

The Migratory Environmental History of Freshwater Resident Flathead Mullet *Mugil cephalus* L. in the Tanshui River, Northern Taiwan

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Chia-Hui Wang, Chih-Chieh Hsu, Chih-Wei Chang, Chen-Feng You, and Wann-Nian Tzeng (2010) The migratory environmental history of freshwater resident flathead mullet *Mugil cephalus* L. in the Tanshui River, northern Taiwan. *Zoological Studies* 49(4): 504-514. The diversity of the migratory environmental history of freshwater resident flathead mullet *Mugil cephalus* was examined by analyzing the otolith elemental composition of mullet ($n = 31$) collected in midstream of the Tanshui River, northern Taiwan by laser-ablation inductively coupled plasma mass spectrometry. The mullet averaged 300 ± 33 mm in fork length and 410 ± 141 g in body weight, and all were sexually immature at 1+ and 2+ yr old. Strontium (Sr)/calcium (Ca) and barium (Ba)/Ca ratios in the otolith core indicated that the mullet in the river were from 2 different groups. Group 1 (18%) fish had higher Sr/Ca ratios and lower Ba/Ca ratios in the otolith core region. Group 2 fish (82%) were opposite to group 1, with higher Sr/Ca ratios and lower Ba/Ca ratios in the otolith core region, indicating that these mullet had resided in offshore waters in early life. Beyond the core region, the Sr/Ca and Ba/Ca ratios alternately varied, indicating that the mullet migrated between brackish and freshwater habitats after the juvenile stage. This study demonstrates that *M. cephalus* occupied diverse environments and migrated among marine, brackish, and freshwater habitats. More study is needed to determine whether the mullet in the Tanshui River of Taiwan is a local population and its relationship to the well-known offshore migratory population that migrates to southwestern Taiwan to spawn in Dec. <http://zoolstud.sinica.edu.tw/Journals/49.4/504.pdf>

Key words: Mullet, Freshwater fish, Otolith elemental composition, Migratory environmental history.

The flathead mullet (*Mugil cephalus* L.) is a cosmopolitan euryhaline fish, distributed in coastal, lagoon, and estuarine waters between 42°N and 42°S latitudes (Thomson 1966). It is economically important worldwide for both capture fishery and aquaculture. More attention has been focused on wild populations of mullet due to significant decrease since 1980 because of overharvesting for the roe (Chang and Tzeng 2006). The population of flathead mullet in waters adjacent to Taiwan migrates annually from the feeding grounds in coastal waters of China to offshore waters of both southwestern and northeastern Taiwan to

spawn in Dec. at the age of 3-4 yr (Chen and Su 1986, Huang and Su 1989, Chang et al. 2004a, Hsu et al. 2007). After a process of passive dispersal for approximately 1 mo after spawning, they metamorphose from larvae to juveniles and are actively recruited to the estuarine nursery area (Blaber 1987, Chang et al. 2000, Hsu et al. 2009). Whether juveniles enter freshwater environments is controversial. Some hypothesized that all juveniles and post-spawning adults might migrate northward to coastal waters of the Chinese mainland to feed until the spawning migration (Tung 1981), while some proposed that a few juveniles

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resided in coastal waters around Taiwan without a northward migration (Liu 1986). Immature mullet of various sizes are found year round in coastal waters, estuaries, and bays of Taiwan (Chen et al. 1989, Lee 1992, Huang et al. 2001). However, the detailed migrations and habitat movements of freshwater resident flathead mullet have never been investigated, nor has information on their connections and contributions to the known offshore migratory population, that is important for sustainable fisheries management for flathead mullet, been established.

Several tagging and marking techniques, such as satellite-transmitting archival tags, coded wire tags, chemical markers, radio telemetry, acoustic tags, and otolith elemental signatures can be used to study the migration history and population structure of fishes (Campana et al. 2000, Loher 2008). Otolith elemental signatures serve as a sensitive natural tag and can be used to track the entire life history of a fish. The otolith is located in the inner ear and functions for hearing and balance of the fish. It is a biomineralized aragonite crystalline structure mainly composed of CaCO_3 with a minor organic substrate. Also, a number of elemental impurities, e.g., sodium (Na), strontium (Sr), potassium (K), zinc (Zn), magnesium (Mg), manganese (Mn), lead (Pb), barium (Ba) etc., are co-precipitated in the otolith growth increments as the fish grows (Campana 1999). Elemental deposition in the otolith is influenced by both physiological and environmental factors, particularly the concentration of elements in the ambient water. The chemicals deposited represent a permanent record of the environmental conditions experienced by the fish at a particular time (Ruttenberg et al. 2005). In addition, otolith growth increments are periodically deposited in daily and annular rhythms which allow one to determinate the daily and annual age of a fish (Pannella 1971). Mullet otolith daily increment and annular periodicity deposition rhythms were validated (Chang et al. 2000, Hsu and Tzeng 2009). Accordingly, the daily and annual age determination of mullet, together with the measurement of trace elements in the increment, can be used as a biological tracer to reconstruct the migratory environmental history and habitat use of the fish (Campana et al. 2000). Alkaline earth metals, such as Sr and Ba, have similar ionic radii as Ca, and may substitute for Ca in the orthorhombic aragonite lattice of fish otoliths (Campana 1999), so their concentrations are higher in otoliths compared to other trace metals.

Also, concentrations of Sr and Ba vary between fresh water and seawater, and are useful as natural indicators for understanding diadromous fish migration behavior. The Sr concentration is approximately 100 times higher in seawater than in fresh water (Campana 1999). Rearing experiments showed that otolith Sr/Ca ratios of *M. cephalus* are positively correlated with the salinity of the ambient water (Chang et al. 2004b). The Ba/Ca ratio in otoliths is another candidate which can be used to reconstruct the history of fish movements (Elsdon and Gillanders 2004 2005). Barium is sourced terrestrially in rock, soil, and aquatic suspended particles, and the dissolved Ba concentration is higher in river water than in seawater (Edmond et al. 1985, Chester 2000). Thus, elemental ratios such as Sr/Ca and Ba/Ca in otoliths, along with the fish age, are widely used to reconstruct the past migratory environmental history of fish (Fowler et al. 2005, Morales-Nin et al. 2005, Bradbury et al. 2008).

This study used the otolith elemental composition as a natural tag to study the migratory environmental history of the freshwater resident mullet *M. cephalus* in the Tanshui River, northern Taiwan. The otolith elemental composition was measured by laser-ablation inductively coupled plasma mass spectrometry (ICP-MS). Temporal changes in the elemental composition in otoliths permit reconstruction of the migratory environmental history of freshwater resident mullet and examination of the diversity of mullet migratory patterns in northern Taiwan.

MATERIAL AND METHODS

Sample collection

In total, 31 *M. cephalus* individuals were collected from midstream of the Tanshui River, the largest river in northern Taiwan, on 3rd-5th Oct. 2006 (Fig. 1). The sampling site is near the Fu-Ho Bridge, close to the conjunction of the Tanshui River and one of its major tributaries, the Shindien River, and is a freshwater environment (0 psu). After collection, the fish were immediately preserved on ice, and their fork length (FL, mm), body weight (BW, g), and gonad weight (GW, g) were measured fresh within 24 h (Table 1). Sexes of the fish were determined from the external features of the gonads (Hsu et al. 2007). The maturation stage of each fish was determined by the gonadosomatic index (GSI) which was

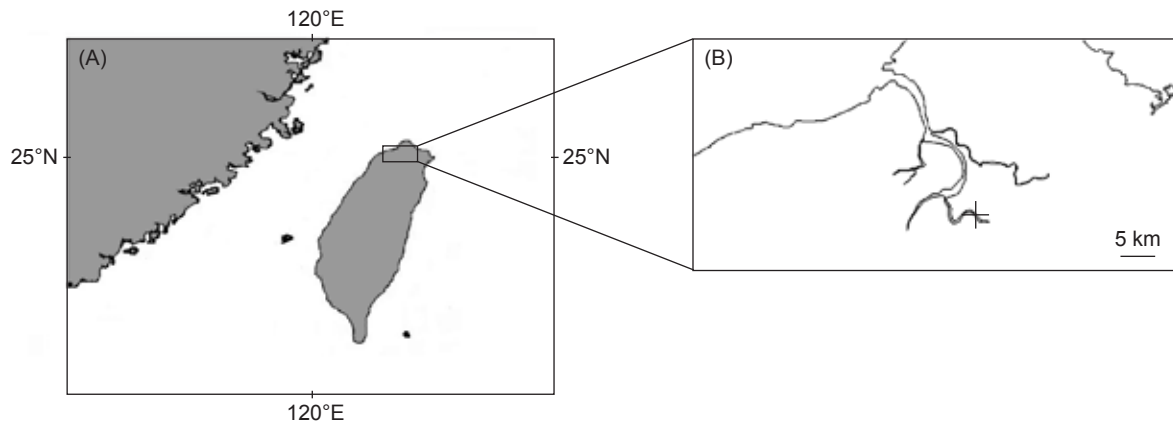


Fig. 1. Sampling location of *Mugil cephalus* (marked with "+") midstream in the Tanshui River, northern Taiwan.

Table 1. Sampling date, sample size (F, female; M, male; -, undetermined), age (yr), mean (\pm SD) fork length (FL, mm), body weight (BW, g), and gonadosomatic index (GSI, %) of *Mugil cephalus* collected from the Tanshui River, northern Taiwan. Otoliths that underwent LA-ICPMS analyses are marked with a V

No.	Date	FL	BW	GSI	Sex	Age	LA-ICPMS
1	3rd Oct. 2006	380	833	3.09	M	2+	V
2	3rd Oct. 2006	312	439	2.81	M	1+	V
3	3rd Oct. 2006	303	452	0.67	F	2+	V
4	3rd Oct. 2006	262	281	-	-	2+	-
5	3rd Oct. 2006	237	189	-	-	2+	V
6	3rd Oct. 2006	283	350	3.55	F	2+	V
7	3rd Oct. 2006	268	275	-	-	1+	-
8	4th Oct. 2006	371	734	2.57	F	2+	V
9	4th Oct. 2006	307	436	0.40	M	2+	V
10	4th Oct. 2006	322	470	2.05	F	1+	V
11	4th Oct. 2006	277	331	-	-	1+	V
12	4th Oct. 2006	290	398	0.43	M	1+	V
13	4th Oct. 2006	283	325	0.17	F	1+	V
14	4th Oct. 2006	318	487	0.83	F	1+	V
15	4th Oct. 2006	294	356	0.11	F	1+	V
16	4th Oct. 2006	280	339	0.68	M	1+	V
17	4th Oct. 2006	281	344	0.25	F	1+	V
18	4th Oct. 2006	294	355	0.10	M	1+	-
19	4th Oct. 2006	266	280	-	-	1+	-
20	5th Oct. 2006	287	322	-	-	1+	V
21	5th Oct. 2006	356	541	1.31	F	2+	V
22	5th Oct. 2006	316	457	-	-	1+	V
23	5th Oct. 2006	342	629	0.58	F	2+	V
24	5th Oct. 2006	297	365	2.44	M	1+	-
25	5th Oct. 2006	353	692	0.69	F	2+	V
26	5th Oct. 2006	295	390	0.18	F	1+	V
27	5th Oct. 2006	292	351	-	-	1+	-
28	5th Oct. 2006	291	358	-	-	1+	-
29	5th Oct. 2006	273	296	-	-	-	-
30	5th Oct. 2006	296	381	0.43	M	1+	V
31	5th Oct. 2006	262	249	-	-	1+	-

calculated as follows:

$$\text{GSI} = [\text{GW} / (\text{BW} - \text{GW})] \times 100.$$

Otolith preparation

Sagittal otoliths of the mullet were removed, ultrasonically cleaned with distilled water, and air-dried. The otoliths were then embedded in epoxy resin (Epofix, Struers), and cut transversely with a low-speed diamond saw to obtain a section of approximately 300 μm thick, which was polished to expose the primordium. The polished otolith thin sections were cleaned with distilled water and then etched with a 5% HCl solution for 10 s to enhance the annuli in the otolith. The otolith was photographed with a digital camera under a light microscope (Nikon) with reflected light. The fish age was determined by counting the annuli, and the distance of each annulus to the otolith core was measured for comparison with the otolith elemental analyses so as to understand their temporal changes (Fig. 2). Nine of the mullet otoliths were slightly over-polished such that the primordium was missing. The annuli number could be counted from the edge but those samples were not included in further elemental composition analyses.

Otolith elemental composition measurement and analyses

An otolith section was re-polished, washed in 5% H_2O_2 , triple-rinsed with distilled water, and air-dried. The elemental composition of the otolith was measured along its dorsoventral axis by ICP-MS (Finnigan MAT ELEMENT 2, Thermo Electron Corp., Waltham, MA, USA) equipped with a laser

microprobe (Merchantek LUV 266 Nd: YAG UV, New Wave Research, Fremont, CA, USA) which was pulsed at 20 Hz with a spot size of 150 μm in diameter. The laser beam was moved along the line transect (Fig. 1) at a speed of 15 $\mu\text{m}/\text{s}$ to continuously measure the change in the otolith elemental composition. Isotopes of ^{44}Ca , ^{88}Sr , and ^{138}Ba were measured because the sensitivities of these isotopes remained at least 10 times higher than background levels. Standard (National Institute of Standards and Technology 612 glass standard) counts were also measured before measurement of every 3 or 4 otoliths to calibrate the otolith elemental concentration and to monitor instrument analytical precision. At the start of each otolith analysis, background counts were collected for 30 s, and the average was subtracted from sample counts to correct for background levels. The ablation chamber was purged for 60 s after sampling each otolith. All counting data were expressed as ratios of an element to the Ca concentration (ppm/ppm) by estimating the relative response factor of the instrument to the known concentration in the standard (NIST 612). Counts of the elements measured along the line transect of the otolith were collected every 2.11 s at an interval of approximately 32 μm in spatial resolution.

Data analysis

The core region of the mullet otolith was deposited during the larval stage. The duration of the mullet larval stage was estimated to be approximately 14 d, and the otolith diameter at that age was about 150 μm (Chang 2003). Thus the average value of the 3 points taken from the core section (approximately 96 μm in radius around

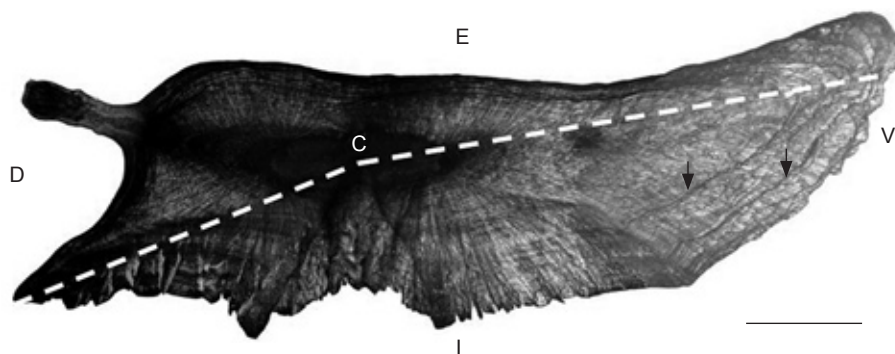


Fig. 2. Thin transverse section of a *Mugil cephalus* otolith (2+ yr old) under reflected light after etching with HCl. The dotted line indicates the analyzed LA-ICPMS transect. Black arrow, annulus; C, core; D, dorsal; E, external; I, internal; V, ventral. Scale bar = 500 μm .

the primordium) (Fig. 2) of the otolith represented the habitat signature for each individual in the early larval stage. The otolith core values were examined by a principal component analysis (PCA) to test whether mullet collected in midstream of the Tanshui River were of a similar origin. The otolith element: Ca concentration ratios were examined by *t*-test for differences in origin using STATISTICA® vers. 6.1. Ba: Ca ratios were log-transformed for all statistical analyses in order to meet the hypotheses for a normal distribution and homogeneity of the variance.

RESULTS

Size composition

Mugil cephalus specimens contained 12 females, 8 males, and 11 undetermined individuals, and the fork length and weight ranged 237-380 mm and 189-833 g, respectively. Fork lengths were

317 ± 32 (mean \pm SD), 307 ± 31 , and 276 ± 21 mm for female, male, and undetermined individuals, and total weights were 481 ± 141 , 443 ± 162 , and 308 ± 69 g, respectively. Fish ages were between 1+ and 2+ yr, and 65% of fish ($n = 20$) were 1+ yr old. The mean (\pm SD) GSI was 1.2 ± 1.1 with the highest GSI values at 3.1 (for males) and 3.6 (for females). The GSI and age composition indicated that all 31 fish collected from the freshwater habitat of the river were immature.

Elemental composition in the otolith edge

Sr/Ca and Ba/Ca ratios in the mullet otolith edges were used as the baseline of a freshwater habitat. The mean Sr/Ca and Ba/Ca ratios were $(3.0 \pm 1.1) \times 10^{-3}$ and $(153 \pm 66) \times 10^{-6}$, respectively (Fig. 3). The otolith Sr/Ca ratio in freshwater-habitat mullet was in the same range at around 3.0×10^{-3} from a tank-rearing experiment (Chang et al. 2004b), so this was set as the threshold value for discriminating fresh and brackish water.

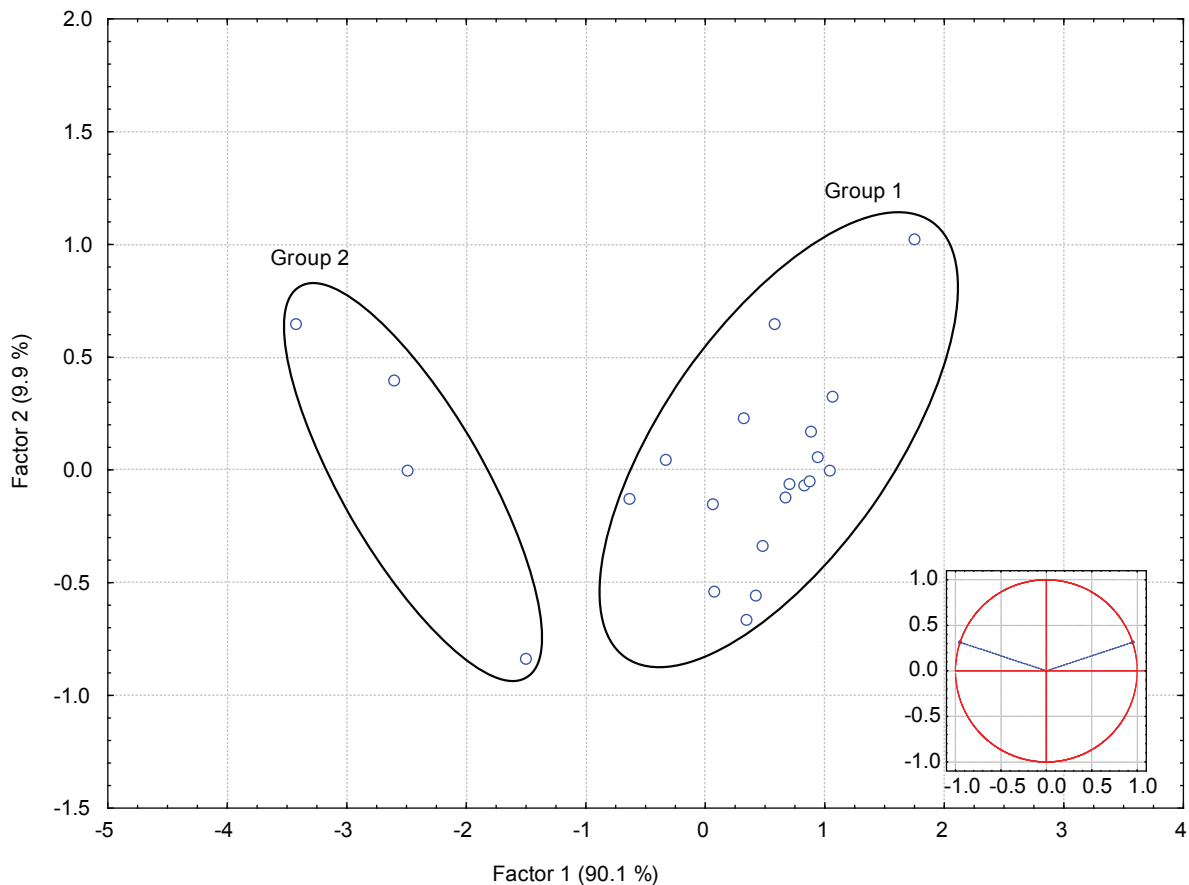


Fig. 3. The principle components analysis (PCA) grouping into 2 types of *Mugil cephalus* from the Tanshui River classified by the otolith core elemental composition and relative contributions of each elemental ratio. The elliptical circle indicates the 95% confidence limit of each group.

Ba/Ca ratios showed larger variations, and the value of 124×10^{-6} , which was the lower limit of the mean, was set as the threshold value.

Elemental composition within the otolith core

The PCA classified the elemental composition in the core region of mullet collected in fresh water in the Tanshui River into 2 groups (Fig. 4). The Sr/Ca and Ba/Ca ratios in the core regions of the otoliths significantly differed between the groups (t -test, $t = -6.908$, $d.f. = 20$, $p < 0.001$ for Sr/Ca; $t = 5.530$, $d.f. = 20$, $p < 0.001$ for log Ba/Ca) (Fig. 3). For group 1, the core and edge Sr/Ca and

Ba/Ca ratios significantly differed (t -test, $t = -7.429$, $d.f. = 38$, $p < 0.001$ for Sr/Ca; $t = 13.692$, $d.f. = 38$, $p < 0.001$ for log Ba/Ca); while for group 2, they were similar (t -test, $t = -2.071$, $d.f. = 24$, $p = 0.049$ for Sr/Ca; $t = -2.521$, $d.f. = 24$, $p = 0.018$ for log Ba/Ca). Group 1 fish comprised 82% ($n = 18$) of the fish examined and were characterized by higher Sr/Ca ratios at $(5.6 \pm 1.0) \times 10^{-3}$ and lower Ba/Ca ratios of $(12 \pm 8) \times 10^{-6}$ in the otolith core, indicating that they inhabited a marine environment during the early larval stage. The otolith core of group 2, which comprised 18% ($n = 4$) of the mullet examined, was characterized by a significantly lower Sr/Ca ratio of $(1.9 \pm 0.3) \times 10^{-3}$ (mean \pm SD) and a higher Ba/Ca ratio of $(67 \pm 25) \times 10^{-6}$ (Fig. 3), indicating that these fish (numbers 17, 20, 21, and 25) inhabited a different environment from group 1 during the early larval stage.

Temporal changes in otolith Sr/Ca and Ba/Ca ratios beyond the core region

Temporal changes in the otolith Sr/Ca and Ba/Ca ratios of the mullet differed between individuals and were divided into 2 types. Temporal changes of 6 otoliths (type I) had higher Sr/Ca ratios ($> 3 \times 10^{-3}$), and the Ba/Ca ratios were consistently $< 20 \times 10^{-6}$ before the annulus near the otolith edges (Fig. 5). For example, fish no. 3 showed consistently high otolith Sr/Ca ratios beyond the core to the position of the 2nd annuli, then dropped to the lower brackish and freshwater level of $< 3 \times 10^{-3}$ after the 2nd annulus formed. In some fish (nos. 3, 6, and 23), the Sr/Ca ratios were high at the 2 annuli and lower in between them. Otolith Ba/Ca ratios remained at a low level ($< 20 \times 10^{-6}$) until the 2nd annulus, after which the Ba/Ca ratio suddenly increased and reached a peak at the otolith edge ($> 120 \times 10^{-6}$). The otolith chemical signatures of high Sr/Ca and low Ba/Ca ratios indicated that those fish inhabited a marine environment during the juvenile and most of the subadult stages and entered fresh water just before being captured.

Type II fish had fluctuating Sr/Ca ratios beyond the core region (Fig. 6). In most of those individuals, the otolith Sr/Ca ratios decreased to a freshwater level of $\leq 3 \times 10^{-3}$ beyond the core region (Fig. 6). Some otolith Sr/Ca ratios fluctuated, but some stayed consistently low before deposition of the 1st annulus. The Ba/Ca ratios were low in the otolith core, then gradually increased and finally fluctuated between $(100$ and $200) \times 10^{-6}$ (Fig. 6). Those individuals stayed

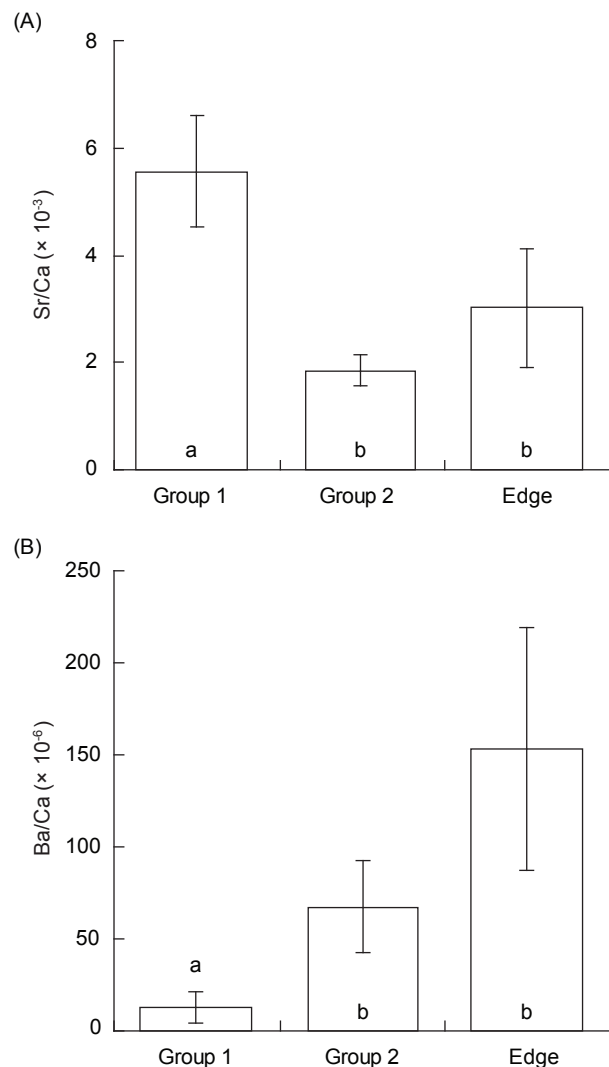


Fig. 4. Otolith Sr/Ca (A) and Ba/Ca (B) ratios at the edge of all individual mullet otolith cores (Edge) and the cores of the 2 groups of *Mugil cephalus* from the Tanshui River. The same letter indicates that otolith ratios are homogeneous among the groups.

in fresh- and brackish-water habitats and never returned to the marine environment again after being recruited into the estuary at the juvenile stage. Individuals with lower Sr/Ca ratios in the otolith core (group 2 in Fig. 4) were all classified into this type.

DISCUSSION

Validation of otolith Sr/Ca and Ba/Ca ratios as an indicator of mullet habitat

The mullet were collected in the middle of the river where the salinity at the bottom was close to 0, although the water level was influenced by tidal fluctuations. The chemical signatures with low Sr/Ca (3.0×10^{-3}) and high Ba/Ca (124×10^{-6}) ratios at the otolith edge indicated that the mullet had recently inhabited fresh water, as indicated by their location of capture. This conclusion was supported by previous studies that otolith Sr/Ca ratios are $< 3 \times 10^{-3}$ when mullet are in fresh water (at salinities of 0-5 psu) and are $> 3 \times 10^{-3}$

in saline water (5-35 psu) (Chang et al. 2004a b). Accordingly, as for other diadromous fishes, the otolith Sr/Ca ratios can be used to indicate fish movement between fresh and saline (estuarine and seawater) environments (Tzeng et al. 2002). Otolith Ba/Ca ratios for mullet in relation to the ambient water salinity have not been validated. However, otolith Ba/Ca ratios were reported to be higher in fresh than in seawater for other fishes (Elsdon and Gillanders 2004 2005). In this study, we found that Ba/Ca ratios in otoliths were higher when the mullet were in fresh water (124×10^{-6}) and lower in seawater ($\sim 20 \times 10^{-6}$, the higher limit of the mean of the core in group 1 (Fig. 4). Therefore, by combining otolith Sr/Ca and Ba/Ca ratios, we could reconstruct the mullet migratory environmental history in greater detail. Low otolith Sr/Ca ratios ($< 3.0 \times 10^{-3}$) can be used to discriminate freshwater from marine habitats, while low otolith Ba/Ca ratios ($< 20 \times 10^{-6}$) can be used to discriminate marine from freshwater habitats. When the chemical signatures fall between the 2 criteria, then an estuarine habitat is suggested.

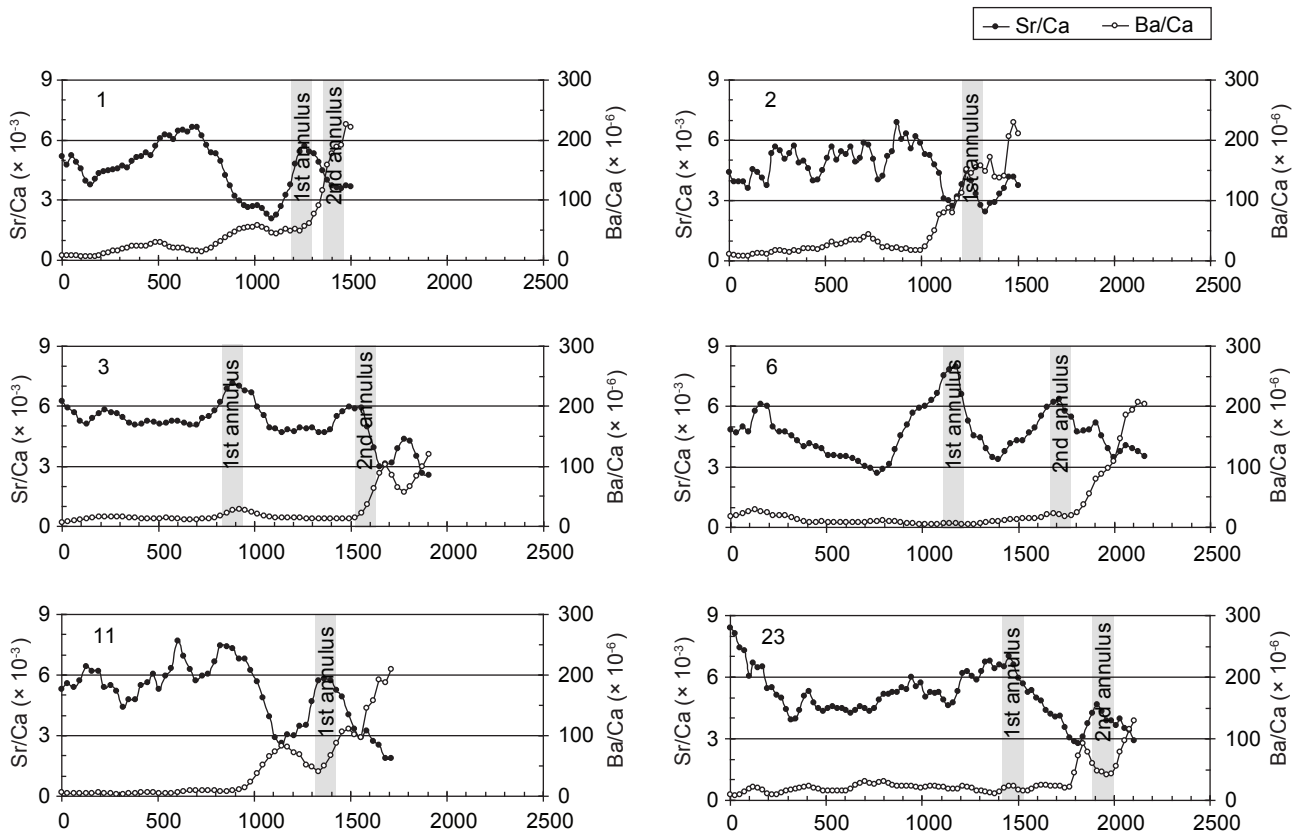


Fig. 5. Temporal changes in otolith Sr/Ca and Ba/Ca ratios for migratory type I *Mugil cephalus* collected from the Tanshui River.

Identification of habitat use during the larval stage by Sr/Ca and Ba/Ca ratios

After spawning off southwestern or northeastern Taiwan (Chen and Su 1986, Huang and Su 1989, Chang et al. 2004a, Hsu et al. 2007), larvae of *M. cephalus* passively disperse to estuarine nursery areas (Chang et al. 2000, Hsu et al. 2009). Larvae younger than 14 d old do not actively swim (Tung 1967, Liao 1975); therefore, these larvae would still be resident in the water mass where they hatched. Accordingly, the elemental composition of the 1st 3 measurement points in the otolith (approx. 48 μm from the primordium) was regarded as characteristic of the ambient water of the spawning

ground. In the present study, 2 different signals near the otolith core were found (Fig. 4). Group 1 was characterized by higher Sr/Ca ratios and comprised larger percentages (82%) of freshwater resident mullet, while Sr/Ca ratios in the core of group 2 fish were $< 3 \times 10^{-3}$. This indicated that group 2 fish spent their early life in a water mass that differed from that of group 1. The lower Sr/Ca ratios in group 2 otolith cores suggest that their parents spawned in a low-salinity water mass, or the distance for larval dispersal to the estuarine nursery area was short, thus making the average core Sr/Ca value significantly lower than that of the other group. It is not known whether mullet spawn outside the known spawning grounds of southwestern and northeastern Taiwan. A 3rd

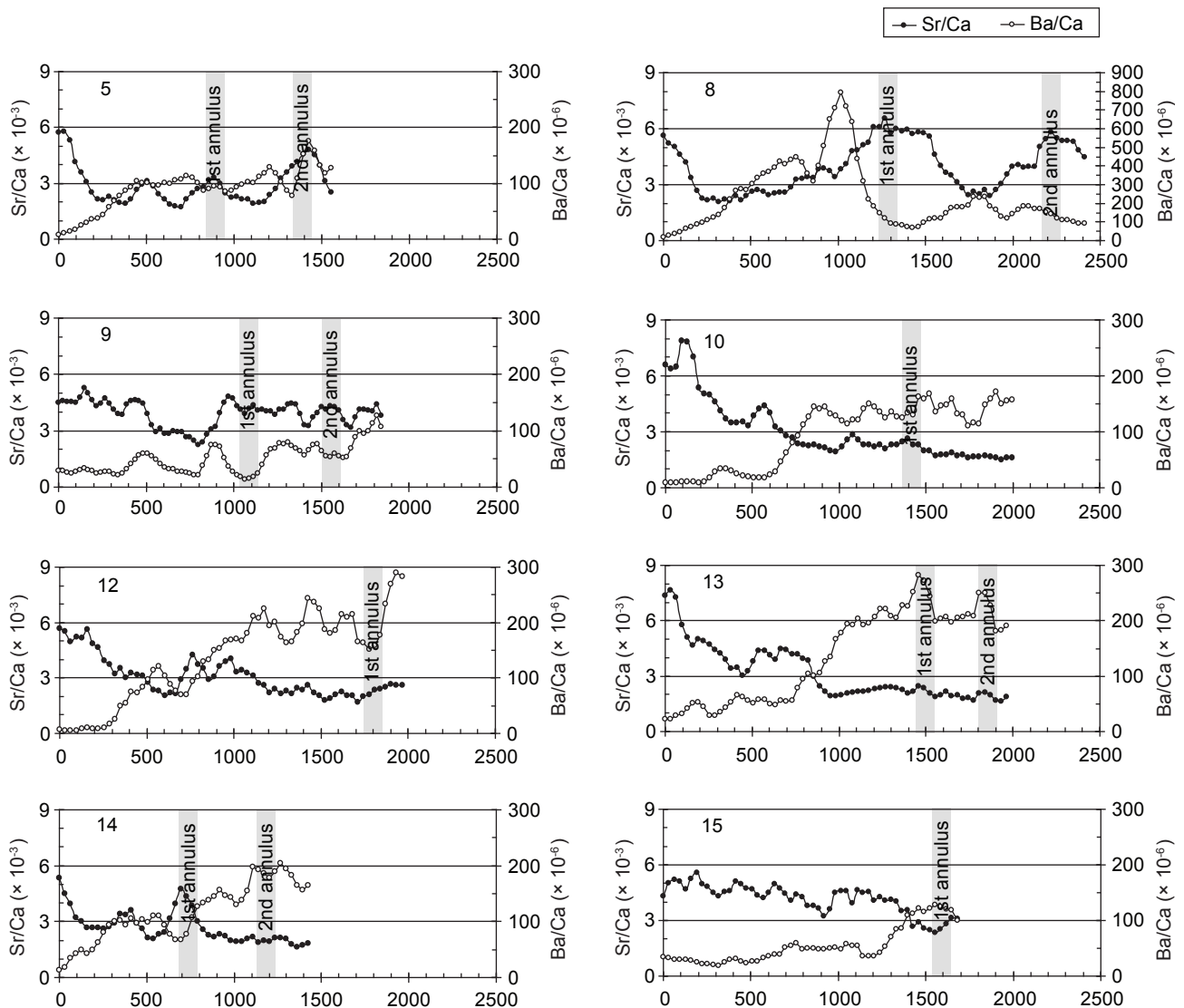


Fig. 6. Temporal changes in otolith Sr/Ca and Ba/Ca ratios for migratory type II *Mugil cephalus* collected from the Tanshui River.

spawning population of *M. cephalus* may explain the appearance of mullet juveniles in estuaries between autumn and the following spring, while juveniles of the traditional spawning population are only recruited during midwinter in northern and southern waters of Taiwan (Chang et al. 2000, Hsu et al. 2007). Our new discovery of different otolith core signatures implies that another spawning population of *M. cephalus*, with different life history traits, exists in the waters of Taiwan and East Asia. Elliott and Dewailly (1995) indicated that *M. cephalus* spend their entire lives in the estuary and do not enter offshore waters. Our results indicated that *M. cephalus* has more-diverse spawning and nursery ground behaviors than expected.

The migratory environmental history of freshwater resident mullet

By analyzing temporal changes in the otolith Sr/Ca and Ba/Ca ratios of freshwater resident mullet, 2 migratory types were found (Figs. 5, 6). Type I mullet (27%) contained high Sr/Ca and low Ba/Ca ratios, indicating that those fish mostly inhabited marine environments. Type II mullet (73%) had fluctuating Sr/Ca and Ba/Ca ratios beyond the core region, indicating that individuals stayed in freshwater and estuarine habitats and never returned to the marine environment again after the juvenile stage. Migratory environmental histories of mullet caught in estuarine, nearshore, and offshore waters were studied by Chang et

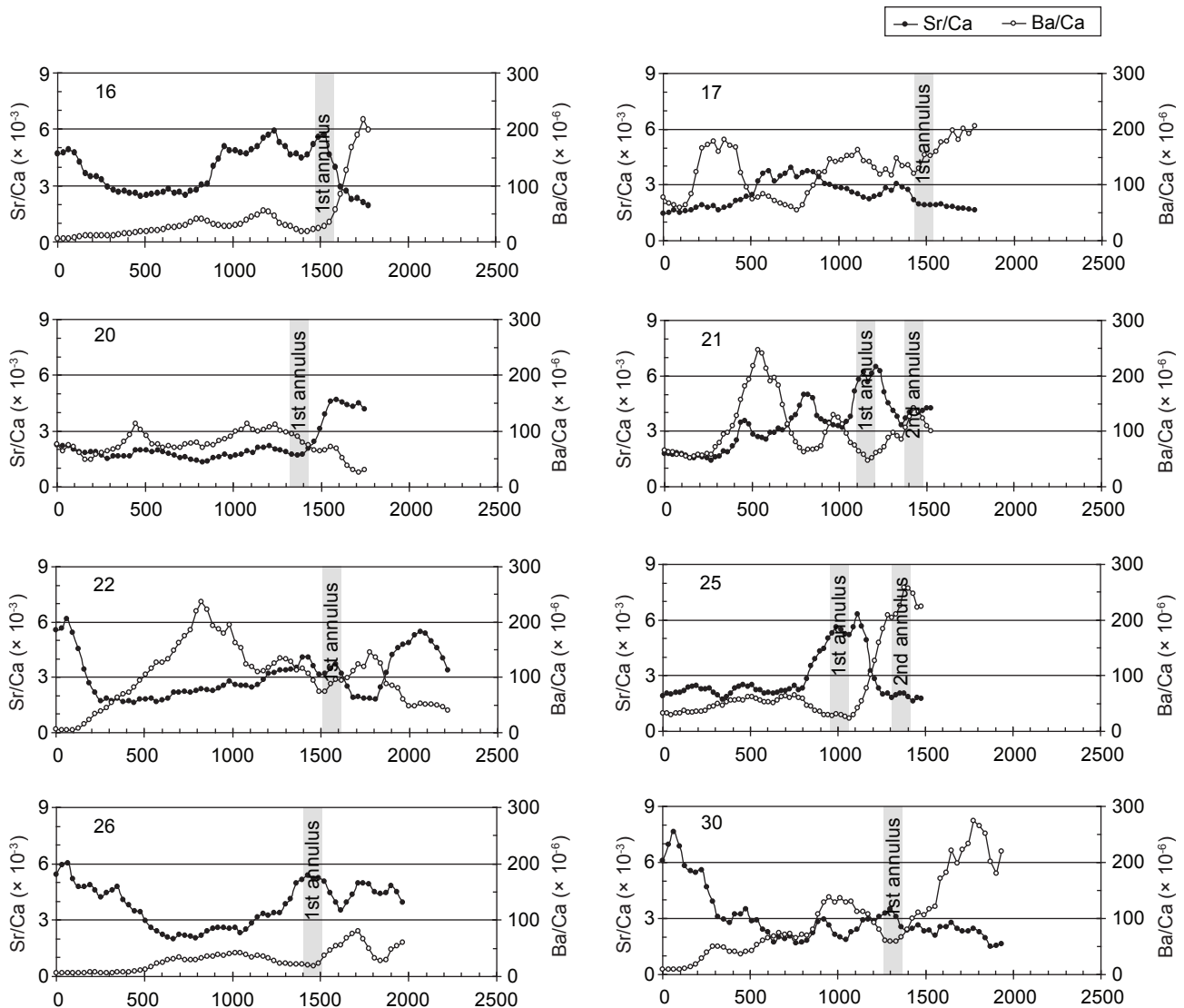


Fig. 6. (continued)

al. (2004a), and similar migratory types were identified. However, mullet from high-salinity water comprised 67% of type I and only 33% of type II. If only the traditional spawning population caught during midwinter was considered, an even lower percentage of type II remained (11.1%) (Chang et al. 2004a). In our study, mullet collected in fresh water were predominantly type II (estuarine and freshwater), and some individuals showed a different pattern in the otolith core (group 2 in figure 4). This indicates that there is a mixture of migratory and resident mullet near Taiwan. Although most individuals recruited from the southeastern Taiwan spawning area may return to the sea after the juvenile phase, a resident mullet population may exist that inhabits estuarine and freshwater habitats and seldom returns to the sea. Juveniles may reside in estuaries and freshwater rivers around Taiwan and not join the northerly maturation migration. Thus the migratory patterns of freshwater mullet may differ from those of nearshore and offshore areas.

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