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Otolith Microchemistry Assessment: Evidence of Migratory *Coilia nasus* of Yangtze River Living in the Shengsi Sea Area

Yu Li [1,2], Jianhua Chen [2], Guangpeng Feng [1,2,3,*], Jian Yang [4], Feng Zhao [1], Chenchen Shen [1,3], Chao Song [1] and Tao Jiang [4]

[1] East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China; jouliyu@yeah.net (Y.L.); zhaof@ecsf.ac.cn (F.Z.); 18936152362@163.com (C.S.); songc@ecsf.ac.cn (C.S.)
[2] Jiangsu Key Laboratory of Marine Biotechnology, College of Marine Science and Fisheries, Jiangsu Ocean University, Lianyungang 222005, China; chenjianhuazsu@163.com
[3] College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China
[4] Wuxi Fisheries College, Nanjing Agricultural University, Wuxi 214081, China; jiany@ffrc.cn (J.Y.); billow_jiang@yeah.net (T.J.)
* Correspondence: fenggp@ecsf.ac.cn

Abstract: At present, the resources of anadromous fish in the world are drastically reduced and one of the solutions to promote its recovery may be to identify and protect its feeding grounds. To determine whether the feeding grounds of anadromous *Coilia nasus* in the Yangtze River are still in the sea area, the quantitative line analysis of Sr and Ca contents (Sr/Ca ratios) and the area distribution analysis of Sr content in the otoliths of *C. nasus* in three water areas (Poyang Lake, Yangtze River estuary and Shengsi Sea area) were carried out by electron probe microanalysis (EPMA) to analyze the habitat use and the migratory patterns of different estuarine *C. nasus*. These results showed that the Sr/Ca ratios of all otoliths fluctuated significantly. By comparison, it was found that *C. nasus* of Yangtze River might exist in the Shengsi Sea area, and the Shengsi Sea area might be one of the feeding grounds of migratory *C. nasus*. Therefore, it is urgent to carry out a comprehensive investigation on the distribution of related groups in the Shengsi Sea area and the influence of potential threat factors, such as fishing and harsh habitat, to effectively protect the resources and habitats of *C. nasus* in the Shengsi Sea area.

Keywords: *Coilia nasus*; otolith; microchemistry; Shengsi Sea area; Yangtze River

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1. Introduction

Coilia nasus is a widely distributed species in the Northwest Pacific, including the marine areas of China, Japan and Korea [1]. In China, the Yangtze River basin is one of the most important habitats for the long supermaxilla and short supermaxilla phenotype estuarine tapertail anchovy *C. nasus* [2]. According to the length of supermaxilla, *C. nasus* could be divided into three different ecotypes: anadromous migratory type, freshwater resident type and landlocked type [3]. However, it is not reliable to judge the ecotype of *C. nasus* only based on the characteristics of maxillary length. In general, adult anadromous *C. nasus* has long supermaxilla and needs to migrate for spawning from early February to late April, which depends on early or late upstream populations from sea areas [2]. During this period, *C. nasus* could mature rapidly and breed annually in the lakes and rivers [4]. Anadromous *C. nasus* used to be one of the most important commercially harvested species in the Yangtze River estuary, but *C. nasus* had been suffering overfishing in recent years due to its distinctive aroma, high nutritional value and high price (USD/kg 1000). Moreover, due to several human activities causing water pollution and habitat degradation, the population of anadromous *C. nasus* has dropped dramatically [5]. For anadromous *C. nasus*, the Yangtze River is a main habitat (migration channel, spawning ground and early baiting ground), but offshore is also a main habitat (feeding grounds) [6]. At present, most researches focused on the *C. nasus* population in the Yangtze River, while less on the offshore feeding grounds [7,8]. The Shengsi Sea area is one of the significant marine fishery areas in China, which is the junction of Qiantang River, Yangtze River and the East China Sea [9]. Moreover, the Shengsi Sea area was a famous feeding ground for *C. nasus* in history [10,11]. Therefore, we hypothesized that the Shengsi Sea area should still be one of the important feeding grounds of *C. nasus* hatching in the Yangtze River, even with overfishing and water conservancy projection construction. Meanwhile, *C. nasus* also exists in the Qiantang River, so it deserves to further judge the migratory groups of *C. nasus* [12].

In previous studies, relying solely on morphological and biomolecular analysis to judge the life experience of *C. nasus* had empirical limitations, which is inefficient [13]. Fortunately, some scholars have proved that the trace elements in *C. nasus* otolith are a medium and efficient natural label and detection method, which provides an efficient tool for the accurate search of feeding grounds of Yangtze *C. nasus* [14,15]. Previous studies have shown that the characteristics of strontium (Sr) and calcium (Ca) in otoliths (mainly from water absorbed by gills) could be used as an accurate basis for judging migration history, because Sr and Sr/Ca ratios in otoliths are powerful markers for different salinity habitats of freshwater, brackish water and seawater [16–22]. The main mineral composition of otoliths is relatively pure calcium carbonate and some trace elements are also present (e.g., Sr, S, Cu Al, N, Cl, P and Si, Fe, Pb, Mg, Zn, Co, Cd, Na, K) [20,21]. Otoliths are born and then grow with fish, recording the whole life history and habitat process [17]. In recent years, many scholars have successfully reconstructed the information of migratory ecology through otolith microchemical analysis [23–27]. The composition and content of strontium in seawater and fresh water are significantly different, especially in some sea areas where the strontium content is 100-times or even 5000-times higher than that in fresh water [28]. Therefore, the changes in strontium content and strontium/calcium ratios in fish otoliths have been widely used to trace the movement between fresh water and marine habitats [29]. It has also been proved to be an accurate tool for other similar studies of *C. nasus* [9,14,22]. All these findings indicated that the Sr/Ca ratios of otoliths were related to salinity and these natural markers could objectively and accurately analyze the life pattern of *C. nasus*. In addition, some Chinese scholars used the electron probe microanalyzer (EPMA) to study the fish populations in the Yellow Sea, Poyang Lake and Dongting Lake, which proved that this method is very effective to understand the life mode of *C. nasus* [30–32].

This study was aimed at determining the life history characteristics of *C. nasus* in the Shengsi Sea area and the possible connectivity between the populations of *C. nasus*. In this study, EPMA was used to analyze the otolith Sr and Ca of *C. nasus* in three water areas (Poyang Lake, Yangtze River estuary and Shengsi Sea area). The results might contribute to the determination of *C. nasus* in the Shengsi Sea area and will strengthen the management and protection of the water area, playing a vital role in the resource recovery of *C. nasus*.

2. Materials and Methods

2.1. Samples Collection

In total, 21 fish samples (Figure 1) were investigated, which were collected by trammel nets from Shengsi Sea area, Yangtze River estuary and Poyang Lake (Figure 2). Six individuals were collected in Shengsi Sea area in April 2021 (30°81′ N 122°90′ E, S group, specimens S1, S2, S3, S4, S5 and S6). Eight individuals were collected at the Yangtze River estuary in May 2021 (31°27′ N 121°95′ E, E group, specimens E1, E2, E3, E4, E5, E6, E7 and E8). Seven individuals were collected in Poyang Lake in September 2021 (29°15′ N 116°27′ E, P group, specimens P1, P2, P3, P4, P5, P6 and P7). Before otolith extraction, fish samples were routinely measured by electronic vernier caliper and electronic balance to obtain the values of weight, total length and jaw length/head length. Age was determined by otolith analysis, and sexual maturity was determined by gonadal observation [32,33].

Figure 1. Anadromous *Coilia nasus*.

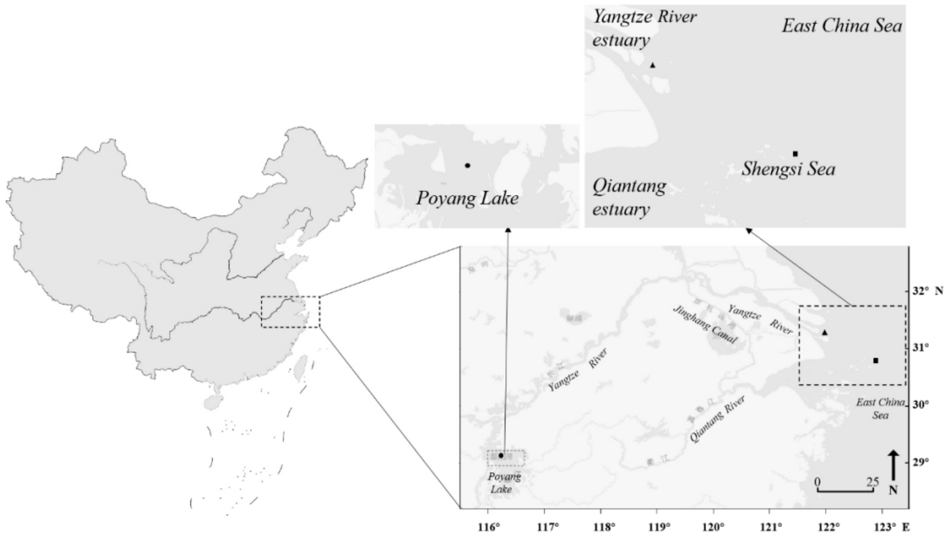

Figure 2. Map showing the *Coilia nasus* sampling sites: Shengsi Sea area (S group) around 30°81′ N 122°90′ E, Yangtze River estuary (E group) around 31°27′ N 121°95′ E and Poyang Lake (P group) around 29°15′ N 116°27′ E).

2.2. Otolith Treatment and Microchemical Analysis

In this study, the right sagittal otoliths were used for pre-treatment and microchemical analysis. The otoliths were embedded in epoxy resin (Epofix, Struers, Copenhagen, Denmark) and polished with an automatic polishing wheel (Discoplan-TS mill, Struers, Copenhagen, Denmark) to expose the core. Then, all otoliths were polished by the LaboPol-35 grinding and polishing machine (Struers, Copenhagen, Denmark) to eliminate the scratches on the otolith surface. All otoliths were washed in an ultrasonic bath with Milli-Q water for 5 min and then rinsed also with Milli-Q water 6 times. After drying in oven at 40 °C, all otoliths were coated by vacuum coating machine (JEE420, JEOL Ltd., Tokyo, Japan) (36 A, 25 s). According to previous study [1], the life history transect analysis on Sr and Ca concentrations (Sr:Ca ratios) and the X-ray intensity map of Sr content were carried out by EPMA (JXA-8100, JEOL Ltd., Tokyo, Japan). Calcite ($CaCO_3$) and Tausonite ($SrTiO_3$) were used as standards for calibration of Ca and Sr measurements, respectively. These parameters of EMPA were programmed (Table 1). After microchemical analysis, otoliths were repolished to remove the surface carbon film and the age was read in photoscope after 5% EDTA acid etching [34].

2.3. Data Analysis

The line charts were drawn by Excel 2020, where *X*-axis was the radius length of the otolith and *Y*-axis was Sr:Ca ratios which were customarily calculated and expressed as Sr/Ca × 1000 [15,30]. To reflect the trend of Sr/Ca ratios of otoliths more directly, the results of quantitative analysis were trend-shifted by STARS (Sequential *t*-test Analysis of Regime Shifts) with a confidence p = 0.05, a truncation length of 5 and a Huber weight of 1.

To detect the significant differences in Sr/Ca ratios of otoliths, IBM SPSS Statistics v. 23.0 was used for nonparametric test (Mann–Whitney *U* test).

Table 1. The parameter values of EMPA analysis.

Analysis Item	Accelerating Voltage/kV	Beam Current/A	Direct Beam Spot/μm	Residence Time/s	Interval/Pixel/μm
Life history transect analysis	15	2×10^{-8}	5	15	10
X-ray intensity mapping analysis	15	5×10^{-7}	5	0.03	7×7

The Sr:Ca ratios of otolith could effectively correspond to the characteristics of salinity differences in different habitats (fresh water, brackish water and seawater): (1) In fresh water, the Sr:Ca ratio was generally less than 3, which corresponds to the blue spectrum in the area distribution analysis of Sr content; (2) in brackish water, the Sr:Ca ratio was generally between 3 and 7, which corresponds to the green-yellow spectrum in the area distribution analysis of Sr content; (3) in seawater, the Sr:Ca ratio was generally greater than 7, which corresponds to the red spectrum in the area distribution analysis of Sr content [15]. Moreover, freshwater coefficient (F_c) refers to the ratio of the time spent in freshwater environment to the whole life history of fish in the early life history [25]. The formula $F_c = L_f/L_T$ was used to calculate freshwater coefficient, where L_f was the diameter of the first blue region near the core and L_T was the analytical diameter of the entire otolith.

3. Results

3.1. Age and Sexual Maturity

The age determination based on otoliths showed that all *C. nasus* were 2 years old. The ratios of jaw length/head length were all greater than 1. The sexual maturity of *C. nasus* was the highest in the Poyang Lake, followed by Yangtze River estuary and the Shengsi Sea area (Table 2).

Table 2. Basic information of *C. nasus* in different water areas.

Sample Code	Weight/g	Total Length/mm	Jaw Length/Head Length	Sexual Maturity	Age
S1	66.50	275.90	1.34	♂I	2
S2	84.50	304.74	1.31	♀II	2
S3	83.50	306.93	1.14	♂I	2
S4	77.30	303.86	1.30	♀I	2
S5	103.20	347.46	1.43	♀I	2
S6	63.20	267.56	1.25	♀I	2
E1	129.74	337.42	1.25	♂II	2
E2	135.20	339.66	1.29	♀II	2
E3	106.96	329.81	1.36	♂II	2
E4	110.99	335.19	1.20	♀II	2
E5	167.58	380.38	1.32	♀II	2
E6	129.79	360.70	1.36	♀III	2
E7	121.34	355.46	1.28	♂II	2
E8	143.33	334.76	1.49	♀II	2
P1	91.60	271.71	1.39	♀IV	2
P2	66.60	248.19	1.28	♀IV	2
P3	69.40	243.75	1.34	♂IV	2
P4	94.00	282.90	1.25	♀IV	2
P5	91.80	269.00	1.39	♂IV	2
P6	112.00	298.54	1.31	♂III	2
P7	103.80	290.28	1.16	♂IV	2

3.2. The Quantitative Line Analysis of Sr and Ca Contents (Sr/Ca Ratios)

Otoliths of *C. nasus* from the three sites (Shengsi Sea area, Yangtze River estuary and Poyang Lake) showed various Sr/Ca ratios. Group E (Yangtze River estuary) and group P (Poyang Lake) were quite similar, with two phases distinguished by the values of the life—history transects and the X-ray intensity maps. The otolith Sr/Ca ratios showed dramatic changes along the life—history transects, with significantly lower mean ± S.D. Sr/Ca ratios of 1.60 ± 0.80 (E6) to 2.38 ± 0.63 (S4) from the core to the point c. 370–1250 µm to the outermost regions (Mann–Whitney *U* test, $p < 0.05$) (Table 3, Figures 3–5).

Table 3. Change in Sr/Ca value in otolith of *C. nasus* in different water areas.

Water Area	Sample Number	Otolith Sr/Ca Value Change Stage	Length from Otolith Core Radius/µm	Number of Element Points (N)	Sr/Ca Value (Mean ± SD) *
Shengsi Sea area	S1	1	0–310	31	2.28 ± 0.70 [a]
		2	310–1830	152	4.62 ± 0.90 [b]
	S2	1	0–1040	104	1.47 ± 0.76 [a]
		2	1040–1710	67	4.00 ± 0.68 [b]
	S3	1	0–710	71	2.08 ± 0.67 [a]
		2	710–2150	144	4.70 ± 1.27 [b]
	S4	1	0–270	27	2.38 ± 0.63 [a]
		2	270–1850	158	5.25 ± 1.41 [b]
	S5	1	0–830	83	1.41 ± 0.62 [a]
		2	830–1860	103	3.60 ± 0.99 [b]
	S6	1	0–770	77	2.13 ± 0.80 [a]
		2	770–2140	137	4.93 ± 1.68 [b]
Yangtze River estuary	E1	1	0–830	83	1.50 ± 0.80 [a]
		2	830–1830	100	3.20 ± 1.07 [b]
		3	1830–2030	20	1.55 ± 0.93 [a]
	E2	1	0–810	81	1.67 ± 0.68 [a]
		2	810–1730	92	3.90 ± 0.99 [b]
		3	1730–2000	27	1.42 ± 0.90 [a]
	E3	1	0–770	77	1.52 ± 0.77 [a]
		2	770–1900	113	5.54 ± 1.17 [b]
		3	1900–2520	62	3.33 ± 0.75 [b]
	E4	1	0–980	98	1.32 ± 0.66 [a]
		2	980–1900	92	4.35 ± 1.30 [b]
		3	1900–2120	22	3.35 ± 0.74 [b]
	E5	1	0–370	37	2.35 ± 0.84 [a]
		2	370–1730	136	5.06 ± 1.35 [b]
	E6	1	0–1250	125	1.60 ± 0.80 [a]
		2	1250–1890	64	4.47 ± 1.14 [b]
		3	1890–1910	2	2.80 ± 0.06 [a]
	E7	1	0–700	70	3.37 ± 0.77 [b]
		2	700–2180	148	4.57 ± 1.36 [a]
		3	2180–2200	2	2.22 ± 0.44 [b]
	E8	1	0–860	86	1.93 ± 0.81 [a]
		2	860–1900	104	5.75 ± 1.16 [b]
		3	1900–2220	32	1.90 ± 1.00 [a]

Table 3. *Cont.*

Water Area	Sample Number	Otolith Sr/Ca Value Change Stage	Length from Otolith Core Radius/μm	Number of Element Points (N)	Sr/Ca Value (Mean ± SD) *
Poyang Lake	P1	1	0–860	86	1.68 ± 0.69 [a]
		2	860–1910	105	4.26 ± 0.72 [b]
		3	1910–2190	28	1.16 ± 0.51 [a]
	P2	1	0–570	57	2.72 ± 0.89 [a]
		2	570–1700	113	4.21 ± 1.33 [b]
		3	1700–2300	60	2.06 ± 1.02 [b]
	P3	1	0–940	94	1.80 ± 0.76 [a]
		2	940–1510	57	4.99 ± 1.16 [b]
		3	1510–1600	9	1.73 ± 0.77 [a]
	P4	1	0–810	81	1.57 ± 0.59 [a]
		2	810–1720	91	3.34 ± 0.90 [b]
		3	1720–1850	13	1.23 ± 0.41 [a]
	P5	1	0–730	73	1.45 ± 0.61 [a]
		2	730–1670	94	4.76 ± 1.01 [b]
		3	1670–1800	13	1.14 ± 0.70 [a]
	P6	1	0–850	85	1.82 ± 0.68 [a]
		2	850–1840	99	4.38 ± 1.07 [b]
		3	1840–1920	8	1.43 ± 0.86 [a]
	P7	1	0–840	84	1.46 ± 0.59 [a]
		2	840–1880	104	4.27 ± 0.97 [b]
		3	1880–2060	18	1.38 ± 0.54 [a]

Note: * represented significant differences at 0.05 level; different superscript letters indicated significant differences (Mann–Whitney *U-test* $p < 0.05$).

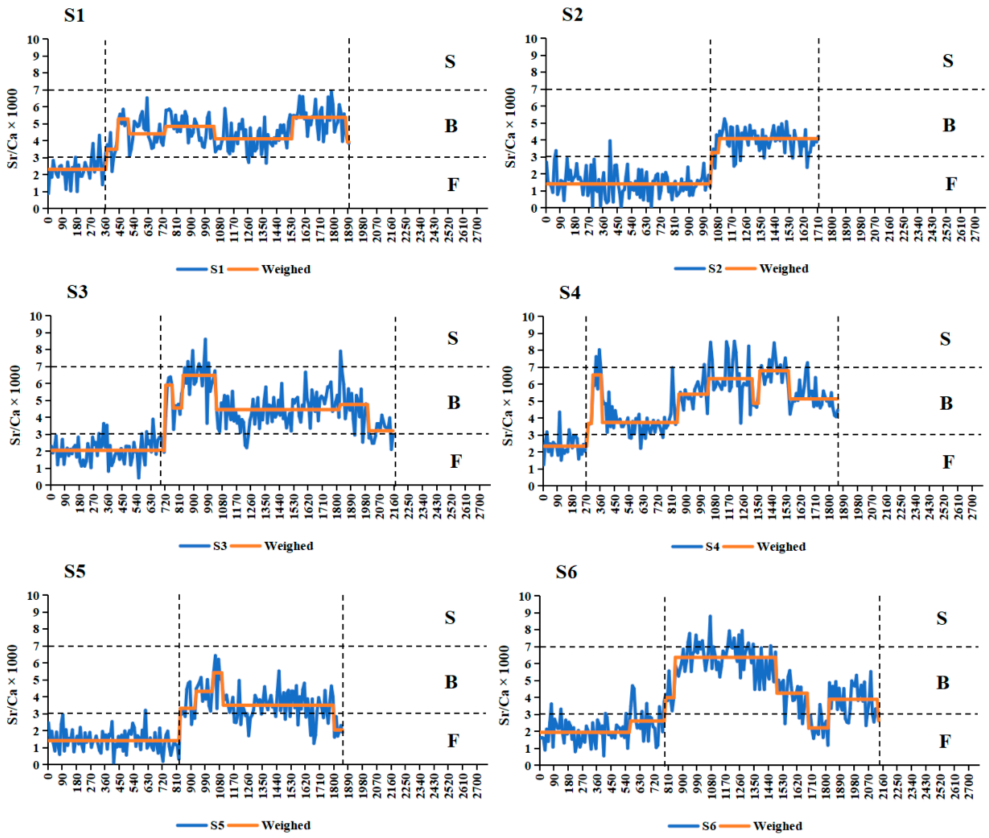

Figure 3. Fluctuation in otolith Sr/Ca ratios along line transects from the core (0 μm) to the edge in the sagittal plane of the otoliths of different *Coilia nasus* groups along Shengsi Sea area. Orange solid line represents the Sr/Ca ratios after trend conversion; blue solid lines represent actual Sr/Ca ratios. S: seawater, B: brackish water, F: fresh water.

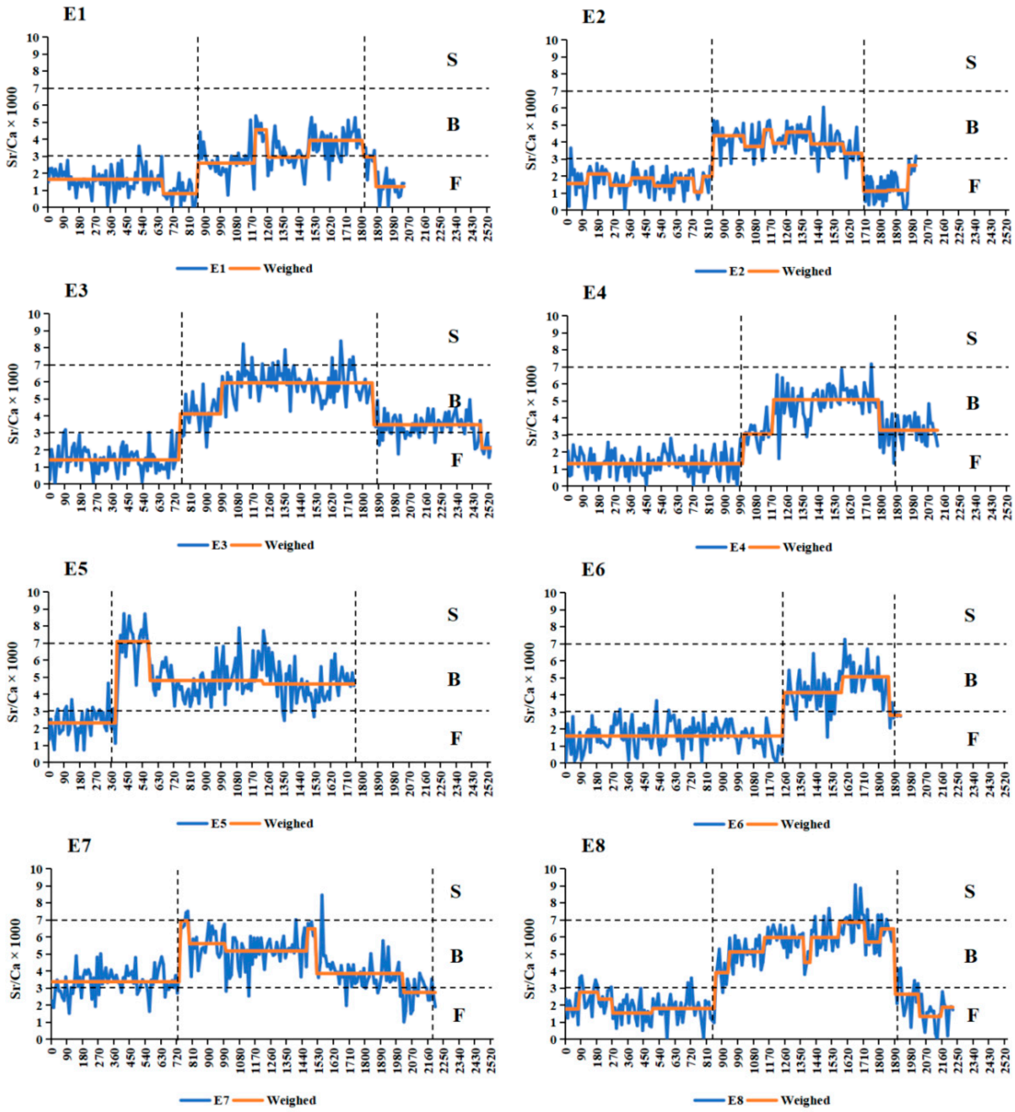

Figure 4. Fluctuation in otolith Sr/Ca ratios along line transects from the core (0 μm) to the edge in the sagittal plane of the otoliths of different *Coilia nasus* groups along Yangtze River estuary. Orange solid line represents the Sr/Ca ratios after trend conversion; blue solid lines represent actual Sr/Ca ratios. S: seawater, B: brackish water, F: fresh water.

3.3. The Area Distribution Analysis of Sr Content

According to previous studies on the sagittal otolith of *C. nasus*, the red-yellow, green and blue regions were characterized by seawater (high salinity), brackish water (medium salinity) and freshwater (low salinity), respectively. All samples of group E and group P had similar blue central regions, suggesting that they were born in freshwater, namely anadromous *C. nasus*. Then, they alternated between yellow or green or even red bands, corresponding to the migration of these adults between offshore, estuarine and freshwater habitats (Figures 6 and 7). All samples had a similar length of Sr/Ca ratios phase (841 ± 148 μm), apart from E5, which had an unusually small blue central region (370 μm), and the periphery turned into a red band with Sr/Ca ratios more than 7 (Figures 3–5). Compared with group E and P, the samples in group S were significantly divided into two groups. Among them, S2, S3, S5 and S6 had the same blue central region (770–1040 μm) on the sagittal plane as those in group E and group P. S1 and S4 had significantly smaller blue central regions (270–310 μm) than the other groups and the same green-yellow or even red bands adjacent to the blue central region appeared alternately. The Sr/Ca ratios continued to be high (>3). According to the results of the quantitative line of Sr/Ca ratios for the otolith, the average length of the blue central region varied greatly among groups (Table 3). Additionally, the values of F_C in group S (0.347 ± 0.169), group E (0.394 ± 0.130) and group P (0.417 ± 0.099) had significant differences ($p < 0.05$), and the Fc of S1 (0.20) and S4 (0.15) was less than that of S2, S5 and S6 (Table S1, Figure 8).

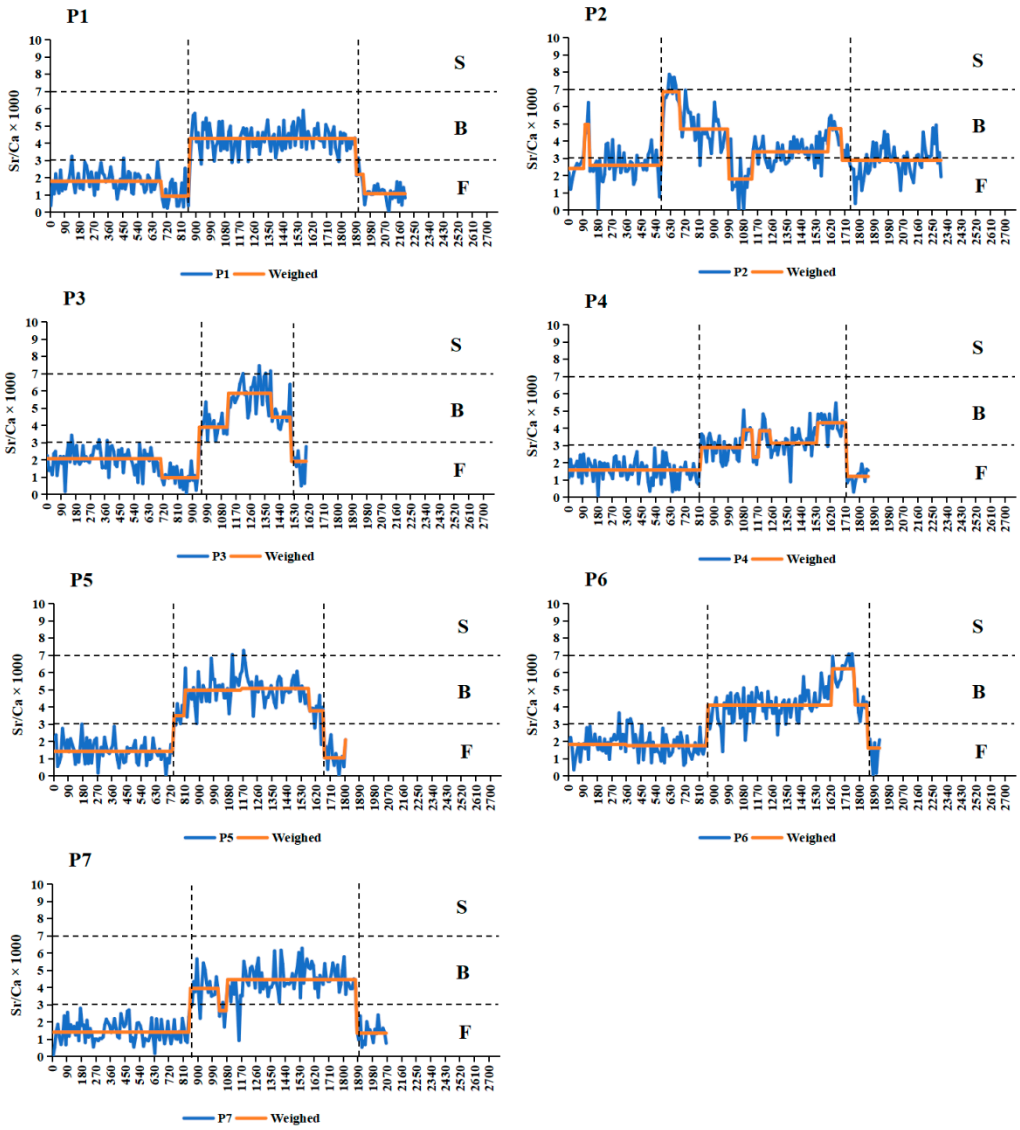

Figure 5. Fluctuation in otolith Sr/Ca ratios along line transects from the core (0 μm) to the edge in the sagittal plane of the otoliths of different *Coilia nasus* groups along and Poyang Lake. Orange solid line represents the Sr/Ca ratios after trend conversion; blue solid lines represent actual Sr/Ca ratios. S: seawater, B: brackish water, F: fresh water.

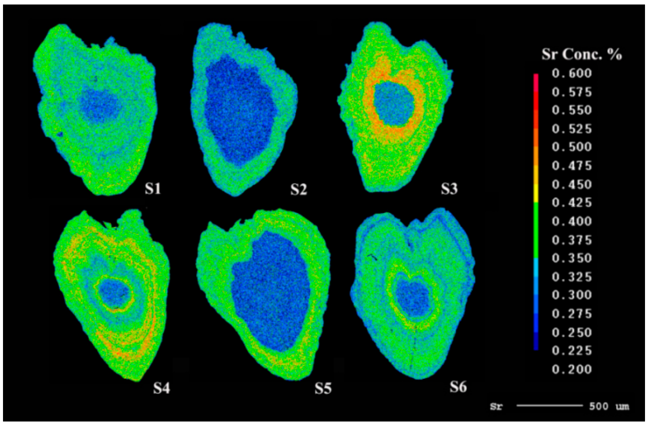

Figure 6. Two-dimensional imaging using X-ray electron microprobe analysis of the Sr concentrations in the sagittal plane of the otoliths of individual *Coilia nasus* from Shengsi Sea area, China. The values corresponding to Sr concentrations are represented by 16 colors from red (highest) through yellow and green, to blue (lowest).

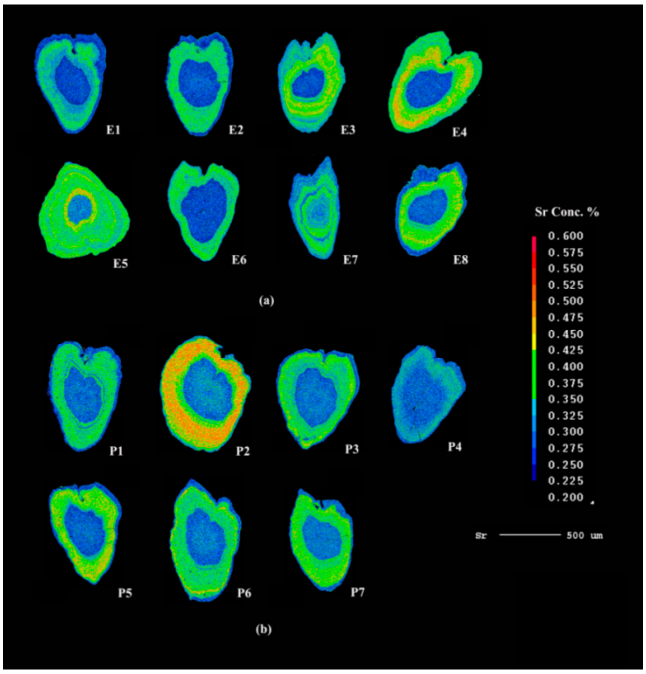

Figure 7. Two-dimensional imaging using X-ray electron microprobe analysis of the Sr concentrations in the sagittal plane of the otoliths of individual *Coilia nasus* from Yangtze River estuary (**a**) and Poyang Lake (**b**), China. The values corresponding to Sr concentrations are represented by 16 colors from red (highest) through yellow and green, to blue (lowest).

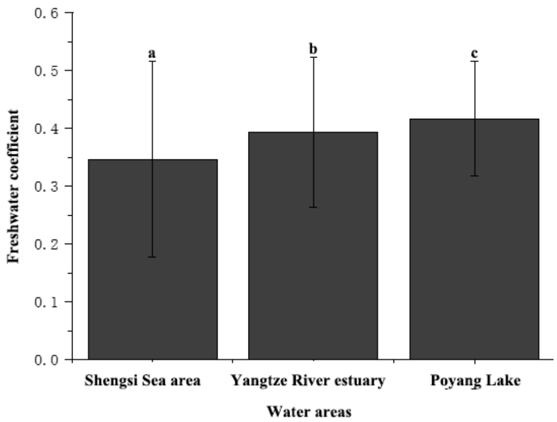

Figure 8. Freshwater coefficient of *Coilia nasus* in each water area (different letters indicate significant differences, $p < 0.05$).

4. Discussion

Physical characteristic is a way to judge the ecological type of *Coilia nasus*. In this study, all *C. nasus* samples (the ratios of jaw length/head length > 1) belonged to the long supermaxilla phenotype, conforming to the physical characteristic of migratory *C. nasus* [5]. Moreover, the maturity of gonads in *C. nasus* increased with the increase in migration distance, which met the criteria of previous studies on *C. nasus* [1,8]. The gonadal development degree of *C. nasus* in the Shengsi sea area was the lowest, indicating that *C. nasus* is still in the gonadal development stage. The Shengsi sea area might provide plenty of food for *C. nasus* to mature.

Previous studies on otoliths find that *C. nasus* has different habitat resume patterns in different waters. In this study, all samples from group S, E and P have a similar pattern and the central area of each sagittal otolith has an obvious blue color (Sr/Ca ratios ≤ 3), which indicates that these individuals were born in fresh water and their fresh water life history might cover a long period of time. Additionally, the calculated *Fc* parameter in the present study also indicates some differences in the freshwater dependency among *C. nasus* in the Shengsi Sea area, Yangtze River estuary and Poyang Lake. The *Fc* parameter suggests the ratio of the first part of freshwater life history to the whole in otolith of fish with similar ages [12]. All individuals in this study were 2 years old, so individuals in the Shengsi sea area have a shorter early life in freshwater than Yangtze River estuary and Poyang Lake on the whole. Notably, group S can be divided into two clusters on the basis of *Fc*: one cluster of low *Fc* included S1 and S4; the other cluster of high *Fc* included S2, S5 and S6. It's reported that the population of *C. nasus* in the Qiantang River had a shorter phase of freshwater initial life history than the population in the Yangtze River [35], so S1 and S4 might belong to the Qiantang River and the other cluster might belong to the Yangtze River.

According to previous studies on the habitat of *C. nasus*, the individual spawning ground of the Yellow River is 400 km from Dongping Lake to the estuary, but the individual spawning ground of the Yangtze River is 800 km from Poyang Lake to the estuary. The longest habitat time of migratory *C. nasus* in freshwater was significantly different between the two rivers [36,37]. Therefore, the individuals in the Yellow River and Yangtze River are significantly different. In this study, the diameter and distance for the central region (Sr/Ca ratios ≤ 3) of otoliths in groups (S, E and P) were between 700 μm and 1250 μm, except S1, S4 and E5, so over 90% of samples might experience similar freshwater life history in early life stages. For S1, S4 and E5, the diameter and distance of the central region (Sr/Ca ratios ≤ 3) of otoliths were between 270 and 370 μm. Compared with other samples, they might have experienced a relatively short freshwater life history in early life. It's reported that *C. nasus* in Qiantang River had bluish central core regions with lower Sr/Ca ratios of otoliths, between 10 and 390 μm, and the phase of freshwater initial life history for *C. nasus* in Qiantang River was usually shorter than individuals in the Yangtze River [35].

According to the short length of otolith radius and the narrow bluish central core regions corresponding to the first stage (Sr/Ca ratios ≤ 3) of S1 and S4, it is speculated that S1 and S4 individuals might not belong to *C. nasus* of the Yangtze River. Moreover, the Shengsi Sea area is the intersection of the Qiantang River, the Yangtze River and the East China Sea [38], so S1 and S4 individuals might belong to the Qiantang River. In addition, the phase of freshwater initial life history for *C. nasus* in the Yangtze River estuary was usually shorter than individuals in Poyang Lake [39]; therefore, E5 individuals might belong to the Yangtze River estuary group. Of course, E5 individuals also might belong to other water areas, as S1 and S4, which requires further research. The similarity between individuals in three water areas was different, suggesting that the Yangtze River *C. nasus* and the Qiantang River *C. nasus* might have multiple populations. Nevertheless, the overall results still showed that *C. nasus* of the Yangtze River might exist in the Shengsi Sea area, and the Shengsi Sea area might be one of the feeding grounds for migratory *C. nasus*.

At present, the ecological problems are serious in the Shengsi Sea area (feeding ground). First, the rapid economic development has led to serious environmental pollution. Secondly, with the increasing material needs, fishing intensity continues to rise, resulting in serious environmental damage [40]. These factors have restricted the recovery of *C. nasus* resources in the Yangtze River. Meanwhile, although the long-term and high-intensity development of the Shengsi Sea area, which is located in the Qiantang River and the Yangtze River, has caused a reduction in fishery resources and the pollution of the ecological environment, the Shengsi Sea area still maintains a high level of primary aquaculture capacity due to a large number of planktonic microorganisms and nutrient inflow [41]. Therefore, in order to better restore the Shengsi Sea area for the recovery of *C. nasus* resources, the fishing intensity should be strictly controlled; the environmental regulation of coastal water and the fishery management should be strengthened; and a unique marine protection area should be established. As a typical diadromous fish species, the resource recovery proposals of *C. nasus* are helpful to restore the traditional diadromous fish stocks caused by human factors in other countries. It is worth noting that the loss and degradation of feeding grounds of diadromous fishes (e.g., sturgeons, alosine clupeids, eels and salmons) have the most profound influence on them, so more attention should be paid to restoration and protection [42]. In fact, poor ecological connectivity in the North American and European basins and the North Atlantic ecosystems leads to a reduction in diadromous fishes [26]. Effective and accurate determination of the feeding grounds of *C. nasus* in each open sea river can effectively help its resource recovery. Therefore, on the basis of this study, it is necessary to cooperate with various aspects (such as otolith morphology, habitat water quality detection, pollution detection) to develop corresponding methods to make the otolith method more targeted [43,44]. In this study, a typical diadromous species and otolith microchemical method were used to study the recovery of diadromous fishes, and the method of detecting feeding grounds was also expanded, which can provide reference for the recovery of other river-sea migratory fish resources in the future.

5. Conclusions

Based on the advantages of fish otolith microchemistry, this study used otolith microchemistry to analyze *C. nasus* in the Shengsi Sea area, for the first time, and obtained the habitat history of *C. nasus* intuitively and accurately. These results suggest that the Shengsi Sea area might be one of the feeding grounds for migratory *C. nasus.* Although this present study only investigated one water area (Shengsi Sea area) in the sea area, the results confirmed that the population of *C. nasus* in the Yangtze River and the population in the Shengsi Sea area should have connectivity. Therefore, in the next step, we will expand the scope of investigation, increase the number of samples and further confirm and delimit the scope of feeding grounds of *C. nasus* in the Shengsi Sea area and its surrounding water areas. Moreover, it is necessary to combine otolith microchemistry with biomolecule technology to improve the accuracy of population distribution of *C. nasus*. At present, under the implementation of the ten-year Yangtze River fishing ban plan and the great ecological protection of the Yangtze River, the resource amount and habitat ecological environment of *C. nasus* in the Yangtze River have developed in a good direction. Notably, the protection of the feeding ground (Shengsi Sea area) should be valued to accelerate the recovery of *C. nasus* resources. These results also provide a scientific reference for other migratory aquatic organisms in the Yangtze River and objective theoretical basis for ecological restoration of the Yangtze River.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes7040172/s1, Table S1: Freshwater coefficient of *Coilia nasus* in each water area.

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Institutional Review Board Statement: The experiments comply with current laws of China. All the samples in this study were obtained from legal commercial fisheries, and the samples were dead when they were obtained.

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