	キョウ　セイケン
氏名（本籍地）	姜　生健（中国）
学位の種類	博士（農学）
学位記番号	農博第 1291 号
学位授与年月日	令和 4 年 3 月 25 日
学位授与の要件	学位規則第 4 条第 1 項
研究科，専攻	東北大学大学院農学研究科（博士課程）資源生物科学専攻
論文題目	Understanding environmental influences on microchemistry and morphology of otoliths in Tokyo Bay flounder (魚類耳石の微量元素組成と形態に対する環境影響に関する研究)
博士論文審査委員	（主査）教授　片山知史
	教授　大越和加
	教授　落合芳博

論文内容要旨

Understanding environmental influences on microchemistry and morphology of fish otoliths

(魚類耳石の微量元素組成と形態に対する環境影響に関する研究)

東北大学大学院農学研究科
資源生物科学専攻
水産資源生態学分野
姜　生健

指導教員
片山 知史　教授

Chapter 1 Introduction

Environmental changes have been drawing attentions of scientists for years, and within marine ecosystem, two of the most concerned problems are hypoxia and ocean acidification (OA). Hypoxia, defined as low or depleted oxygen in water body (dissolved oxygen less than 2 mg/L in water), has increased area and duration in recent decades around the world. Lack of oxygen has been shown to affect marine organisms in multiple ways, such as habitat selection, reproduction and metabolism. OA is the decrease of pH caused by increased amount of discharged CO_2 absorbed into the oceans, and also causes disorders in fish in terms of settlement success and perception of predators. Both problems have been reported to affect fish otoliths, structures that facilitate fish hearing and balancing and are composed mainly of calcium carbonate.

Thus, this study aimed to use marbled flounder to study the potential influences of environmental changes such as hypoxia and ocean acidification (OA) in Tokyo Bay on fish otoliths. As one of the most important fishery sites in Japan, Tokyo Bay has been suffering from summer hypoxia for years, and potentially influenced by OA soon. Marbled flounder, as a demersal fish, was chosen due to its wide distribution. The main focuses of this study were: 1) microchemistry difference between otoliths of marbled flounder captured from Tokyo Bay and the outside open sea; 2) correlation between water and otolith Mn; 3) influences of pH on otolith morphology.

Chapter 2 Otolith microchemistry in Tokyo Bay

Studies have shown that hypoxia increases $[Mn/Ca]_{otolith}$ in multiple fishes, and other elements such as Mg, Ba, and Sr might be correlated to environmental factors like temperature and salinity. Thus, otolith microchemistry was analyzed to evaluate the environmental factor differences among in and outside of the Tokyo Bay areas.

Marbled flounder captured from in Bay (Funabashi, Kaneda, and Takeoka) and outside (Choshi) were dissected (Fig. 1), and otoliths were sectioned for Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA ICPMS) analysis. Values of Mn, Ca, Mg, Ba, and Sr were measured, and $[Element/Ca]_{otolith}$ were boxplotted among locations. $[Mn/Ca]_{otolith}$ of samples in age 0 and 1 captured from Choshi and Tokyo Bay locations were directly plotted to see the seasonal trend, and $[Mn/Ca]_{otolith}$ of all four locations was analyzed by Analysis of Variance (ANOVA). Age 0 and 1 were selected because only these two age stages were completed by all samples.

No difference seemed to be found in $[Mg/Ca]_{otolith}$, $[Ba/Ca]_{otolith}$, and $[Sr/Ca]_{otolith}$, but $[Mn/Ca]_{otolith}$ showed significant difference in age 0 and 1 data (Fig. 2 & 3). The pattern of age 0 matched the expectation that the inner Bay experienced most severe hypoxia and showed the highest $[Mn/Ca]_{otolith}$, followed by the mouth area of the Bay, and finally the outside of the Bay, which did not have hypoxia, and showed the lowest value of $[Mn/Ca]_{otolith}$. These results, together with the seasonal trend revealed by

Tokyo Bay samples (Fig. 4), showed the potential to use $[Mn/Ca]_{otolith}$ as an indicator of hypoxia, especially when the otoliths measured were from young fish (Fig. 3).

Chapter 3 Relationship of Mn concentrations between water and otolith

Although $[Mn/Ca]_{otolith}$ has been shown to correlate with hypoxia, the mechanism is not clear. The most commonly mentioned explanation is that more soluble form of Mn^{2+} was left for otolith uptake when low oxidation happened under hypoxia. In this case, the direct cause seemed to be water Mn concentration. This chapter aimed to examine this correlation between water and otolith Mn, and to explore the mechanism behind elevated $[Mn/Ca]_{otolith}$ under hypoxia.

Juvenile marbled flounder were reared under three different concentrations (control, 30x and 50x) of water Mn for two months, with six fish contained in each treatment, and later another 10 fish were reared under weekly increasing concentrations (from 30x - 190x) for 11 weeks. Otolith and water chemistry were analyzed by ICPMS, and the data was compared among treatments. Samples of the field experiments were compared as well.

The constant concentration did not show strong connection between water and otolith Mn ($Pr = 0.0057$, Tukey's test Control: b, Mn 30: a, Mn 50: ab), and this result was supported by the weekly increasing concentration which revealed that $[Mn/Ca]_{otolith}$ under treatment from 30x to 190x was not higher than normal Mn concentration provided by the institute for stock enhancement before arriving laboratory (Fig. 5). The comparison among treatments and locations between laboratory and field data on maximum $[Mn/Ca]_{otolith}$ suggested the same. Maximum represents the highest value fish could get under a specific environment, and can avoid the seasonal changes in field samples by averaging. However, results revealed that with pretty low $[Mn/Ca]_{water}$ which was close to the control level, field samples showed a much higher $[Mn/Ca]_{otolith}$ even than 50x treatment at laboratory (Table 1, Fig. 6). Results in this chapter indicated that the mechanism of high $[Mn/Ca]_{otolith}$ under hypoxia could not be solely explained by water chemistry.

Chapter 4 Otolith morphology under ocean acidification (OA)

Otoliths have been found to grow larger under OA in former studies, however, the roughness of otolith surface has not been well described yet. This chapter focused on the impacts of pH on otolith morphology.

Juvenile marbled flounder were reared under three pH treatments for two months. Otolith area, perimeter, solidity (defines as area/convex hull area), length, width, and thickness were measured through photos by ImageJ (Fig. 7). Data was first analyzed by principle component analysis (PCA), then ANOVA was applied on the most contributed principle components to reveal difference.

A separation occurred between Control and two pH treatments along Dimension 2 (Fig. 8), and the most contributed variables were solidity, area and thickness. Tukey's test suggested that area and thickness increased under low pH, and solidity became larger, indicating smoother surface but the changes of roughness might have threshold (Fig. 9).

Conclusions

Marbled flounder captured in Tokyo Bay showed a higher $[Mn/Ca]_{otolith}$ than those from the outside, together with the seasonal trend, supporting the occurrence of hypoxia in Tokyo Bay in summers. However, the rearing experiments and the comparison between laboratory and field data revealed that the main reason for elevated $[Mn/Ca]_{otolith}$ under hypoxia was not the change of solubility of Mn irons. Thus, the mechanism requires further studies. OA seemed to affect otoliths and resulted in larger size and smoother surface, and solidity might be used as an indicator to the roughness of otoliths.

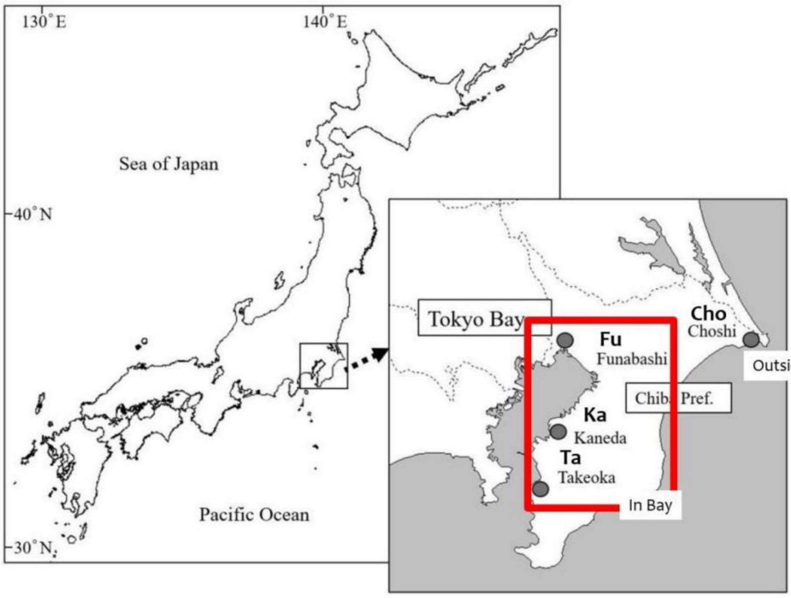

Fig. 1 Map of capture locations. Fish samples were collected from Tokyo Bay (Fu, Ka and Ta shown within the red box) and outside (Cho), belonging to Chiba Prefecture of Japan. White area in detailed map shows land and gray area is water. Water sample was collected near Fu.

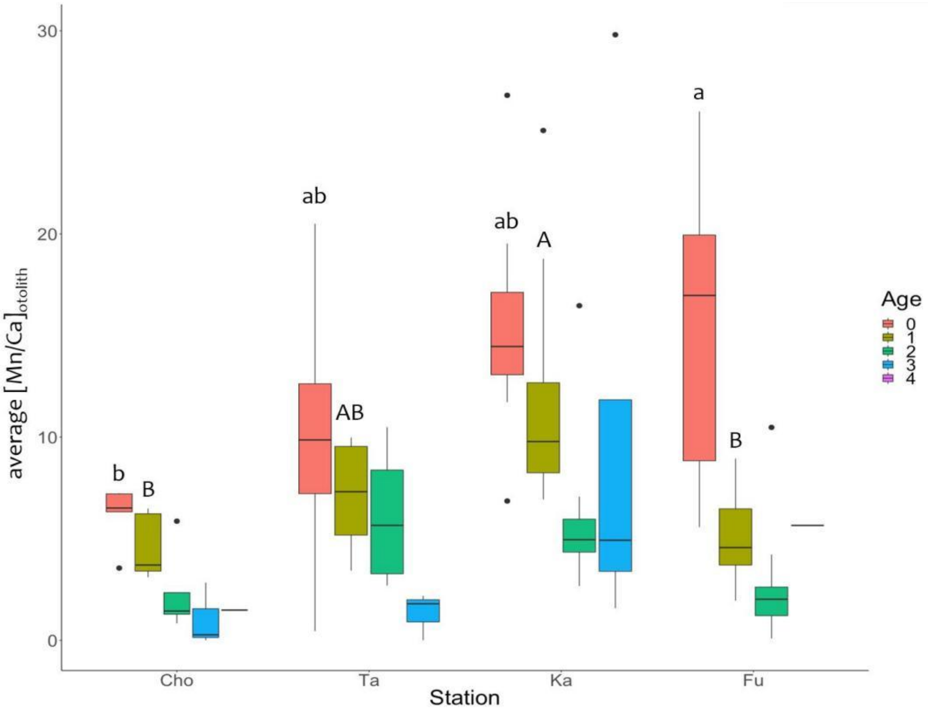

Fig. 3 Boxplots of average $[Mn/Ca]_{otolith}$ (μmol/mol) of otoliths from outside (Cho) to mouth (Ta and Ka) and inner Bay (Fu). Each box showed the $[Mn/Ca]_{otolith}$ averaged for different ages within each station. Colors represented ages, and significant differences of the Tukey's test on Age 0 and 1 were shown on top of the boxes as Age 0: a, ab, b; Age 1: A, AB, B.

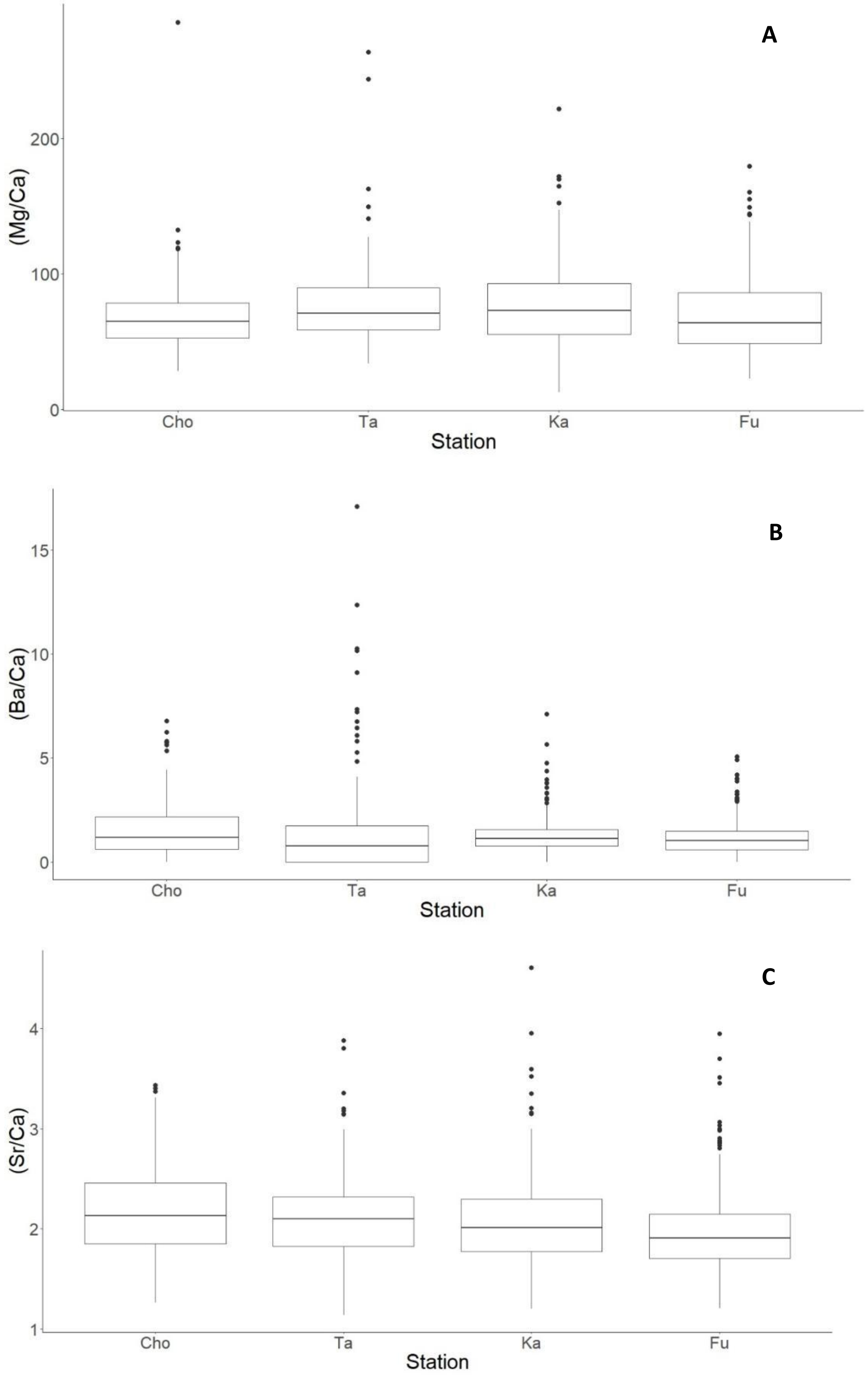

Fig. 2 Boxplots of average $[Element/Ca]_{otolith}$ compared by locations. A: $[Mg/Ca]_{otolith}$ (μmol/mol); B: $[Ba/Ca]_{otolith}$ (μmol/mol); and C: $[Sr/Ca]_{otolith}$ (mmol/mol).

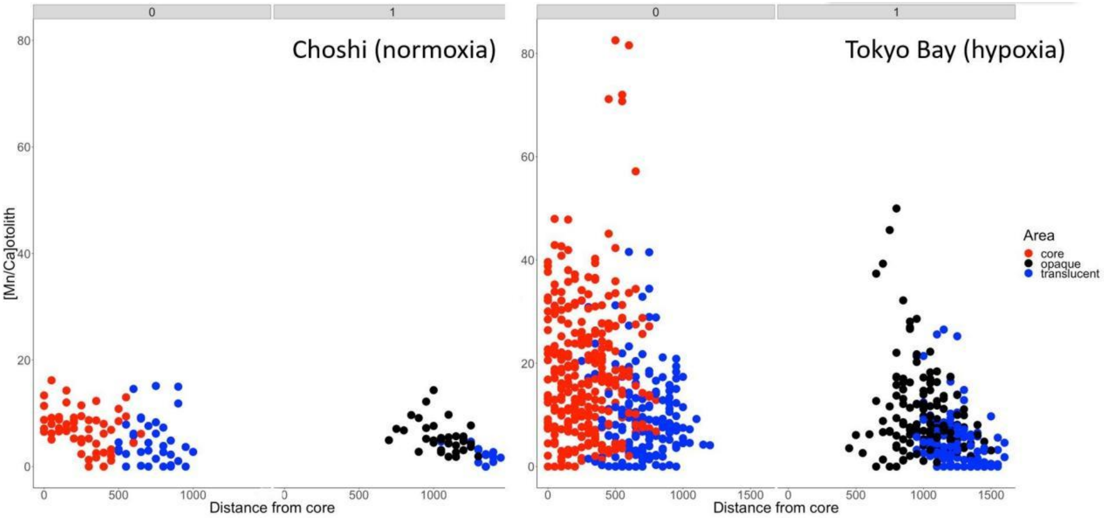

Fig. 4 Seasonal trend in [Mn/Ca]$_{otolith}$ (µmol/mol) of Tokyo Bay samples (Fu, Ka, & Ta) at age stage 0 and 1 compared to outside of the Bay (Cho/Choshi on the left). The x-axis represents the distance from core to edge on otolith (µm). Colors indicate different structures as core (the center), opaque (white and unclear areas), and translucent (clear areas).

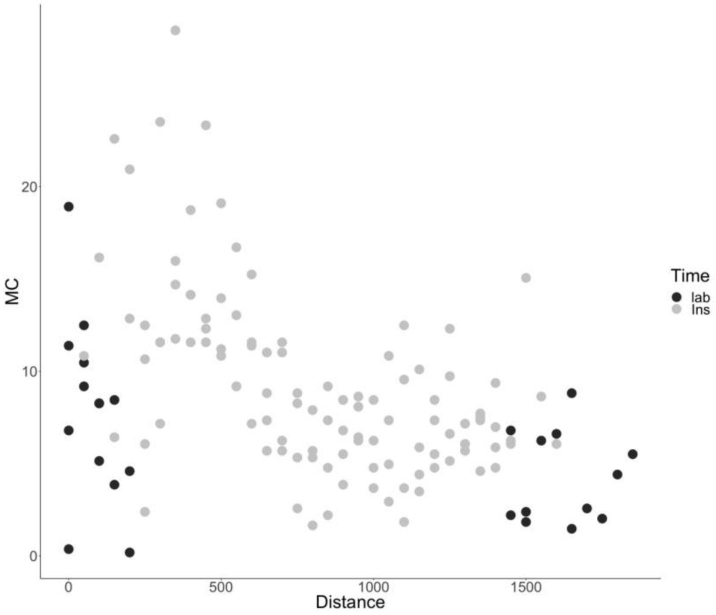

Fig. 5 Distribution of [Mn/Ca]$_{otolith}$ in the Exp. Weekly increasing Mn. The x-axis is the diameter of otoliths (µm), and the y-axis is the [Mn/Ca]$_{otolith}$ (µmol/mol) along this diameter. The dark dots represent microchemistry formed under treatment, and the gray dots were formed at the institute for stock enhancement before arriving laboratory. Symbols in figure: MC is [Mn/Ca]$_{otolith}$, Lab is laboratory, and Ins is institute.

Table 1 Water chemistry of the laboratory and field experiments. Control, Mn 30 and Mn 50 were collected from the rearing experiment weekly, and water samples of the field experiment were collected near Fu. Values are represented as mean ± standard deviation (SD).

	Mn (mmol)	Ca (mol)	Mn/Ca (mmol/mol)	Mg (mol)	Ba (mmol)	Sr (mmol)
Control	0.50±0.16	4.45±0.68	0.12±0.06	87.8±15.7	3.27±0.57	81.8±10.9
Mn 30	6.94±1.10	4.48±0.84	1.57±0.20	87.0±16.5	2.92±0.55	82.0±14.5
Mn 50	10.4±1.92	4.23±0.74	2.47±0.10	81.4±15.4	2.76±0.71	76.4±13.1
Tokyo Bay	0.30±0.01	2.85±0.34	0.11±0.01	47.0±5.5	0.06±0.01	77.6±4.8

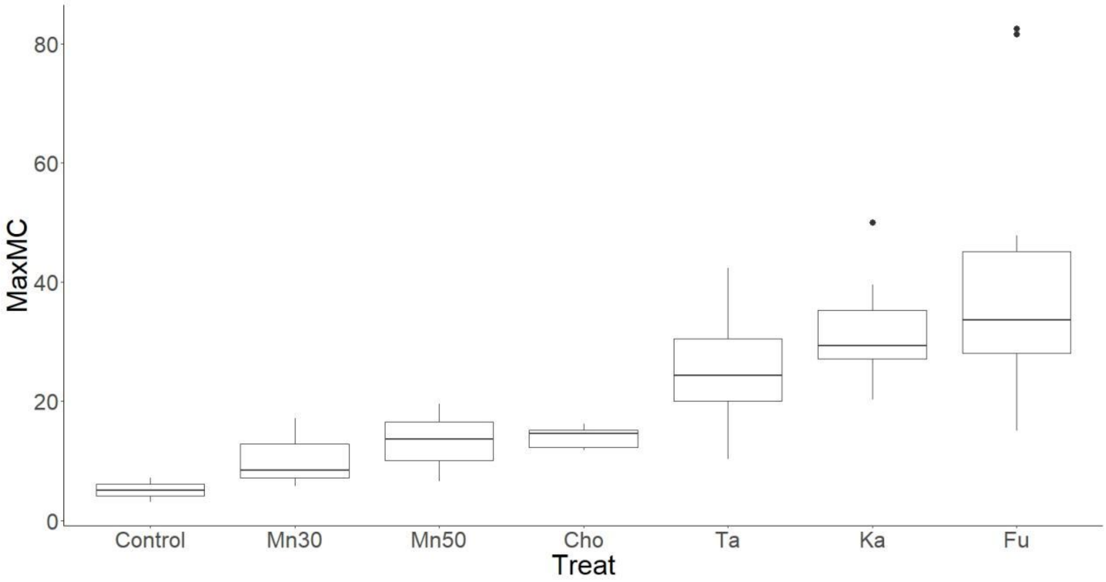

Fig. 6 Boxplot of maximum $[Mn/Ca]_{otolith}$ (µmol/mol) of all treatment groups from the laboratory experiment and locations from the field experiment. The maximum value of $[Mn/Ca]_{otolith}$ in each sample (y-axis) was compared between the rearing experiment (Control, Mn 30 and Mn 50) and field experiment (normoxic: Cho; hypoxic: Ta, Ka, and Fu).

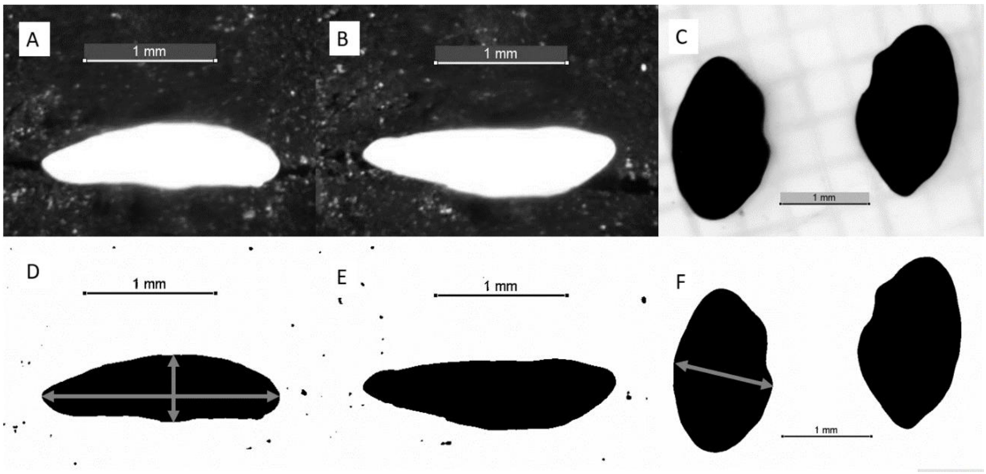

Fig. 7 Otolith measurements used as variables in this study. All photos taken were of sample 24 (pH 7.5) on lateral face (A, B, D, E) and flat surface (C, F). A, D, and otoliths on the left in C and F are eye-side otoliths, and B, E, and the right ones in C and F are blind-side otoliths. A and B were taken using an anatomical lens. C is background reversed by Paint. D, E, and F are the final analyzed versions in Image J The solidity, area, and perimeter of each dark area in D, E, and F were directly measured by ImageJ, and the arrows represent the lateral length (long arrow on D), thickness (short arrow on D), and width (arrow on F).

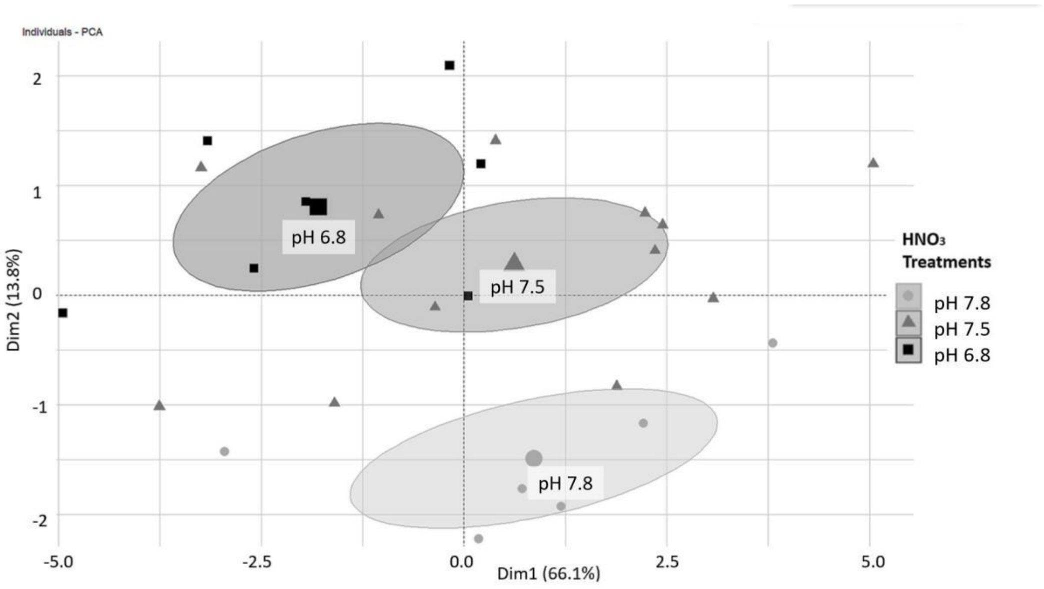

Fig. 8 Individual plot of PCA on otolith measurements together with fish SL. Shapes represent the pH treatments, with the small icons being individual samples, and the big circle, triangle and square in the central area of each ellipse being the average of each treatment group. A 95% confidence ellipse is used for each treatment to highlight data separation.

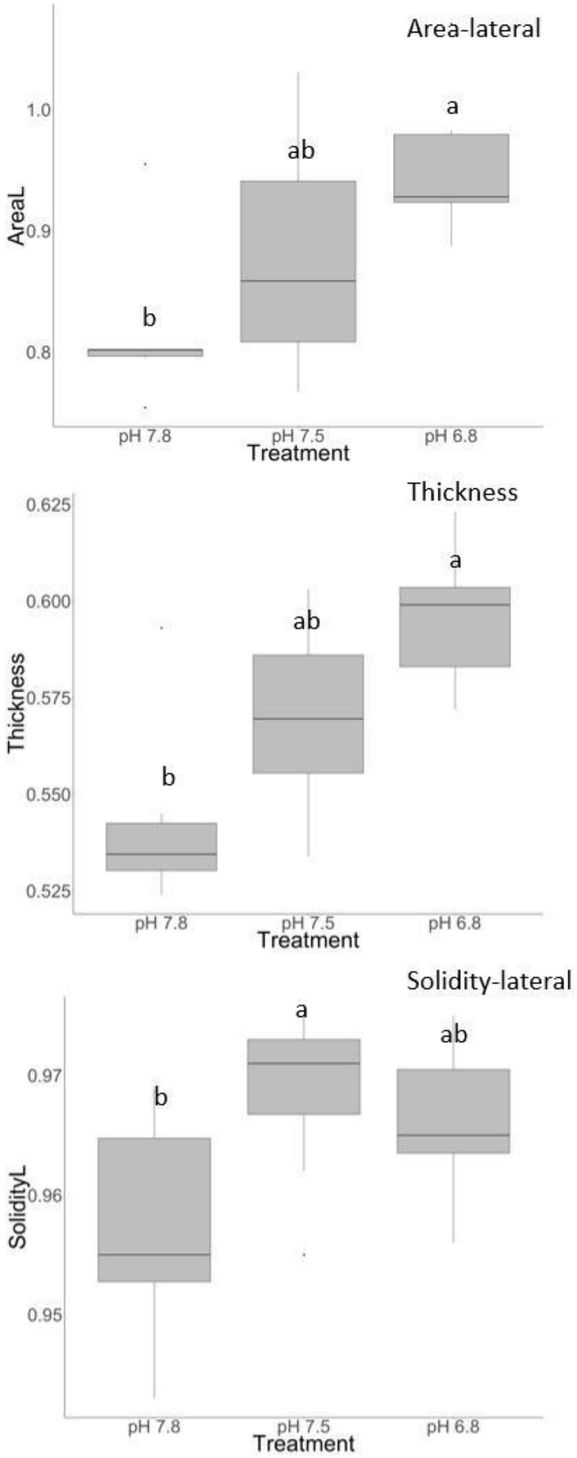

Fig. 9 Boxplot of the most contributed variables on Dimension 2. Letters represented significant differences among the HNO_3 treatments revealed by Tukey's test.

論文審査の結果の要旨及び担当者

氏名	姜　生健
審査委員	主査：教授　片山知史 副査：教授　大越和加　　教授　落合芳博
学位論文題目	Understanding environmental influences on microchemistry and morphology of fish otolisths（魚類耳石の微量元素組成と形態に対する環境影響に関する研究）

論文審査の結果の要旨

耳石の微量元素については、Sr が塩分の指標となることが知られている。その他、Mn, Mg, Fe 等が存在するがそれらの含有量を左右する環境要因は不明であった。東京湾のマコガレイのMnを調べたところ、外房（銚子）には極めて少なかったが、湾奥（船橋）、内房（竹岡、金田）においては、特に春季、夏季に大きく増加することがわかった。海底底質中のMnが、貧酸素・還元状態で溶出し増加することに起因すると考えられた。

この仮説を検証するために、飼育下におけるMn添加実験を行った。耳石Mn密度はコントロールに比べて有意に増加したが、水中Mn濃度とは相関しなかった。したがって、内湾の貧酸素条件等に加えてストレス等が耳石Mnを増加させるものと考えられた。これらの結果より、耳石 Mn が内湾環境の指標となり得る可能性が示された。

また地球温暖化ガスの二酸化炭素濃度増加がもたらす海洋酸性化の影響を明らかにするために、pH 低下と耳石外部形態の関係をマコガレイの飼育実験によって調べたところ、pH7.5, 6.8においては、耳石のサイズが大きくなり凹凸が減少した。

本研究によって明らかにされた、耳石の微量元素（Mn）への環境影響は、種々の内湾資源が貧酸素環境を利用しながら生活史を全うしている沿岸生態系理解に大きく寄与すると考えられ、博士（農学）に相応しい研究内容であると判断される。