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Life History Variations Among Different Populations of *Coilia nasus* Along the Chinese Coast Inferred from Otolith Microchemistry

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The habitat use and the migratory patterns of different estuarine tapertail anchovy *Coilia nasus* populations along the Chinese coast were studied by examining the environmental signatures of strontium and calcium in their otoliths using electron probe microanalysis (EPMA). The results showed that the life patterns were quite similar between the individuals from the Huanghe (Yellow) River and Changjiang (Yangtze) River and considerably different from those of the Qiantang River and Oujiang River. Most of the anchovies were typical anadromous fish, but several individuals were brackish–water residents. Our results also suggest that *C. nasus* may probably be a natal homing fish.

Key words: *Coilia nasus*; strontium and calcium signature; life history; Chinese coast

INTRODUCTION

Estuarine tapertail anchovy *Coilia nasus* is a widely distributed species in the Northwest Pacific, including the sea areas close to China, Korea and Japan. As an anadromous fish, most adults migrate for spawning to the Changjiang (Yangtze) River starting from early February until the end of April (Yuan *et al*., 1980; Li *et al*., 2011). The anchovy species used to be one of the most important commercially harvested species in the Changjiang River estuary but it has been suffering overfishing these years due to its delicacy and high price (US$1000/kg). In addition, due to several human activities that caused water pollution and habitat degradation, *C. nasus* suffered a sharp decline (Chen *et al*., 1999; Chen and Gu, 2012). Today, most of the research focuses on the Changjiang River population while less on population from different regions, like Oujiang River, Qiantang River, Huanghe River (Crossin *et al*., 2007; Li *et al*., 2007; Zhong *et al*., 2007). However, the knowledge on the spatial and temporal dynamics of these populations is quite necessary for the resources understanding as well as the stock assessments, which are very useful to manage and conserve this important fish species.

In previous study, only some life stages have been included due to the empirical limitations of the catch analysis method to monitor the patterns of habitat used by *C. nasus*, which still remains inefficient (Ge and Zhong, 2010). Fortunately, trace elements in the teleost fish otolith have been proved to be an effective natural tag and the detecting methods (like EPMA), have provided researchers with a powerful tool to precisely estimate the migratory history of these anadromous species. Previous studies have already revealed that strontium (Sr) and calcium (Ca) signature in fish otolith (primarily derived from water through gill uptake) could be used as a useful scalar to estimate the migratory history, as otolith Sr and Sr/Ca ratio are robust markers of different salinity habitats of freshwater, brackish water and sea water (Campana, 1999; Secor and Rooker, 2000; Arai *et al*., 2002; Arai *et al*., 2003; Limburg *et al*., 2011; Tsukamoto *et al*., 2011; Dou *et al*., 2012). Also, using EPMA we have studied the populations from the Taihu Lake, Changjiang River estuary as well as some sea areas in Yellow Sea, which proved to be very effective in understanding the life patterns of *C. nasus* (Yang *et al*., 2006; Jiang *et al*., 2012; Jiang *et al*., 2013).

In this study, we estimated for the first time the migratory history of *C. nasus* from different regions along the Chinese coast (especially near estuaries) by analyzing their otolith Sr and Ca using EPMA to determine the different habitats they experienced as well as the knowledge on complement resources and stock movement of *C. nasus*.

MATERIALS AND METHODS

A total of 41 fish samples were investigated (Table 1). Ten individuals with total length 33.0±1.2 cm (L_T, mean±S.D.) were collected on September 9, 2009 in the Huanghe River estuary (37°36´N 118°32´E, DYCE group, specimen codes of DYCE–01, –02, –03, –04, –05, –08, –09, –10, –11, –12). Nine individuals of 28.7±1.1 cm L_T were collected on March 5, 2009 in the Changjiang estuary or estuary around the Chongming Island (31°23´N 121°57´E, CMCE group, specimen codes of CMCE–01, –02, –03, –04, –05, –06, –08, –09, –10). Twelve individuals were collected around the Zhoushan Islands, among

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Table 1. Biological characteristics of *Coilia nasus* used for otolith microchemistry analyses

Group Name	Sampling Location	Sampling date	No. fish examined	Age (year)	Total length (cm)	
					Mean±S.D.	Range
DYCE	37°36´N 118°32´E	9 Sept. 2009	10	2^+–3^+	33.0±1.2	30.2–35.1
CMCE	31°23´N 121°57´E	2 Mar. 2009	9	2^+–3^+	28.7±1.1	27.0–30.5
ZSCE	30°15´N 121°44´E	20 May 2010	10	2^+	28.0±0.9	27.1–29.2
ZDCE	30°07´N 121°52´E	29 May 2009	2	2^+	23.1±0.4	22.8–23.4
OJCE	27°57´N 120°58´E	7 Nov. 2008	10	1^+–2^+	18.3±3.9	14.1–26.3

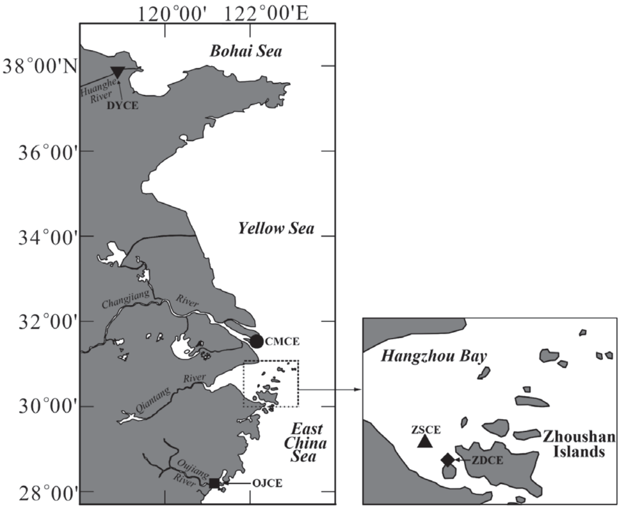

Fig. 1. Map showing the *Coilia nasus* sampling sites along the Chinese coast (DYCE group located around 37°36´N 118°32´E, CMCE group around 31°23´N 121°57´E, ZSCE group around 30°15´N 121°44´E, ZDCE group around 30°7´N 121°52´E, and OJCE group around 27°57´N 120°58´E).

of which ten (28.0±0.9 cm L_T) were collected on May 20, 2010 around 30°15´N 121°44´E (ZSCE group, specimen codes of ZSCE–58, –60, –62, –63, –64, –66, –67, –68, –69, –70), while the rest (23.1±0.4 cm L_T) were collected on May 29, 2009 around 30°7´N 121°52´E (ZDCE group, specimen codes of ZDCE–01, –02). Ten individuals (18.3±3.9 cm L_T) were collected on November 7, 2008 in the Oujiang estuary (27°57´N 120°58´E, OJCE group, specimen codes of OJCE–01, –02, –03, –04, –06, –07, –08, –09, –11, –12) (Fig. 1).

The sagittal otoliths were extracted and embedded in epoxy resin (Epofix; Struers, Copenhagen, Denmark) in the frontal plane. All otoliths were ground to expose the core with an automated polishing wheel (Roto Pol–35; Struers, Copenhagen, Denmark). After that, all samples were cleaned in an ultrasonic bath and rinsed with Milli–Q water. For EPMA measurements, all otoliths were carbon coated by a high–vacuum evaporator (JEE–420, JEOL Ltd, Tokyo Japan).

The otoliths were used for life history transect analysis on Sr and Ca concentrations, which were measured along a line down the longest axis of each otolith from the core to the edge by a wavelength dispersive X–ray electron microprobe (JXA–8100, JEOL Ltd, Tokyo Japan). Calcite ($CaCO_3$) and Tausonite ($SrTiO_3$) were purchased from Chinese Academy of Geological Sciences and used as standards to judge the precision and accuracy of Sr and Ca concentrations (Arai *et al.*, 2011; Yokouchi *et al.*, 2011). The accelerating voltage and beam current were 15 kV and 2×10^{-8} A, respectively. The electron beam was focused on a point 5 μm in diameter, with measurements spaced at 10 μm intervals. X–ray intensity maps of both elements were made of the representative otoliths using the same microprobe in accordance with the aforementioned life history transect. The beam current was 5×10^{-8} A, counting time was 40ms, pixel size was 7×7 μm, and the electron beam was focused on a point 5 μm in diameter. Statistical analysis was performed using IBM SPSS Statistics v.19.0 (IBM Corp., Armonk, NY, U.S.A.).

RESULTS

Otoliths of *C. nasus* from the five sites along the Chinese coast showed variable Sr:Ca ratios. Among of them, DYCE, CMCE and ZDCE groups were quite similar with two phases distinguished by the values of the life–history transects and the X–ray intensity maps. Their otolith Sr:Ca ratios showed dramatic changes along the life–history transects, with significantly lower mean ±S.D. Sr:Ca ratios of $1.55\times10^{-3}\pm0.74\times10^{-3}$ (DYCE04) to $2.52\times10^{-3}\pm0.86\times10^{-3}$ (CMCE08) from the core to the point *c.* 210–1200 μm to the outermost regions (Mann–Whitney *U*–test, $P<0.001$) (Fig. 2). Based on the previous studies on sagittal otoliths of *C. nasus* which indicated bluish, greenish, yellowish and reddish regions were characteristics of freshwater (low salinity), brackish water (medium salinity) and sea water (high salinity), respectively (Yang *et al.*, 2006). All samples (DYCE, CMCE and ZDCE groups) had similar bluish central regions, which meant that they were born in freshwater, i.e., anadromous *C. nasus*. Thereafter they alternated among greenish or yellowish even reddish bands, corresponding to the migration of these adults between the estuarine and freshwater habitats (Fig. 3).

All the other samples had a similar length of low Sr:Ca ratios phase (980±111 μm, mean±S.D.) except CMCE08. Not like the others, CMCE08 had an unusually small bluish central regions (210 μm), adjacent to which it suddenly turned into a reddish band (Fig. 3) with a Sr:Ca ratios more than 7 (Fig. 2).

In contrast to DYCE, CMCE and ZDCE groups, the

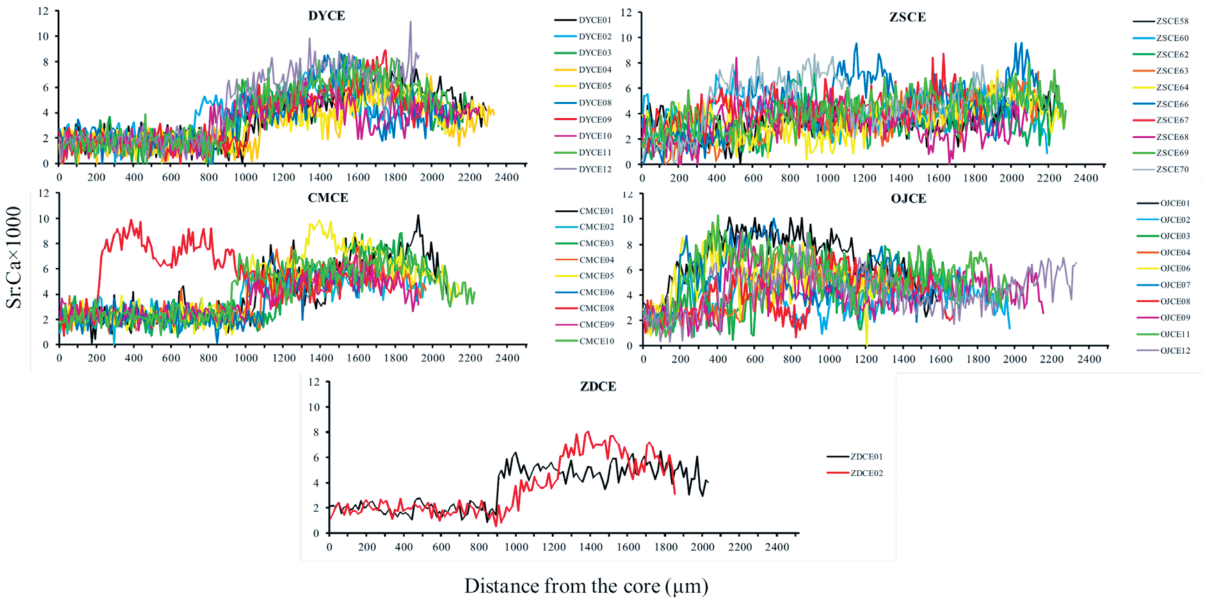

Fig. 2. Fluctuation of otolith Sr:Ca ratios along line transects from the core (0 μm) to the edge in the sagittal plane of the otoliths of different *Coilia nasus* groups along the Chinese coast.

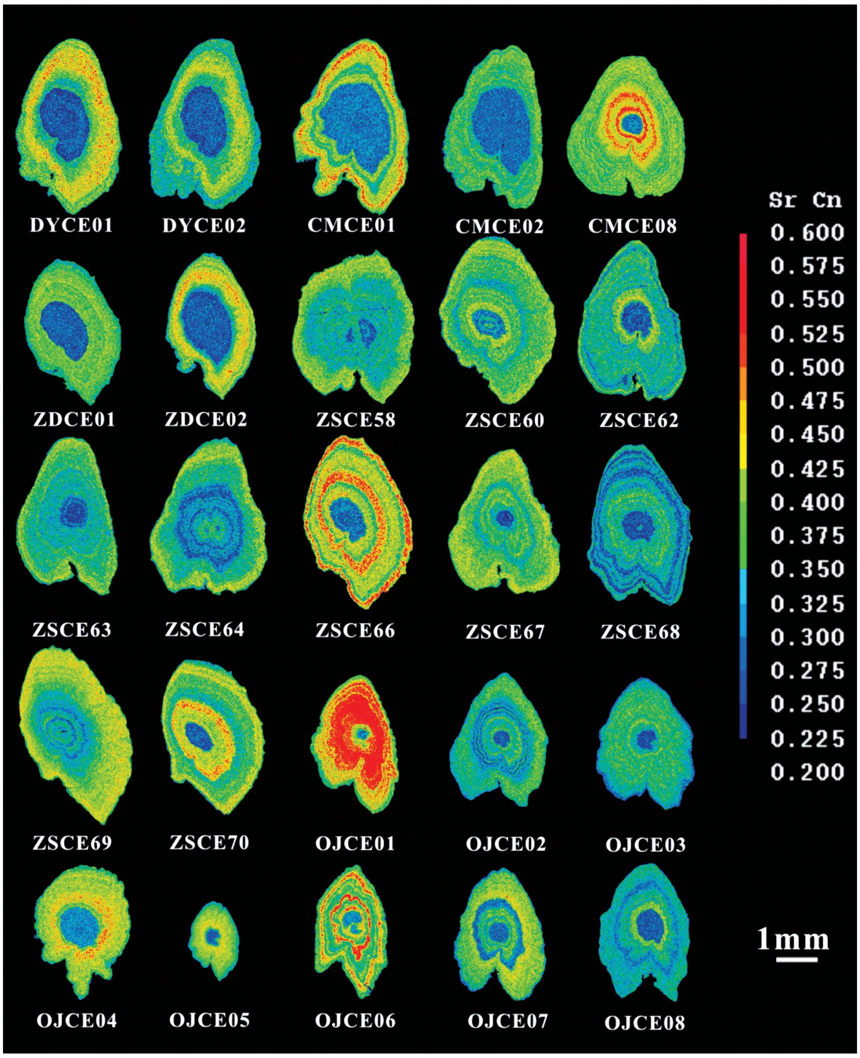

Fig. 3. Two–dimensional imaging using X–ray electron microprobe analysis of the Sr concentrations in the sagittal plane of the otoliths. The values corresponding to Sr concentrations are represented by 16–colors from the blue (lowest) through green, yellow to red (highest).

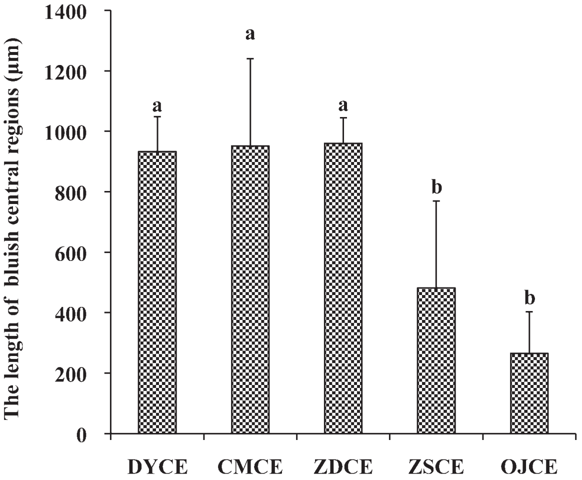

Fig. 4. The mean length of bluish central regions of DYCE, CMCE, ZDCE, ZSCE, and OJCE groups. Those with different alphabet letters are significantly different at $P<0.01$ (one–way ANOVA). ZSCE–58, –60, –64, –68, –69 were not counted for the greenish core regions.

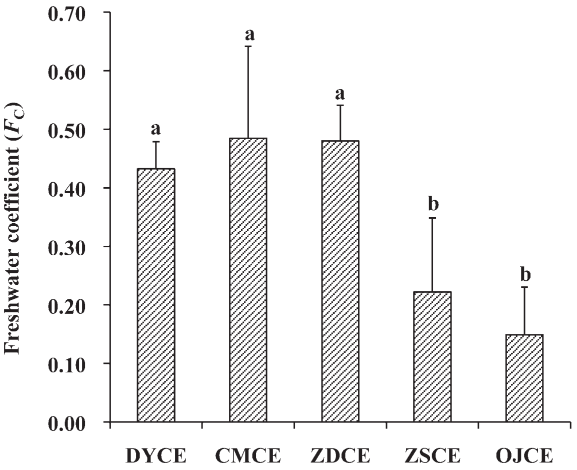

Fig. 5. The freshwater coefficient (FC) of ZDCE, CMCE, DYCE, and OJCE groups. Those with different alphabet letters are significantly different at $P<0.01$ (one–way ANOVA). ZSCE–58, –60, –64, –68, –69 were not counted for the greenish core regions.

samples of OJCE group had a significantly smaller bluish central regions (130–540 μm) (one–way ANOVA, $P<0.01$) (Fig. 4), after which there were some quite variable color bands. Among them, OJCE–01,–03,–04,–05 and –06 alternated among greenish and yellowish even reddish bands adjacent to the bluish central regions (Fig. 3) with sustaining high Sr:Ca ratios (more than 3) (Fig. 2) just like those of DYCE, CMCE and ZDCE groups, while there were some obvious outer bluish (i.e. freshwater) regions (Fig. 3) with lower Sr:Ca ratios (less than 3) in the otoliths of OJCE–02,–07 and –08.

Compared to those of typical anadromous *C. nasus* described each of which had a bluish central region and some other color bands of higher Sr:Ca ratios adjacent to it, there were not only some usual *C. nasus* (ZSCE–62, –63, –66, –67, –70) with a similar lower Sr:Ca ratios central region from the core to 70–770 μm but also some new types (ZSCE–58, –60, –64, –68, –69) with greenish core regions (Fig. 3). Like OJCE group, the usual samples (ZSCE–62, –63, –66, –67, –70) had significantly smaller bluish central regions (70–770 μm) than those of DYCE, CMCE and ZDCE groups (one–way ANOVA, $P<0.01$). Among the unusual samples (ZSCE–58, –60, –64, –68, –69), there was a wide variety of Sr concentration patterns but still some quite obvious bluish regions adjacent to the core regions, especially ZSCE68 (Fig. 3). Interestingly, the Sr:Ca ratios in the otoliths of most ZSCE group varied quickly (Fig. 2), especially to those of unusual ones (ZSCE–58, –60, –64, –68, –69). The X–ray intensity maps (Fig. 3) showed that there were many bluish and greenish tight concentric rings, which could also be found in the samples of OJCE (–02, –03, –07, –08).

Based on the results of otolith Sr:Ca ratios along line transects, the mean length of bluish central regions seemed to be quite different among these groups (Fig. 4). To standardize the data, F_C (Freshwater Coefficient) was used and can be expanded as follows:

$$F_C = \frac{L_f}{L_T}$$

where L_f was the first low stage from the core, in other words the length of bluish central regions, and L_T was the total length, both of which were based on the fluctuation of otolith Sr:Ca ratios (Fig. 2). The results show that the values of F_C of DYCE (0.43±0.05), CMCE (0.48±0.16) and ZDCE (0.48±0.06) groups were significantly higher than those of ZSCE (0.22±0.13) and OJCE (0.15±0.08) groups (Mean ± S.D.) (one–way ANOVA, $P<0.01$) (Fig. 5). Because some individuals of ZSCE group were quite unusual with greenish core regions, these samples (ZSCE–58, –60, –64, –68, –69) were not calculated.

DISCUSSION

While the mineral composition of fish otolith is relatively pure calcium carbonate (92–96%), a number of minor and trace elements are present (e.g., Sr, Na, K, S, N, Cl, P and Mg, Zn, Si, Fe, Cu Al, Pb,Co, Cd) (De Pontual H and Geffen A, 2002; Sweeting *et al.*, 2004). At present, there have been many successful studies on migration history reconstruction of diadromous or anadromous teleosts by otolith elemental microchemistry, especially Sr contents and Sr:Ca ratios (Tsukamoto *et al.*, 2001; Arai *et al.*, 2002; Kotake *et al.*, 2003; Gillanders, 2005; Arai and Hirata, 2006; Brown and Severin, 2009; Limburg *et al.*, 2011; Tsukamoto and Arai, 2011), as variability of the latter in fish otoliths have been widely applied as tracers of movement between freshwater and marine habitats, with a direct relationship between Sr/Ca in the otoliths and the water, across a range of estuarine salinities (Kraus and Secor, 2004;Yang *et al.*, 2011). It has also been proved to be a powerful tool for other similar studies of *C. nasus* (Yang *et al*., 2006; Zhong *et al*., 2007; Dou *et al*., 2012; Jiang *et al*., 2012; Jiang *et al*., 2013). All these findings have clearly showed that salinity has to do with the migration and should be recorded as the Sr:Ca ratios in otoliths and that these natural tags would be able to be used for the estimation of *C. nasus* life patterns.

In the present study, all samples from DYCE, CMCE, ZDCE and OJCE groups shared a similar pattern with obviously bluish areas (low Sr) in the central region of each otolith, showing that these individuals were born in fresh water and that their freshwater life–history would cover a considerable period of time. Some researchers have found a strong relationship between fish age and otoliths length (e.g., radius/diameter along the longest axis) and weight (Ilkyaz *et al*., 2011) which meant that the otoliths length might relate to the growth time over the lifespan. Among these individuals, the time spent in fresh water seemed to be quite different and for group OJCE might be significantly shorter than for groups DYCE, CMCE and ZDCE (one–way ANOVA, $P<0.01$) (Fig. 4).

Based on previous studies on *C. nasus* from the Yellow Sea (Jiang *et al*., 2012), all individuals of DYCE, CMCE and ZDCE groups, with low Sr:Ca ratios length of $980\pm111\,\mu$m, might have spent their first year in the freshwater except CMCE 08. Compared with the length of low Sr:Ca of these samples, the significantly shorter length of CMCE08 (210 μm) and those of OJCE group (130–540 μm) showed that they might have experienced a quite shorter freshwater life history in their early life. This period seems to be very important to juveniles because soon they would get into estuary where the water is brackish and would lead to dysmorphia or even death if the young fish could not develop and survive enough in such an environment with higher salinity (Matsui *et al*., 1986). In the Changjiang River, adults are gathered into the estuary and start migration in a regular period starting around late April and peaks in early and middle May (Fish Laboratory, Institute of Hydrobiology, Hubei Province, 1976). This period is corresponding to relatively stable lengths of low Sr:Ca ratios of CMCE. By this, it seemed that individuals of OJCE group had developed into a quite different population from those of DYCE, CMCE and ZDCE groups and the former might develop faster than the latter in their early life.

After the freshwater life history, all of them appeared to have moved into the estuary (brackish water), because the bands adjacent to the central bluish region turned into greenish or yellowish. Not like the bluish central regions, the patterns were diverse of greenish or yellowish bands. In the X–ray intensity maps and among different individuals there were different patterns of bluish, greenish, yellowish or reddish concentric rings (Fig. 3). All of these provided the evidence that they would gather into separate groups when they swam down to the estuary and sea. Some of them, such as CMCE02, ZDCE01 and OJCE03, where the outer areas of the central bluish regions were stably greenish, seemed to stay near the shore or estuary, instead of swimming far away where the water has higher salinities, such as DYCE01 and OJCE02, ZDCE02, OJCE01, OJCE04 and OJCE06 which appeared to remain in higher salinities for longer periods of time (Fig. 3). In contrast to the individuals of DYCE group, there seemed to be more patterns for those of CMCE group (Fig. 2). One of the reasons might be that there were certain islands (such as the Zhoushan Islands) near the Changjiang River estuary while there were not near the Huanghe River estuary. These islands could change not only the water conditions around them but they could also supply some valuable habitats. Comparing life patterns, the individuals of ZDCE group were quite like those of CMCE group in contrast to the ZSCE group, which showed that some individuals from the Changjiang River might, swam south to the sea area around the Zhoushan Islands of East China Sea. Meanwhile, other groups from the Changjiang River could swim north into the Yellow Sea and some of which might live near the shore and others off shore (Jiang *et al*., 2012).

Additionally, it was quite similar for the samples of OJCE group because there were some islands on the north of the Oujiang estuary, which could separate them into some smaller groups. Based on the result of life–history transects of the otolith Sr:Ca ratio and otolith map of Sr distribution, it seems that there were at least more than two groups. One group could swam far away from the freshwater until maturation and initiation of the anadromous migration (OJCE04, –05and –06), while the other to migrate between freshwater and brackish water (OJCE02,–07 and –08) and showing in the otoliths some obvious bluish bands outer the bluish central region (Fig. 3). Another interesting evident was that the size of the bluish central regions was different (Fig. 3) as for the minimum length of the bluish central regions of 130 μm (OJCE 01) and the maximum of 540 μm (OJCE04). This phenomenon of these fish implied that natal river was very important.

By comparison with the individuals of DYCE, CMCE, ZDCE and OJCE groups, the situation of ZSCE group was quite different. Some of them, such as ZSCE62, –63, –66, –67, –68 and –70, were just similar to those that stayed from birth in freshwater for some time but others (ZSCE58, –60, –64, 68, 69) were quite different with otolith greenish core regions, meaning that their parents had laid the eggs in the higher salinity water (brackish water). Some of them (ZSCE68) seems to have been born in brackish water and soon got into freshwater, for they had a little greenish core region (20 μm), with considerable wide bluish bands (370 μm) (Fig. 3). The rest (ZSCE58, –60, –64, –69) seemed to move frequently between waters with different salinity (Fig. 3), which were also found by Dou *et al*. (2012). However, there were few differences in the color of the central regions to some estuary born individuals, which were relatively greenish to yellowish although they turned frequently between bluish and greenish in those samples of the present study. Our estimation was that it would be hard to believe that the larvae or juveniles of *C. nasus* could migrate between fresh and brackish water with the main reason for that to be the environmental changes. We should clarify that the Qiantang River has a length of about 688 km, is longer than the Oujiang River, quite shorter than the Changjiang River, and with an estuary breadth of about 100 km. Therefore, every year, the tide here is so great and there are no large streams or lakes along the river creating unstable environmental conditions for *C. nasus*. As about the typical anadromous ones of ZSCE groups the F_C values showed some differences from DYCE and CMCE groups (Fig. 5), which was just like that from Oujiang River, and suggesting that shorter natal river might lead to smaller bluish central regions. Based on these evidences, CMCE08 could be one from the Qiantang River with smaller bluish core regions than that of CMCE group from the Changjiang River.

Although Qiantang estuary is close to Changjiang River estuary, fish from these two rivers might, at the same time, share the Zhoushan Islands (ZDCE group), and they can easily discriminated as well by the otolith Sr level probed with EPMA, meaning they might be two different populations (Yuan and Qin, 1984). In addition, it was also found that *C. nasus* might be like other anadromous fish such as salmon (Crossin *et al.*, 2007) with the propensity to home to the natal rivers for spawning.

In the present study, the calculated F_C parameter suggested some difference among several *C. nasus* population along the Chinese coast. Based on the F_C, it could be stressed that ZDCE group (0.48±0.06) might belong to the population of CMCE group (0.48±0.16). The advantage of F_C was that the individual difference need not be considered and the disadvantage of F_C was that it could lead to deviation due to the length of otolith (*Lo*) because the elder male or female fish should have a bigger otolith meaning the *Lo* is bigger and might lead to the reduction of F_C. In our research, most of samples were about 2 and 3 years old except the 1 year old individuals of OJCE group, which might not seriously affect these results and give an explanation as well on why the F_C results seem quite similar to the bluish central regions (Figs. 4 & 5) although both coefficients were quite different. The bluish central regions length reflected the time the young fish had spent in freshwater during the first year, while F_C suggested the ratio of the first year freshwater life history to the whole. Furthermore, the former reflected a relative time related to the individual and the latter to the characteristics of the population, which can also be used as an index of the natal river. In our future work, F_C coefficient will be improved especially for the samples with quite different age.

In conclusion, there were different populations of *C. nasus* that possibly had their own, clear life patterns along the Chinese coast (Fig. 6). The natal river dependency may probably be one of the most important reasons for their differentiation. The distribution of Sr concentrations and Sr:Ca ratios were possibly a powerful tool not only for reconstructing life history patterns, but also for estimating natal homing and the population structure. In addition, it could be better than those of meta–population, for genetic approaches may not have sufficient resolution to quantify natal homing unless stray ones could be negligible over evolutionary time scales (Thorrold *et al.*, 2001). However, the distribution of Sr concentration or Sr:Ca ratios were not enough for ZDCE group and CMCE08 and, thus, not able to exclude all the rest possibilities. Future research needs to be undertaken to verify the possibility of natal river dependency of *C. nasus* by further analysis of more elements otolith (especially near the core), utilizing alternative approaches (e.g., LA–ICPMS).

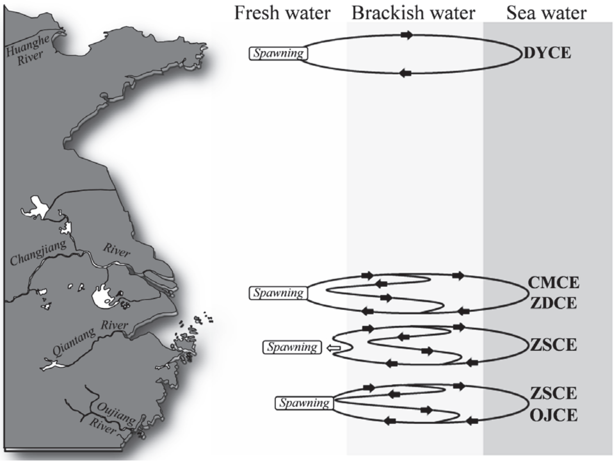

Fig. 6. Diagrammatic sketch of the life cycle of different populations of *Coilia nasus* along the Chinese coast based on the otolith Sr concentration or Sr:Ca ratio analyses along the life–history transects. The line with arrows represents the possible dispersion patterns during the non–spawning migratory phases.

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