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Migratory environmental history of the grey mullet *Mugil cephalus* as revealed by otolith Sr:Ca ratios

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ABSTRACT: We used an electron probe microanalyzer (EPMA) to determine the migratory environmental history of the catadromous grey mullet *Mugil cephalus* from the Sr:Ca ratios in otoliths of 10 newly recruited juveniles collected from estuaries and 30 adults collected from estuaries, nearshore (coastal waters and bay) and offshore, in the adjacent waters off Taiwan. Mean (±SD) Sr:Ca ratios at the edges of adult otoliths increased significantly from $6.5 \pm 0.9 \times 10^{-3}$ in estuaries and nearshore waters to $8.9 \pm 1.4 \times 10^{-3}$ in offshore waters ($p < 0.01$), corresponding to increasing ambient salinity from estuaries and nearshore to offshore waters. The mean Sr:Ca ratios decreased significantly from the core ($11.2 \pm 1.2 \times 10^{-3}$) to the otolith edge ($6.2 \pm 1.4 \times 10^{-3}$) in juvenile otoliths ($p < 0.001$). The mullet generally spawned offshore and recruited to the estuary at the juvenile stage; therefore, these data support the use of Sr:Ca ratios in otoliths to reconstruct the past salinity history of the mullet. A life-history scan of the otolith Sr:Ca ratios indicated that the migratory environmental history of the mullet beyond the juvenile stage consists of 2 types. In Type 1 mullet, Sr:Ca ratios range between 4.0×10^{-3} and 13.9×10^{-3}, indicating that they migrated between estuary and offshore waters but rarely entered the freshwater habitat. In Type 2 mullet, the Sr:Ca ratios decreased to a minimum value of 0.4×10^{-3}, indicating that the mullet migrated to a freshwater habitat. Most mullet beyond the juvenile stage migrated from estuary to offshore waters, but a few mullet less than 2 yr old may have migrated into a freshwater habitat. Most mullet collected nearshore and offshore were of Type 1, while those collected from the estuaries were a mixture of Types 1 and 2. The mullet spawning stock consisted mainly of Type 1 fish. The growth rates of the mullet were similar for Types 1 and 2. The migratory patterns of the mullet were more divergent than indicated by previous reports of their catadromous behavior.

KEY WORDS: *Mugil cephalus* · Otolith · Sr:Ca ratio · Migration · Environmental history

INTRODUCTION

Grey mullet (*Mugil cephalus* Linnaeus, 1758) are distributed circumglobally between 42° N and 42° S, and are an economically important species for both commercial fisheries and aquaculture because their roes have a high culinary value (Thomson 1966, Nash & Shehadeh 1980). The adult mullet migrates annually from the coastal waters of mainland China to the offshore waters of SW Taiwan to spawn during the winter (December through January) when 3 to 4 yr old (Tung 1981, Chen & Su 1986, Huang & Su 1989). Their eggs and larvae drift with the coastal current to estuaries along the SW to NE coasts of Taiwan where they become juveniles at the age of 1 to 2 mo post-hatching (Tung 1981, Chang & Tzeng 2000, Chang et al. 2000).

Current knowledge of the migratory history of the mullet has been interpreted fragmentally from the spatio-temporal distribution of the migrating spawners and estuarine-recruited juveniles. The lifetime migratory history, especially between estuarine arrival and spawning migration, is not completely understood.

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Tung (1981) speculated that all the juveniles and adults after spawning might migrate northward to the coastal waters of mainland China to feed until the spawning migration. Liu (1986) postulated that a few juveniles did not migrate northward but resided in the coastal waters around Taiwan. Chen et al. (1989) and Lee (1992) also found immature mullet of various sizes in estuaries and bays of Taiwan. The mullet was also believed to be catadromous, migrating from estuarine brackishwaters to high salinities offshore for breeding (Breder & Rosen 1966, De Silva 1980, Torricelli et al. 1982). However, the definition of catadromy for the mullet remains controversial because its use of freshwater habitats is not clear (Blaber 1987, McDowall 1988). Thus, the determination of whether the mullet enters freshwater before spawning is critical to clarify the extent of its catadromy.

The fish otolith is composed of calcium carbonate deposited rhythmically as aragonite crystals in a protein matrix (Degens et al. 1969, Pannella 1971). Except for their physiological functions in balance and hearing, fish otoliths also function as a chronological and environmental recorder of the fishes' past life history (Campana & Neilson 1985, Campana 1999). The ratios of strontium (Sr) to calcium (Ca) in otoliths are positively correlated to ambient salinities (Secor et al. 1995, Tzeng 1996, Kawakami et al. 1998). The temporal changes of Sr:Ca ratios in otoliths have been widely applied to determine the migratory environmental histories of diadromous fishes between freshwater and marine environments, including anadromous salmonids (Kalish 1990, Howland et al. 2001, Limburg et al. 2001), striped bass (Secor 1992, Secor & Piccoli 1996) and catadromous freshwater eels (Tzeng 1994, 1995, 1996, Tzeng & Tsai 1994, Tzeng et al. 1997, 1999, 2000, 2002a, 2003, Tsukamoto & Arai 2001, Jessop et al. 2002). Accordingly, the analysis of Sr:Ca ratios in otoliths, in combination with age data, makes possible the reconstruction of the migratory environmental history of the mullet and the clarification of its freshwater habitat use.

The aims of the present study were: (1) to validate whether otolith Sr:Ca ratios could be used to determine retrospectively the migratory environmental history of the mullet, (2) to elucidate its lifetime migratory environmental history, particularly freshwater habitat use, and (3) to reconsider the definition of catadromy as applied to the mullet.

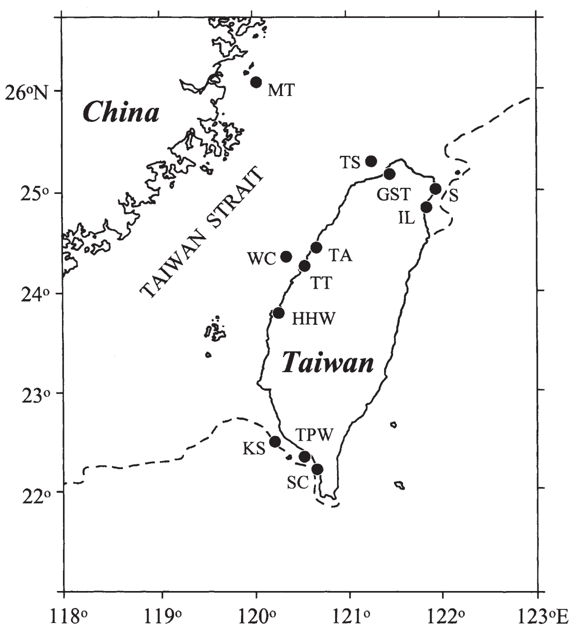

Fig. 1. *Mugil cephalus*. Sampling sites of grey mullet. Estuary = IL: I-lan River; S: Shuang Creek; GST: Gong-shy-tyan Creek; TA: Ta-an Creek; TT: Ta-tu River; HHW: Hsin-hu-wei Creek; SC: Szu-chung Creek. Nearshore = MT: Ma-tzu; TPW: Ta-peng-wan Bay. Offshore = TS: Tan-shui; WC: Wu-chi; KS: Kan-shan. Dashed line: 200 m depth contour

MATERIALS AND METHODS

Sampling design. We collected 10 newly recruited juvenile mullet from the estuaries of Shuang Creek (S), Ta-an Creek (TA) and Szu-chung Creek (SC) on the northern and western coasts of Taiwan from December 1998 through February 1999 (Fig. 1, Table 1).

In addition, 30 adults were collected from the estuaries, nearshore (coastal waters and bay) and offshore across the dispersal range of the fish (Fig. 1). Of these, 15 were collected from the estuaries of I-lan River (IL), Gong-shy-tyan Creek (GST), Ta-tu River (TT) and Hsin-hu-wei Creek (HHW); 3 were taken from

Table 1. *Mugil cephalus*. Biological characteristics of juveniles used for otolith Sr:Ca ratio analysis

Site, Sampling date	Fork length (mm)	Weight (g)
Shuang Creek (S)		
Dec 23, 1998	25.32	0.13
Jan 24, 1999	30.31	0.27
Feb 22, 1999	32.98	0.33
Ta-an Creek (TA)		
Dec 25, 1998	31.40	0.25
Jan 25, 1999	31.87	0.25
Feb 25, 1999	35.79	0.47
Feb 25, 1999	33.76	0.36
Szu-chung Creek (SC)		
Dec 11, 1998	24.83	0.14
Jan 17, 1999	28.66	0.20
Feb 9, 1999	24.09	0.12

Ta-peng-wan bay (TPW) on the NE and western coasts of Taiwan; 3 were collected from coastal waters influenced by freshwater runoff off Ma-tzu (MT) on the SE coast of mainland China; and 9 were collected during their spawning migration offshore of Tan-shui (TS), Wu-chi (WC) and Kan-shan (KS) on the western coast of Taiwan. Sampling sites and dates, sex, length, weight, gonadosomatic index and age of the mullet are given in Table 2.

Salinities at the sampling sites were obtained from Su & Jane (1974), Anonymous (1998), Cheng et al. (1990) and Tzeng et al. (2002b).

Otolith preparation and microprobe analysis. Sagittal otoliths of the mullet were used for the measurement of Sr and Ca concentrations. The otoliths were extracted, cleaned, air-dried and embedded in epoxy resin (Epofix, Struers). The lateral view of the mullet otolith is oval in shape in the juvenile stage but becomes oblong in the adult stage. The otolith grows fastest along the antero-posterior axis, moderately along the dorso-ventral axis and slowest along the disto-proximal axis. The otoliths were polished along the sagittal plane for juveniles, but along the frontal plane for adults because the adult otolith curves and elongates dorsally and is concave distally and convex proximally (Fig. 2a). Each otolith was polished using a Metaserv grinder-polisher (Buehler) equipped with various grit sandpapers and 0.05 µm alumina slurry and polishing cloth. After polishing, the otoliths were rinsed with deionized water, air-dried and coated with carbon.

The concentrations of Sr and Ca in the otoliths were measured with an electron probe microanalyzer, EPMA (JXA-8900R, JEOL) from the primordium to the otolith edge along the posterior maximum growth axis. The beam condition of the EPMA was similar to that used by Tzeng et al. (2002a), but the beam currents, beam sizes, intervals and peak counting times were differed for juveniles (3 nA, 5 µm, 10 µm and 120 s, respectively, for Sr) and adults (5 nA, 10 µm, 20 µm and

Table 2. *Mugil cephalus*. Biological characteristics of adults used for otolith Sr:Ca ratio analysis. Sampling sites abbreviated as in Fig. 1. FL: fork length; Wt: weight; GSI: gonadosomatic index; –: sex undetermined

Code	Site	Sampling date	Sex	FL (mm)	Wt (g)	GSI	Age (yr)
Estuary							
E1	IL	May 24, 2000	–	207	109	<0.01	1+
E2	IL	May 24, 2000	–	292	324	<0.01	2+
E3	IL	Nov 23, 2000	♂	297	296	2.80	1+
E4	IL	Nov 23, 2000	♀	393	549	15.16	4+
E5	GST	Dec 29, 1997	–	235	168	<0.01	1+
E6	GST	Jun 23, 1998	–	237	149	<0.01	1+
E7	GST	Oct 30, 1997	–	376	730	0.22	3+
E8	GST	Oct 8, 1998	♀	423	940	0.34	4+
E9	TT	Feb 27, 1998	♂	268	270	<0.01	1+
E10	TT	Feb 27, 1998	♂	292	364	0.03	2+
E11	TT	Feb 27, 1998	♂	333	485	<0.01	2+
E12	TT	Feb 27, 1998	♀	376	626	0.28	3+
E13	HHW	May 15, 2001	–	72	4	<0.01	0+
E14	HHW	May 15, 2001	–	91	8	<0.01	0+
E15	HHW	May 15, 2001	–	269	216	<0.01	1+
Nearshore							
N1	MT	Apr 19, 1998	♀	424	934	0.48	3+
N2	MT	Apr 19, 1998	♀	468	1147	1.29	4+
N3	MT	Apr 19, 1998	♀	471	1061	0.96	5+
N4	TPW	Dec 15, 1997	♂	260	215	0.02	1+
N5	TPW	Dec 15, 1997	♂	280	308	0.02	1+
N6	TPW	Dec 15, 1997	♂	440	1120	0.03	5+
Offshore							
O1	TS	Dec 16, 1997	♂	421	1000	0.32	4+
O2	TS	Dec 16, 1997	♀	422	945	11.57	4+
O3	TS	Dec 16, 1997	♀	446	1200	0.93	4+
O4	WC	Dec 25, 1997	♀	453	1198	11.82	4+
O5	WC	Dec 25, 1997	♀	488	1321	14.09	4+
O6	WC	Dec 25, 1997	♀	491	1466	12.58	5+
O7	KS	Jan 12, 1997	♀	448	1254	20.08	4+
O8	KS	Jan 12, 1997	♂	481	1309	8.70	5+
O9	KS	Jan 12, 1997	♀	500	1525	2.45	5+

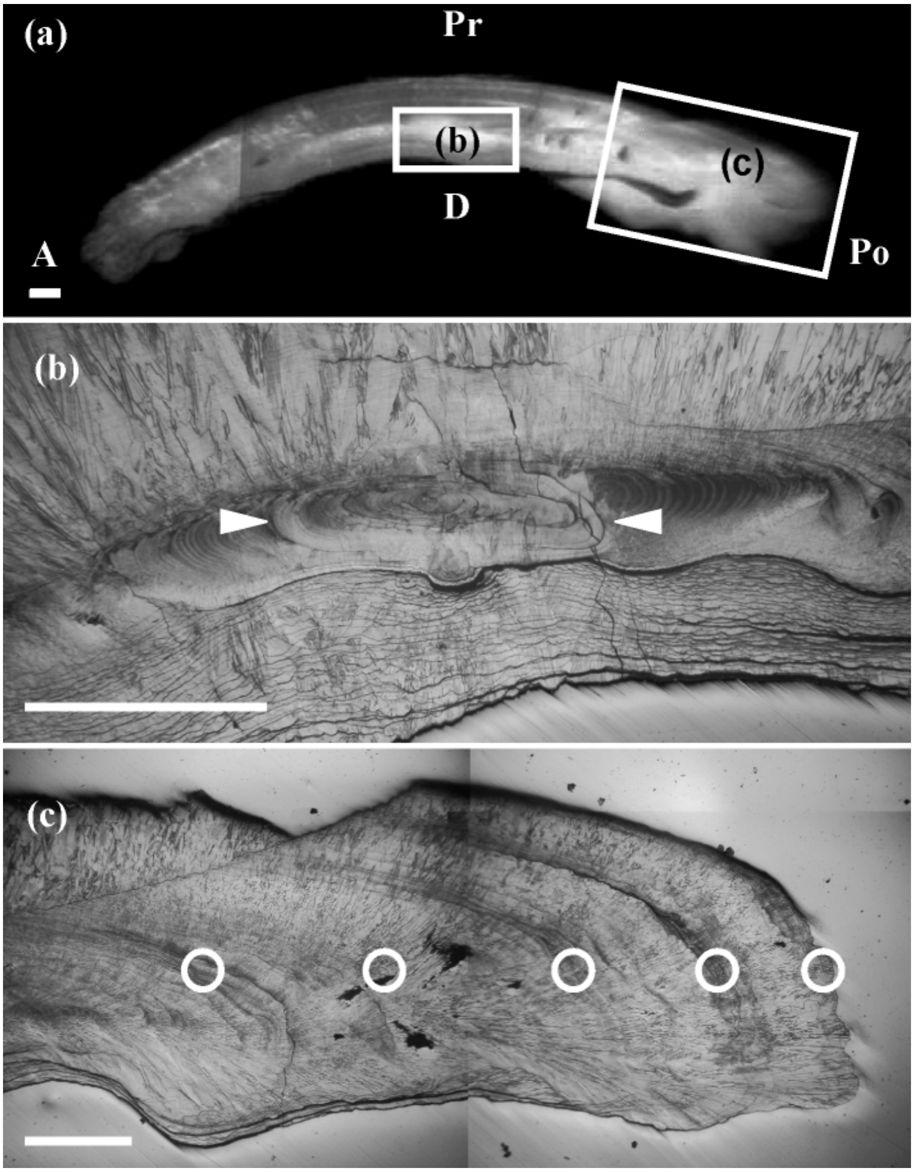

Fig. 2. *Mugil cephalus*. Frontal section of otolith photographed with reflected light. (a) Whole otolith; (b) estuarine mark (arrowheads) (c) annuli (circled); (b) and (c) magnified from (a). A: anterior; Po: posterior; D: distal; Pr: proximal. Scale bar = 500 µm

90 s, respectively, for Sr). Strontianite ($SrCO_3$; National Museum of Natural History [NMNH] R10065) and calcite ($CaCO_3$; NMNH 136321) were used as standards for the calibration of Sr and Ca concentrations in otoliths (Jarosewich & White 1987). The quantitative data were calculated by the ZAF method (Z: atomic number; A: absorption; F: fluorescence correction; Philibert & Tixier 1968) and Ca was used to normalize the concentration of Sr. The temporal changes in Sr:Ca ratios in the otoliths were similar with the same beam condition (e.g. see Mullet Nos. E11, N2, N6 and O6 in Fig. 5) and similar with different beam conditions for the same otolith (e.g. see E15 in Fig. 5 and E13 and 14 in Fig. 6). This indicates that the measurements of Sr:Ca ratios in the mullet otoliths were stable and repeatable.

After electron probe analysis, the otoliths were repolished to remove the carbon coating and etched with 5% ethylenediaminetetraacetate (EDTA) to reveal annulus marks for age determination. The otolith Sr:Ca ratios were correlated with life history events including estuarine arrival and annulus deposition.

Data analysis. The migratory environmental histories of the adult mullet were classified into 2 types according to age and the temporal changes in the Sr:Ca ratios in the otoliths. Differences in frequency distributions among habitats and among migration patterns for the different mullet environmental history types were examined by a *G*-test (Siegel 1956). Differences in otolith mean radius and Sr:Ca ratios among life stages, age groups and salinity habitations were tested by analysis of variance (ANOVA) and a Tukey multiple-comparison test at a significance level of $\alpha = 0.05$ (Winer 1971).

In addition, the growth curve of the mullet was fitted with the von Bertalanffy growth equation (Ricker 1958). The mean lengths at annulus formation were compared between migratory types by a *t*-test.

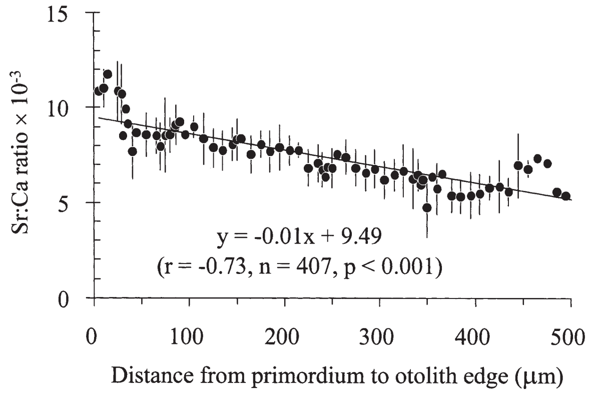

Fig. 3. *Mugil cephalus*. Changes in mean otolith Sr:Ca ratios from core to otolith edge of 10 juveniles. Vertical lines = ±SD

RESULTS

Otolith microstructure

Estuarine checks and annuli in otoliths from adult mullet were discernible with reflected light after EDTA etching (Fig. 2b,c). The mean (±SD) radius of the estuarine check in adult mullet otoliths was 435.4 ± 76.8 µm (range 318.6–595.0 µm). The radius of the estuarine check in adult otoliths was close to the mean (±SD) otolith radius of newly recruited juveniles (387.0 ± 73.9 µm, range 280.0–510.0 µm, Table 3). Thus, the estuarine check in otoliths of the adult mullet are deposited during their estuarine arrival at the juvenile stage and can be used as a marker to identify the estuarine arrival of the adult.

Otolith Sr:Ca ratios in relation to habitat salinity

The Sr:Ca transect pattern indicated that Sr:Ca ratios in the otoliths of juvenile mullet significantly decreased from the core to the otolith edge ($r = -0.73$, $p < 0.001$, Fig. 3). The mean (±SD) Sr:Ca ratios in the core of the otolith ($11.2 \times 10^{-3} \pm 1.2 \times 10^{-3}$) were significantly greater than that in otolith edge ($6.2 \times 10^{-3} \pm 1.4 \times 10^{-3}$, $p < 0.001$, Table 3). The decrease in Sr:Ca ratios from core to edge of the otolith was consistent with larval dispersal from the high-salinity offshore spawning ground to the low-salinity estuarine nursery ground.

The Sr:Ca ratios at the estuarine check of the adult mullet otoliths averaged $5.1 \times 10^{-3} \pm 1.0 \times 10^{-3}$, did not differ significantly among sampling sites, and were similar to those at the juvenile otolith edge ($6.2 \times 10^{-3} \pm 1.4 \times 10^{-3}$; Table 3). This indicates that the adult mullet probably migrated to a low-salinity estuary at the juvenile stage.

The mean salinity in the sampling sites changed from 23.5‰ (range from 1.0 to 34.9‰) in the estuaries and 23.9‰ (14.3–33.5‰) in the nearshore coastal zone to 34.4‰ (34.0–34.8‰) in the offshore (Fig. 4a). Maximum salinities were similar among sampling sites, but their mean values increased from estuary and nearshore to offshore. Similarly, the mean (±SD) Sr:Ca

Table 3. *Mugil cephalus*. Mean (±SD) radius and Sr:Ca ratios at otolith core and edge for juveniles and of estuarine check and annulus (A_{1-5}) of otoliths for adults. HG: homogeneous group; same letters indicate that mean values were homogeneous among areas

Otolith area	n	Otolith radius (µm) Range	Mean ± SD	CV	HG	Sr:Ca ratio × 10^{-3} Range	Mean ± SD	CV	HG
Juveniles									
Core	10	11.3–16.3	14.5 ± 1.6	10.7	a	9.4–13.5	11.2 ± 1.2	10.5	d
Edge	10	280.0–510.0	387.0 ± 73.9	19.1	b	3.9–8.0	6.2 ± 1.4	22.9	a
Adults									
Check	30	318.6–595.0	435.4 ± 76.8	17.6	b	2.9–7.0	5.1 ± 1.0	20.3	a
A_1	28	2840.0–4030.0	3421.1 ± 349.5	10.2	c	3.6–10.7	7.9 ± 1.8	23.2	b
A_2	20	3680.0–4760.0	4283.0 ± 283.6	6.6	d	5.3–11.2	8.6 ± 1.6	18.9	bc
A_3	17	4260.0–5220.0	4927.1 ± 266.8	5.4	e	7.7–13.9	10.0 ± 1.5	15.2	cd
A_4	14	4600.0–5900.0	5383.6 ± 331.5	6.2	f	5.7–12.4	9.7 ± 1.7	17.8	cd
A_5	5	5600.0–6280.0	5864.0 ± 259.8	4.4	g	8.1–11.7	9.8 ± 1.4	14.0	bcd

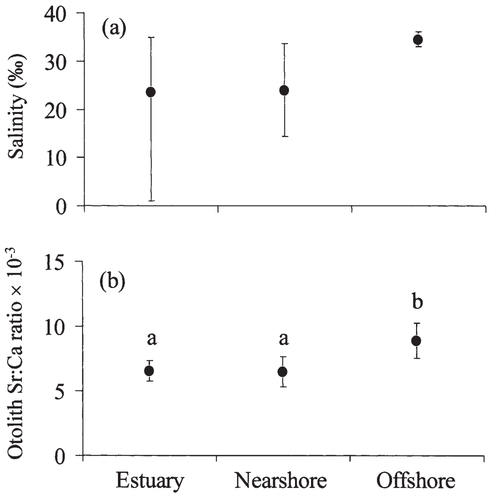

Fig. 4. *Mugil cephalus*. Mean (±SD) salinity and the mean (±SD) Sr:Ca ratios at otolith edge of adults collected in estuaries (i.e. Sites IL, GST, TT, HHW), nearshore coastal zone (Sites TPW, MT) and offshore (Sites TS, WC, KS) (site abbreviations as in Fig. 1). Same letters above data points indicate that mean Sr:Ca ratios at otolith edge were homogeneous among designated sites

ratios at the otolith edge of the adult mullet of ≥3 yr old significantly increased from $6.5 \times 10^{-3} \pm 0.8 \times 10^{-3}$ (sample size, n = 4) in the estuaries (Sites IL, GST, TT, HHW) and $6.5 \times 10^{-3} \pm 1.2 \times 10^{-3}$ (n = 4) nearshore (Bay Site TPW and Coastal Site MT) to $8.9 \times 10^{-3} \pm 1.4 \times 10^{-3}$ (n = 9) offshore (Sites TS, WC, KS) ($p < 0.01$, Fig. 4b). The correspondence between the Sr:Ca ratios at the edge of adult mullet otoliths and the ambient salinity between estuary and offshore sites indicates that the use of Sr:Ca ratios in mullet otoliths to reconstruct their migratory salinity history is reliable.

The upper and lower limits of the 95% confidence interval (CI) of the mean (±SD) Sr:Ca ratios of $5.1 \times 10^{-3} \pm 1.0 \times 10^{-3}$ at the estuarine check of the adult mullet otoliths were 3×10^{-3} and 7×10^{-3}, respectively, and were used as criteria to define the migratory environmental history of the mullet between freshwater and seawater. In other words, the fish was expected to have migrated to the high-salinity offshore sites if the otolith Sr:Ca ratios were greater than the upper limit of the CI, to have migrated to freshwater sites if the ratios were less than the lower limit of the CI, and to have migrated to brackish estuaries if the ratios fluctuated between the upper and lower limits of the CI.

Temporal changes in Sr:Ca ratios in adult otoliths

The migratory environmental history of the mullet was divided into 2 types based on temporal changes in otolith Sr:Ca ratios:

Type 1. Most Sr:Ca ratios beyond the estuarine check in the otoliths of Type 1 mullet adults fluctuated between 4×10^{-3} and 13.9×10^{-3} and were seldom less than 3×10^{-3} (Fig. 5), indicating that after the juvenile stage the mullet had migrated between estuary and offshore waters and had rarely entered freshwaters. The ages of Type 1 mullet ranged from 1 to 5 yr old, but most were aged 4 to 5 yr (Fig. 5, Table 2).

Type 2. Sr:Ca ratios beyond the estuarine check in the otoliths of Type 2 adult mullet fluctuated between 0.4×10^{-3} and 12.5×10^{-3} (Fig. 6), indicating that they had migrated between freshwater and offshore waters. The recruitment time of the mullet to freshwater differed among individuals, lasting from 1 to 2 yr. In some cases, the mullet all directly entered freshwater after estuarine arrival (e.g. Mullet Nos. E2, 5, 13, 14, and N1 and O4 in Fig. 6). Among these, Mullet E2 and O4 remained in freshwater for approximately 1 yr and then migrated to higher-salinity waters, whereas Mullet N1 remained in freshwater for a short time only (ca. 1–2 mo) and then migrated to higher salinities. Mullet E5 migrated between freshwater and brackishwater in the first year. Mullet E13 and E14 remained in freshwater after estuarine arrival until they were captured. In other cases, the mullet delayed entering freshwater after estuarine arrival (e.g. Mullet Nos. E1, 6, 9, 10 in Fig. 6). Among these, Mullet E1 and E10 resided in brackish or highly saline waters for approximately 6 mo and then migrated between freshwater and brackishwaters. Mullet E6 and E9 had migrated between brackish and high-salinity waters for 1 yr and then entered freshwater. Thus, the Type 2 mullet migrates to freshwater after the juvenile stage, but the duration of freshwater residence differs among individuals.

Composition of migratory patterns

The composition of Types 1 and 2 mullet differed significantly among habitats ($p < 0.001$, Table 4). The pro-

Table 4. *Mugil cephalus*. Percent composition of Types 1 and 2 as a function of migratory status and sampling sites. Site abbreviations as in Fig. 1

Season, Sites	n	Composition (%) Type 1	Type 2
Non-spawning season			
Estuary (IL, GST, TT, HHW)	15	46.7	53.3
Nearshore (MT, TPW)	6	83.3	16.7
Spawning season			
Offshore (TS, WC, KS)	9	88.9	11.1
Total	30	66.7	33.3

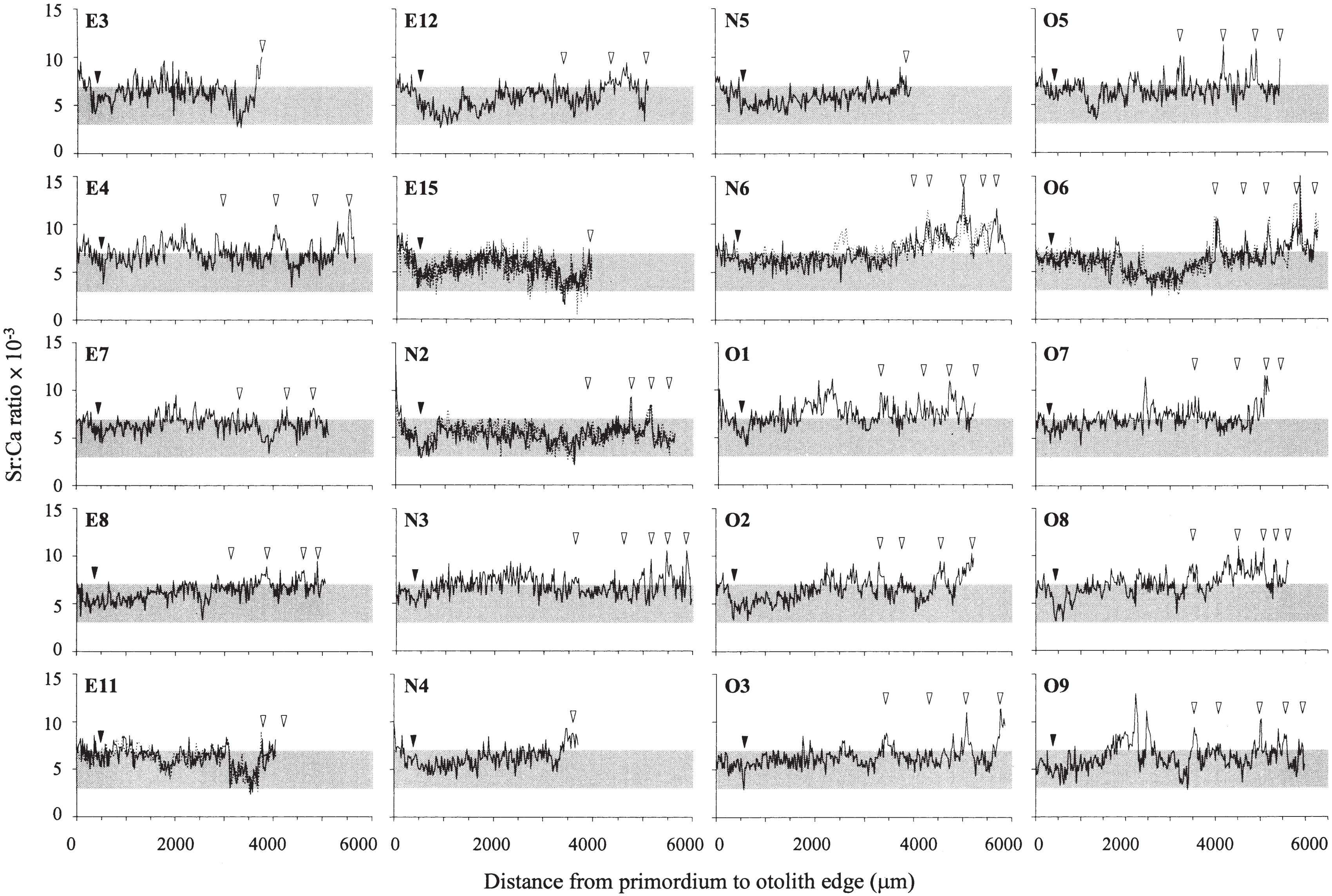

Fig. 5. *Mugil cephalus.* Changes in Sr:Ca ratios from core to otolith edge in otoliths of Type 1 adults. (▼) estuarine check; (▽) annuli. Sr and Ca concentrations in Mullet Nos. E11, N2, 6 and O6 were measured for fish from 2 transects with the same electron probe microanalyzer (EPMA) beam conditions and those in Mullet E15 were with different beam conditions. Grey bands between the Sr:Ca ratios 3 to 7×10^{-3} indicate migration into brackishwaters

portion of Type 1 mullet was significantly higher in the nearshore (83.3%) and offshore (88.9%) sites irrespective of season (spawning or non-spawning), but the proportion of Type 2 mullet was slightly higher in the estuaries (53.3%) during non-spawning season. In other words, the proportion of mullet entering freshwaters (Type 2) was lower for the nearshore and offshore populations than the estuarine population.

Conversely, the proportion of Type 1 mullet (88.9%) was higher than Type 2 during the spawning season (11.1%, Table 4) suggesting that the spawning stock of the mullet was mostly composed of Type 1 fish that did not enter freshwater.

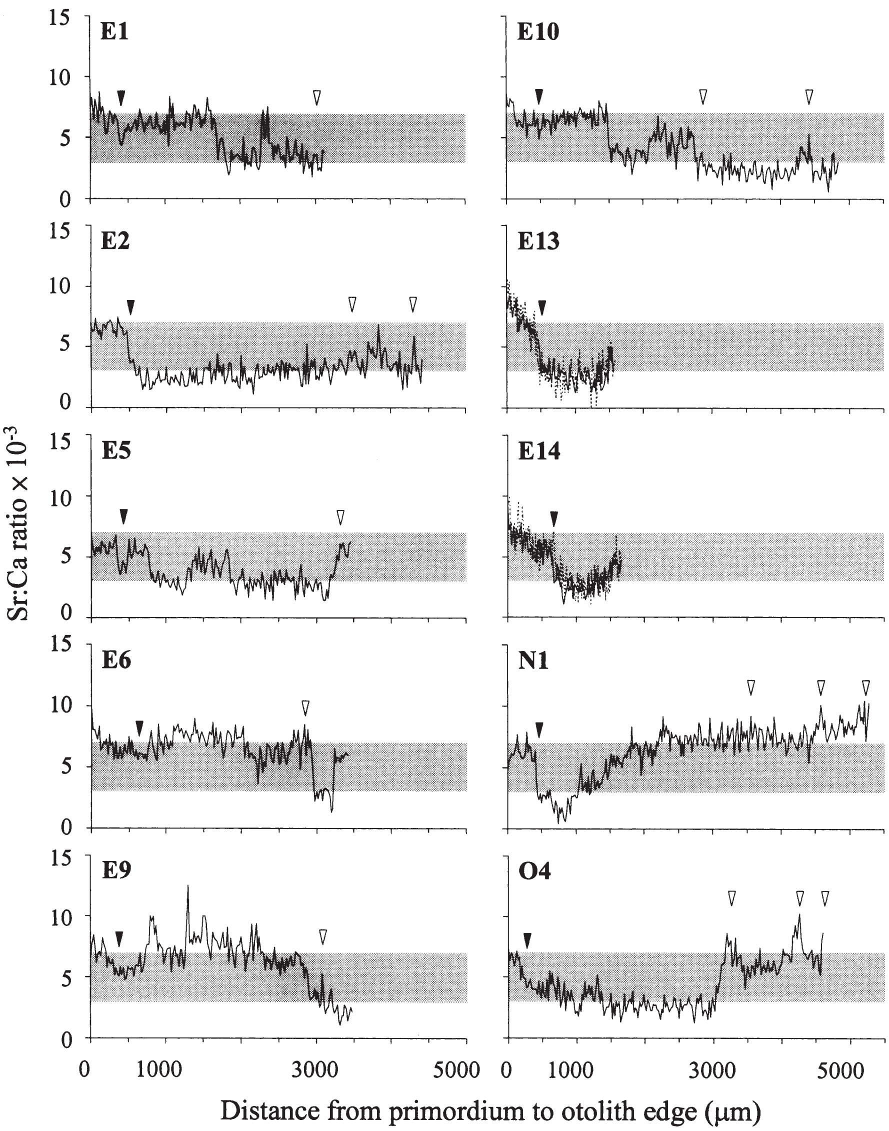

Fig. 6. *Mugil cephalus*. Changes in Sr:Ca ratios from core to otolith edge in the otoliths of Type 2 adults. (▼) estuarine check; (▽) annuli. Sr and Ca concentrations in Mullet Nos. E13 and E14 were measured for fish from 2 transects with different electron probe microanalyzer (EPMA) beam conditions. Grey bands between the Sr:Ca ratios 3 to 7×10^{-3} indicate migration in brackishwaters

Age-related habitat use

The habitat use of the mullet changed with age (Fig. 7). At Age 1 yr, about 26.7% of mullet inhabited freshwater; this decreased to 10.5% at Age 2 yr, and to none at Age 3 yr and older. In contrast, the proportion of mullet inhabiting offshore waters increased from 13.3% in the first year to 41.7% in the fifth year. Thus, a small portion of mullet inhabited freshwater and the number of mullet migrating offshore from freshwaters and the estuary increased with age.

Beyond the estuarine check in adult otoliths, the peak Sr:Ca ratios corresponded to annuli (Figs. 5 & 6). Mean (±SD) Sr:Ca ratios at the annuli ranged from $7.9 \pm 1.8 \times 10^{-3}$ to $10.0 \pm 1.5 \times 10^{-3}$ and were significantly greater than at the estuarine check in otoliths of both adult ($5.1 \pm 1.0 \times 10^{-3}$) and juvenile mullet ($6.2 \pm 1.4 \times 10^{-3}$, Table 3). The frequency distribution of peak Sr:Ca ratios could be roughly divided into 2 groups, one comprising the first and second annuli and the other the third to fifth annuli. The mean peak Sr:Ca ratios were larger in the latter than in the former group (Fig. 8). The mullet presumably tended to migrate to higher-salinity offshore waters after their third year.

Growth

The maximum otolith radius and fork length of the mullet were linearly correlated (Fig. 9), indicating that otolith growth reflects the somatic growth of the fish. The mean radius of the estuarine check and the successive annuli in otoliths of the adult differed significantly (Table 3). Annual increments within the otoliths were largest (ca. 3500 μm) within the first year and decreased dramatically to ca. 850 μm within the second year and ca. 650 μm to <500 μm within the third to fifth years, indicating that mullet growth was fastest in the first year and declined with age. The coefficient of variation (CV) values of the otolith radius at both the estuarine check and first annulus were larger than for succeeding annuli for adults, indicating greater variability of growth rate in the early life stage.

The mean lengths of the mullet at annulus formation were not significantly different between migratory history types (Fig. 10). This indicated that migratory environments did not affect growth rates of the mullet. The age–length data of different migratory types were combined and a theoretical growth curve was fitted with the von Bertalanffy growth equation as follows:

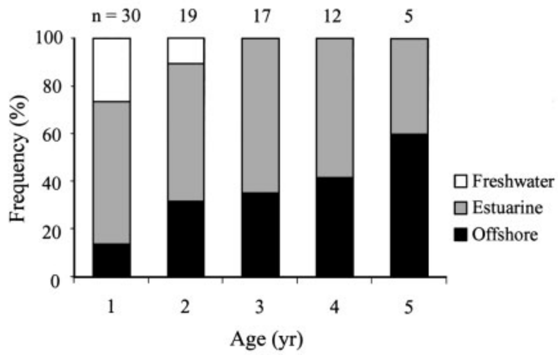

Fig. 7. *Mugil cephalus*. Frequency distribution of age-related habitat use. n = sample size

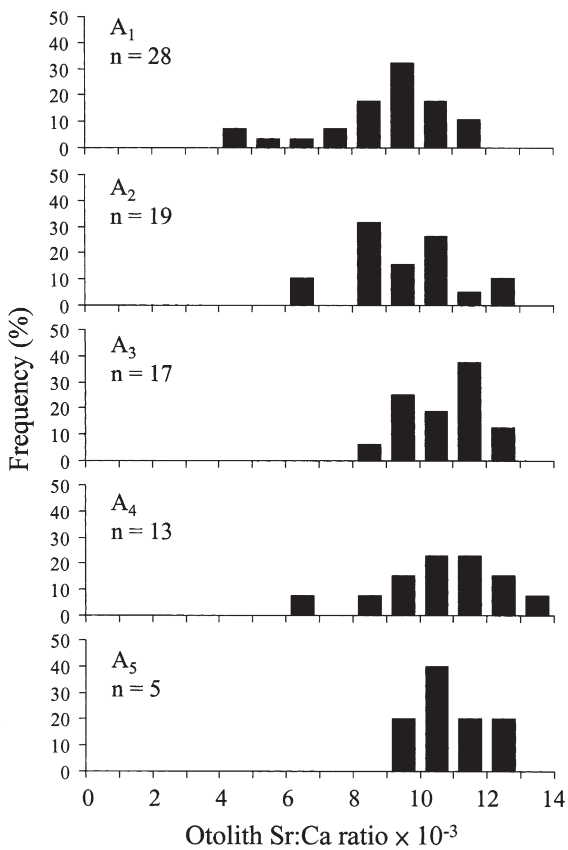

Fig. 8. *Mugil cephalus*. Frequency distribution of Sr:Ca ratios at annulus (A_{1-5}) in otoliths. n = sample size

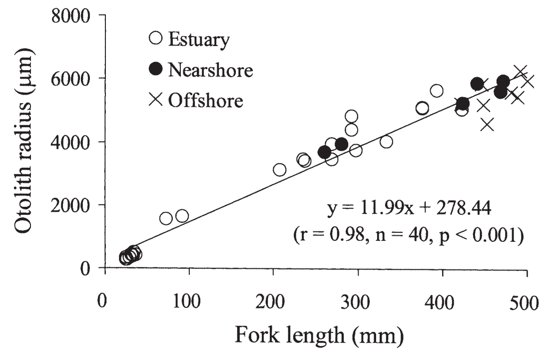

Fig. 9. *Mugil cephalus*. Relationship between fork length and otolith radius

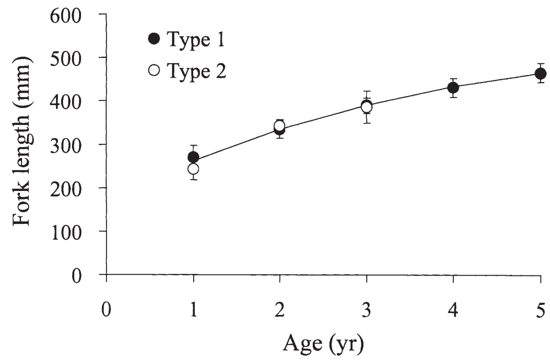

Fig. 10. *Mugil cephalus*. Relationship between fork length and age. Vertical lines = ±SD

$$L_t = 571.3\ (1 - e^{(-0.27[t + 1.29])})$$

where L_t was the theoretical length at age t, the maximum estimated length was 571.3 mm and the growth coefficient was 0.27 yr^{-1}.

DISCUSSION

Sr:Ca otolith ratios as indicator of environmental salinity

The Sr:Ca ratios in otoliths of both juvenile and adult mullet were positively correlated with the salinities of the sampling sites (Fig. 4). Thus, the Sr:Ca ratios in otoliths can be used to reconstruct the migratory environmental history of the mullet, as for other fishes (Tzeng & Tsai 1994, Secor et al. 1995, Secor & Rooker 2000, Limburg et al. 2001).

Based on our findings, the Sr:Ca ratios in otoliths of the mullet decreased to less than 3×10^{-3} when the fish migrated to freshwater, fluctuated between 3 and 7×10^{-3} in estuarine brackishwaters, and were greater than 7×10^{-3} in high-salinity offshore waters. Secor & Rooker (2000) found a similar relationship between otolith Sr:Ca ratios and environmental salinity. They divided estuarine fishes into freshwater (salinity range of 0 to 5‰; mean Sr:Ca ratios of 1.97×10^{-3}), estuarine (5 to 25‰; 5.03×10^{-3}) and marine (>25‰; 7.43×10^{-3}) habitats. Tsukamoto & Arai (2001) divided the Japanese eel *Anguilla japonica* into river, estuarine and sea eels when otolith Sr:Ca ratios were $<2.5 \times 10^{-3}$, between 2.5 and 6×10^{-3} and $>6 \times 10^{-3}$, respectively. Jessop et al. (2002) divided the American eel *A. rostrata* into freshwater and estuarine residents when otolith Sr:Ca ratios were $<4 \times 10^{-3}$ and $>5 \times 10^{-3}$, respectively. Tzeng et al. (2003) used the Sr:Ca ratio of 4×10^{-3} in otoliths of the Japanese eel as a criterion to distinguish freshwater and seawater contingents of the eel. Otolith Sr:Ca ratios relative to ambient salinity seem similar among different species suggesting that their use to reconstruct the past salinity history of a fish is reliable.

Sr:Ca otolith ratios and their implication in migratory history of the mullet

The migratory environmental histories of juvenile mullet collected in the estuaries and those of the adult mullet collected in the estuaries, nearshore and offshore, were reconstructed from the temporal changes of the Sr:Ca ratios in their otoliths. The Sr:Ca ratios in juvenile otoliths decreased from the core to the otolith edge (Fig. 3), indicating that the mullet larvae dispersed away from the high-salinity offshore spawning ground and grew to juveniles in the estuaries, as expected (Tung 1981, Chang et al. 2000). The Sr:Ca ratios beyond the estuarine check in the otoliths of Type 2 mullet (e.g. Mullet Nos. E2, 5, 13, 14, N1 and O4 in Fig. 6) indicated that the fish resided in freshwater in Taiwan during the first year. The Sr:Ca ratios at the edge of adult mullet otoliths collected in Taiwanese estuaries were lower (e.g. E1, 6, 9, 10 in Fig. 6), indicating that the mullet resided in freshwater after 1 yr. The temporal change in Sr:Ca ratios in otoliths of Type 2 mullet supports the hypothesis that some mullet did not migrate to the coastal waters of mainland China but resided in the coastal waters of Taiwan (Liu 1986).

The mullet migrated to the spawning ground at approximately 3 to 5 yr of age (Table 2). The Sr:Ca ratios in otoliths of mullet older than 3 yr were found to have oscillated annually, with a peak Sr:Ca ratio at the annulus check (Figs. 5 & 6). The annuli in otoliths of adult mullet are formed in the winter during spawning (Ibáñez-Aguirre & Gallardo-Cabello 1996). Thus, the peak Sr:Ca ratio and annulus in adult mullet otoliths were deposited simultaneously in the winter, as in the European eel (Tzeng et al. 1999). In other words, the fluctuations in the Sr:Ca ratios in adult mullet otoliths corresponded to their migration between feeding grounds in the low-salinity coastal waters of mainland China and the high-salinity offshore spawning ground off the SW coast of Taiwan, which is influenced by the Kuroshio Current in winter (Tung 1981, Huang & Su 1989). Also, a few adult mullet also showed a peak Sr:Ca ratio 1 and/or 2 yr of age (e.g. Mullet Nos. O5, 6, 8 in Fig. 5 and O4 in Fig. 6). Some immature mullet might migrate with the spawners to the spawning ground, as reported by Hwang (1986) and Huang & Su (1989).

On the other hand, the feeding activity of mullet may decrease and their growth rates become lower in the winter. The deposition rate of Sr in the otolith may be negatively correlated to both water temperature (Radtke 1989, Townsend et al. 1992, Tzeng 1994) and growth rate of the fish (Sadovy & Severin 1992). Therefore, the temperature and fish growth rate may interact with salinity to regulate the Sr:Ca ratios in mullet otoliths. Interactive effects of temperature and salinity on otoliths were also found for black bream *Acanthopagrus butcheri* (Elsdon & Gillanders 2002).

Divergent migratory behaviour of the mullet

The mullet was shown to comprise 2 coexisting migratory types in coastal waters of Taiwan (Table 4). Consequently, we do not completely agree with the hypothesis that the mullet is a catadromous fish spending most of its life in freshwater before migrating offshore for spawning (De Silva 1980, Torricelli et al. 1982).

Type 1 mullet is not a typical catadromous fish, because it does not migrate to freshwater but moves between estuarine and offshore waters (Fig. 5). One of the Type 2 mullet may have been catadromous, as are mullet in Australia (Thomson 1966) and the USA (Shireman 1975), which reside in freshwater before maturation at 3 to 4 yr of age (e.g. Mullet No. E10 in Fig. 6). Conversely, 2 of the Type 2 mullet emigrated from the freshwater habitat before 1 yr of age and did not remain in freshwater until maturation (i.e. N1 and O4 in Fig. 6) as do mullet in freshwaters of South Africa (Bok 1979) and western Australia (Chubb et al. 1981). Strictly, these 2 mullet could be only categorized as marine amphidromous because they emigrated before maturation (McDowall 1988). The remaining 7 mullet of the Type 2 mullet also could not be categorized as catadromous or amphidromous because they resided in freshwater only at an early stage (≤1 yr old). Thus, the migratory pattern of mullet is more plastic and complicated than previously thought. The term 'catadromy' is insufficient to represent the diverse migratory patterns of mullet, which seem to be more a diadromous continuum (McDowall 1988) in a sequential succession of 4 ecophenotypes, namely marine, euryhaline, amphidromous and catadromous, as in the directional evolution of diadromous fishes as proposed by Gross (1987).

Mullet can inhabit freshwater, estuarine and offshore waters, but tend to inhabit high-salinity offshore waters after maturation (Figs. 7 & 8). This phenomenon was also found in the Mediterranean grey mullet, in which the mature mullet prefer polyhaline areas and strongly avoid freshwater sites (Cardona 2000). Young mullet may have greater phenotypic plasticity and more divergent migratory patterns than older mullet. The migratory patterns of mullet collected from estuaries were more divergent than those from nearshore and offshore coastal areas (Table 4). The salinity was more varied and productivity was higher in the estuary than offshore (Haedrich & Hall 1976). The mullet is a euryhaline fish and can tolerate a wide range of salinity. Consequently, the divergent migratory behaviour of the mullet might be simply an expression of its euryhaline nature and/or an attempt to seek the maximum evolutionary fitness for foraging (Gross 1987).

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