response in the early juvenile stages can be responsible for the response of biomass to the temperature variations. Because the inter-annual variations of temperature were not necessarily associated by cool or warm temperature events, however, other potential drivers, such as predations, need to be considered to fully understand the mechanism of the population fluctuations.

Finally, a method to estimate migration history from otolith δ^{18}O profile by combining numerical simulation was developed, which will be valuable in future studies. Tracking the movement of migratory fish is of great importance in marine biology. Although otolith δ^{18}O has been a potential alternative to tagging and electronic loggers that could not be attached to small fish, the poor resolution of conventional δ^{18}O analysis and the longitudinal homogeneity of open ocean environments have prevented from estimation of migration history. First, using micro-volume carbonate analysing system, otolith δ^{18}O profiles with 10–30 days resolution through entire lives of 6 unmatured

Japanese sardines captured in the offshore Oyashio region, were obtained. An individual-based model with random swimming behaviour in a realistic environment generated by a data assimilation model FRA-ROMS was run to search the routes that are consistent with the otolith δ^{18}O profiles. Although otolith δ^{18}O profiles themselves did not show apparent signs of migration, the analysis combined with simulations successfully showed clear northward migration routes heading for the capture point in the Oyashio region. This method will be a valuable for revealing migration routes in early life stages, thereby providing crucial information to understand population structures and the environmental cause of recruitment variabilities, and to validate and improve fish movement models.

The comparison of sardines in South Africa and North Pacific revealed the general features of *Sardinops* spp. that sardines living in upwelling regions experience lower temperature in the early life stage than those in western boundary current regions, and larval growth are enhanced in warmer waters. Therefore, the basic environment for sardine is different between upwelling and warm current regions, which may be the key to understand why response of the abundance to the temperature variation are opposite between those regions. Overall, I demonstrated that the high-resolution otolith δ^{18}O is capable of answering scientific questions regarding sardine stocks that need to be solved for effective fisheries managements and also that it may be more powerful when combined with numerical simulations. As the temperature dependence of δ^{18}O is a general feature of fish otoliths, the technique and the methods described here will be valuable for studying ecology of numerous fishes, thereby leading to better relationships between human and marine living resources in the future world.