

# Otolith microchemical evidence revealing multiple spawning site origination of the anadromous tapertail anchovy (*Coilia nasus*) in the Changjiang (Yangtze) River Estuary

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## Abstract

The estuarine tapertail anchovy (*Coilia nasus*) is a high-value commercial fish. Estimating the spawning site or hatchery origin and habitat is essential for its conservation. This study aimed to determine the habitat use and life history characteristics of *C. nasus* from the Changjiang River Estuary. We investigated the environmental signatures of strontium (Sr) and calcium (Ca) in the otoliths of the collected specimens using electron probe microanalysis; additionally, we examined their gonadal maturity stage. Our results indicate that the 31 adult *C. nasus* specimens used in this study could be classified into two types based on their otolith Sr:Ca concentration ratios and their gonadal maturity stage. The long freshwater early life history type (Type LF) had wider central region in the otolith with low Sr:Ca concentration ratios ranging from  $1.24 \pm 0.62$  to  $1.92 \pm 0.78$  and a bluish pattern of low Sr content level. These fish are of riverine origin and had a relatively long early life history in freshwater and low gonadal maturation when captured. The short freshwater early life history type (Type SF) had a shorter central region in the otolith with low Sr:Ca concentration ratios ranging from  $1.35 \pm 0.5$  to  $2.82 \pm 0.97$  and a correspondingly bluish pattern. These fish also had a relatively short-term early life history in freshwater and high gonadal maturation when captured. The results of the otolith microchemical analysis indicated that Type LF and Type SF originated in spawning/hatching sites far from and close to the estuary, respectively. The mature gonads of Type SF fish indicated that they may breed in areas close to the estuary, whereas the immature gonads of Type LF fish indicated that they may breed in areas far from the estuary. This study is the first to reveal that the Changjiang River Estuary contains stocks of anadromous *C. nasus* originating in different spawning sites during the same season. The estuarine habitat plays a critical role in the connectivity between freshwater recruitment and the marine resources available to adult spawners of this commercially valued species. From a conservation perspective, this study provides important information for identifying anadromous *C. nasus* stocks originating in different spawning sites in the Changjiang River Basin.

**Key words:** *Coilia nasus*, otolith, migration, connectivity, spawning site

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## 1 Introduction

The estuarine tapertail anchovy *Coilia nasus*, an anadromous fish, is widely distributed in coastal waters, estuaries, and rivers in East Asia, such as the East China Sea, Yellow Sea, Changjiang River, Huanghe River, Qiantang River, and Oujiang River in China as well as river estuaries around the Ariake Sea of Japan (Yang et al., 2006; Jiang et al., 2012; Suzuki et al., 2014; Liu et al., 2018). In China, the anadromous *C. nasus* has high commercial value owing to its nutritional value and the fact that it is consumed as a delicacy of cultural significance (Jiang et al., 2017). As *C. nasus* is an anadromous species, during its adult stage, it migrates upstream from estuaries and coastal waters to freshwater areas, such as lakes or slow-flow water bodies, during the spawn-

ing season (ranges between February and October) (Yuan, 1987). After spawning, the larvae and juveniles drift with the river into estuaries and adjacent marine waters, which serve as their growth habitats, and live there until the onset of sexual maturation, after which they move upstream to their spawning area (Yuan, 1987; Yang et al., 2006; Jiang et al., 2012). Jiang et al. (2016) reported that a certain stock of anadromous *C. nasus* in the Poyang Lake may have natal homing capabilities as its members can move upstream from their growth habitats (i.e., the Changjiang River Estuary and Yellow Sea) to return to their natal Poyang Lake to spawn. The natal homing capability indicates that the spawning site has an irreplaceable effect on the anadromous *C. nasus* stock. Once the natal spawning grounds were destroyed, it

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was difficult to prevent the extinction risk threatening the stock (Hall et al., 2012; Huang et al., 2013; Littrell et al., 2018).

Adult *C. nasus* (mostly 1–2 years old) has been reported to spawn in many lakes in the Changjiang River Basin, such as the Dongting Lake and Poyang Lake, which are connected to the Changjiang River through its middle and lower reaches (Yuan, 1987; Zhu, 1992; Jiang et al., 2017) as well as in slow-flow water or backwater bays along the mainstream of the Changjiang River in its middle and lower reaches (Zhu, 1992). During the past 60 years, however, extensive environmental degradation caused by anthropogenic activities, such as overfishing, water pollution, farmland reclaiming, gravel abstraction, and river-lake connectivity losses, resulted in significant biodiversity declines in aquatic organisms and even in the local extirpation of some migratory fish species, such as clupeids, tetraodontidae, anguillids, acipenserids, and cyprinids in the Changjiang River Basin (Huang et al., 2013; Huang and Li, 2016; Liu et al., 2019; Jiang et al., 2020). Likewise, stocks of the anadromous *C. nasus* in the Changjiang River have declined dramatically since the late 1970s (Li et al., 2007; Liang and Pauly, 2017). For instance, the destruction of the tapertail anchovy's spawning habitats or the loss of spawning and nursery habitats owing to gravel abstraction, farmland reclaiming, and prevention of access to spawning sites through widespread hydraulic construction may have played an important role in the dramatic decline in the anadromous *C. nasus* abundance (Yuan, 1988). Therefore, the discovery and conservation of potential *C. nasus* spawning sites could play a prominent role in the preservation of this treasured species (Jiang et al., 2017). Currently, spawning sites can only be confirmed in Poyang Lake, owing to the capture of sexually mature adult anadromous *C. nasus* (Jiang et al., 2017). There is an urgent need to clarify whether there are other spawning sites and breeding groups in Changjiang River and to accurately assess and protect these different breeding groups originating in different spawning sites.

In recent years, the importance of the Changjiang River Estuary to *C. nasus* has received much attention. The Changjiang River Estuary acts as a feeding and nursing ground for *C. nasus* larvae and juveniles as well as an essential migration channel for their seaward dispersion and the anadromous migration of *C. nasus* adults (Ge and Zhong, 2010; Gao et al., 2018; Dai et al., 2020). Ge and Zhong (2010) estimated the hatching date of *C. nasus* based on the otolith microstructural patterns of *C. nasus* larvae and juveniles in the surf zone of the Changjiang River Estuary. They found that larvae and juveniles began to appear in the surf zone approximately 7 days after hatching and speculated that there may be a spawning site near the Changjiang River Estuary. Dou et al. (2012) discovered the existence of two different groups, one of freshwater origin and one of estuary origin, among the young-of-the-year *C. nasus* of the Changjiang River Estuary, using otolith Sr:Ca concentration ratios. Jiang et al. (2015) found that two groups of *C. nasus* juveniles originated in different hatcheries, based on the characteristics of C and O stable isotopes in their otoliths; one hatchery may have been located far from the Changjiang River Estuary and the other close to it.

Another species inhabiting the same riverine reaches and river-connected lakes as *C. nasus* is *C. brachygnathus*. Individuals with a supermaxilla extending beyond the end of the gill cover have been identified as *C. nasus*, whereas individuals with a supermaxilla that does not reach the end of the gill cover have been identified as *C. brachygnathus* (Yuan et al., 1976). It has commonly been thought that *C. brachygnathus*, unlike the anadromous *C. nasus*, resides in freshwater and does not migrate to seawater. Nevertheless, the presence of both migratory *C. brachygnathus* and non-migratory *C. nasus* in Changjiang River

(Li et al., 2017; Chen et al., 2017) creates a difficulty in determining if fish and fertilized eggs are from anadromous or freshwater-resident ecotypes in the Changjiang River, using morphological indicators and molecular biology methods, even though molecular single nucleotide polymorphism markers can distinguish between anadromous and landlocked *C. nasus* populations in closed lakes (Cheng et al., 2019).

Fortunately, otolith microchemical technology provides an opportunity to solve these problems. Otoliths are metabolically inert and are not reabsorbed; they grow incrementally throughout the entire life history of a fish as they accumulate elements from the surrounding water. Therefore, otoliths can record environmental changes throughout the life history of fish. A strong relationship between ambient salinity and the element:Ca concentration ratio (particularly the Sr:Ca concentration ratio) in the water and fish otoliths has been discovered (Brown and Severin, 2009; Keller et al., 2018; Taddese et al., 2019). Therefore, otolith microchemistry is a powerful tool that can be used to distinguish between different ecotypes (e.g., anadromous and non-anadromous), to elucidate the spatial movement trajectory of anadromous fish individuals, and to assess population structure/dynamics (Ložys et al., 2017; Santana et al., 2018; Jiang et al., 2019). In previous studies, otolith Sr:Ca signatures that can identify freshwater, brackish water, and marine habitats of *C. nasus* were established (Yang et al., 2006; Jiang et al., 2017; Khumbanyiwa et al., 2018; Liu et al., 2018) and used for the discovery of a spawning area of anadromous *C. nasus* in the Poyang Lake, China (Jiang et al., 2017).

Based on data regarding larvae that hatched after approximately 7 days, as reported by Ge and Zhong (2010), the estuary origin of the young-of-the-year *C. nasus* from the Changjiang River Estuary reported by Dou et al. (2012), and the *C. nasus* juveniles that originated in different hatcheries reported by Jiang et al. (2015), we posit that *C. nasus* in the Changjiang River Estuary originate in different spawning grounds; at least one of these spawning grounds is located far from the Changjiang River Estuary, while some spawning grounds are potentially located near the estuary. However, this hypothesis lacks validation in terms of adult fish breeding populations. The physiological characteristics of adult fish, especially the degree of gonadal development, can also be used as a basis for discriminating among the breeding populations. The capture of highly mature individuals can provide an approximate location of the spawning ground (Karakulak et al., 2004; Kayaba et al., 2014; Filina and Budanova, 2015). In order to test our hypothesis, we employed otolith microchemistry to analyze the otoliths of *C. nasus* adults in the Changjiang River Estuary, to reconstruct their life histories, and to measure the distance from their hatching areas to the estuary. At the same time, we recorded the gonadal maturity stage of anadromous *C. nasus* adults to estimate the distance from the sampling site to their potential breeding areas.

## 2 Materials and methods

### 2.1 Sampling site and fish specimens

A total of 31 adult *C. nasus* specimens were collected [total length (mean±SD): (308.35±26.03) mm] using commercial drifting gill net fishery in a sampling site approximately 31°26'30"–31°26'50"N and 121°51'50"–121°52'30"E at the lower part of the Changjiang River Estuary near the Chongming Island. Out of the 31 samples, 21 were collected on May 5, 2017 and referred to as 17CMCN01–21, and the remaining 10 were collected on May 8, 2017 and referred to as 17TJSCN01–10 (Fig. 1). We measured the total length, body weight, and supermaxilla:head length ratio of

all the samples. The gender and gonadal maturity stage of all specimens were determined by examining the gonads visually, using the criterion proposed by Li et al. (2007) and Xu et al. (2012). Individuals at Stages IV or V were defined as mature fish (Li et al.,

2007; Xu et al., 2012). In addition, 17 out of the 31 *C. nasus* specimens collected were highly mature individuals with Stages IV or V gonads, whereas the gonads of the remaining 14 *C. nasus* specimens were in the Stages II or III (Table 1). The former fish with

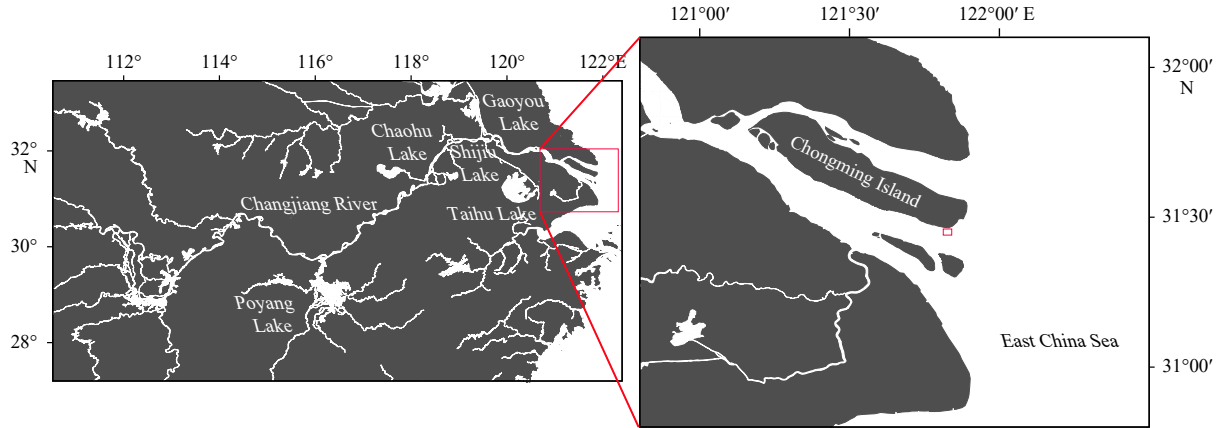


Fig. 1. Map of the sampling site of *Coilia nasus* in the Changjiang River Estuary. The red box in the right figure indicates the sampling range.

Table 1. The proportions of *Coilia nasus* specimens collected from the waters of the Changjiang River Estuary, throughout their freshwater life history

Type	Individual code	Total length/mm	Body weight/g	Supermaxilla:Head length ratio	Sex	Age <sup>a</sup>	Gonadal maturity stage	$L_f^b/\mu\text{m}$	$L_T^c/\mu\text{m}$	$F_C^d$
Type LF	17CMCN08	387	140.00	1.09	♂	2+	II	910	2 290	0.40
	17CMCN12	314	98.11	1.12	♀	2+	II	550	1 770	0.31
	17CMCN13	309	104.01	1.24	♀	2+	II	1 100	2 310	0.48
	17CMCN14	310	94.61	1.30	♂	2+	II	980	2 020	0.49
	17CMCN15	317	103.62	1.26	♂	2+	II	980	2 100	0.47
	17CMCN16	300	97.79	1.20	♀	2+	II	910	2 100	0.43
	17CMCN17	322	115.69	1.18	♂	2+	II	660	2 160	0.31
	17CMCN18	320	116.83	1.13	♀	2+	II	970	2 100	0.46
	17CMCN19	294	94.36	1.21	♂	2+	II	690	1 960	0.35
	17CMCN20	315	107.69	1.16	♀	3	II	510	1 830	0.28
	17CMCN21	310	99.81	1.16	♀	2+	II	1 130	2 190	0.52
Type SF	17TJSCN03	323	111.89	1.15	♀	2+	III	920	2 390	0.38
	17TJSCN06	275	67.44	1.13	♂	2	III	890	1 920	0.46
	17TJSCN08	333	155.13	1.22	♂	2+	II	1 690	2 270	0.74
	17CMCN01	325	115.81	1.24	♀	2+	IV	220	2 320	0.09
	17CMCN02	230	31.48	1.29	♂	2+	V	290	1 860	0.16
	17CMCN03	306	100.01	1.27	♀	2+	IV	480	2 250	0.21
	17CMCN04	281	71.55	1.12	♀	2+	IV	650	1 950	0.33
	17CMCN05	302	70.71	1.31	♂	2+	IV	660	2 270	0.29
	17CMCN06	305	93.59	1.16	♂	2+	IV	440	2 080	0.21
	17CMCN10	333	126.22	1.17	♀	2+	IV	270	1 930	0.14
17CMCN11	312	123.67	1.20	♂	2+	IV	670	1 990	0.37	
17TJSCN01	322	148.16	1.14	♀	3+	IV	200	2 200	0.09	
17TJSCN02	305	98.13	1.23	♀	2+	IV	200	2 370	0.08	
17TJSCN04	278	72.32	1.22	♂	2	IV	270	2 120	0.13	
17TJSCN05	295	89.19	1.35	♀	2+	IV	200	2 060	0.10	
17TJSCN09	305	98.28	1.21	♂	2	IV	370	1 940	0.19	
17TJSCN10	331	124.57	1.22	♂	3+	IV	150	2 350	0.06	
17CMCN07	293	74.21	1.17	♀	2+	IV	0	2 160	0	
17CMCN09	331	109.87	1.19	♀	3+	V	0	2 120	0	
17TJSCN07	276	50.18	1.24	♀	2+	IV	0	1 970	0	

Note: Type LF: the long freshwater early life history type; Type SF: the short freshwater early life history type. Age<sup>a</sup> is estimated using the otolith rings;  $L_f^b$ : the length of the first freshwater stage of the low Sr:Ca concentration ratio line of the otolith microchemical line analysis or the length of the low Sr bluish central regions of the Sr mapping analysis;  $L_T^c$ : the radius of the entire otolith microchemical line analysis measurement line along the line down the longest axis of each otolith from the core;  $F_C^d$ : freshwater coefficient.

highly mature gonads were categorized as mature fish (17CMCN01–07, 17CMCN09–11, 17TJSCN01, 17TJSCN02, 17TJSCN04, 17TJSCN05, 17TJSCN07, 17TJSCN09, and 17TJSCN10), whereas the latter were categorized as immature fish (17CMCN08, 17CMCN12–21, 17TJSCN03, 17TJSCN06, and 17TJSCN08). The total length, body weight, and supermaxilla: head length ratio of all samples are shown in Table 1.

## 2.2 Otolith microchemical analysis

In this study, only the left sagittal otolith of each specimen was used when possible. The *C. nasus* otoliths were subjected to electron probe microanalysis (EPMA) according to the methods described by Khumbanyiwa et al. (2018) and Liu et al. (2018).

The otoliths were extracted, cleaned, and embedded in epoxy resin (EpoFix; Struers, Copenhagen, Denmark) in the frontal plane. The individually embedded otoliths were then ground with an automated grinding machine equipped with a diamond cup wheel (Discoplan-TS, Struers) to expose the core. Next, all otoliths were further polished with silica suspension using an automated polishing wheel (LaboPol-35, Struers). The otoliths were then cleaned in an ultrasonic bath and rinsed with Milli-Q water. All otoliths were coated with carbon under a high vacuum evaporator (JEE-420, JEOL Ltd., Tokyo, Japan) for the EPMA (Khumbanyiwa et al., 2018; Liu et al., 2018).

The life history transect analysis was performed with a wavelength dispersive X-ray electron microprobe (JXA-8100, JEOL Ltd.) by analyzing quantitatively the Sr and Ca concentrations in the otolith samples (Jiang et al., 2012), while calcite ( $\text{CaCO}_3$ ) and tausonite ( $\text{SrTiO}_3$ ) were used as standards. Line analysis was performed along a line from the otolith center (core) to the edge. The analysis was performed under the following conditions: the accelerating voltage was 15 kV, the electron beam was focused on a point 5  $\mu\text{m}$  in diameter, and measurements were spaced at 10  $\mu\text{m}$  intervals (Khumbanyiwa et al., 2018; Liu et al., 2018). The X-ray intensity maps of the Sr concentrations were created based on the representative otoliths, using the same microprobe and in accordance with the aforementioned life history transect. The beam current was  $5 \times 10^{-7}$  A, the counting time was 30 ms, the pixel size used was 8  $\mu\text{m} \times 8 \mu\text{m}$ , and the electron beam was focused onto a point 5  $\mu\text{m}$  in diameter (Khumbanyiwa et al., 2018; Liu et al., 2018).

## 2.3 Age determination

According to the methods of Jiang et al. (2016) and Khumbanyiwa et al. (2018), the otoliths that underwent microchemical analysis were repolished to remove the carbon coating from the otolith surface and then etched with 5% ethylenediaminetetraacetate (EDTA) to reveal the annuli of the otolith samples. The ages of the individual samples were determined from the annuli. A digital microscope camera (Olympus BX51, Olympus Corporation, Tokyo, Japan) was used to image the otoliths and measure the length from the core of the otoliths to the annulus marks along the measurement line used in the line analysis of otolith microchemistry.

## 2.4 Statistical analysis

In this study, the Sr:Ca ratios of the concentrations based on the molecular weights of SrO and CaO were calculated as Sr:Ca $\times$ 1 000 (hereinafter referred to as the Sr:Ca ratios).

In order to identify significant changes between the current mean Sr:Ca ratio and that of a consecutive point that could indicate the life history transition, we applied a sequential regime shift algorithm (Rodionov and Overland, 2005). The inputs of the re-

gime shift index were: cutoff length=10, probability level=0.1, and Huber's weight parameter=1 (Rodionov and Overland, 2005; Liu et al., 2018; Jiang et al., 2019). According to the results of previous studies (Yang et al., 2006; Jiang et al., 2014; Chen et al., 2017), we categorized the *C. nasus* habitats into freshwater (Sr:Ca ratio  $\leq 3$ ), brackish (Sr:Ca ratio 3–7), and seawater habitats (Sr:Ca ratio  $\geq 7$ ). In the X-ray intensity map of the Sr content for confirmation of the *C. nasus* habitats, blue corresponded to freshwater habitats, green or yellow corresponded to brackish habitats, and red corresponded to sea habitats.

The freshwater coefficient ( $F_C$ ) was estimated using the formula  $F_C = L_f/L_T$  (Jiang et al., 2014; Chen et al., 2016; Khumbanyiwa et al., 2018), where  $L_f$  is the length of the first freshwater stage of the low Sr:Ca ratio line of the otolith microchemical line analysis or the length of the low Sr bluish central regions of the Sr mapping analysis, and  $L_T$  is the radius of the entire otolith microchemical line analysis measurement line along the line down the longest axis of each otolith from the core. This coefficient represented the ratio of the time spent in the freshwater environment in the early life history of individual fish to their entire life history duration.

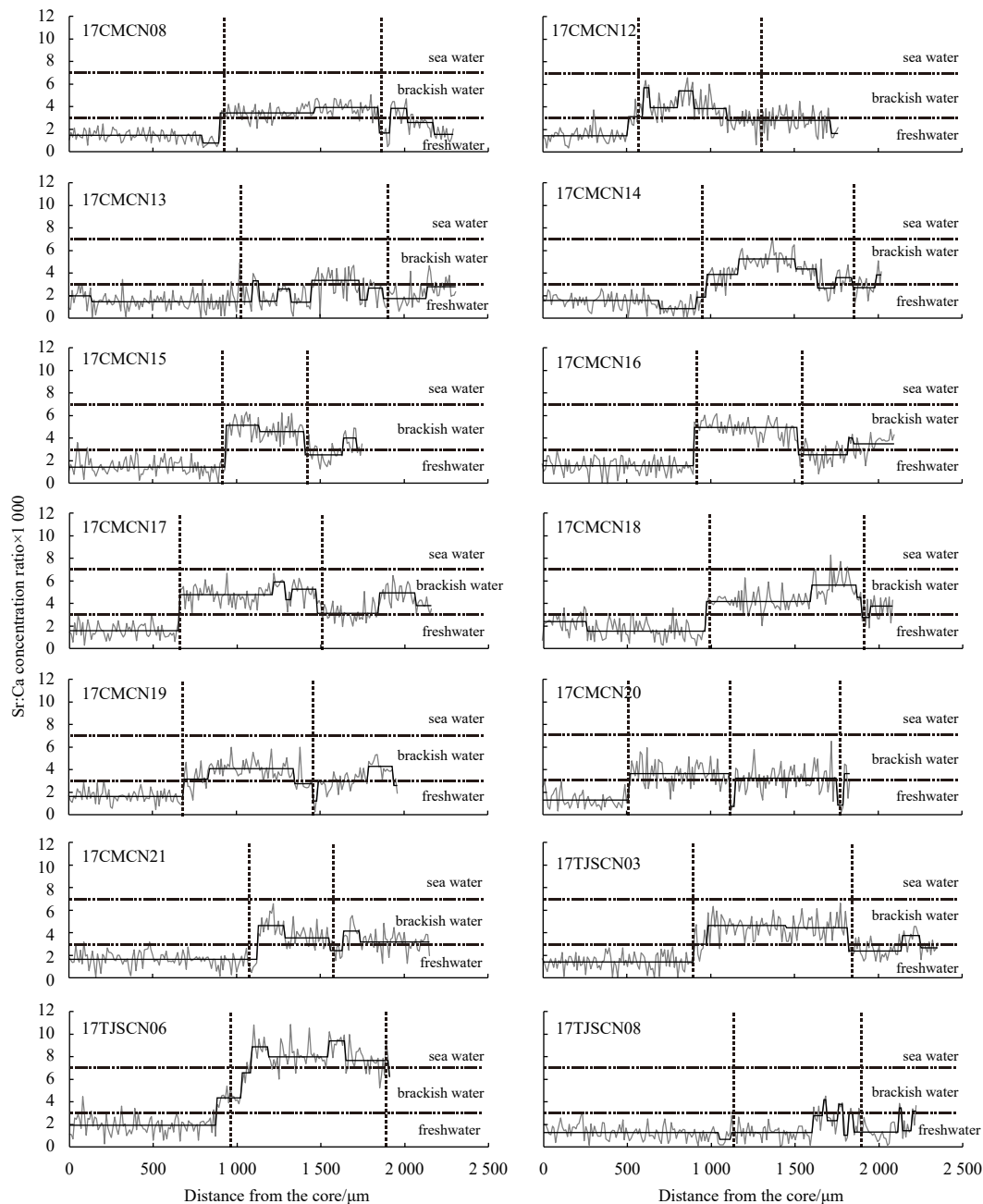
## 3 Results

### 3.1 Quantification of changes in otolith Sr and Ca microchemical profiles

In this study, *C. nasus* showed remarkably different life history phases. Based on the length of the first freshwater stage along the measurement line of the otolith microchemical line analysis of each specimen (i.e., the width of the bluish central core regions), the *C. nasus* in this study could be classified into two types: Type LF and Type SF.

Type LF fish included 17CMCN08, 17CMCN12–21, 17TJSCN03, 17TJSCN06, and 17TJSCN08, which were the same as the fish categorized as immature earlier (see Section 2.1). In this type, the first stage of the Sr:Ca ratios (mean $\pm$ SD) ranged from 1.24 $\pm$ 0.62 (17TJSCN08) to 1.92 $\pm$ 0.78 (17TJSCN06), and the length of the first low Sr:Ca ratio stage ranged from 550  $\mu\text{m}$  (17CMCN12) to 1 690  $\mu\text{m}$  (17TJSCN08) (Fig. 2), corresponding to wider bluish central core regions in the X-ray intensity map. The long low Sr:Ca ratio in the central region indicated a long early life history in freshwater. The high Sr:Ca ratio stage ( $>3$ ) corresponding to the greenish or yellowish concentric bands followed the first low Sr:Ca ratio stage and showed that *C. nasus* entered the estuary brackish water or offshore areas. In the Type LF group, the high Sr:Ca ratio stage of most fish was larger than 3 and did not reach 7, thereby indicating that they had lived in the estuary's brackish water or in medium-salinity waters and did not enter the marine environment. Only 17TJSCN06 individuals had a high Sr:Ca ratio stage of 7 or higher, thereby indicating that these individuals had entered high-salinity seawater (Fig. 2).

Type SF fish included 17CMCN01–07, 17CMCN09–11, 17TJSCN01, 17TJSCN02, 17TJSCN04, 17TJSCN05, 17TJSCN07, 17TJSCN09, and 17TJSCN10 (Fig. 3), which were the same fish categorized as mature earlier. Within Type SF existed two groups based on the length range of the first low Sr:Ca ratio stage. In the first group, the length range of the first low Sr:Ca ratio stage was between 150  $\mu\text{m}$  (17TJSCN10) and 650  $\mu\text{m}$  (17CMCN04), whereas the Sr:Ca ratio range of this stage was between 1.35 $\pm$ 0.5 (17CMCN04) and 2.82 $\pm$ 0.97 (17TJSCN10), corresponding to shorter bluish central core regions in the X-ray intensity map. The first low Sr:Ca ratio stage was followed by the high Sr:Ca ratio stage with an Sr:Ca value larger than 3, corresponding to the



**Fig. 2.** Fluctuation (grey line) and shift (black line) of the otolith Sr:Ca concentration ratios along line transects from the core (0  $\mu\text{m}$ ) to the edge of the sagittal plane of Type LF *Coilia nasus*, which bred far from the Changjiang River Estuary. The position of the vertical dashed lines represents the annulus.

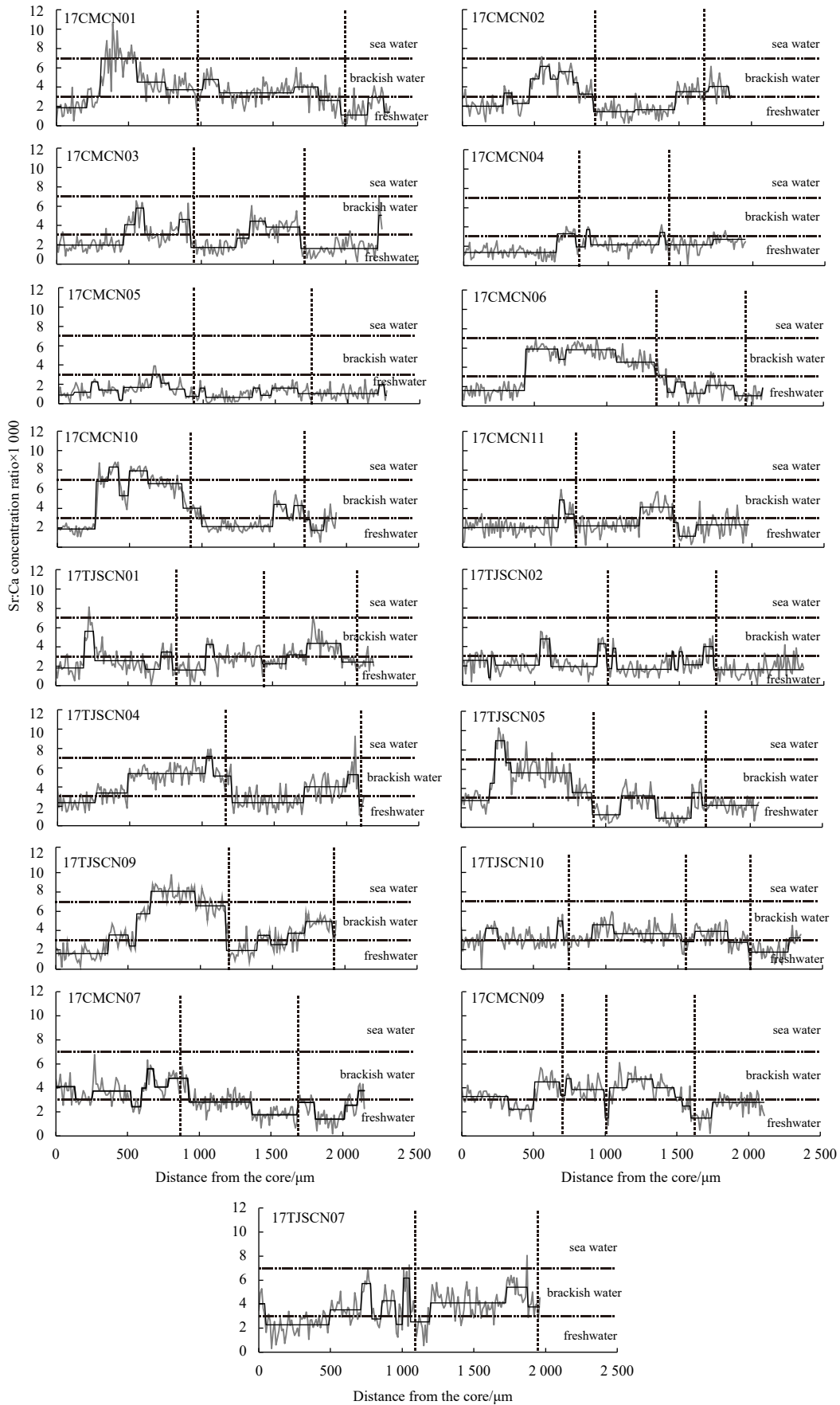
greenish, yellowish, or red concentric bands. In this group, 17CMCN01, 17CMCN10, 17TJSCN04, 17TJSCN05, and 17TJSCN09 had a high Sr:Ca ratio stage of 7 or higher (Fig. 3) and corresponded to red concentric bands (Fig. 4). The second group included three highly mature individuals (17CMCN07, 17CMCN09, and 17TJSCN07). Their first Sr:Ca ratios were  $>3$  and  $<7$  (medium Sr:Ca ratios). The lengths of the first medium Sr:Ca ratios were 520  $\mu\text{m}$ , 330  $\mu\text{m}$ , and 40  $\mu\text{m}$ , respectively, while the Sr:Ca ratio of the first stage ranged from  $3.26 \pm 0.45$  (17CMCN09) to  $4.07 \pm 0.97$  (17TJSCN07) (Fig. 3). The medium Sr:Ca ratios were in the central region, thereby indicating that these fish were born in brackish water. The first Sr:Ca ratio ( $>3$ ), was followed by a low Sr:Ca ratio ( $<3$ ) that corresponded to bluish concentric bands,

which, however, alternated between greenish and bluish (Fig. 4).

### 3.2 Freshwater coefficient

Age determination was performed using the otolith rings (Table 1); the position of the rings along the line transects is shown in Figs 2 and 3. The  $F_C$  of each sample is shown in Table 1. The  $F_C$  of Type LF was 0.31–0.74. The transition of Type LF fish from a freshwater to a brackish water environment occurred close to and even after the first annulus formation. The  $F_C$  of Type SF was 0.00–0.37. The transition of Type SF fish from freshwater to brackish water environment occurred earlier than the first annulus formation.

The group average and standard deviation of  $F_C$  were  $0.43 \pm$



**Fig. 3.** Fluctuation (grey line) and shift (black line) of the otolith Sr:Ca concentration ratios along the line transects from the core (0 μm) to the edge of the sagittal plane of Type SF *Coilia nasus*, which bred close to the Changjiang River Estuary. The position of the vertical dashed lines represents the annulus.

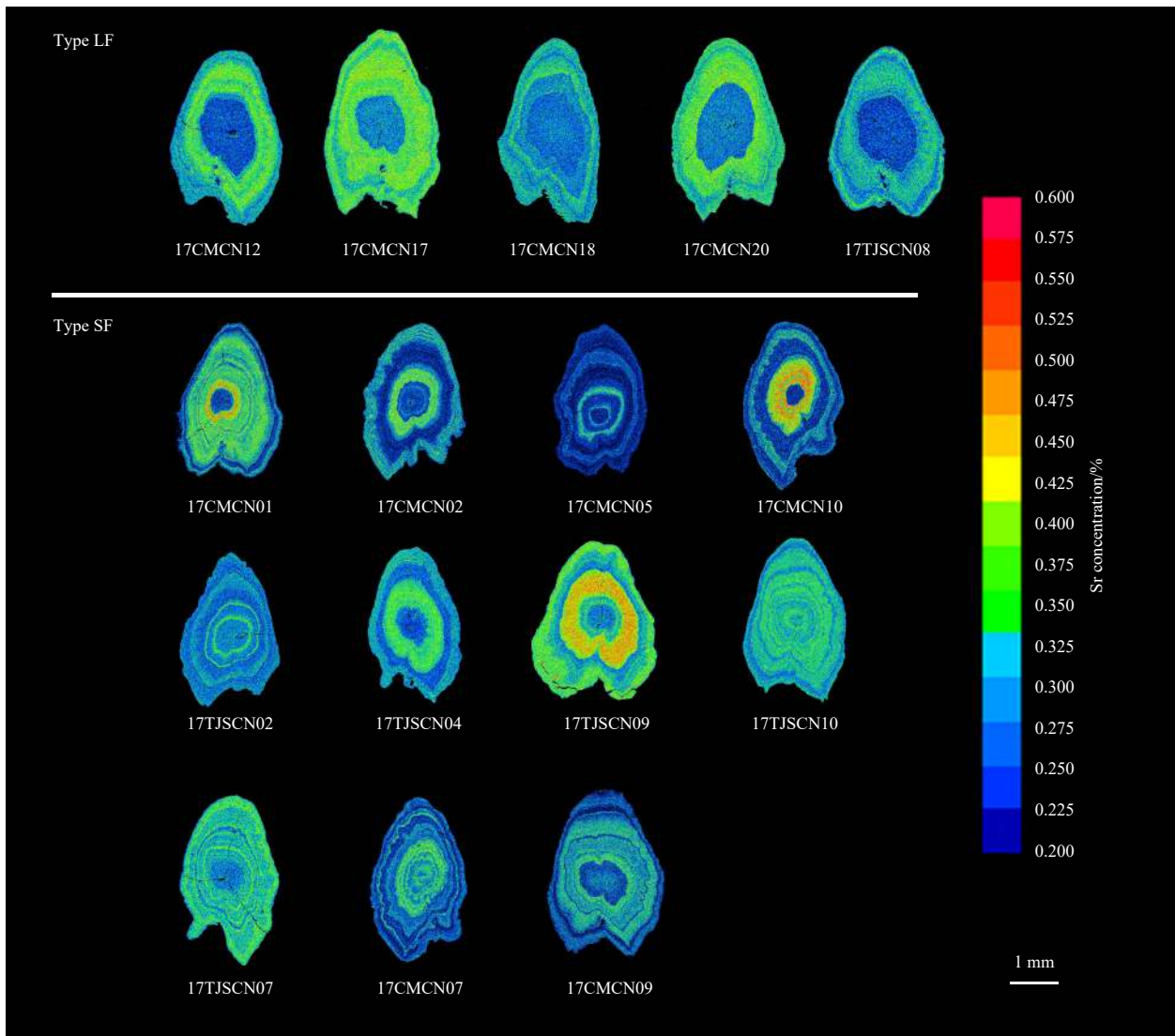


Fig. 4. Two-dimensional strontium (Sr) concentration imaging of the sagittal otoliths of two *Coilia nasus* types using X-ray electron microprobe analyses.

0.12 and  $0.14 \pm 0.26$  for Type LF and Type SF fish, respectively (Fig. 5). A single factor analysis of the  $F_c$  of different individuals in the two types showed that the  $F_c$  of Type LF ( $0.43 \pm 0.12$ ) was significantly higher than that of Type SF ( $0.14 \pm 0.26$ ), and significant differences (one-way ANOVA,  $P < 0.01$ ) were found between the two groups (Fig. 5).

#### 4 Discussion

##### 4.1 Life history reconstruction

The length of the central core low Sr:Ca ratio ( $< 3$ ) regions of *C. nasus* specimens indicates the duration of the initial freshwater life history of *C. nasus* before their initial downstream migration into the estuary (Jiang et al., 2014; Khumbanyiwa et al., 2018). *Coilia nasus* eggs are pelagic and can drift with the current (Suzuki et al., 2014). The swimming ability of *C. nasus* larvae and juveniles is very weak, thereby making it difficult for individuals to select their own habitat (Jiang et al., 2014, 2015b). Therefore, the duration of the initial freshwater life history mainly depends on the distance traveled by parent fish from the estuary to spawning sites (Jiang et al., 2014; Khumbanyiwa et al., 2018). The dura-

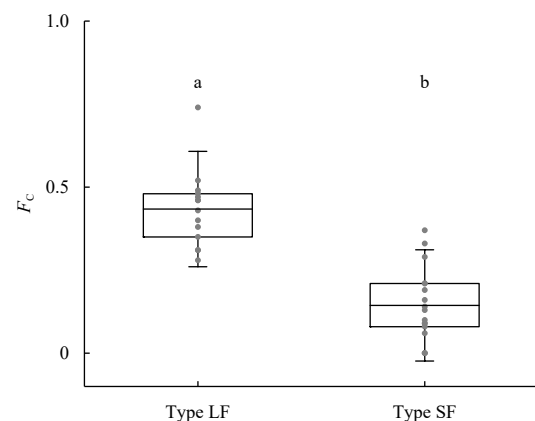


Fig. 5. The freshwater coefficient ( $F_c$ ) of two *Coilia nasus* types from the Changjiang River Estuary. The different letters indicate significant differences at  $P < 0.01$  (one-way ANOVA). Type LF: the long freshwater early life history type; Type SF: the short freshwater early life history type.

tion of freshwater or low-salinity water residency (i.e., freshwater residency duration) correlates positively with the distance to sea in blueback herring (*Alosa aestivalis*) in several spawning runs along the coast of Maine, USA (Wynne et al., 2015). Research on *C. nasus* from the Huanghe, Changjiang, Qiantang, and Oujiang Rivers suggests that shorter natal rivers may lead to shorter central core low Sr:Ca ratio (<3) regions (Jiang et al., 2014; Khumbanyiwa et al., 2018). The results of this study suggest that Type LF hatching sites were far from the Changjiang River Estuary, while Type SF hatching sites were close to the Changjiang River Estuary.

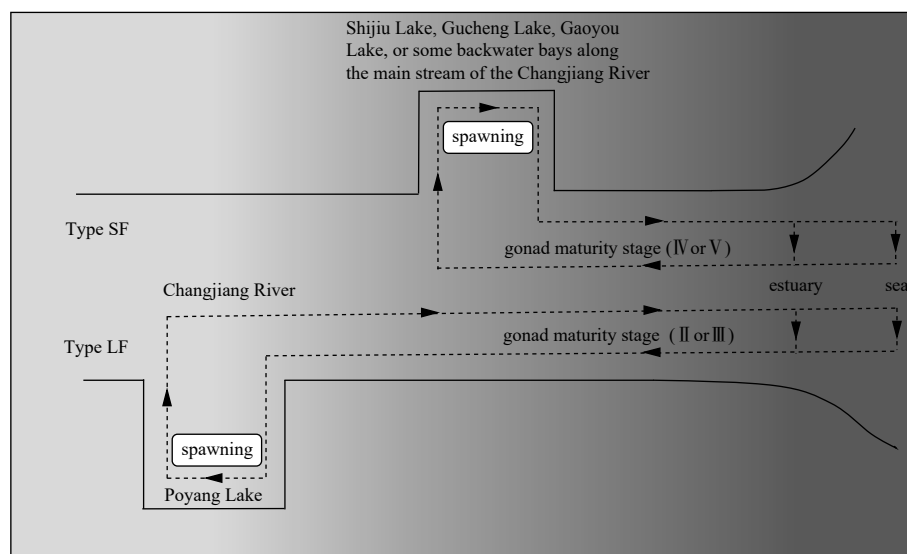
Moreover, Type LF individuals that hatched far from the Changjiang River Estuary were all at a low stage of gonadal maturity. According to previous studies, a high stage of gonadal maturity is not necessary for anadromous *C. nasus* to commence migrating upstream in order to spawn in freshwater. In fact, many *C. nasus* individuals are usually less sexually mature in downstream reaches and develop gonadal maturity during the long-distance upstream migratory process (Chen et al., 2017; Jiang et al., 2017; Sokta et al., 2020). Therefore, Type LF individuals with low gonadal maturity are more likely to continue developing and reach gonadal maturity and spawn in areas far from the Changjiang River Estuary, while they are less likely to waste energy wandering near the Changjiang River Estuary until they reach sexual maturity. In brief, Type LF originated in hatching sites far from the estuary and may enter spawning grounds that are far from the estuary (Fig. 6). This also coincides with the natal homing ability proposed by Jiang et al. (2016). The length of the initial low Sr:Ca ratio central region of Type LF was similar to the pattern of the anadromous *C. nasus* in the Poyang Lake, which was the only confirmed spawning ground for anadromous *C. nasus* (Jiang et al., 2017). Therefore, we infer that Type LF in this study consisted of anadromous *C. nasus* from the Poyang Lake that would enter the Poyang Lake for breeding (Fig. 6).

The Type SF pattern was similar to that of some individuals examined in the studies of Yang et al. (2006) (CE9) and Jiang et al. (2014) (CMCN08), while the latter authors presumed that such individuals may originate in a shorter river, such as Qiantang River, near Changjiang River. However, Type SF tapertail an-

chovies were mature, suggesting that they were close to their spawning site. Moreover, according to Jiang et al. (2016), individuals hatching in the Poyang Lake can move approximately 800 km upstream of the Changjiang River Estuary to return to the Poyang Lake for spawning, thereby indicating that anadromous *C. nasus* may have natal homing capabilities. Therefore, in this study, the mature Type SF was more likely to have originated in rather than have strayed into Changjiang River.

The distribution of individuals with a high degree of gonadal development can be used to determine their spawning grounds. This method has been used in many species (Karakulak et al., 2004; Kayaba et al., 2014; Filina and Budanova, 2015). Fish with stage larger than IV gonads are considered fully mature, whereas females reaching the Stage V can be considered to be at the “spawning stage” (Filina and Budanova, 2015). Jiang et al. (2017) estimated the spawning grounds of anadromous *C. nasus* in Poyang Lake by capturing fully mature females. In this study, the Type SF population had reached full gonadal maturity, which suggests that the spawning grounds of Type SF individuals were upstream areas not far from the Changjiang River Estuary. Moreover, there were three Type SF individuals (17CMCN07, 17CMCN09, and 17TJSCN07) different from the typical *C. nasus* of riverine origin. The Sr:Ca ratio of the central region was larger than 3, which indicated the brackish water origin of these fish. *Coilia nasus* is generally of migratory riverine origin (Yuan, 1987). However, *C. nasus* individuals that hatch in brackish water have also been found in the Qiantang River Estuary (Jiang et al., 2014; Khumbanyiwa et al., 2018), river estuaries around the Ariake Sea of Japan (Liu et al., 2018), and the Changjiang River Estuary (Dou et al., 2012). Sporadic *C. nasus* that hatched in brackish water may have evolved adaptations to cope with high-salinity environments; the mechanism behind such adaptations needs to be studied further.

Although we know which three individuals were born in brackish water, there is still uncertainty regarding the exact hatching location of these individuals. One of the possibilities is that these *C. nasus* individuals hatched in the estuary brackish water area, similar to the specimens of estuarine origin examined in the study of Dou et al. (2012), wherein the young-of-



**Fig. 6.** Diagrammatic model of *Coilia nasus* migration from the Changjiang River Estuary based on Sr:Ca concentration ratio analyses. The lines with the arrows represent possible dispersion patterns. Type LF: the long freshwater early life history type; Type SF: the short freshwater early life history type.

the-year fish that originated in the estuary and resided in the estuary after birth until they were captured (the distance from the core to the edge of the otolith was 950–1 490  $\mu\text{m}$ ). Nevertheless, in this study, the central high Sr:Ca ratio area of *C. nasus* larvae was small (40–520  $\mu\text{m}$ ), and after the early life history in brackish water stage, their environment changed to freshwater. It is difficult for *C. nasus* larvae with weak swimming ability to proactively change their living environment by moving upstream of the estuary to freshwater. However, the Sr:Ca ratios of tapertail anchovies from the Gaoyou Lake are higher than those of anchovies from other lakes owing to the higher degree of mineralization of the Gaoyou Lake than the Poyang Lake, the Taihu Lake, and other freshwater lakes adjacent to Changjiang River (Sokta et al., 2020). Moreover, in recent years, salt intrusion has frequently affected Changjiang River, resulting in ambient salinity changes (Xu et al., 2018). Therefore, we could attribute the changing Sr:Ca ratios to the alternating chemistry of the ambient environment of *C. nasus* larvae. In other words, these individuals may have hatched in lakes, such as Gaoyou Lake, which is located near the estuary, or the mainstream of Changjiang River affected by salt intrusion, and the ambient salinity was brackish when these *C. nasus* larvae hatched and changed from brackish to fresh after a period of time. In this scenario, the spawning grounds of the three Type SF individuals (17CMCN07, 17CMCN09, and 17TJ-SCN07) were close to the estuary. In summary, the Type SF population both originated in and spawned near the Changjiang River Estuary, thereby indicating that this was a breeding population located near the Changjiang River Estuary.

#### 4.2 Spawning site/hatchery origin estimation

Recently, Jiang et al. (2015) used C and O stable isotopes in otoliths and found that two groups of *C. nasus* juveniles originated in different hatcheries; one was far from and the other close to the Changjiang River Estuary. However, it was difficult to locate the exact location of the spawning grounds. Furthermore, Ge and Zhong (2010) found that the larvae and juveniles began to appear in the surf zone approximately 7–30 days after hatching and speculated that there may be spawning sites near the Changjiang River Estuary. The anchovies reached the Changjiang River Estuary at different times, thereby indicating that they originated in different spawning sites. Consistent with this, the diversity of the early freshwater life history duration of Type SF fish also indicates that these anchovies may have originated in different spawning sites near the Changjiang River Estuary. According to previous research, *C. nasus* historically spawns in lakes, such as the Shijiu Lake, Gucheng Lake, and Gaoyou Lake, which are connected to the Changjiang River in its middle and lower reaches (Yuan, 1987, 1988), and in slow-flow water or backwater bays along the mainstream of the Changjiang River in its middle and lower reaches (Zhu, 1992). The Type SF population in this study may have originated in these lakes or some backwater bays in the mainstream of the lower Changjiang River (Fig. 6); however, further studies are urgently required to determine its exact point of origin.

The loss of spawning habitat is the highest contributing factor to the local extirpation of fish species, especially migratory ones, as exemplified by the dramatic decline of North Atlantic diadromous fish (Limburg and Waldman, 2009). In fact, the local extirpation of the alewife along the Atlantic coast of North America and the extinction of the anadromous *Macrura reevesi*, which is of economic and cultural value in China, from the Changjiang River Basin, have both been attributed primarily to the loss of spawning sites caused by dam construction (Hall et al., 2012; Huang et

al., 2013; Littrell et al., 2018). It should be noted that although anadromous *C. nasus* used to breed in many lakes along the middle and lower reaches of the Changjiang River, it is now unlikely that this kind of migratory anchovy will be able to utilize in most lakes, owing to the extensive dam construction, land reclamation, sand mining, and other activities underway in the lakes of the Changjiang River Basin (Sokta et al., 2020). Recently, the Poyang Lake was confirmed to be the only spawning site of the anadromous *C. nasus*, as evidenced by otolith microchemistry (Jiang et al., 2017; Sokta et al., 2020). However, the results of this study showed that there are still anadromous *C. nasus* breeding grounds near the Changjiang River Estuary. The existence of different breeding groups can reduce the risk of extinction and help the recovery of anadromous *C. nasus*. There is no doubt that our findings provide an important reference for the protection of the migratory *C. nasus* resources. Furthermore, a high degree of homing that brings offspring back to their natal habitat, as shown in many diadromous Salmonidae, tends to reduce the gene flow among populations, thereby allowing the development of genetic structuring and local stocks that adapt to local conditions (McDowall, 2001; Aykanat et al., 2015; Asaduzzaman et al., 2020). Therefore, whether the different *C. nasus* breeding populations in the Poyang Lake and near the Changjiang River Estuary were genetically divergent and formed different, locally adapted stocks deserves to be studied further.

At present, China has ramped up its efforts to implement a policy for the protection of Changjiang River, by introducing, in 2020, a 10-year fishing ban on the river in order to curb the decline of the river's ecosystem and avert the biodiversity crisis (Mei et al., 2020). This policy may prove beneficial for the recovery of anadromous *C. nasus* populations. For migratory fish, however, the fishing ban is only part of the conservation effort. The protection of spawning, nursery, and feeding grounds, as well as of the migratory channels of migratory fishes and their connectivity, are also highly essential (Hall et al., 2012; Mattocks et al., 2017). The local extirpation of the alewife along the Atlantic coast of North America and the extinction of *M. reevesi* from the Changjiang River Basin can both be attributed primarily to the loss of spawning sites (Hall et al., 2012; Huang et al., 2013; Littrell et al., 2018). These occurrences highlight the importance of protecting spawning grounds as they constitute resources for anadromous fish. This study proved that there were anadromous *C. nasus* spawning grounds in the area near the Changjiang River Estuary, implying that this species may have evolved adaptations to cope with high-salinity environments. However, this is one of the highly urbanized and industrialized areas in China, while the anthropogenic transformation of natural landscapes, such as rivers and lakes, is also very serious (Hou et al., 2020). Under such circumstances, it is imperative to study further the impact of human activities on the reproduction of migratory *C. nasus* and to consider the importance of these spawning grounds for the population of migratory *C. nasus* when formulating protection policies for the Changjiang River.

In summary, the results of this study showed that the spawning/hatching sites of Type SF fish were near the Changjiang River Estuary. On the contrary, Type LF fish originated far from the Changjiang River Estuary. The migratory *C. nasus* resources of the Changjiang River are nearly depleted. From the perspective of conservation, this study provides important information for identifying anadromous *C. nasus* stocks originating in different spawning sites in the Changjiang River Basin. The estuarine habitat plays a critical role in the connectivity between freshwater recruitment and the marine resources available to adult spawners.

In addition, further studies are warranted to accurately determine the location of spawning sites using alternative ways and identify the aforementioned high-salinity mechanism using genetic approaches to provide a reference for the resource protection of this commercially valued species.

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