

Behavioral migration diversity of the Yangtze River Japanese Eel, *Anguilla japonica*, based on otolith Sr/Ca ratios

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Abstract: The Yangtze River estuary is the main production area of *Anguilla japonica* in China, as well as the only existing fishery area for adult eels. Japanese eels are distributed in the main rivers and many tributaries from the Yangtze River estuary to the upper Jinsha River, which extend to nearly 3 000 km. However, their migration behaviors remain relatively unknown. We analyzed the biological characteristics of 153 specimens of silver eels collected from the Jingjiang section of Yangtze River (31°30'N, 120°42'E) between September and November, 2008, and tested the sagittal Sr/Ca ratios of 27 specimens. Among the 153 specimens examined, 85 were female and 68 were male, which translated to a female-male ratio of 1 : 0.8. The ages of the female specimens ranged from 3 to 7 a (average 5.52) with an average total length (TL) of (669±80) mm, average body weight (BW) of (555±229)g, average condition factor of 1.77±0.22, and average gonad somatic index (GSI) of 1.32±0.31. The ages of the males ranged from 3 to 5 a (average:4.38) with an average TL of (518±51) mm, average BW of (234±76) g, average condition factor of 1.62±0.18, and average GSI of 0.21±0.11. All biological parameters of females were significantly larger than those of the male specimens ($P < 0.05$). According to the average Sr/Ca ratio $(7.99 \pm 1.05) \times 10^{-3}$ of the elver mark of sagitta, 17 individuals (62.96%) were river eels and 10 individuals (37.04%) were estuarine eels. Of 16 females, 13 individuals (81.25%) were river eels and 3 were estuarine eels, while of 11 males, 36.36% were river eels and 63.64% were estuarine eels. The analysis on Sr/Ca ratios for every growth layer group (GLG) indicated there were no significant differences between second-age males and females. However, significant differences were observed between the third-age, fourth-age, and migration-age male and female specimens. This was likely related to the fact that second-age eels of both sexes stay in the same inhabitation waters; however, as they grow older, they move to different areas.

Key words: Sagitta; Age composition; Growth parameter; *Anguilla japonica*; Silver eel; Yangtze River estuary

据于矢耳石 Sr/Ca 值的长江日本鳗鲡迁移格局多样性分析

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摘要: 长江口是中国日本鳗鲡苗的主要产区和仅存的成鳗渔业水域。日本鳗自长江河口至上游金沙江近 3000 km 干流及许多支流中都有分布, 但其迁移行为却不为人了解。该文分析了 2008 年 9~11 月采自长江靖江段 (31°30'N, 120°42'E) 的 153 尾银色鳗样本的生物学特征, 测定了其中 27 尾标本的矢耳石 Sr/Ca 值。结果显示, 153 尾样本中有雌性 85 尾、雄性 68 尾, 雌雄性比 1 : 0.8。雌性由 3~7(平均 5.52) 龄组成, 平均体长 (669±80) mm, 体重 (555±229) g, 丰满度 1.77±0.22, 性腺指数 (GSI) 1.32±0.31。雄性由 3~5(平均 4.38) 龄组成, 平均体长 (518±51) mm, 体重 (234±76) g, 丰满度 1.62±0.18, GSI 0.21±0.11。雌性的这些生物学参数均显著大于雄性 ($P < 0.05$)。依据矢耳石

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线鳗标志轮平均 Sr/Ca 值 $(7.99 \pm 1.05) \times 10^{-3}$ 进行判断, 有 17 尾 (即 62.96% 个体) 为“淡水型”, 10 尾 (即 37.04% 个体) 为“河口型”。16 尾雌性中有 13 尾 (即 81.25%) 为“淡水型”, 3 尾为“河口型”。11 尾雄性中仅 36.36% 为“淡水型”, 63.64% 为“河口型”。对每个生长层组的 Sr/Ca 值分析表明, 雌雄间 2 龄时无显著差异, 但 3 龄、4 龄和洄游龄组都有显著或极显著的差异, 预示着 2 龄时两者的栖息水域比较一致, 但后来出现了明显栖息地分化。

关键词: 矢耳石; 年龄组成; 生长参数; 日本鳗鲡; 银色鳗; 长江口

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The Japanese eel, *Anguilla japonica*, is a facultatively catadromous species (McDowall, 1988) found in the freshwater areas of the Western North Pacific from Hokkaido, Japan, to northern Philippines. Spawning sites are located in the southern part of the West Mariana Ridge west of the Mariana Islands (Tsukamoto, 1992, 1996, 2006). The newly-hatched transparent leaf-like larvae, called leptocephali, drift to the continental shelf of China, South Korea, and Japan along the North Equatorial and Kuroshio Currents, and metamorphose into glass eels near the coast (Tsukamoto, 1992) and develop into elvers in neighboring estuaries. After migrating into the freshwater rivers, they further grow into yellow eels. Once they become sexually mature, they gradually metamorphose into silver eels on their migration downstream. They die when they finish spawning at the spawning site (Tzeng et al, 2003; Tzeng, 2004). During their life cycle Japanese eels migrate from sea water to fresh water and back to sea water. These life histories are widely accepted in literature on their biology and Japanese eels have long been considered the typical representative of catadromous fish. However, recent studies on sagittal Sr/Ca ratio have discovered that some of these eels can finish their life cycle without freshwater immigration (Tsukamoto et al, 1998; Tsukamoto & Arai, 2001; Tzeng et al, 2002). Consequently, eel behavior appears to be diversified to some extent.

Japanese eels are distributed in all rivers open to the sea in China. Although Japanese eel is a famous and precious species with high economic value, the number of adults is on the decline. With 6300 km in length, the Yangtze River is the third longest river in the world and the longest in Asia. The Yangtze River estuary is the main fishing area for Japanese eel elvers as well as the only adult eel fishery exists in China. Every fall, processional fishermen fish in this estuary for adult eels (yellow and silver eels) their downstream migration. The Yangtze River is the leading area for Japanese eel spawning stock, and the estuary is the main place where spawning stock are protected. As early as last century, scholars in China began to study the biological

characteristics of eels inhabiting Taiwan, Mulanxi in Fujian province, and Pearl River estuary (Lin et al, 1977; Li et al, 1992; Guan et al, 1994; Fu et al, 1996; Han & Tzeng, 2000; Tzeng et al, 1997, 2000, 2002; Xie et al, 2002; Shiao et al, 2003; Kotake et al, 2003; Chino & Arai, 2009). So far, however, no relevant studies on the behavioral characteristics of adult eels have been conducted. To determine the migratory pattern behaviors of Japanese eels in the Yangtze River, we analyzed the sagittal Sr/Ca ratios of silver eels collected at the Yangtze River estuary during their downstream migration to provide basic biological information for their protection.

1 Materials and Methods

1.1 Fish

From September to November, 2008, during the traditional local catching season, a total of 153 silver eels were obtained from commercial fisheries in the Jingjiang section of Jiangsu province in the upper area of the Yangtze River estuary (32°01'27"N, 120°25'56"E; about 162 km from the estuary mouth of the Yangtze River; Fig. 1) with composite-type eel nets. The nets are 10–11 m wide, 4.5 m high, and 20–25 m long. The net

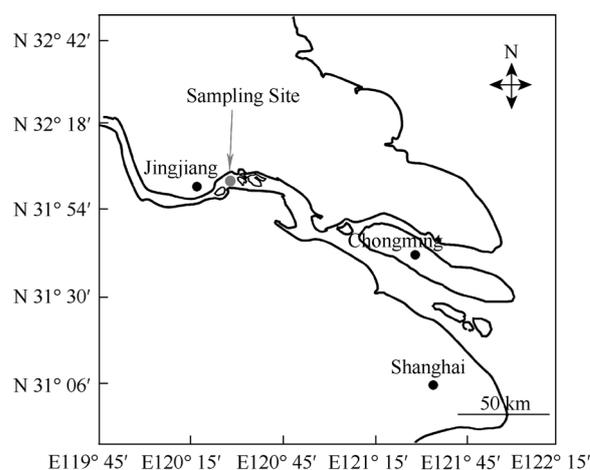


Fig. 1 Sampled sites

Eels were captured at Jingjiang section of Yangtze River (32°01'27"N, 120°25'56"E; about 162 km away from the entrance of Yangtze River estuary).

mesh reduced gradually from 3 cm × 3 cm at the net mouth to 1 cm × 1 cm at the bottom. Catching positions were fixed to waters around 2 km from the north bank of the Yangtze River. The salinity of the surrounding waters was 0‰. The bottoms of the nets were 4.5 m under water while the top remained at water level.

1.2 Biological measurement and preparation of sagitta

After the live specimens were taken to the laboratory and anesthetized through MS-222 anesthetic, their total length (TL) and body weight (BW) were measured to accuracies of 1 mm and 1 g, respectively. They were then anatomized and their sexes were determined according to the morphological features of their gonads: the reproductive glands in the shape of crisp long ribbon are ovaries while those with lobes are testis (Lin et al, 1977). The silver eels were identified according to their body colors and gonads developmental conditions. The specimens used in the study were all silver eels. The gonad weights were measured to determine the gonad somatic index (GSI) of each eel. The GSI value was calculated as follows: $GSI = \text{gonad weight (g)}/BW \text{ (g)} \times 100$.

Condition factor was calculated according to the formula: $\text{Condition factor} = BW \text{ (g)}/TL \text{ (mm)}^3 \times 1000$.

The left sagitta was removed and the envelopes and phlegm on its surface were cleaned with an ultrasonic cleaner. After it dried, the sagitta was placed in a plastic sharpener box and the already prepared acrylic resin was slowly poured in to embed it. After the resin hardened, it was successively ground with 240 mesh, 600 mesh, 1 200 mesh, and 1500 mesh waterproof abrasive paper on the grinder with care until close to the otolith core. After grinding on one side was finished, the other side was ground with the same steps and methods. When both sides were ground to thicknesses near the core, 2 000 mesh abrasive paper was used to accurately grind them. Finally, otolith slices with cores exposed on both sides, clearly visible age layers and thicknesses of 10–15 μm were made. Under OLYMPUS BX41 stereoscope, the structures of these slices were observed and photos were taken for records. Conventional methods were adopted to confirm and count the growth layer groups (GLG) on the otolith (Yin, 1995). We calculate 0⁺ age by the first GLG, 1⁺ age by the second GLG, and so forth.

Sagitta slices of 27 specimens were randomly selected, placed in an ultrasonic cleaner, and thoroughly cleaned with ultra-pure water. After drying, they were fixed onto specimen target frames with glue intended for

adhesive purposes. The target frame took the Mylar film as the underlay. The absorption sheet had aluminum galvanized on one side and was 4 μm thick. The slice was fixed upon the galvanized aluminum side of the absorption sheet.

1.3 Otolith PIXE analysis

The test was conducted on the NEC 9SDH-2 tandem accelerator at the Modern Physics Institute of Shanghai Fudan University. Measurement was made through PIXE (Proton Induced X Ray Emission). The voltage at the side of the accelerator was regulated from 0.3–3.0 mV. The accelerated particles could enter five different pipes through magnetic analyzers. In this test, south 30° pipes were used. After repeated tests, we found that the most suitable accelerator voltage was 2 mV. The proton beams generated by the accelerator became beam spots with 900 pA intensity and about 10 μm × 15 μm in size through the PIXE target chamber to bombard the specimens. The detector used was the ORTEC Si (Li) model; its relative sensitivity against X rays was 10⁻⁹–10⁻⁶ magnitude; and the calculation accuracy (credibility) of X rays yield was 2%–5%.

The PIXE control and data collection were completed by OMDAQ computer software. Plane scanning mode was first selected to obtain scanning images of the sagitta slice outlines and determine the core positions and the major semi-axis endpoint according to the scanning range of size adjustment of the tested otolith slices (maximum: 3 mm × 3 mm). The plane scanning mode was then switched to the line scanning mode. Fixed point and line scanning were subsequently initiated along the long radius of each otolith and the PIXE energy spectrums of Sr and Ca were available. The PIXE energy spectrum analysis was finished by GUPIX software (Espen et al, 1979) and calculation and processing of data and graphs were conducted through Excel 2007 and Origin pro 8.0.

2 Results

2.1 Biological characteristics

Among the 153 specimens analyzed, 85 were females and 68 were males, accounting for 55.56 % and 44.44 % of all the specimens, respectively. The females ranged from 476–910 mm in total length (TL) with an average value ± SD of 669 ± 80 mm, and 246–1 506 g in body weight (BW) with an average value ± SD of (555 ± 229) g. The males ranged from 367–634 mm in TL, (518 ± 51) mm on average, and from 80–432 g in BW, (234 ± 76) g on average (Tab. 1). The values for the females were all

Tab. 1 Characteristics of Japanese eel Collected at Jingjiang section of Yangtze River

	No. of specimen	TL (mm) Range (Mean± SD)	BW (g) Range (Mean± SD)	Condition factor Range (Mean± SD)	GSI Range (Mean± SD)
Female	85	476 – 910 669 ± 80	555 ± 229 246 – 1506	1.77 ± 0.22 1.28 – 2.87	1.32 ± 0.31 0.62 – 2.05
Male	68	367 – 634 518 ± 51	234 ± 76 80 – 432	1.63 ± 0.18 1.33 – 2.16	0.21 ± 0.11 0.004 – 0.73
Total	153	367 – 910 602 ± 102	413 ± 239 80 – 1506	1.71 ± 0.21 1.28 – 2.87	0.83 ± 0.61 0.004 – 2.05

significantly larger than those for the males. The ANOVA test differences were significant ($P < 0.05$) (Fig. 2).

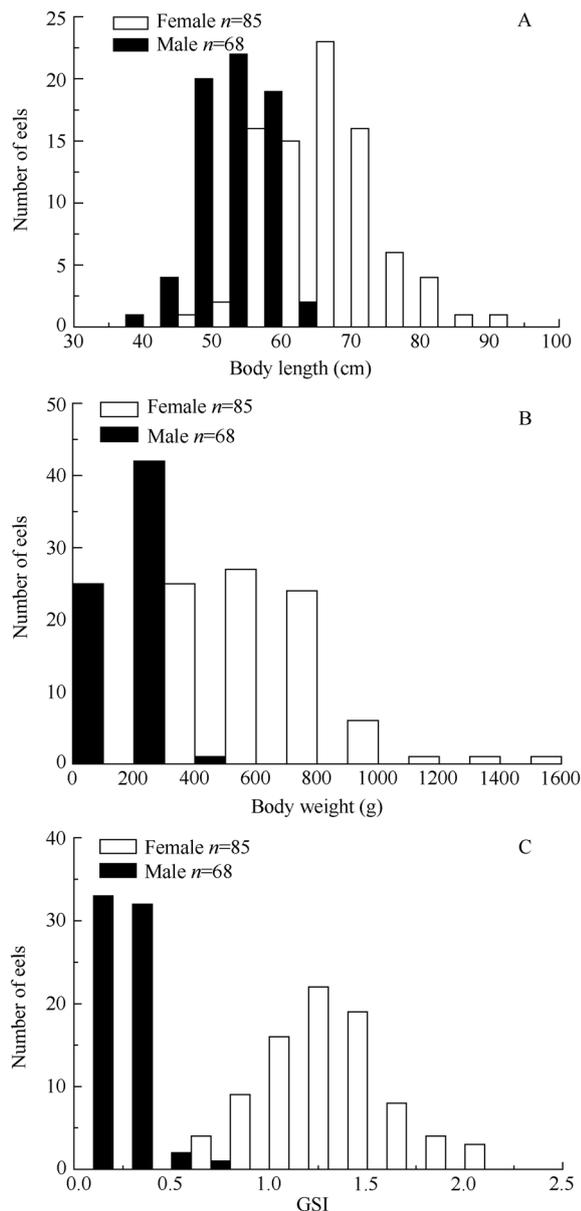


Fig. 2 Total length (A), body weight (B) and GSI (C) of eels collected at Jingjiang section of Yangtze River
Solid and open bars indicate male and female eels, respectively.

The condition factor of females ranged from 1.28 to 2.87, with an average of 1.77 ± 0.22 , while males ranged from 1.33 to 2.16, with an average of 1.62 ± 0.18 . The difference between the two was very significant (ANOVA, $P < 0.05$). The GSI for females ranged from 0.62 – 2.05, averaging 1.32 ± 0.31 (mean ± SD), while males ranged from 0.004 – 0.73, averaging 0.21 ± 0.11 . The differences between the two were significant ($P < 0.05$; Fig.2).

According to the age characteristics of sagitta slices (Fig. 3), the ages of 125 specimens, including 64 females and 61 males, were determined. There were five age groups for females, i.e. the age of the females ranged from 3–7 a whereas males ranged from 2–6 a (Fig. 4).



Fig. 3 Characteristic of sagittal otoliths of the Japanese eel collected at Jingjiang section of Yangtze River

The above figure shows a sagitta slice of 5⁺ age. The arrows point to the core, elver mark, the first GLG, second GLG, third GLG, fourth GLG, and fifth GLG, respectively (from left to right).

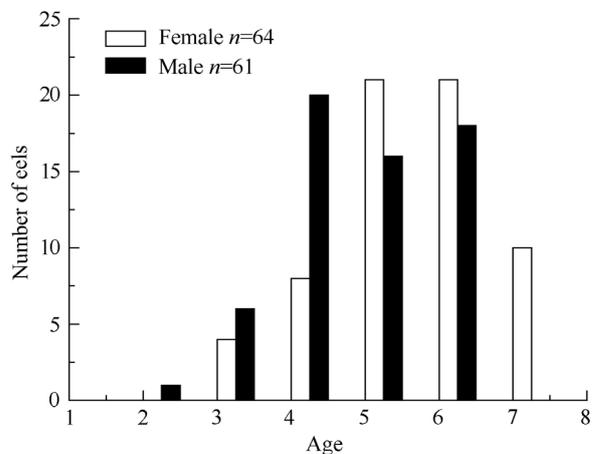


Fig. 4 Age of eels collected at Jingjiang section of Yangtze River

2.2 Elver mark of sagitta and its Sr/Ca ratio

Microscopy showed an elver mark on the outside of the core of each sagittal slices (Fig. 3). The distances between elver marks and the cores, i.e. the radii of elver marks, were 128.45 – 172.28 μm and averaged (mean \pm *SD*) (148.52 \pm 11.51) μm (Tab. 2). The marks radii of females were 129.09 – 165.71 μm , averaging (mean \pm *SD*) (149.34 \pm 11.52) μm , while the marks radii of males

were 128.45 – 172.28 μm , averaging (mean \pm *SD*) (147.31 \pm 11.94) μm . These results indicate there was no significant difference in the radii of the elver marks between females and males ($P > 0.05$) (Fig. 5). These were consistent with the radii of sagittal otoliths of elvers (mean \pm *SD*) (Wei, 2009) which only migrate to the Yangtze River estuary from the ocean.

The PIXE analysis conducted on the sagittal slices

Tab. 2 Radius in elver mark and its Sr/Ca ratio

	Elver mark radius (μm)		Sr/Ca ratio in elver mark ($\times 10^{-3}$)		No. fish analyzed
	Mean \pm <i>SD</i>	Range	Mean \pm <i>SD</i>	Range	
Females	149.34 \pm 11.52	129.09 – 165.71	8.06 \pm 1.04	6.25 – 9.67	16
Males	147.31 \pm 11.94	128.45 – 172.28	7.88 \pm 1.06	6.28 – 9.57	11
Total fish	148.52 \pm 11.51	128.45 – 172.28	7.99 \pm 1.05	6.25 – 9.67	27

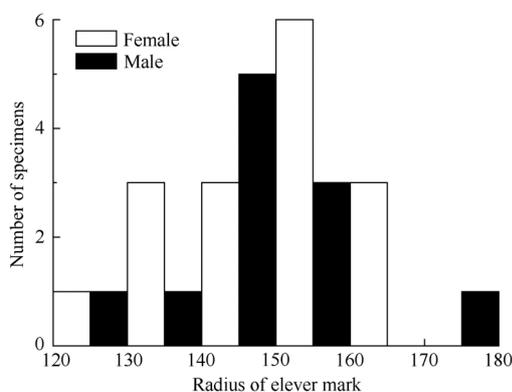


Fig. 5 Frequency distribution of mean radius of elver mark in sagitta

of the 27 specimens indicated that the average Sr/Ca ratio (\pm *SD*) at the elver marks was (8.06 \pm 1.04) $\times 10^{-3}$ for females and (7.88 \pm 1.06) $\times 10^{-3}$ for males (Tab. 2). There was no significant difference between the two ($P > 0.05$). Total average Sr/Ca ratio (\pm *SD*) was (7.99 \pm 1.05) $\times 10^{-3}$. The elver period is a stage during the Japanese eel life cycle when they migrate to the estuary. Therefore, the value 8.0×10^{-3} can be roughly taken as the reference value for the ratio between depositing elements Sr and Ca in sagitta when Japanese eel live in the estuary. Based on this reference value, their life history can be inferred according to the changes in Sr/Ca ratios in every growth layer group outside the elver mark. If Sr/Ca values outside the elver marks were equal or close to 8.0×10^{-3} implies that the eels that remained in the Yangtze estuary or moved frequently between freshwater and estuary were “estuarine eels”; if the Sr/Ca values were higher than 8.0×10^{-3} implies that the eels that once migrated to the sea and then returned to the estuary were “sea eels”;

and if the Sr/Ca values were lower than 8.0×10^{-3} and consistently low along their otolith transects with no increase implies the eels that stayed in the freshwater of Yangtze River until their capture were “river eels” (Fig. 6).

2.3 Migration pattern

The Sr/Ca ratios in the transects along the radius of each otolith showed that from the core to the elver mark (corresponding to the leptocephalus period), the Sr/Ca ratios of all specimens showed the same trend of change: from the core, the Sr/Ca ratios increased gradually until the peak value of higher than 20.00×10^{-3} . This indicated that Japanese eel live in the ocean during their leptocephali and early glass eel period. After the peak, the Sr/Ca ratios gradually decreased to the estuary reference value 8.00×10^{-3} . This indicated that these individuals migrated to the estuary and metamorphosed to elvers (Fig. 6).

In every GLGs behind the elver mark, the Sr/Ca ratio changes displayed two patterns of change.

1) The Sr/Ca ratios in the GLGs outside the elver marks were very low, usually below 5×10^{-3} . This indicated that such individuals remained in the freshwater areas after the elver stage after they drifted into the Yangtze River. Hence they were river eels. Out of the 27 specimens tested 17 (62.96%) were river eels, among which 13 were female, accounting for 81.25% of the total female number, and 4 were male, accounting for 36.36% of the total male number (Fig. 7).

2) The Sr/Ca ratios in the GLGs from the elver marks to the otolith edge fluctuated between 4×10^{-3} and 8×10^{-3} . One or more peak values that approximated or exceeded the estuary marker value 8.00×10^{-3} appeared.

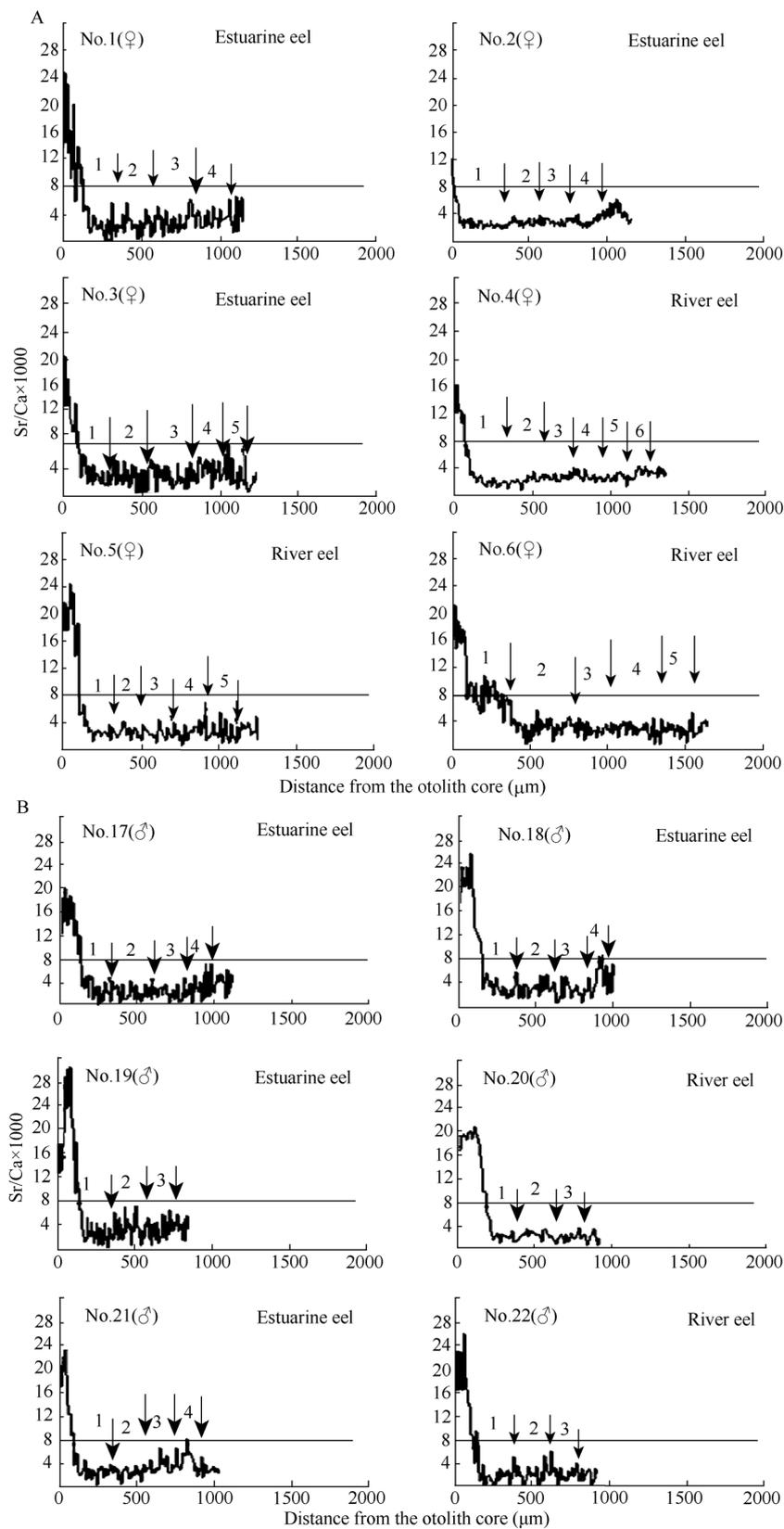


Fig. 6 Typical changes in otolith Sr/Ca ratio along line transects from the core (0 μm) to the edge in the frontal plane of sagittal otoliths of specimens collected at Jingjiang section of Yangtze River

A: female eel; B: male eel.

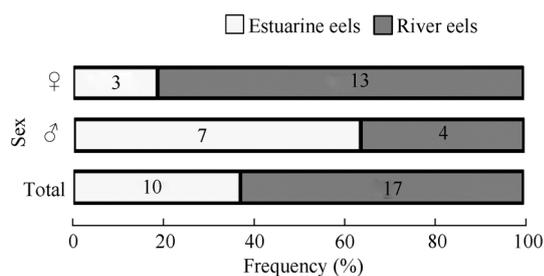


Fig. 7 Frequency composition of the two designated types of eels with different migratory histories (river eel and estuarine eel) based on mean Sr/Ca ratios outside the elver mark in the otolith of all specimens examined, and for the specimens separated into sex (female and male).

Numbers within each bar indicate the number of eels belonging to each type.

These were estuarine eels, who may have stayed in a river section not far from the estuary after the elver stage when they drifted into the Yangtze River and reached high-salinity areas outside the estuary many times. Of the 27 specimens, 10 (37.04%) were estuarine eels, including 7 males and 3 females (Fig. 7). Tab. 3 lists the estuarine eels' sex, age, and GLG when Sr/Ca ratios of otolith approximated the estuary marker value. All these individuals appeared at the estuary when they were at least 2-a old.

2.4 Sex differences in otoliths Sr/Ca contents in the same age layer group

In the specimens analyzed, the females were from

Tab. 3 Sex, age, and ghosted estuarine time (a) of estuarine eels

No.	Sex	Age	Ghosted estuarine Time (a)
No.1	♀	4 ⁺	4-4 ⁺
No.2	♀	4 ⁺	4 ⁺
No.3	♀	5 ⁺	4-5
No.17	♂	4 ⁺	4-4 ⁺
No.18	♂	4 ⁺	2, 3, 4, 4 ⁺
No.19	♂	3 ⁺	2, 3, 3 ⁺
No.21	♂	4 ⁺	3, 4
No.24	♂	3 ⁺	3, 3 ⁺
No.26	♂	4 ⁺	3, 4, 4 ⁺
No.27	♂	4 ⁺	4, 4 ⁺

four to seven a old while the males were four to five a old. Except for the first-age layer with very high Sr/Ca values, the values of the female and male groups of other growth layer groups (GLG) are described in Tab. 4.

Tab. 4 ANOVA and difference Sr/Ca ratio of second age layer, third age layer, fourth age layer and outmost layer between female and male eel in sagitta

Layer	Sr/Ca ratio ($\times 10^{-3}$)		n	ANOVA	
	Mean \pm SD	Range			
Second	Female	3.11 \pm 0.58	2.44 - 4.93	16	0.480
	Male	3.28 \pm 0.63	2.33 - 4.65	11	
	Total	3.18 \pm 0.60	2.33 - 4.93	27	
Third	Female	3.02 \pm 0.34	2.44 - 3.75	16	0.046
	Male	3.50 \pm 0.81	2.25 - 5.03	11	
	Total	3.22 \pm 0.61	2.25 - 5.03	27	
Forth	Female	3.14 \pm 0.42	2.42 - 3.81	16	0.000
	Male	4.38 \pm 0.57	3.90 - 5.12	5	
	Total	3.44 \pm 0.70	2.42 - 5.12	21	
Outmost	Female	3.34 \pm 0.65	1.95 - 4.30	16	0.042
	Male	4.13 \pm 1.25	1.95 - 5.64	11	
	Total	3.66 \pm 1.00	1.95 - 5.64	27	

The Sr/Ca values of the males were generally higher than those of females, although there was no significant difference in the Sr/Ca values between the second-age GLG female and male individuals ($P=0.480>0.05$). However, significant or very significant differences appeared between female and male individuals in the third GLG, fourth GLG, and the migration GLG (outmost GLG). This indicated that second-age female individuals lived in the same environment on the Yangtze River as second-age male individuals. However, male individuals older than three a preferred to stay in waters near estuaries and with relatively high salinity, while females tended to stay in upstream river sections with low salinity. The average GLG Sr/Ca ratios for all ages are shown in Fig. 8.

3 Discussion

3.1 Migration behavior of *Anguilla japonica* in the Yangtze River

The Japanese eel, *A. japonica*, is generally considered a catadromous fish species (McDowall, 1988). After recent analysis of otolith Sr/Ca ratios, however, *A. japonica* (Tsukamoto et al, 1998; Arai et al, 2003), *A. anguilla* (Tsukamoto et al, 1998; Tzeng et al, 2000), and *A. rostrata* (Jessop et al, 2002; Morrison et al, 2003; Lamson et al, 2006) do not easily fall into the category of seawater and freshwater migration fish; their life has certain diversity.

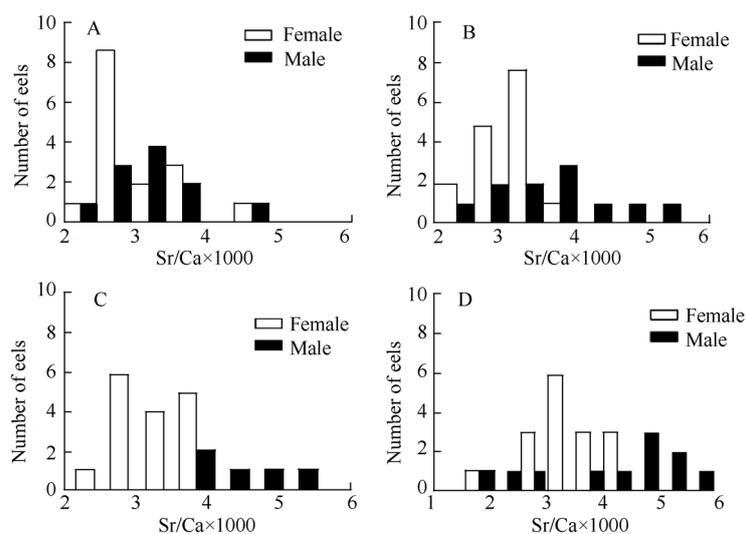


Fig. 8 Frequency distribution of mean Sr/Ca ratios

Sr/Ca ratio in GLG of second age layer (A), third age layer (B), fourth age layer (C), and from the last age mark to the otolith edge in otoliths (D).

Both Sr and Ca are divalent ions of alkaline earth metals. Their ionic radii, charges on ions, and electron orbits are very similar. Thus Sr can easily replace Ca in the otolith deposits of fish. The concentration of Sr in sea water is dozens to hundreds of times higher than that in fresh water. The Sr/Ca ratios of sagitta are positively correlated with water salinity and their time series changes can trace the migration history of fish between the sea and fresh water. Tsukamoto & Arai (2001) analyzed 61 *A. japonica* sagitta specimens taken from south of Japan including fresh water, estuaries and oceans, and they discovered three types of life history. Among them, sea eels that had always lived in the ocean without migration to fresh water accounted for 20% of all; estuarine eels that inhabited the estuaries after the elver stage or migrated between fresh water and sea water accounted for 57% of all; and river eels that had always lived in fresh water accounted for only 23% of all. Among seven specimens collected from the fresh water, six were river eels and one was an estuarine eel.

Using electron spectra, Kotake et al (2003) also analyzed 25 *A. japonica* otolith specimens collected near Amakusa Island in Japan during migration season. They found that 52% were estuarine eels, 28% were sea eels, and 20% were river eels.

Japanese eel are distributed in the main rivers and many tributaries, from the Yangtze River estuary to the upper Jinsha River extending nearly 3 000 km. However, the migration behaviors of elvers during the several years after they drift into the Yangtze River are almost unknown.

In this research, advanced PIXE technology was applied to analyze 27 silver eel specimens collected from the Jingjiang section of the lower reaches of the Yangtze River (fresh water). Of the 27 specimens, 62.96% were river eels that had always lived in fresh water and 37.04% were estuarine eels that frequently migrated between fresh water and estuarine water. No sea eels were found.

3.2 Difference in migration behavior between the two sexes

Of the 16 female specimens studied, 13 (81.25% of all) were river eels (Fig. 7) and only 3 were estuarine eels. Of the 11 male specimens, only 4 (36.36% of all) were river eels and 7 (63.64% of all) were estuarine eels. These results indicated that female groups tended to migrate upstream and stay in the upper stream of Yangtze River estuary, whereas the male groups preferred to stay near the river section of the estuary.

Further analysis showed no significant difference in the Sr/Ca ratios between the second GLG female and male individuals; however, significant differences appeared between female and male individuals of third-age, fourth-age, and migration-age (outmost GLG). This indicates that the second-age eels of both sexes stayed in the same inhabitation waters. As they grow older (three or more than three years old), however, they begin to inhabit different areas.

Our results reasonable predicted the migration behaviors of *A. japonica* on the Yangtze River. Elvers entered the Yangtze River estuary at the age of 130 d

(Wei, 2009); most female individuals migrated upstream as far as the Jinsha River, some 3 000 km from the estuary; after they sexually matured, they evolved into silver eels and migrated back to the estuary. However, most male individuals live in fresh water areas near the

estuary and often return to the estuary after they are two years old.

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