

Unravelling habitat use of *Coilia nasus* from the Rokkaku River and Chikugo River estuaries of Japan by otolith strontium and calcium

LIU Hongbo¹, JIANG Tao¹, YANG Jian^{1*}

¹ Key Laboratory of Fishery Ecological Environment Assessment and Resource Conservation in Middle and Lower Reaches of the Yangtze River, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi 214081, China

Received 13 September 2017; accepted 13 October 2017

© Chinese Society for Oceanography and Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

The migratory history of the engraulid fish *Coilia nasus* in the Rokkaku and Chikugo River estuaries of the Ariake Sea, Japan was assessed using otolith strontium (Sr) X-ray intensity maps and strontium:calcium (Sr:Ca) ratio life history transects by an electron probe microanalyzer (EPMA). The results showed that seven of the ten specimens from the Rokkaku River Estuary (LJC) and all 15 specimens collected in the Chikugo River Estuary (ZHC) had low Sr:Ca ratios (≤ 3) at the central otolith area, indicating their riverine origin and initial freshwater residence. After the first regime shift adjacent to natal regions, the Sr level mapping displayed a wide variety of color patterns, and the Sr:Ca ratios obtained by the line transect analysis could be divided into one to six significantly different phases indicative of gradual life history transition. The other three specimens from the Rokkaku River Estuary had high Sr:Ca ratios (3–6.7) at the central otolith area but showed alternating changes between low and high values outside the natal region, suggesting that estuarine-origin individuals occurred in the Rokkaku River Estuary. The two-dimensional maps of the Sr level and average of the otolith Sr:Ca ratios along the life-history transects could be used as effective tools for reconstruction of past habitat use of the tapertail anchovy in estuaries of the Ariake Sea, Japan.

Key words: *Coilia nasus*, otolith microchemistry, habitat use, Rokkaku River, Chikugo River

Citation: Liu Hongbo, Jiang Tao, Yang Jian. 2018. Unravelling habitat use of *Coilia nasus* from the Rokkaku River and Chikugo River estuaries of Japan by otolith strontium and calcium. Acta Oceanologica Sinica, 37(6): 52–60, doi: 10.1007/s13131-018-1190-8

1 Introduction

The estuarine tapertail anchovy *Coilia nasus* Temminck et Schlegel, 1846 (junior synonym *Coilia ectenes* Jordan et Seale, 1905) is commonly distributed in East Asia including China, Korea, and Japan. *Coilia nasus* is a famous traditional delicacy, and is a highly commercial anadromous species present in the estuaries of several main rivers (e.g., Changjiang River (Yangtze River), Huanghe River (Yellow River), Qiantangjiang River, Oujiang River) in China (Jiang et al., 2014), and the estuaries of rivers (especially the Chikugo River) flowing into the Ariake Sea of Kyushu in Japan (Suzuki et al., 2014). As *C. nasus* is an endemic and restricted species in the Ariake areas of Japan, it has been listed as a threatened species in the Japan Red Data Book of Japan's Ministry of the Environment and placed in the category of "vulnerable" species by Japan's Fisheries Agency. Importantly, this species is regarded from its habitat as an Asian continental relict (Yagi et al., 2011; Simanjuntak et al., 2015) in Japan, as only the Ariake Sea retained an environment of large tidal range and vast tidal flats similar to those in the Yellow Sea and East China Sea (Yagi et al., 2011). Therefore, life histories between the *C. nasus* populations in China (e.g., the Changjiang River) and Japan (e.g., the Chikugo River) may be comparable.

So far, there have been a considerable number of studies on distribution patterns, feeding habits and spawning areas of *C. nasus* in the estuary of the Chikugo River, which is the largest river discharging into the Ariake Sea (e.g., Takita and Masutani, 1979; Matsui, 1986a, b; Suzuki et al., 2014; Simanjuntak et al., 2015). However, the spatial and temporal dynamics are still poorly known for this species during the life history in the estuaries of other rivers (e.g., the Rokkaku River) along the coast of the Ariake Sea. Noteworthy, otoliths of teleost fish grow continuously throughout entire life history (Campana, 1999) and otolith core region corresponds to the larval period (Secor et al., 1998). Moreover, previous studies have already demonstrated that otolith strontium (Sr) and calcium (Ca) signatures can be applied as a useful scalar to trace environmental history of fish, since the deposition of Sr and the Sr:Ca ratio positively correlates with the salinities of freshwater, brackish water, and sea water (Secor and Rooker, 2000; Zimmerman, 2005; Yang et al., 2011). Recently, based on otolith microchemical analysis of Sr:Ca ratio and Sr content, a number of previously unknown features of life history patterns were revealed in *C. nasus* from the Changjiang River in China. These include the composition of an apparent spawning population by individuals with a variety of migratory patterns, es-

Foundation item: The National Natural Science Foundation of China under contract No. 31372533; China Central Governmental Research Institutional Basic Special Research Project from Public Welfare Fund under contract No. 2016PT01; the "948" Program of the Ministry of Agriculture under contract No. 2014-S6.

*Corresponding author, E-mail: jiany@ffrc.cn

tuarine or freshwater resident phenotypes of *C. nasus*, and the tendency of different natal riverine homing of *C. nasus* in different water areas along the Chinese coast (Yang et al., 2006; Dou et al., 2012; Jiang et al., 2014, 2016; Chen et al., 2017). These updated studies provide more accurate and detailed evidence for the need for further assessment of quantity and resource composition of the anchovies with different migratory patterns, as well as the need to develop a plan of habitat conservation and nature reserve settlement. Furthermore, an improved understanding of diverse mechanisms to adapt to different salinity environment of this fish in Japan and China is desirable. Therefore, we hypothesized that *C. nasus* in river estuaries around the Ariake Sea of Japan must have much more diverse and flexible patterns of life history among habitats of fresh, brackish and sea water. Otolith microchemical analysis can give new insights to the ecological study and conservation of this endemic and continental relict species. To test the above hypothesis, in this pilot study we examined the otolith Sr and Ca microchemistry of *C. nasus* in the estuaries of the Chikugo River and the Rokkaku River.

The tapertail anchovy has been confirmed to inhabit both the Chikugo River and Rokkaku River although the spawning sites may only be located in the Chikugo River (Takita and Masutani, 1979). The Rokkaku River Estuary is a brackish, highly turbid water estuary which is located in the northern portion of the Ariake Sea (Yagi et al., 2011), while the Chikugo River is the largest river flowing into the Ariake Sea which receives strong tidal currents in the north-eastern part of the inner Ariake Bay (Islam et al., 2007; Suzuki et al., 2014).

The objectives of the present study were to validate the environmental markers of Sr and Ca in the otoliths of *C. nasus* from the estuaries of the Rokkaku River and Chikugo River around the Ariake Sea and accumulate information regarding the spatial and temporal dynamics in habitat use of the *C. nasus*. The information will be important for effective conservation and management of the valuable species.

2 Materials and methods

The target field sites of this study are the Rokkaku River and Chikugo River estuaries (Fig. 1). Table 1 provides the background information of *C. nasus* collected from different regions.

Ten specimens of *C. nasus* (total length (L_T): range, 15.7–23.8 cm; mean \pm SD, (20.2 \pm 2.3) cm; similarly hereafter) aged 1–2 years were captured in the Rokkaku River Estuary (LJC) in Saga Prefecture on 15 June and 30 August of 2011. The other fifteen *C. nasus* (L_T : 26.3–32.5 cm, (29.2 \pm 1.8) cm) aged 2–3 years were collected in the Jojima section of the Chikugo River (ZHC) in Fukuoka Prefecture on 30 June of 2015.

Methods of preparing *C. nasus* otoliths for use in electron probe microanalysis (EPMA) measurement were described by Jiang et al. (2017) and Chen et al. (2017). The sagittae otoliths were extracted first. After cleaning with deionized water, all left otoliths were embedded in epoxy resin (EpoFix; Struers, Copenhagen, Denmark) in the frontal plane until solidification. The embedded otoliths were ground to expose their core with a grinding machine equipped with a diamond cup wheel (Discoplan-TS; Struers, Copenhagen, Denmark). Each otolith was further polished on an automated polishing wheel (LaboPol-35; Struers, Copenhagen, Denmark). The otoliths were then cleaned in an ultrasonic bath and rinsed with deionized water. Finally, all otoliths were dried and then carbon coated by a high vacuum evaporator (JEE-420, JEOL Ltd., Tokyo, Japan) for further examination.

EPMA was used to study the otolith elements Sr and Ca, based on the method described by Chen et al. (2017) but with a slight modification. Samples were measured along a line of elements detectable in each otolith from the core to the edge (i.e., otolith radius) using a wavelength dispersive X-ray electron probe microanalyzer (JXA-8100; JEOL Ltd.). Commercial standards of tausonite (SrTiO₃) and calcite (CaCO₃) (Institute of Mineral Resources, the Chinese Academy of Geological Sciences, Beijing, China) were used for calibrating the Sr and Ca contents in the otolith samples. The accelerating voltage and beam current were 15 kV and 2 \times 10⁻⁸ A, respectively. The electron beam

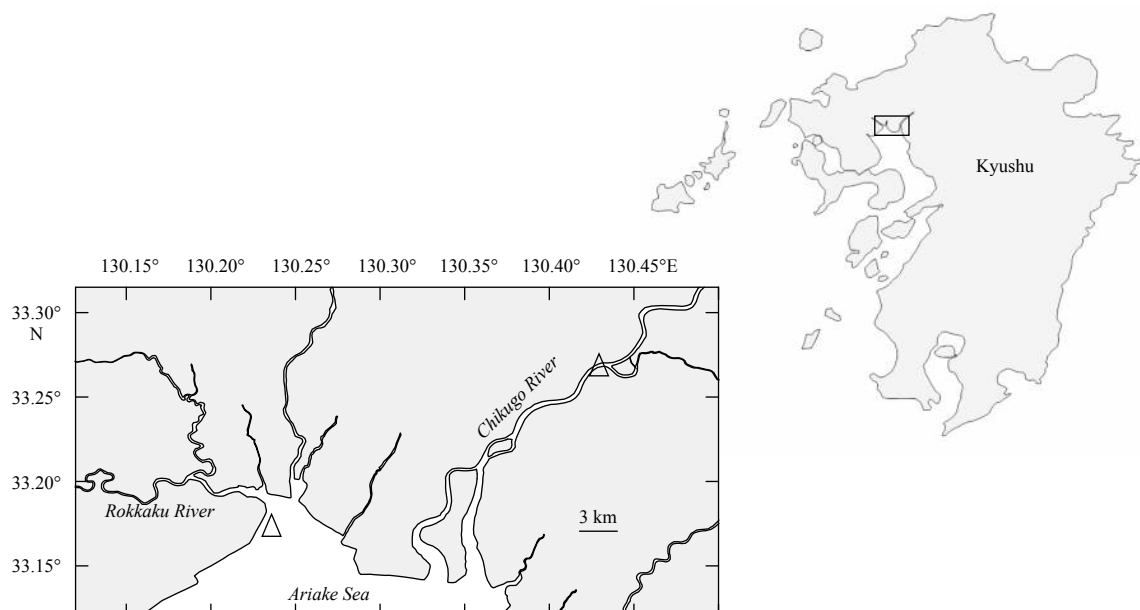


Fig. 1. Sampling sites (Δ) for *Coilia nasus* in the Rokkaku River and Chikugo River estuaries along the coast of the Ariake Sea, Kyushu, Japan.

Table 1. Estuarine tapertail anchovy *Coilia nasus* used in the present study

Sampling location	Sample code	Total length /mm	Estimated age
The Rokkaku River Estuary	LJC01	198	1+
	LJC02	238	1+
	LJC03	184	1+
	LJC04	204	1+
	LJC05	157	1+
	LJC06	187	1+
	LJC07	207	1+
	LJC08	211	1+
	LJC09	229	1+
	LJC10	209	1+
Jojima section of the Chikugo River	ZHC01	315	2+
	ZHC02	297	2+
	ZHC03	265	2+
	ZHC04	308	2+
	ZHC05	305	2+
	ZHC06	268	2+
	ZHC07	278	2+
	ZHC09	284	2+
	ZHC10	294	2+
	ZHC12	263	2+
	ZHC14	293	2+
ZHC15	325	2+	
ZHC17	302	2+	
ZHC19	289	2+	
ZHC20	293	2+	

was focused on a point 5 μm in diameter, with measurements spaced at 10 μm intervals. The X-ray intensity mapping of Sr was performed with 15 kV and 5×10^{-7} A of accelerating voltage and beam current, respectively. The counting time was 30 ms and the electron beam was focused on a point 5 μm in diameter. The pixel size was 7 $\mu\text{m} \times 7 \mu\text{m}$ and 6 $\mu\text{m} \times 6 \mu\text{m}$ for otoliths of fish from LJC and ZHC, respectively. Based on our previous studies (Yang

et al., 2006; Jiang et al., 2014; Chen et al., 2017) on otoliths of *C. nasus* which indicated bluish, greenish-yellowish and reddish regions, these were characteristics of freshwater (low salinity), brackish water (medium salinity) and sea water (high salinity), respectively, by corresponding 16 color map patterns of Sr concentration from blue (lowest) through green and yellow, to red (highest).

By conventional otolith research, Sr:Ca ratios of concentrations were expressed as the ratio of Sr to Ca amplifying by 1 000 by a simple conversion based on the molecular weights of SrO and CaO. Statistical analysis was performed using Excel 2013 (Microsoft, Seattle, WA, USA) and IBM SPSS Statistics v.19.0 (IBM Corp., Armonk, NY, USA). The Mann-Whitney *U*-test was used to test the differences between otolith Sr:Ca ratios. In addition, a sequential regime shift algorithm was applied to identify significant changes between the current mean of Sr:Ca ratio data and that of a consecutive point, an indication of the life history transition (Rodionov, 2004; Altenritter et al., 2015). The inputs of the regime shift index (RSI) were established as follows: cut-off length=10, probability level=0.1, and Huber's weight parameter=1 (Rodionov and Overland, 2005).

3 Results

3.1 *Coilia nasus* from the Rokkaku River

In the ten specimens of *C. nasus* from the Rokkaku River Estuary, two patterns of otolith in the life history transect were observed (Table 2, Fig. 2). In the first pattern, the Sr:Ca ratios in the central regions of seven fish (LJC01, LJC02, LJC05, LJC07, LJC08, LJC09, and LJC10) otoliths possessed low values (≤ 3 , mean \pm SD: 1.98 ± 0.91 to 2.75 ± 0.82 , similarly hereafter) within a distance of 50–1 630 μm from the otolith core, probably corresponding to larval (LJC01, LJC02, LJC09) or juvenile (LJC05, LJC07, LJC08, and LJC10) stages. The X-ray intensity map of Sr in the remaining otolith regions displayed a wide variety of color patterns (from blue-lowest, to green to red-highest, Fig. 3). The Sr:Ca ratios obtained by the line transect analysis could be divided into one (LJC07 and LJC08) to five (LJC05) significantly different phases indicative of

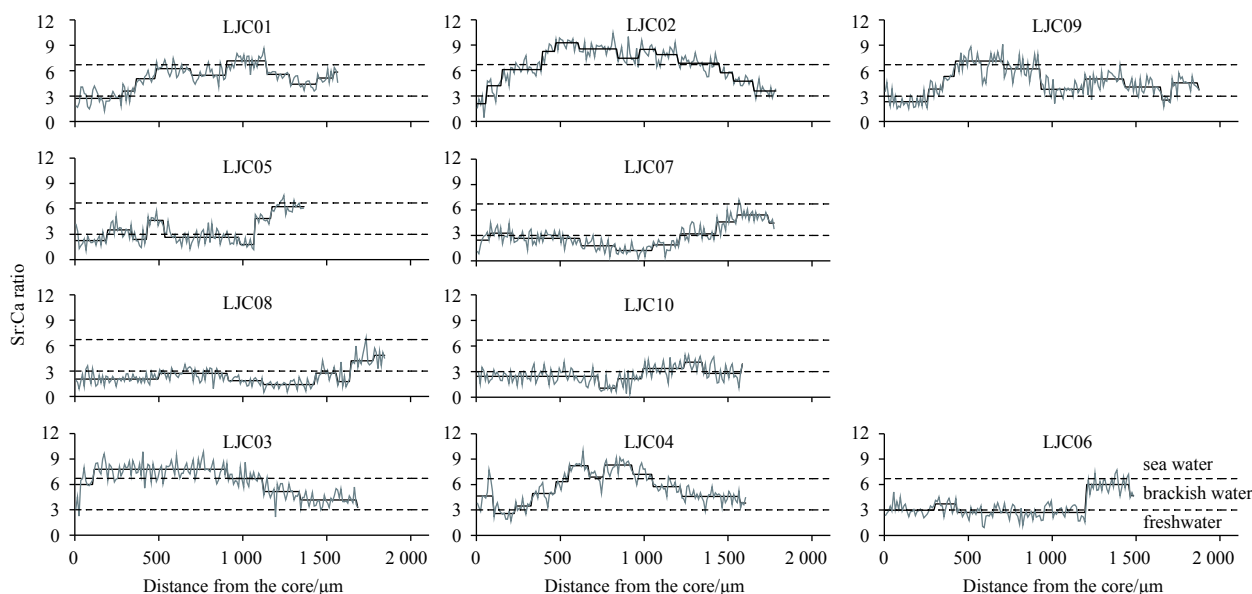
**Fig. 2.** Fluctuation (grey line) and shift (black line) of Sr:Ca ratios (the ratio of Sr to Ca amplifying by 1 000) along line transects from the core (0 μm) to the edge of sagittal otoliths of *Coilia nasus* collected from the Rokkaku River Estuary.

Table 2. Fluctuation of Sr and Ca microchemistry in otoliths of *Coilia nasus* from the Rokkaku River Estuary, Kyushu, Japan

Sample code	Distance from the core/ μm	Sr:Ca ratio	Fluctuation phases of otolith Sr:Ca ratio
LJC01	0–270	2.75 \pm 0.82	1 ^a (FW)
	270–890	5.41 \pm 1.08	2 ^b (BW)
	890–1 130	7.13 \pm 0.74	3 ^c (SW)
	1 130–1 560	5.06 \pm 0.72	4 ^d (BW)
LJC02	0–50	1.98 \pm 0.91	1 ^a (FW)
	50–380	5.54 \pm 1.22	2 ^b (BW)
	380–1 190	8.38 \pm 0.89	3 ^c (SW)
	1 190–1 780	5.5 \pm 1.45	4 ^b (BW)
LJC03	0–100	5.62 \pm 1.68	1 ^a (BW)
	100–900	7.77 \pm 0.9	2 ^b (SW)
	900–1 680	5.13 \pm 1.32	3 ^a (BW)
LJC04	0–90	4.84 \pm 1.49	1 ^a (BW)
	90–220	2.61 \pm 0.61	2 ^b (FW)
	220–510	4.6 \pm 1.1	3 ^a (BW)
	510–1 040	7.71 \pm 0.92	4 ^c (SW)
	1 040–1 600	4.88 \pm 0.86	5 ^a (BW)
LJC05	0–180	2.3 \pm 0.76	1 ^a (FW)
	180–330	3.57 \pm 0.73	2 ^b (BW)
	330–420	2.4 \pm 0.73	3 ^a (FW)
	420–520	4.66 \pm 0.55	4 ^c (BW)
	520–1 060	2.53 \pm 0.78	5 ^a (FW)
LJC06	1 060–1 360	5.8 \pm 0.85	6 ^d (BW)
	0–420	3.23 \pm 0.72	1 ^a (BW)
	420–1 190	2.68 \pm 0.7	2 ^b (FW)
LJC07	1 190–1 480	5.9 \pm 0.92	3 ^c (BW)
	0–1 200	2.18 \pm 0.9	1 ^a (FW)
LJC08	1 200–1 770	4.37 \pm 1.18	2 ^b (BW)
	0–1 630	2.11 \pm 0.74	1 ^a (FW)
LJC09	1 630–1 840	4.55 \pm 0.98	2 ^b (BW)
	0–250	2.44 \pm 0.83	1 ^a (FW)
	250–410	4.5 \pm 0.96	2 ^b (BW)
	410–700	7.06 \pm 1.13	3 ^c (SW)
LJC10	700–1 870	4.6 \pm 1.27	4 ^b (BW)
	0–980	2.24 \pm 0.81	1 ^a (FW)
	980–1 340	3.58 \pm 0.78	2 ^b (BW)
	1 340–1 570	2.84 \pm 0.94	3 ^c (FW)

Note: Sr:Ca ratio is expressed as the ratio of Sr to Ca amplifying by 1 000. Phases in one otolith sample having the same superscript letter indicate no significant differences ($P>0.05$); whereas different superscript letters indicate significant differences ($P<0.05$). FW, BW and SW indicate the phases of freshwater, brackish water and sea water, respectively.

life history transition based on the aforementioned sequential regime shift algorithm (Table 2, Fig. 2). Particularly, mean values of the Sr:Ca ratios could be as high as >7 in certain areas of the otolith regions in the individuals of LJC01, LJC02, LJC09 (Table 2) and generally remained at high levels (>4.50) in the other regions.

In the second pattern, the Sr:Ca ratios in the central regions of three fish (LJC03, LJC04, and LJC06) otoliths had high values (>3 , 3.23 ± 0.72 to 5.62 ± 1.68) within a distance of 90–420 μm from the otolith core, although those in the other regions varied drastically at low (2.61 ± 0.61 to 2.68 ± 0.70) or high levels (4.60 ± 1.10 to 7.77 ± 0.90) (Table 2, Fig. 2). The X-ray intensity maps of the Sr-level distribution in the otoliths showed patterns similar to those of the Sr:Ca ratios in the above life history transects with a wide variety of color patterns (Fig. 3).

Generally, all otolith samples of LJC01, LJC02, LJC05, LJC07, LJC08, LJC09, and LJC10 had similar bluish central regions, sug-

gesting that the fish were born in freshwater. Thereafter, the color patterns alternated among greenish or yellowish or even reddish bands, corresponding to the migration of these fish in the estuarine and even offshore sea habitats (e.g., LJC01, LJC02, and LJC09) (Fig. 3). In contrast, otolith samples of LJC03, LJC04, and LJC06 had greenish or yellowish central regions. The color patterns for the other regions of these fish otoliths varied among bluish, greenish, yellowish or reddish bands, which implied that the fish were not born in freshwater but were more likely born in estuarine brackish water.

3.2 *Coilia nasus* from the Chikugo River

In the fifteen specimens of *C. nasus* captured from the Chikugo River Estuary, the life-history transect analyses (Table 3, Fig. 4) and X-ray intensity maps (Fig. 5) showed that all otoliths had a similar bluish color patterns and low Sr:Ca ratios (≤ 3) in their central regions, which were consistent with a presumed

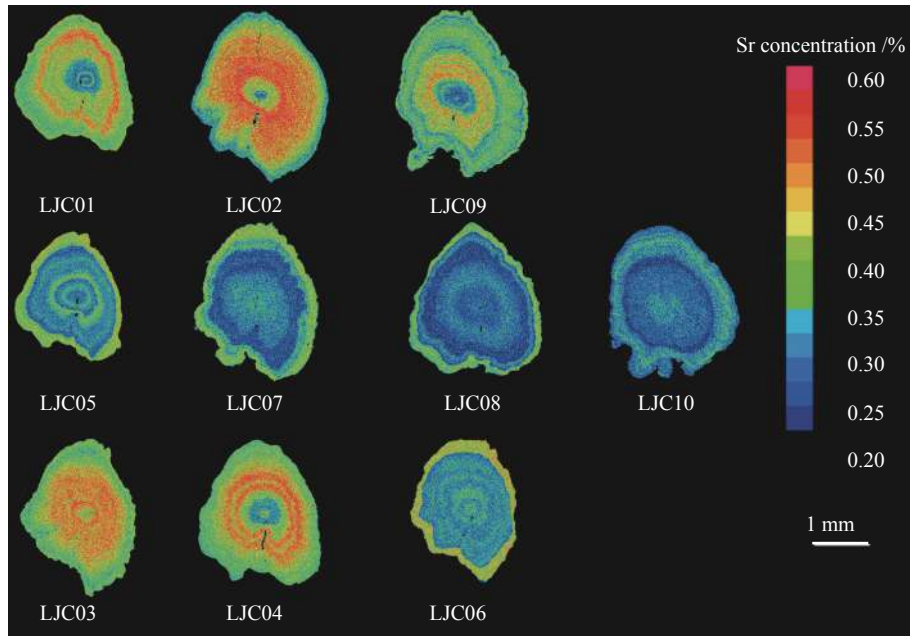


Fig. 3. Strontium (Sr) imaging of *Coilia nasus* collected from the Rokkaku River Estuary using otolith mapping analysis.

Table 3. Fluctuation of Sr and Ca microchemistry in otoliths of *Coilia nasus* from Jojima section of the Chikugo River, Kyushu, Japan

Sample code	Distance from the core/ μm	Sr:Ca ratio	Fluctuation phases of otolith Sr:Ca ratio
ZHC01	0–80	1.97 ± 0.79	1 ^a (FW)
	80–790	3.68 ± 0.86	2 ^b (BW)
	790–1 010	2.61 ± 0.52	3 ^c (FW)
	1 010–1 780	4.12 ± 0.98	4 ^d (BW)
	1 780–1 970	2.2 ± 0.8	5 ^{ac} (FW)
	1 970–2 060	3.68 ± 1.02	6 ^{bd} (BW)
ZHC02	0–230	1.68 ± 0.57	1 ^a (FW)
	230–530	3.42 ± 0.92	2 ^b (BW)
	530–710	2.67 ± 0.74	3 ^c (FW)
	710–1 390	4.68 ± 0.92	4 ^d (BW)
ZHC03	0–130	1.66 ± 0.57	1 ^a (FW)
	130–370	3.48 ± 1.04	2 ^b (BW)
	370–1 030	2.41 ± 0.99	3 ^c (FW)
	1 030–1 290	3.59 ± 0.73	4 ^d (BW)
ZHC04	1 290–1 340	1.93 ± 0.5	5 ^{ac} (FW)
	0–800	2.24 ± 0.79	1 ^a (FW)
	800–1 400	3.88 ± 0.8	2 ^b (BW)
ZHC05	1 400–1 740	2.69 ± 0.66	3 ^c (FW)
	0–430	1.95 ± 0.78	1 ^a (FW)
	440–490	3.88 ± 1.27	2 ^b (BW)
ZHC06	500–590	7.35 ± 0.6	3 ^c (SW)
	590–1 350	4.68 ± 1.36	4 ^d (BW)
	1 350–1 450	2.05 ± 0.63	5 ^a (FW)
	1 450–1 550	3.48 ± 0.42	6 ^b (BW)
	1 550–1 670	2.46 ± 1.02	7 ^a (FW)
	0–230	2.05 ± 0.6	1 ^a (FW)
ZHC07	230–1 490	4.07 ± 1.19	2 ^b (BW)
	0–200	2.29 ± 1.05	1 ^a (FW)
	200–440	4.81 ± 1.14	2 ^b (BW)
	440–530	6.28 ± 0.66	3 ^c (BW)
	530–1 140	4.96 ± 1.05	4 ^b (BW)
	1 140–1 710	2.58 ± 0.8	5 ^a (FW)

to be continued

Continued from Table 3

Sample code	Distance from the core/ μm	Sr:Ca ratio	Fluctuation phases of otolith Sr:Ca ratio
ZHC09	0–290	2.21 \pm 0.67	1 ^a (FW)
	290–640	4.88 \pm 1.4	2 ^b (BW)
	640–730	7.52 \pm 0.54	3 ^c (SW)
	730–1 960	4.64 \pm 0.98	4 ^b (BW)
ZHC10	0–390	2.06 \pm 0.85	1 ^a (FW)
	390–960	5.1 \pm 1.19	2 ^b (BW)
	960–1 450	2.29 \pm 0.94	3 ^a (FW)
	1 450–1 950	3.39 \pm 1.1	4 ^c (BW)
	1 950–1 980	2.16 \pm 0.33	5 ^a (FW)
ZHC12	0–240	1.5 \pm 0.57	1 ^a (FW)
	240–560	6.31 \pm 1.01	2 ^b (BW)
	560–1 170	4.47 \pm 0.94	3 ^c (BW)
	1 170–1 770	2.69 \pm 0.76	4 ^d (FW)
ZHC14	0–290	1.83 \pm 0.69	1 ^a (FW)
	290–1 440	4.36 \pm 0.98	2 ^b (BW)
	1 440–1 810	2.47 \pm 0.66	3 ^c (FW)
ZHC15	0–820	2.49 \pm 0.8	1 ^a (FW)
	820–1 060	3.54 \pm 0.63	2 ^b (BW)
	1 060–2 050	2.43 \pm 0.75	3 ^a (FW)
ZHC17	0–710	1.47 \pm 0.6	1 ^a (FW)
	710–1 600	3.49 \pm 0.86	2 ^b (BW)
ZHC19	0–370	2.21 \pm 0.65	1 ^a (FW)
	370–450	3.62 \pm 0.71	2 ^b (BW)
	450–580	6.73 \pm 0.71	3 ^c (SW)
	580–680	4.69 \pm 0.54	4 ^d (BW)
	680–930	6.12 \pm 0.6	5 ^e (BW)
	930–1 480	3.8 \pm 0.93	6 ^b (BW)
ZHC20	1 480–1 830	2.57 \pm 0.85	7 ^a (FW)
	0–1 040	2.02 \pm 0.65	1 ^a (FW)
	1 040–1 220	3.38 \pm 0.57	2 ^b (BW)
	1 220–1 640	1.5 \pm 0.78	3 ^a (FW)

Note: Sr:Ca ratio is expressed as the ratio of Sr to Ca amplifying by 1 000. Phases in one otolith sample having the same superscript letter indicate no significant differences ($P>0.05$); whereas different superscript letters indicate significant differences ($P<0.05$). FW, BW and SW indicate the phases of freshwater, brackish water and sea water, respectively.

freshwater residency period. Adjacent to the bluish regions in the center, patterns in Sr:Ca ratios along microprobe profiles varied among individuals. The concentric rings in the other regions of the otoliths varied from being bluish–greenish (ZHC01, ZHC02, ZHC03, ZHC04, ZHC14, ZHC15, ZHC17 and ZHC20) to bluish–greenish–yellowish–reddish (ZHC05, ZHC06, ZHC07, ZHC09, ZHC10, ZHC12 and ZHC19), which corresponded to the Sr:Ca ratios (≤ 3 , mapping color: blue; 3–6.7, green or yellow; >6.7 , red; Fig. 5). Mean Sr:Ca values observed were significantly different between two subsequent phases of Sr:Ca ratio and this phenomenon should be associated with transitions between water sources of different salinity (Table 3, Fig. 4). Interestingly, no high levels of the Sr:Ca ratios could be found in the central regions of the otoliths from all Chikugo *C. nasus* captured, unlike the situation observed in aforementioned Rokkaku *C. nasus*.

4 Discussion

Coilia nasus is traditionally believed to be one species of anadromous fish in Japan and China. During a long spawning season ranging from May to August, adults ascend to the river from the sea for spawning (Whitehead et al., 1988). Reproduction of the *C. nasus* population appears to greatly depend on the oligohaline region of the estuaries as the eggs and larvae of this

fish might have a much better chance to survive in waters with very low salinity (Takita and Masutani, 1979; Suzuki et al., 2014), i.e., freshwater habitat. However, fluctuation (gray line) of Sr:Ca ratios along line transects from the core (0 μm) to the edge (Table 2, Fig. 2) and Sr imaging using otolith mapping analysis of sagittal otoliths (Fig. 3) indicated that the habitat use of Rokkaku *C. nasus* was much more variable and complex than previously thought. Although the first pattern of otolith microchemistry in seven fish (LJC01, LJC02, LJC05, LJC07, LJC08, LJC09, and LJC10) showed a typical freshwater origin or anadromous pattern with obviously different phases of low-center high-edge of Sr:Ca ratio and Sr content map, the second pattern in the otoliths of three fish (LJC03, LJC04 and LJC06) with a high center region of Sr:Ca ratio (>3) and Sr content map (greenish or yellowish color) suggested that not all of the *C. nasus* anadromously migrated to the freshwater area to spawn. These results suggest that these individuals might all be of estuarine origin based on our previous studies of the otolithic salinity markers of freshwater, brackish water and seawater habitats by Sr:Ca ratios of wild *C. nasus* (Yang et al., 2006, 2011; Jiang et al., 2014). Unfortunately, it is still impossible to know the exact salinity requirement for the spawning sites of *C. nasus* in the Rokkaku River, due to a lack of corresponding studies so far. Although the eggs and larvae of *C. nasus*

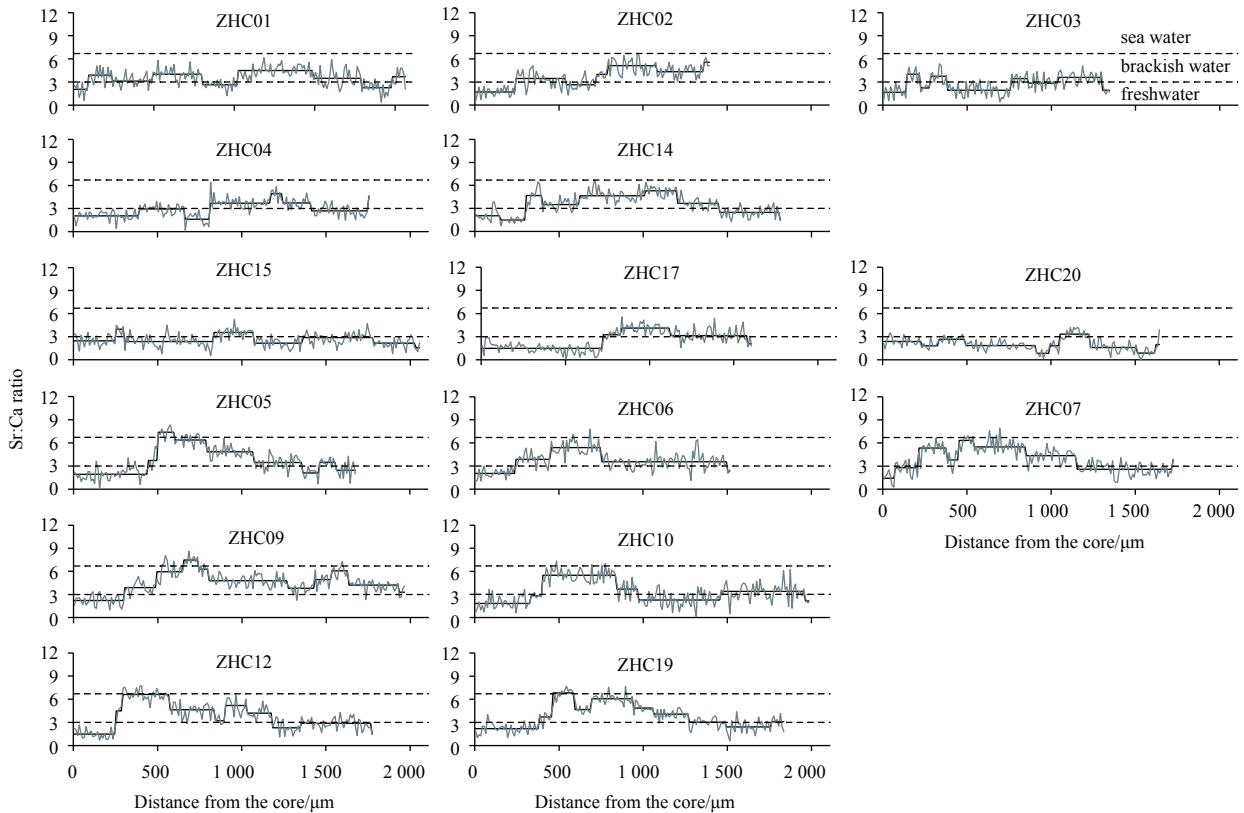


Fig. 4. Fluctuation (grey line) and shift (black line) of Sr:Ca ratios (the ratio of Sr to Ca amplifying by 1 000) along line transects from the core (0 μm) to the edge of sagittal otoliths of *Coilia nasus* collected from the Chikugo River Estuary.

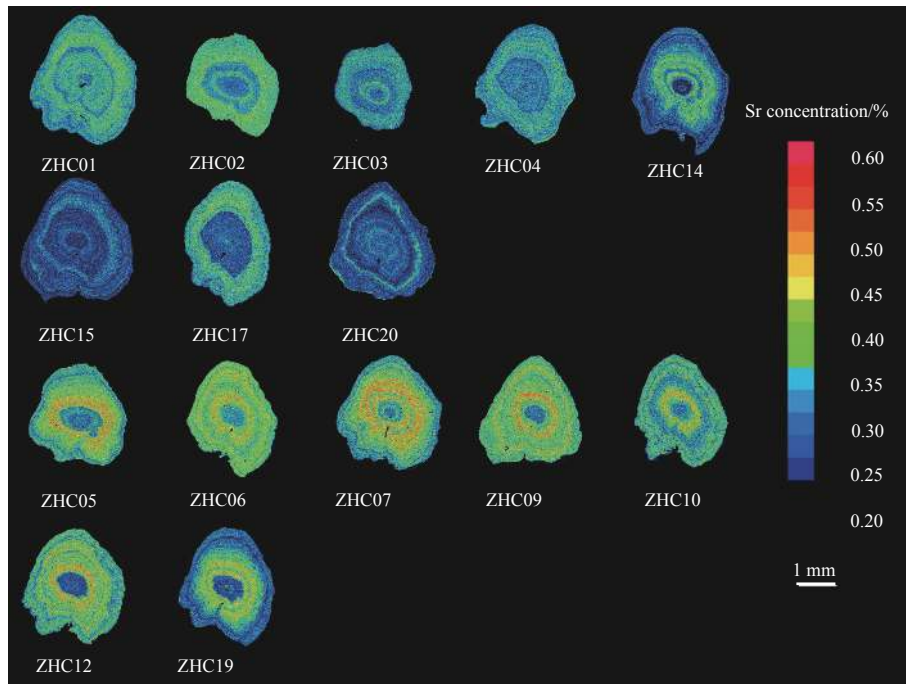


Fig. 5. Strontium (Sr) imaging of *Coilia nasus* collected from the Chikugo River Estuary using otolith mapping analysis.

originating from the Chikugo River were believed to be highly vulnerable to high-salinity brackish water (Takita and Masutani, 1979; Suzuki et al., 2014), this phenomenon will not be fully correct in those from the Rokkaku River. The otolith microchemical

results of LJC03, LJC04 and LJC06 in the present study suggested that sporadic reproduction of *C. nasus* in the mesohaline zone of the Rokkaku River Estuary might be not impossible, i.e., some individuals could tolerate saltwater during early life stages (e.g.,

eggs, larvae) or even during their whole life history (like LJC03). Spawning of parent *C. nasus* in brackish water was possibly contributing to future population recruitment. Similar cases were found in some of those *C. nasus* from estuaries of several other rivers (e.g., the Changjiang River, Qiantangjiang River) along the coasts of the East China Sea (Dou et al., 2012; Jiang et al., 2014), indicating that they have the potential to develop into a non-anadromous stock in this region, as was the case of *C. nasus* found in the Changjiang Estuary by Dou et al. (2012).

In contrast, the results of both otolith life-history transect analyses (Table 3, Fig. 4) and X-ray intensity maps (Fig. 5) suggested that all fifteen specimens of *C. nasus* captured from the Chikugo River Estuary were of freshwater origin, unlike the aforementioned individuals of estuarine origin in *C. nasus* from the Rokkaku River. Nevertheless, the habitat use of Chikugo *C. nasus* was still more variable and complex than previously thought, considering their whole life history. All otoliths of the anchovies had a similar bluish color pattern and low Sr:Ca ratios (≤ 3) in their central regions, which were consistent with a presumed freshwater residency period (Yang et al., 2006; Jiang et al., 2014). Adjacent to the bluish regions in the center, patterns in Sr:Ca ratios along microprobe profiles varied among individuals. This variation in otolith Sr concentrations indicated that the anchovies from the same apparent spawning population in the Jojima section of the Chikugo River might have experienced a variety of movement patterns among fresh, brackish and sea waters in the period of life history after egg-larval stages, and, furthermore, even showed relatively more riverine-estuarine (e.g., ZHC01, ZHC02, ZHC03, ZHC04, ZHC14, ZHC15, ZHC17 and ZHC20), or estuarine-marine dependencies (ZHC05, ZHC06, ZHC07, ZHC09, ZHC10, ZHC12 and ZHC19), respectively.

Like the results of Yangtze *C. nasus* (Chen et al., 2017), the change in otolith Sr:Ca ratio and transition in concentric color rings (from blue to green, or further to red) corresponded to the salinity requirement of Rokkaku and Chikugo *C. nasus* during their migration from the spawning ground, from egg hatching to larval nursery, and to juvenile rearing habitats. It was unexpected to find that some *C. nasus* showed tendencies of freshwater or oligohaline requirement during their whole life histories. More specifically, the individuals of LJC08, LJC10, ZHC15 and ZHC20 almost consistently stayed in freshwater habitat except for entering brackish water for a very short time. This special strategy of habitat use implies that there may be unknown benefits (e.g., better feeding conditions) that can contribute substantially to the recruitment success and higher juvenile growth in endemic and restricted species like *C. nasus*, as Suzuki et al. (2014) indicated. Therefore, the current study suggests that *C. nasus* from both estuaries of the Rokkaku River and Chikugo River have a flexible capability of salinity habitat use with a high degree of migratory plasticity to decide whether to spend more or their life histories in freshwater, brackish, or seawater habitats. As previously emphasized by Kraus and Secor (2004) in their research on otolith microchemistry of white perch (*Morone americana*), we believe that a mosaic of habitats and developmental plasticity could be critical elements that have ecological significance, and both contingents play important roles in the population dynamics of *C. nasus*.

Many complex reasons are involved in the evolution and occurrence of migratory plasticity in diadromous fishes (Tsukamoto et al., 1998; Dou et al., 2012; Chen et al., 2017). Besides the natural ones, those from anthropogenic activity cannot be neglected. According to a previous study by Simanjuntak et al. (2015), a reclamation dike of the Ariake Bay has dramatically

changed the environment and caused ecological problems associated with growth, breeding and systematics of *C. nasus*, including the occurrence of landlocked and non-anadromous *C. nasus* populations in the water areas inside the dike in the Ariake Bay.

Based on the results of microchemical analysis of the otolith Sr:Ca ratios and Sr-level map, the present study provides more objective and intuitive evidence and the most recent information on discriminating migratory patterns, and traces the habitat utilization patterns of *C. nasus* from estuaries of the Rokkaku River as well as the Chikugo River. This study reveals that either the former and/or the latter can play an important role as spawning and nursery grounds for *C. nasus*, and recognizes the availability of estuarine-origin individuals of this fish in the Rokkaku River Estuary, apart from the traditional riverine-origin individuals. The aforementioned results might be used for future effective management and artificial breeding of *C. nasus* in both Japan and China. Further studies need to be conducted for a better understanding of the spatial and temporal dynamics during the life history of *C. nasus*, in all river estuaries around the Ariake Sea, and the population connectivity of *C. nasus* between these rivers, the Chikugo River or the Rokkaku River.

Acknowledgements

The authors thank Atsuko Yamaguchi (Nagasaki University) and Yuji Oshima (Kyushu University) for providing the *C. nasus* samples from Rokkaku River and Chikugo River, respectively. The authors are also grateful to Davison D. Khumbanyiwa for critically reading and providing comments that improved the manuscript.

References

- Altenritter M E, Kinnison M T, Zydlewski G B, et al. 2015. Assessing dorsal scute microchemistry for reconstruction of shortnose sturgeon life histories. *Environmental Biology of Fishes*, 98(12): 2321–2335
- Campana S E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188: 263–297
- Chen Tingting, Jiang Tao, Liu Hongbo, et al. 2017. Do all long Supermaxilla-type estuarine tapertail anchovies (*Coilia nasus* Temminck et Schlegel, 1846) migrate anadromously?. *Journal of Applied Ichthyology*, 33(2): 270–273
- Dou Shuozeng, Yokouchi K, Yu Xin, et al. 2012. The migratory history of Anadromous and non-Anadromous tapertail anchovy *Coilia nasus* in the Yangtze River Estuary revealed by the Otolith Sr:Ca ratio. *Environmental Biology of Fishes*, 95(4): 481–490
- Islam M S, Hibino M, Tanaka M. 2007. Tidal and diurnal variations in larval fish abundance in an estuarine inlet in Ariake Bay, Japan: implication for selective tidal stream transport. *Ecological Research*, 22(1): 165–171
- Jiang Tao, Liu Hongbo, Lu Mingjie, et al. 2016. A Possible connectivity among estuarine tapertail anchovy (*Coilia nasus*) populations in the Yangtze River, Yellow Sea, and Poyang Lake. *Estuaries and Coasts*, 39(6): 1762–1768
- Jiang Tao, Liu Hongbo, Shen Xinqiang, et al. 2014. Life history variations among different populations of *Coilia nasus* along the Chinese coast inferred from Otolith microchemistry. *Journal of the Faculty of Agriculture, Kyushu University*, 59(2): 383–389
- Jiang Tao, Yang Jian, Lu Mingjie, et al. 2017. Discovery of a spawning area for anadromous *Coilia nasus* Temminck et Schlegel, 1846 in Poyang Lake, China. *Journal of Applied Ichthyology*, 33(2): 189–192
- Kraus R T, Secor D H. 2004. Dynamics of white perch *Morone americana* population contingents in the Patuxent River estuary, Maryland, USA. *Marine Ecology Progress Series*, 279: 247–259
- Matsui S, Tomishige S, Tsukahara H. 1986a. Ecological studies on the engraulid fish, *Coilia nasus* Temminck et Schlegel (1): Prelim-

- inary report on the ecology of the ascending population. Science Bulletin of the Faculty of Agriculture, Kyushu University (in Japanese), 40(4): 221–228
- Matsui S, Tomishige S, Tsukahara H. 1986b. Ecological studies on the engraulid fish, *Coilia nasus* Temminck et Schlegel (2): the effect of salinity on embryonic development and hatch. Science Bulletin of the Faculty of Agriculture, Kyushu University (in Japanese), 40(4): 229–234
- Rodionov S N. 2004. A sequential algorithm for testing climate regime shifts. Geophysical Research Letters, 31(9): L09204
- Rodionov S, Overland J E. 2005. Application of a sequential regime shift detection method to the Bering Sea ecosystem. ICES Journal of Marine Science, 62(3): 328–332
- Secor D H, Ohta T, Nakayama K, et al. 1998. Use of otolith microanalysis to determine estuarine migrations of Japanese sea bass *Lateolabrax japonicus* distributed in Ariake Sea. Fisheries Science, 64(5): 740–743
- Secor D H, Rooker J R. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes?. Fisheries Research, 46(1–3): 359–371
- Simanjuntak C P H, Kinoshita I, Fujita S, et al. 2015. Reproduction of the endemic engraulid, *Coilia nasus*, in freshwaters inside a reclamation dike of Ariake bay, western Japan. Ichthyological Research, 62(3): 374–378
- Suzuki K W, Kanematsu Y, Nakayama K, et al. 2014. Microdistribution and feeding dynamics of *Coilia nasus* (Engraulidae) larvae and juveniles in relation to the estuarine turbidity maximum of the macrotidal Chikugo River estuary, Ariake Sea, Japan. Fisheries Oceanography, 23(2): 157–171
- Takita T, Masutani H. 1979. Spawning area of Engraulid fish, *Coilia nasus*. Bulletin of the Faculty of Fisheries, Nagasaki University (in Japanese), 46: 7–10
- Tsukamoto K, Nakai I, Tesch W V. 1998. Do all freshwater eels migrate?. Nature, 396(6712): 635–636
- Whitehead P J P, Nelson G J, Wongratana T. 1988. FAO Species Catalogue. Vol. 7. Clupeoid Fishes of the World (Suborder Clupeoidei): an annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies, and wolf-herrings: Part 2. Engraulididae. Rome: FAO Fisheries Synopsis No.125, 460–475
- Yagi Y, Kinoshita I, Fujita S, et al. 2011. Importance of the upper estuary as a nursery ground for fishes in Ariake Bay, Japan. Environmental Biology of Fishes, 91(3): 337–352
- Yang Jian, Arai T, Liu Hongbo, et al. 2006. Reconstructing habitat use of *Coilia mystus* and *Coilia ectenes* of the Yangtze River estuary, and of *Coilia ectenes* of Taihu Lake, based on otolith strontium and calcium. Journal of Fish Biology, 69(4): 1120–1135
- Yang Jian, Jiang Tao, Liu Hongbo. 2011. Are there habitat salinity markers of the Sr: Ca ratio in the otolith of wild diadromous fishes? A literature survey. Ichthyological Research, 58(3): 291–294
- Zimmerman C E. 2005. Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids. Canadian Journal of Fisheries and Aquatic Sciences, 62(1): 88–97