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Preliminary results of Sr:Ca ratios of *Coilia nasus* in otoliths by micro-PIXE

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Abstract

Coilia nasus, distributed in Changjiang River as well as northwest Pacific, has a high economic value owing to its delicacy and nutritional value. Recently, the fishing yields in Changjiang River have decreased dramatically due to excessive fishing and changes in the aquatic ecology. In order to prevent excessive fishing effectively, the life history pattern of *C. nasus* should be known in detail. Ootoliths contain much information about a fish's life history, because elemental concentrations remain unaltered after deposition, and can be analysed. *C. nasus* collected from Jing Jiang (lower reaches of the Changjiang River) and Jiu Duan Sha (the estuary of the Changjiang River) were studied by measuring Sr:Ca ratios in their otoliths using micro-PIXE. On average, the Sr:Ca ratios of estuarine *C. nasus* were found to be higher. The Sr:Ca ratios were higher in the core regions and lower in the outermost marginal regions, and shows fluctuations in certain regions. Possible corresponding life history patterns are discussed.

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Keywords: *Coilia nasus*; Otolith; Sr:Ca ratios; Micro-PIXE

1. Introduction

Coilia nasus, also called Japanese grenadier anchovy, are widely distributed in the northwest Pacific, including the Yellow Sea and East Sea as well as penetrating over 1400 km (middle reaches) up the Changjiang River [1]. However, the *C. nasus* caught from the Changjiang River have a much higher economic value owing to their delicacy and nutritional value. Unfortunately, the abundance of *C. nasus* in Changjiang River had decreased significantly due to excessive fishing and changes in the aquatic ecology. They almost disappeared in the middle reaches of the Changjiang River [1]. Even in the lower reaches of the Changjiang River, the abundance of *C. nasus* had decreased 50% during the last five years [2]. The fish available on the market is mostly caught from the sea. An understanding of the fish's life history patterns is fundamental to conservation and management plans for these fish in the Changjiang River. The species exhibits high genetic diversity [3] and seems to display a range of life history patterns, but detailed information is from current methods (catch analysis, tagging, and hydroacoustics) [4] is quite limited.

The otoliths, with their annular structure, are located in the membranous labyrinth of the inner ear of teleost fish. From core to edge, an otolith contains much information about a fish's life history, because its element concentrations remain unaltered after deposition [5]. It is composed mainly of a calcium carbonate (aragonite) and a protein matrix; but trace elements, e.g. strontium (Sr), can be incorporated into or replace Ca in the otolith. The Ca and trace elements stem mainly from the waters that the fish inhabits. Sr concentrations in seawater and freshwater

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are quite different, and such differences are reflected in the Sr content of otoliths of fish from different water bodies [6]. A positive relationship between otolith Sr and habitat salinities among freshwater, estuarine, and marine taxa have been observed [4]. Therefore, as a tool for the reconstruction of life history patterns, the otolith Sr:Ca ratios appear to be among the best available [7].

Considering the composition and morphology of an otolith, scanning nuclear microprobe (SNM), with several micrometer spatial resolution and ppm detection limit, is apparently a good method for high-resolution sampling of an otolith along a transect. In the present study, the otolith Sr:Ca ratios in the *C. nasus* collected from Jing Jiang and Jiu Duan Sha were measured. Based on the results, the possible maternal condition and life history pattern of the species are discussed.

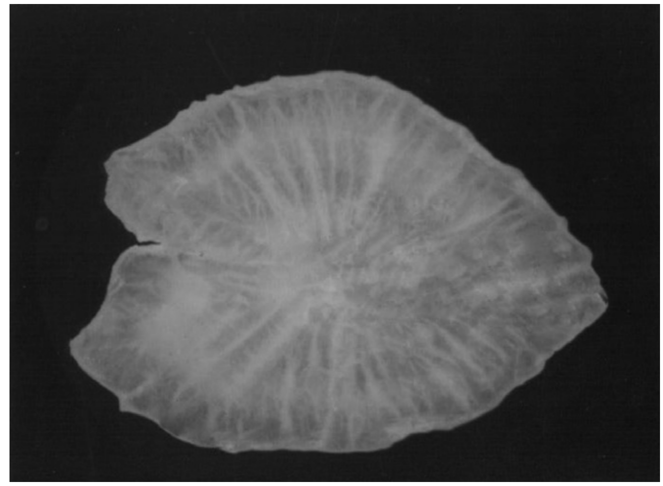

Fig. 2. *Coilia nasus* otolith (radius is 1600 μm).

2. Experimental

2.1. Fish and otolith preparation

Specimens of *C. nasus* were collected from Jing Jiang and Jiu Duan Sha in Spring. Jing Jiang is located at the lower reaches of the Changjiang River while Jiu Duan Sha is the estuary of the Changjiang River, which is connected to the East Sea. The location of these two sites is illustrated in Fig. 1. Otoliths were extracted from each fish. The otoliths were then cut by a homemade machine through the sagittal section and ground with 600 grit silicon carbide abrasive paper to expose the core. After that, they were polished with 2000 silicon carbide abrasive paper. Finally, they were cleaned with deionized water before measurement. The obtained *C. nasus* otolith (Fig. 2) did not show a daily increment as seen in some other species of fish. Only an annual ring was visible. The distance along a transect from the core to the first ring is around 800 μm which is corresponding to one year.

Fig. 1. Sampling sites for *Coilia nasus* in China (the map is taken from http://www.maps-of-china.com/).

2.2. Measurements

Point micro-PIXE analyses were performed by means of the SNM at Shanghai Institute of Nuclear Research, Chinese Academy of Sciences [8]. The 3 MeV proton beam was collimated and focused to a 5–10 μm spot size. The beam current was typical of around 50 pA. The beam caused no apparent damage to the otoliths. Ca and Sr K X-ray intensities versus position along a radius for each otolith were recorded by a Si(Li) detector (ORTEC) with an energy resolution of 150 eV at 5.9 keV (Ka line of ^{55}Fe) and an active area of 70 mm^2. Because a suitable absorber was not available during the PIXE measurements, statistical errors for Sr measurements were between 4% and 9% which should be improved later. Since there was no standard samples in hand, Sr:Ca ratios are merely peak count ratios.

3. Results and discussion

Sr:Ca ratios were measured along a transect from the core to the otolith outermost edge of both the fish collected from Jing Jiang and Jiu Duan Sha. The results are shown in Table 1.

Otolith core Sr:Ca value was 2.2×10^{-4} and 0.96×10^{-4} for Jiu Duan Sha and Jing Jiang, respectively. Otolith core Sr:Ca values could reflect maternal associations with life history pattern, since the core represented the earliest stages of otolith formation. Moreover, disparate Sr:Ca levels characteristic of fresh and seawater were presumably incorporated into the developing ova during yolk deposition, then deposited in the embryonic otolith. The core Sr:Ca values had been used to effectively discriminate between the progeny of anadromous and freshwater resident female salmon by Volka et al. [9]. They had found that fry spawned by females maturing in seawater had otolith

Table 1a
Sr:Ca ratio versus otolith distance from the core (the fish collected from Jing Jiang)

Distance from the core (μm)	Sr/Ca ratio ($\times10^{-4}$)
0	0.96
50	0.91
100	0.85
200	0.97
300	0.94
450	0.81
600	0.74
750	2.53
900	0.57
1050	2.19
1200	1.83
1350	1.71
1500	1.15
1600 (the outer edge)	0.98

Table 1b
Sr:Ca ratio versus otolith distance from the core (the fish collected from Jiu Duan Sha)

Distance from the core (μm)	Sr/Ca ratio ($\times10^{-4}$)
0	2.2
200	2.3
350	2.1
500	2.2
650	2.1
800	2.2
1400	1.7
1600 (the outer edge)	1.3

Sr:Ca values greater than those from their freshwater counterparts. From our results, the core ratio for Jiu Duan Sha is higher than that for Jing Jiang. We cannot confirm at the moment that their maturing female were from two different populations or subspecies [10]. However the results suggest that their maternal life history pattern, environmental condition and spawning location were quite different.

Sr:Ca ratios along the life history transect in the otoliths were remarkably different between the fish caught from Jing Jiang and Jiu Duan Sha. In general, physiological condition, environmental parameters, and water chemistry each has the potential to influence changes of the Sr:Ca ratio in fish otoliths. Accordingly, Sr content in fish otoliths offers the potential to distinguish between time spent in seawater and freshwater and can provide information regarding the environmental history of individual fish [5]. Averagely, the measured otolith Sr:Ca values in sea water fish was roughly two times greater than that from its freshwater counterparts. Obviously this difference corresponds to the ambient Sr concentration, which is higher in seawater than in freshwater. Sr:Ca values of outermost region of the Otolith could reflect the environmental situation when the fish were collected. The outermost Sr:Ca value in Jiu Duan Sha was slightly higher than that from the Changjiang River. Jiu Duan Sha is the estuary of the Changjiang River. Therefore the results are quite reasonable since the Sr content in estuary was relatively lower than in seawater but higher than in freshwater.

The Sr:Ca ratios in Jiu Duan Sha fish had higher levels in the core regions and lower levels in the marginal regions. Campbell et al. observed the same systematics in sea fish [11]. Our results indicate that the fish spent its early life in the sea, later migrated to the estuary where the fish was collected. This is evidence for a migrant rather than a resident life history pattern. Sr:Ca distribution for the Changjiang River *C. nasus* show fluctuations at certain regions. This suggests that the fish spent its first year in the river where the Sr content was relatively low. However, a slight increase in Sr:Ca ratio was found around 750 μm from the otolith core which is corresponding to almost one year. This may indicate that the fish migrated to the sea; a decline in Sr within the same year (annulus) indicates that the fish returned to freshwater. Later , the fish may have migrated to the sea again and then wandered in the estuarine area for several months, suggested by the Sr:Ca ratios in the 1050–1500 μm region. Most anadromous types exhibit annual migratory behavior [6]. It will be very helpful to collect older fish (more annuluses in otolith) to confirm its anadromous.

4. Conclusions

It is critical to establish conservation and management plans for *C. nasus* now, since its abundance in the Changjiang River decreases dramatically. Little is known about the fish's life history patterns, which is fundamental information for effective protection. A project, related to the life history pattern investigation of *C. nasus*, was recently proposed to the Chinese National Science Foundation. Otolith micro-PIXE analysis of Sr:Ca ratio along the life history transect is a useful tool for such investigation. In the present preliminary study, otolith Sr:Ca ratios for the fish in the Jing Jiang and Jiu Duan Sha were examined. The core ratio measurements indicated that the two fish's maternal life history pattern, environmental condition and spawning location were quite different. Sr:Ca ratios along the life history transect in the otoliths were also measured owing to high spatial resolution of SNM. A migrant rather than resident life history pattern was suggested for the fish collected from two different environments. Since most anadromous fish exhibit annual migratory behavior, data from older fish are required to confirm the results and get a clear view of the life history pattern of the two forms of the species.

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