Zoological Studies **47**(6): 676-684 (2008)

Zoological Studies

Differences in Otolith Elemental Composition of the Larval *Rhinogobius giurinus* (Perciformes, Gobiidae) among Estuaries of Taiwan: Implications for larval Dispersal and Connectance among Metapopulation

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(Accepted March 18, 2008)

Mei-Yu Chang, Wann-Nian Tzeng, Chia-Hui Wang, and Chen-Feng You (2008) Differences in otolith elemental composition of the larval *Rhinogobius giurinus* (Perciformes, Gobiidae) among estuaries of Taiwan: Implications for larval dispersal and connectance among metapopulations. *Zoological Studies* **47**(6): 676-684. Trace elements in otoliths can be used as biological tracers to identify fish populations, their migratory environmental histories, and larval origins because the elemental composition of otoliths of fish reflects that of the ambient water. The goby *Rhinogobius giurinus* is one of the dominant species in estuaries of western Taiwan. The seasonal occurrence of larvae of this goby was found to be delayed from south to north. To understand if gobies of different estuaries come from the same population, 89 larvae were collected from the estuaries of Gongshytyan Creek (GST), Tatu River (TT), and Tongkang Creek (TK) in western Taiwan during the period Mar.-Aug. 1998. Ages of the larval goby were examined by examining daily growth increments in otoliths, and the ratios of 12 elements with the Ca concentration of otoliths were analyzed by solution-based inductively coupled plasma mass spectrometry. The daily ages ranged 12-22 d, total lengths 6.37-7.64 mm, and growth rates 0.321-0.548 mm/d, and none of their means significantly differed among estuaries ($p > 0.05$). On the other hand, however, the majority of ratios of elements to Ca measured in otoliths of larvae significantly differed among estuaries and between months (one-way ANOVA, $p = 0.036$ to < 0.0001). Jackknife classification using otolith elemental composition indicated that 93.75% -100% of larvae could be successfully assigned to their original estuary and birth month. These results indicate that *R. giurnus* maintains self-sustaining populations with minimal connection among the estuaries of Taiwan examined.
http://zoolstud.sinica.edu.tw/Journals/47.6/676.pdf

Key words: Goby, Otolith, Elemental composition, Larval dispersal, Estuary.

Otolith (ear stone) is a biomineralized aragonite crystalline structure mainly composed of $CaCO_3$ with a minor organic substrate, and is used for balance and hearing in all teleost fishes. A number of elemental impurities (e.g., Na, Sr, K, Zn, Mg, Mn, Pb, Ba, etc.) have been found to be precipitated in the growth increments of otoliths during fish growth and may be affected by physiological and environmental factors (Campana 1999). In addition, growth increments of otoliths are deposited daily which allows determination of the age of larvae in days (Pannella 1971). Otoliths are metabolically inert, and once the trace element is deposited in the otolith, it presents a permanent record of the environmental conditions experienced by the fish at a particular time (Ruttenberg et

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al. 2005). Accordingly, trace elements together with age information of otoliths can be used as biological tracers, to track the dispersal and origin of fish larvae and the connectance among metapopulations (Rieman et al. 1994, Tzeng and Tsai 1994, Campana et al. 1995, Fowler et al. 1995, Kennedy et al. 1997 2000 2002, Secor et al. 2001, Gillanders et al. 2003, Forrester 2005, Chang et al. 2006, Zentitani and Kimura 2007).

The Gobioidei is a large suborder in the order Perciformes comprising about 268 genera and more than 2000 species (Nelson 1994). More than 190 species of gobies have been recorded in Taiwan (Shao et al. 1997). They are distributed from marine to freshwater environments. According to their migratory behavior and habitat use, gobies are classified into amphidromous and non-amphidromous species (McDowell 1988). Amphidromous species spawn in fresh water. After hatching, their larvae drift downstream to the sea where they live as planktonic larvae and may disperse to neighboring estuaries. After metamorphosing into juveniles, they return to the natal stream to grow until maturation (McDowell 1988 1997). The dispersal of larvae among estuaries is influenced by such biotic factors as spawning strategies, growth rates, and migratory behaviors of the fish and such abiotic factors as freshwater discharge, coastal currents, and tides (Govoni et al. 1989, Cowen et al. 1993, Raynie and Shaw 1994). However, dispersal patterns of most goby species are not clear (Dotu and Mito 1953, Dotu 1955 1961, Ryan 1991, Katoh and Nishida 1994, Shen 1997, Shen et al. 1998).

To better understand the dispersal patterns of gobies in estuaries, the widespread goby *Rhinogobius giurinus* was used in this study. *R. giurinus* is an amphidromous goby, which spawns in fresh water during summer (Kawanabe and Mizuno 1989). After hatching, the larvae drift with downstream to estuarine nursery areas (Tamada 2000). This goby is widely distributed in estuaries of western Taiwan with a time lag of seasonal occurrence from south to north (Tzeng et al. 2002). It is not known whether gobies among various estuaries originate from the same population and drift with coastal currents from south to north and populate each of the estuaries, or each estuary has its own self-sustaining population which spawns with seasonally increasing temperatures from south to north; so the cause leading to this delay is unknown.

This study aims to clarify the possible pattern of larval dispersal and population connectance of *R. giurinus* among estuaries in Taiwan. We proposed 3 conceptual dispersal models for the goby. In type 1, larvae of different estuaries come simultaneously from a single spawning population with minimal connectance among estuaries; in type 2, larvae come from different populations which are self-sustaining with maximal connectance among estuaries; and in type 3, larvae also come from different populations and are self-sustaining but with minimal connectance among estuaries (Fig. 1). To demonstrate these larval dispersal models, the elemental compositions of otoliths of the goby collected from 3 estuaries of western Taiwan were examined by solution-based inductively coupled plasma mass spectrometry (ICPMS) and the age of the goby was determined by daily growth increments in otoliths.

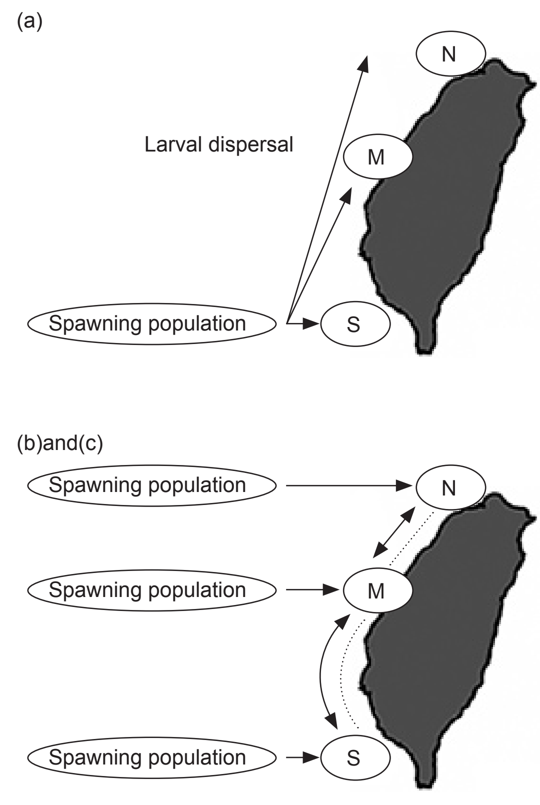

Fig. 1. Three conceptual models of dispersal of larval *Rhinogobius giurinus* among 3 estuaries: (a) type 1, a single spawning population with minimal connectance among metapopulations; (b) type 2, multiple populations with maximal connectance among metapopulations (⟷); and (c) type 3, multiple populations with minimal connectance among metapopulations (……). S, Tongkang Creek, the southern estuary; M, Tatu River, the middle estuary; and N, Gongshytyan Creek, the northern estuary.

MATERIAL AND METHODS

Sampling design

Larval *R. giurinus* were collected by a fyke net set against the tidal current in the estuaries of Gongshytyan Creek (GST), Tatu River (TT), and Tongkang Creek (TK) in western Taiwan during the night-time flood tide from Mar. to Aug. 1998 (Fig. 2). After collection, the gobies were preserved in 95% alcohol. Larval *R. giurinus* was sorted according to the characteristics and graphs described in the illustrated handbook, *An Atlas of the Early Stage Fishes in Japan* (Okiyama 1988). This species has black pigment on the body surface and a fin membrane at the larval stage. Larval *R. giurinus* can be separated from a similar species, *R. brunneus*, based on the shape of the pelvic fin. The pelvic fin of larval *R. giurinus* is shaped like a diamond. Among the fish collected, 19 and 17 larvae from Tongkang Creek collected on 27 Mar. and 26 May, 16 and 20 larvae from Tatu River collected on 23 May and 29 Aug., and 17 larvae from Gongshytyan Creek collected on 6 Aug. were randomly selected for daily age determination by daily growth increment and the measurement of trace elements of otolith by solution-based high-resolution Inductively Coupled Plasma Mass Spectrometry (ICPMS; Finnigan Element 2, Thermo Electron Corp., Bremen).

Sagittae, the largest of 3 pairs of otoliths were extracted with glass probes under a microscope. After removing adhering organic tissues by washing with deionized water (DIW), one of the sagittal otolith was prepared for daily age estimation, and the other was prepared for the ICPMS analysis.

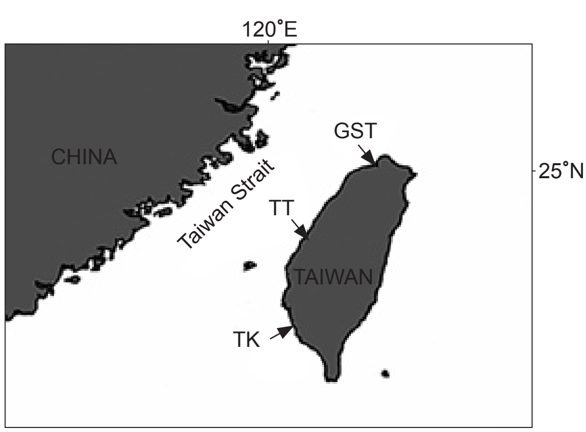

Fig. 2. Sampling sites. GST, Gongshytyan Creek; TT, Tatu River; and TK, Tongkang Creek.

Daily ring and growth rate estimation

Otoliths were embedded with Epofix (Stuers), the frontal section was polished with sandpaper and aluminum oxide (Al_2O_3) powder until the core was exposed, and then was etched with 5% ethylenediaminetetraacetic acid (EDTA) for about 2 mins to enhance the daily growth increments (DGIs). The daily ages of the larval gobies were determined with DGIs which were assumed to be deposited on a daily schedule similar to those other species (Shen 1997, Shiao 1998). The mean growth rate (GR) of the larvae was calculated as follows:

GR (mm/d) = Total length ÷ Daily age

Measurement of elements in otoliths

The procedure for measuring elements in otoliths of the goby was similar to that of a previous study (Chang et al. 2006). Otoliths were soaked with 5% H_2O_2 in vials to dissolve the organic tissues using an ultrasonic cleaner for 5 min, triple-rinsed with DIW, dried overnight in an oven, and weighed to the nearest 0.001 mg. Two milliliters of 0.3 N double-distilled ultrapure HNO_3 was added to the vial to dissolve the otolith, and the vial was weighed to the nearest 0.01 g before being subjected to ultrasonic wave vibrations for at least 2 h to ensure the complete dissolution and solution homogeneity.

A homemade standard solution containing 13 elements (Li, Na, Mg, K, Mn, Fe, Ca, Ni, Cu, Zn, Sr, Ba, and Pb) was used to calculate elemental concentrations of otoliths of gobies measured by ICPMS. The standard was dissolved with 0.3 N HNO_3 in 4 concentrations according to the concentration of Ca, i.e., 0.5, 1, 2.5, and 5 ppm, to produce calibration curves for each element for each run of the ICPMS analysis. Element detection limits were calculated from the concentration of the signal equivalent to 12 times the mean of the blank signal, which were 0.0838 (Li), 0.6121 (Na), 0.1284 (Mg), 0.1461 (K), 0.0813 (Mn), 0.1141 (Fe), 23.4367 (Ca), 0.0854 (Ni), 0.0050 (Cu), 0.6192 (Zn), 0.0189 (Sr), 0.0012 (Ba), and 0.0023 (Pb) ppb for each of the elements. HNO_3 (0.3 N) was used as a blank which was checked every 5 samples during the analysis as well as at the beginning and end of the analysis. The 2.5 ppm standard solution was analyzed after every 10th otolith sample to detect any machine drift in intensity.

Data analysis

All elements measured from otoliths were statistically analyzed irrespective of whether they were metabolically or environmentally related (Campana 1999, Thresher 1999). All otolith elemental concentrations were standardized by the concentration of Ca as element/Ca ratios and were natural log-transformed to fit the normal distribution hypothesis before further statistical analysis. Differences in larval otolith elemental composition among the 3 estuaries and between months were tested with MANOVA (Everitt 1978). Differences in mean total lengths, daily ages, growth rates, and element/Ca ratios in otoliths of larvae among the 3 estuaries and between months were tested with one-way ANOVA followed by multiple comparisons with Tukey's honest significant difference (HSD) test (Tukey 1949). The relative contributions of otolith element/Ca ratios to grouping of larval gobies among estuaries and months were analyzed by a backward stepwise canonical discrimination analysis (Williams and Titus 1988). Classification success, as the percentage of larval gobies assigned to their original estuaries and birth month, was calculated by a Jackknife classification of the discrimination function analysis (Williams and Titus 1988).

RESULTS

Age and growth rates

Ages of larval gobies did not significantly differ among the 3 estuaries with a range of 12-22 d and a mean of 15.0-18.6 d after hatching (p = 0.23). Total lengths ranged 6.4-7.6 mm with a mean of 6.87-7.13 mm, and mean growth rates ranged 0.32-0.55 mm/d with a mean of 0.384-0.460 mm/d, which also did not significantly differ among estuaries (p = 0.23 and 0.36, Table 1).

Differences in otolith elemental compositions between months

MANOVA indicated that the elemental composition of otoliths of larval *R. giurinus* significantly differed between months at both TK (Mar. vs. May) and TT (May vs. Aug.) ($p < 0.0001$). Ratios of the 12 elements (Li, Na, Mg, K, Mn, Fe, Ni, Cu, Zn, Sr, Ba, and Pb) to Ca detected in otoliths significantly differed between months at each site (one-way ANOVA, $p < 0.01$), except for the Mn/Ca and Na/Ca ratios at TT (p = 0.41 and 0.39) and the Li/Ca, Mg/Ca, Zn/Ca, and Sr/Ca ratios at TK (p = 0.26-071, Table 2).

Differences in ratios of elements to Ca among the 3 estuaries

Differences in mean element/Ca concentration ratios of otoliths of larval gobies among estuaries were tested for TT and TK in May and for GST and TT in Aug. because data were not available for all 3 localities in the same month (Table 3). In May, except for the Li/Ca, K/Ca, and Ba/Ca ratios (one-way ANOVA, p = 0.063-0.50), the remaining 9 element/Ca ratios all significantly differed between TT and TK (p = 0-0.31). In Aug., six of the 12 element/Ca ratios (Li/Ca, K/Ca, Mn/Ca, Fe/Ca, Ni/Ca, and Zn/Ca) significantly differed between GST and TT (p = 0-0.018). Overall, except for the Li/Ca and Na/Ca ratios, the remaining 10 element/Ca ratios all significantly differed among 3 estuaries ($p < 0.0001$). Even if the temporal effect of month was ignored, the otolith elemental compositions of larvae also significantly differed among estuaries (MANOVA, $p < 0.0001$). Tukey's HSD test indicated that most of the element/Ca ratios of TK

Table 1. Comparisons of mean daily age, total length, and growth rate of larval *Rhinogobius giurinus* collected from 3 estuaries in western Taiwan in 1998

Location	Sampling date	Sample size (no. for ICPMS)	Daily age (d)		Total length (mm)		Growth rate (mm/d)	
			range	mean ± S.D.	range	mean ± S.D.	range	mean ± S.D.
Gongshytyan Creek	Aug. 6	30 (19)	15-22	18.3 ± 3.51	6.4-7.6	7.13 ± 0.67	0.35-0.49	0.40 ± 0.08
Tatu River	May 23	30 (16)	14-16	15.0 ± 0.82	6.4-7.3	6.88 ± 0.36	0.43-0.52	0.46 ± 0.04
Tongkang Creek	Mar. 27	30 (17)	12-21	18.6 ± 3.38	6.6-7.6	6.87 ± 0.35	0.32-0.55	0.38 ± 0.08
Difference (p value)				0.23		0.23		0.36

ICPMS, inductively coupled plasma mass spectrometry.

were the highest, followed by TT, and then by TS. However, the element/Ca ratios for Li and Na were similar among all sites; Mn was the highest at GST followed by TT and TK; Pb was similar at TT and TK; and K, Cu, and Sr were higher at GST than TT (Table 3).

A backward stepwise canonical discrimination function analysis indicated that the origin of larvae from different estuaries and months could be classified into 5 groups by 6 element/Ca ratios of Li/Ca, Na/Ca, Mg/Ca, Fe/Ca, Sr/Ca, and Ba/Ca (Fig. 3). In other words, the element/Ca ratios of Na/Ca, Li/Ca, Ba/Ca, and Sr/Ca in root 1 of the canonical discrimination function could separate larvae of TK in May (TK05, Fig. 3) from the other 4 groups, and Fe/Ca and Mg/Ca in root 2 could separate the other 4 groups: GST in Aug. (TS08), TT in May (TT05) and Aug. (TT08), and TK in Mar.

Table 2. One-way ANOVA of differences between months in each of the 12 element/Ca ratios of otoliths of larval *Rhinogobius giurinus* collected from the TT (Tatu River) and TK (Tongkang Creek) estuaries in 1998

Element/Ca	TT			TK		
	Mean ± S.D.		p value	Mean ± S.D.		p value
	May (n = 16)	Aug. (n = 20)		Mar. (n = 19)	May (n = 17)	
Li/Ca	$3.5\times10^{-5} \pm 1.7\times10^{-5}$	$2.1\times10^{-4} \pm 9.8\times10^{-5}$	0	$5.3\times10^{-5} \pm 1.0\times10^{-4}$	$4.3\times10^{-5} \pm 2.9\times10^{-5}$	0.70
Na/Ca	$7.4\times10^{-3} \pm 1.3\times10^{-3}$	$7.8\times10^{-3} \pm 1.2\times10^{-3}$	0.39	$1.1\times10^{-2} \pm 1.2\times10^{-2}$	$2.6\times10^{-2} \pm 3.5\times10^{-3}$	0
Mg/Ca	$1.7\times10^{-3} \pm 3.3\times10^{-4}$	$9.7\times10^{-4} \pm 5.1\times10^{-4}$	< 0.001	$2.1\times10^{-3} \pm 2.7\times10^{-4}$	$5.8\times10^{-3} \pm 2.6\times10^{-3}$	0.36
K/Ca	$3.7\times10^{-4} \pm 7.8\times10^{-5}$	$2.9\times10^{-3} \pm 2.6\times10^{-3}$	0.006	$4.5\times10^{-4} \pm 8.7\times10^{-5}$	$2.8\times10^{-3} \pm 3.7\times10^{-3}$	0
Mn/Ca	$4.1\times10^{-4} \pm 1.1\times10^{-5}$	$5.5\times10^{-4} \pm 2.3\times10^{-4}$	0.4053	$3.0\times10^{-4} \pm 8.8\times10^{-5}$	$8.4\times10^{-4} \pm 6.4\times10^{-5}$	0
Fe/Ca	$3.5\times10^{-4} \pm 1.2\times10^{-4}$	$2.1\times10^{-4} \pm 7.8\times10^{-5}$	8×10^{-5}	$7.8\times10^{-4} \pm 8.2\times10^{-5}$	$4.4\times10^{-4} \pm 1.4\times10^{-5}$	0
Ni/Ca	$7.8\times10^{-5} \pm 9.2\times10^{-5}$	$2.6\times10^{-4} \pm 1.8\times10^{-4}$	0.0113	$9.9\times10^{-5} \pm 2.5\times10^{-5}$	$1.3\times10^{-4} \pm 2.3\times10^{-5}$	0.0369
Cu/Ca	$2.6\times10^{-5} \pm 9.9\times10^{-6}$	$3.8\times10^{-5} \pm 4.4\times10^{-6}$	0.0008	$6.9\times10^{-5} \pm 2.7\times10^{-5}$	$1.6\times10^{-4} \pm 9.1\times10^{-5}$	0
Zn/Ca	$1.2\times10^{-3} \pm 4.3\times10^{-4}$	$1.2\times10^{-3} \pm 4.7\times10^{-4}$	0.0007	$3.3\times10^{-3} \pm 1.9\times10^{-3}$	$3.5\times10^{-3} \pm 5.9\times10^{-3}$	0.2646
Sr/Ca	$7.7\times10^{-3} \pm 6.3\times10^{-4}$	$9.3\times10^{-3} \pm 1.9\times10^{-3}$	0.0009	$8.9\times10^{-3} \pm 4.9\times10^{-3}$	$9.2\times10^{-3} \pm 2.5\times10^{-3}$	0.7115
Ba/Ca	$1.7\times10^{-4} \pm 4.0\times10^{-5}$	$3.2\times10^{-5} \pm 7.8\times10^{-6}$	2×10^{-5}	$2.1\times10^{-4} \pm 5.4\times10^{-5}$	$4.6\times10^{-5} \pm 2.6\times10^{-6}$	0
Pb/Ca	$1.2\times10^{-4} \pm 2.1\times10^{-4}$	$4.1\times10^{-5} \pm 6.8\times10^{-5}$	0.0068	$8.7\times10^{-5} \pm 4.0\times10^{-5}$	$3.4\times10^{-5} \pm 3.7\times10^{-5}$	0

$p = 0$ means $p < 0.00001$.

Table 3. Comparison of element/Ca ratios in otoliths of larval *Rhinogobius giurinus* between estuaries (GST, Gongshytyan Creek, TT, Tatu River, and TK, Tongkang Creek) by one-way ANOVA and Tukey's honest significant difference (HSD) test, respectively

Element/Ca	May			Aug.			Tukey's HSD test
	Mean ± S.D.		p value	Mean ± S.D.		p value	
	TT	TK		GST	TT		
Li/Ca	$3.5\times10^{-5} \pm 1.7\times10^{-5}$	$4.3\times10^{-5} \pm 2.9\times10^{-5}$	0.5034	$2.9\times10^{-5} \pm 1.7\times10^{-5}$	$2.1\times10^{-4} \pm 9.8\times10^{-5}$	0	TS = TT = TK
Na/Ca	$7.4\times10^{-3} \pm 1.3\times10^{-3}$	$2.6\times10^{-2} \pm 3.5\times10^{-3}$	0	$7.2\times10^{-3} \pm 6.0\times10^{-4}$	$7.8\times10^{-3} \pm 1.2\times10^{-3}$	0.0943	TS = TT = TK
Mg/Ca	$1.7\times10^{-3} \pm 3.3\times10^{-4}$	$5.8\times10^{-3} \pm 2.6\times10^{-3}$	0.0001	$1.2\times10^{-3} \pm 7.3\times10^{-4}$	$9.7\times10^{-4} \pm 5.1\times10^{-4}$	0.1569	TS < TT < TK
K/Ca	$3.7\times10^{-4} \pm 7.8\times10^{-5}$	$2.8\times10^{-3} \pm 3.7\times10^{-3}$	0.5013	$4.2\times10^{-4} \pm 1.4\times10^{-4}$	$2.9\times10^{-3} \pm 2.6\times10^{-3}$	0	TT < TS < TK
Mn/Ca	$4.1\times10^{-4} \pm 1.1\times10^{-5}$	$8.4\times10^{-4} \pm 6.4\times10^{-5}$	0.0314	$4.5\times10^{-5} \pm 4.6\times10^{-5}$	$5.5\times10^{-4} \pm 2.3\times10^{-4}$	0	TS > TT > TK
Fe/Ca	$3.5\times10^{-4} \pm 1.2\times10^{-4}$	$4.4\times10^{-4} \pm 1.4\times10^{-5}$	0.0290	$3.4\times10^{-4} \pm 2.6\times10^{-4}$	$2.1\times10^{-4} \pm 7.8\times10^{-5}$	0.0179	TS < TT < TK
Ni/Ca	$7.8\times10^{-5} \pm 9.2\times10^{-5}$	$1.3\times10^{-4} \pm 2.3\times10^{-5}$	0.0003	$5.6\times10^{-5} \pm 4.0\times10^{-5}$	$2.6\times10^{-4} \pm 1.8\times10^{-4}$	0	TS < TT < TK
Cu/Ca	$2.6\times10^{-5} \pm 9.9\times10^{-6}$	$1.6\times10^{-4} \pm 9.1\times10^{-5}$	0.0087	$4.2\times10^{-5} \pm 3.2\times10^{-5}$	$3.8\times10^{-5} \pm 4.4\times10^{-6}$	0.3631	TT < TS < TK
Zn/Ca	$1.2\times10^{-3} \pm 4.3\times10^{-4}$	$3.5\times10^{-3} \pm 5.9\times10^{-3}$	0.0005	$8.5\times10^{-4} \pm 5.5\times10^{-4}$	$1.2\times10^{-3} \pm 4.7\times10^{-4}$	0.0055	TS < TT < TK
Sr/Ca	$7.7\times10^{-3} \pm 6.3\times10^{-4}$	$9.2\times10^{-3} \pm 2.5\times10^{-3}$	0.0208	$8.5\times10^{-3} \pm 9.3\times10^{-4}$	$9.3\times10^{-3} \pm 1.9\times10^{-3}$	0.0648	TT < TS < TK
Ba/Ca	$1.7\times10^{-4} \pm 4.0\times10^{-5}$	$4.6\times10^{-5} \pm 2.6\times10^{-6}$	0.0630	$2.9\times10^{-5} \pm 1.1\times10^{-5}$	$3.2\times10^{-5} \pm 7.8\times10^{-6}$	0.0908	TS < TT < TK
Pb/Ca	$1.2\times10^{-4} \pm 2.1\times10^{-4}$	$3.4\times10^{-5} \pm 3.7\times10^{-5}$	0	$2.6\times10^{-5} \pm 3.1\times10^{-5}$	$4.1\times10^{-5} \pm 6.8\times10^{-5}$	0.7707	TS < TT = TK

$p = 0$ means $p < 0.00001$.

(TK03). The Jackknife classification success for larval natal estuaries and birth months was 93.75% in TT05, 94.12% in TK03, and 100% in GST08, TT08, and TK05, respectively (Table 4). This indicates that most of the larvae remained in their natal estuary and there was minimal connectance among estuaries.

DISCUSSION

Dispersal ability and connectance of metapopulation

Compared to other amphidromous gobies, such as *Sicyopterus japonicus* which may have the opportunity to disperse to other estuaries because of a longer marine pelagic larval duration (PLD) with a mean of 163.72 d or approximately 6 mo (Shen et al. 1998), the mean daily age of larval *R. giurinus* collected in this study was only 12-22 d. Consequently, the PLD for larval *R. giurinus* might be too short to disperse very far away. We did not collect juvenile or young *R. giurinus* in this study, and thus we do not know if this goby migrates from one estuary to another after the juvenile stage. However, the Sr/Ca ratio in an otolith of a young 70-d-old *R. giurinus* caught in another stream was < 0.4%, which is used to discriminate between seawater and freshwater residents (Shiao 1998). This indicates that *R. giurinus* does not disperse to seawater and subsequently to adjacent estuaries. In other words, this species might not disperse far from its original estuary during the early larval stage, and the population in the natal estuary is self-sustaining with minimal connectance among metapopulations of different estuaries (type 3 of figure 1).

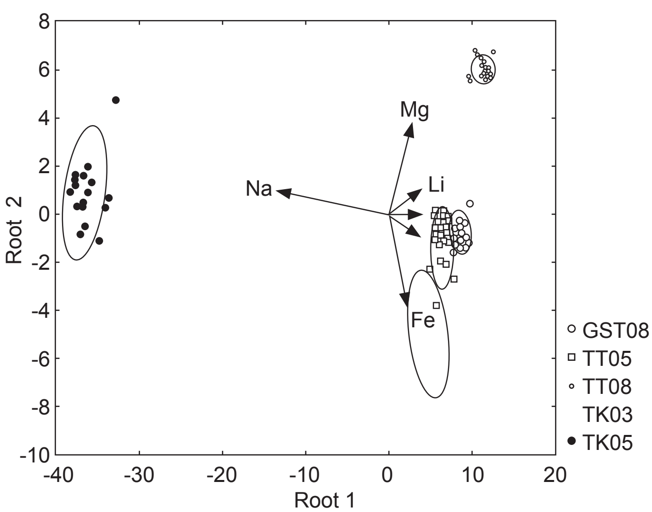

Fig. 3. Plot of canonical scores from discriminant function analysis of otolith elemental compositions of the 89 larval gobies of *Rhinogobius giurinus* collected from 3 estuaries (GST, Gongshytyan Creek; TT, Tatu River; and TK, Tongkang Creek) in Taiwan in Mar. (03), May (05), and Aug. (08) 1998. Ellipses indicate the 95% confidence limits.

Factors affecting the otolith elemental composition of goby among estuaries

Estuaries are tidal mixing areas which receive chemical inputs from both offshore seawater and freshwater river discharges. The chemical composition of seawater was similar among the estuaries studied because of the effect of coastal currents in the Taiwan Strait, but the water chemistry, substratum, and pollutants of the associated rivers differed among the estuaries examined. According to an investigation by the Environmental Protection Administration of Taiwan, GST was lightly polluted in 1998; however TT and TK were moderately polluted. For example, concentrations of Zn, Cu, Cr, Pb, and Cd in

Table 4. Classification success of larval *Rhinogobius giurinus* from different natal estuaries and birth months (GST, Gongshytyan Creek; TT, Tatu River; and TK, Tongkang Creek; 03, 05, and 08, Mar., May, and Aug.)

	Sample size	No. of fish					Classification success (%)
		GST08	TT05	TT08	TK03	TK05	
GST08	19	19	0	0	0	0	100
TT05	16	0	15	0	1	0	93.75
TT08	20	0	0	20	0	0	100
TK03	17	0	1	0	16	0	94.12
TK05	17	0	0	17	0	0	100

estuarine waters were all higher at TT and TK than at GST in 1998. This might explain why the concentrations of most trace elements were higher at TK and TT than at GST. In 2005 and 2006, we also analyzed the water chemistry of these estuaries and found that the water chemistry differed between GST and TT, and Fe, Ni, and Ba concentrations in the water were higher at TT than at GST (unpublished data).

Deposition of trace elements in otoliths is a complicated biomineralization process which is regulated by both physiological and environmental factors (Dove et al. 1996, Brown et al. 2001, Gillanders and Kingsford 2003, Swearer et al. 2003, Chang et al. 2006) as well as the crystalline structure of the otolith (Tzeng et al. 2007). Elements such as Sr, Mg, and Ba in fish otoliths are positively related to their presence in ambient waters (Brown and Harris 1995, Schroder et al. 1995, Farrell and Campana 1996, Tzeng 1996, Bath et al. 2000, Milton and Chenery 2001, Elsdon and Gillanders 2003). Ba, Cd, and Zn may respectively reflect freshwater discharge, river pollution, and metabolic requirements (Bruland 1983, Bath et al. 2000, Alibert et al. 2003, Wells et al. 2003, Elsdon and Gillanders 2003 2005). The concentration of Mn in fish otoliths may be related to physiology or ontogeny rather than abiotic factors (Elsdon and Gillanders 2003, Brophy et al. 2004). Ten of the 12 element/Ca ratios in otoliths of the larval goby *R. giurinus* significantly differed among the estuaries investigated. Except for Mg and K which may be related to physiological conditions, elements such as Cu, Zn, Pb, Mn, Fe, and Ni are pollution related; while Sr and Ba are salinity and fresh water related. The larvae investigated were all at the same stage with similar ages and lengths; therefore ontogenetic and physiological effects on the otolith elemental compositions were negligible among estuaries. Accordingly, differences in elemental compositions of otoliths of the larval gobies among estuaries should reflect the effects of the environment rather than fish physiology.

Classification success of larval natal origin and its implications

It was surprising that 93.75%-100% of larvae collected from the 3 estuaries could be successfully classified into their original estuaries and birth month (Table 4). This indicates that the otolith elemental fingerprints were very sensitive at discriminating the origin of larval *R. giurinus* in estuaries of western Taiwan. The high classification success rate may have been due to contributions of different water chemical compositions among estuaries as well as larval retention. Most rivers in Taiwan are polluted, and pollutants differ among rivers (unpublished water chemistry data for 2005 and 2006). Substrates of these 3 estuaries also differed: TK has a sandy bottom, TT has a muddy bottom, and GST has a mixed rocky and sandy bottom. Natural differences in sediments and anthropogenic pollutants among estuaries may lead to differences in water chemistry among estuaries and may subsequently be reflected in otolith elemental fingerprints of the larvae. Differences in otolith elemental compositions of fish have also been found among estuaries in other areas by similar analyses (Hoff and Fuiman 1995, Gillanders and Kingsford 1996, Kafemann et al. 2000, Thorrold et al. 2001, Ruttenberg and Warner 2006). The elemental composition of larval goby otoliths provides environmental clues to track their natal origin and dispersal pathways of any interchange of larval gobies among estuaries as well as serving as an indicator of environmental change in an estuary. The high classification success rate among estuaries and between months implies that larvae in each estuary are self-sustaining with minimal connectance among estuaries. The non-significant difference in mean daily age among estuaries also suggests that *R. giurinus* in these 3 estuaries may be independently self-sustaining rather than dispersing from other estuaries (type 3 of figure 1). Suppose that larvae among the 3 estuaries had been spawned from a single population and then had been dispersed from south to north by the coastal current; then the mean daily age of the larvae should have gradually increased from south to north. However, it did not. Accordingly, larvae in the 3 estuaries may have been supplied from 3 separate spawning populations. In addition, *R. giurinus* reproduces when water temperatures increase in summer (Tamada 2000). The seasonal occurrence of larval gobies increased from Mar. in TK to Aug. in GST, implying that the spawning season of goby populations in the 3 different estuaries progressed geographically from south to north following the seasonal increase in water temperatures. In practice, this species should be managed independently in each estuary according to their life history characteristics.

CONCLUSIONS

Elemental signatures of otoliths of the larval goby *R. giurinus* are unique natural tags that can be used as biological tracers to determine the natal estuary of the larvae. The otolith elemental compositions differed, but mean daily ages were similar among estuaries, indicating that the goby maintains self-sustaining populations with minimal connectance of metapopulation among estuaries. However, further validation of the metapopulation is needed, including population identification by genetic analyses.

Acknowledgments: This study was financially supported by the Council of Agriculture, Executive Yuan, R.O.C. (COA 94AS-14.1.1-FA-F1). The authors are grateful to assistants and former students of the Fisheries Biology lab of the Department of Life Science (former Department of Zoology), National Taiwan Univ. for field sampling work, Dr. Y.T. Wang for larval species identification, and Mr. B.M. Jessop for helpful comments on the manuscript.

REFERENCES

Alibert C, L Kinsley, SJ Fallon, MT McCulloch, R Berkelmans, F McAllister. 2003. Source of trace element variability in Great Barrier Reef corals affected by the Burdekin flood plumes. Geochim. Cosmochim. Acta. **67:** 231-246.

Bath GE, SR Thorrold, CM Jones, SE Campana, JW McLaren, JWH Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochim. Cosmochim. Acta. **64:** 1705-1714.

Brophy D, TE Jeffries, BS Danilowicz. 2004. Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Mar. Biol. **144:** 779-786.

Brown AL, MS Busby, KL Mier. 2001. Walleye pollock *Theragra chalcogramma* during transformation from the larval to juvenile stage: otolith and osteological development. Mar. Biol. **139:** 845-851.

Brown P, JH Harris. 1995. Strontium batch-marking of golden perch (*Macquaria ambigua* Richardson) and trout cod (*Maccullochella macquariensis* Cuvier). *In*: Secor DH, Dean JM, Campana SE (eds.). Recent Developments in Fish Otolith Research. University of South Carolina Press, Columbia, pp. 693-701.

Bruland KW. 1983. Trace elements in sea-water. Chem. Oceanogr. **8:** 157-220.

Campana SE. 1999. Chemistry and composition of fish otoliths: pathway, mechanisms and applications. Mar. Ecol.-Prog. Ser. **188:** 263-297.

Campana SE, JA Gagne, JW Mclaren. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar. Ecol. Prog. Ser. **122:** 115-120.

Chang MY, CH Wang, CF You, WN Tzeng. 2006. Individual-based dispersal patterns of larval gobies in an estuary as indicated by otolith elemental fingerprints. Sci. Mar. **70:** 165-174.

Cowen RK, JA Hare, MP Fahay. 1993. Beyond hydrography: can physical process explain larval fish assemblages within the Middle Atlantic Bight? Bull. Mar. Sci. **53:** 567-587.

Dotu Y. 1955. The life history of a goby, *Chaenogobius urotarnia* (Hilgendorf). Sci. Bull. Faculty Agric. Kyushu. Univ. **15:** 367-374. (in Japanese with English abstract)

Dotu Y. 1961. The binomics and life history of the gobioid fish, *Rhinogobius giurinus* (Rutter). Bull. Faculty Fish. Nagasaki. Univ. **10:** 120-126. (in Japanese with English abstract)

Dotu Y, S Mito. 1953. Life history of a gobioid fish, *Sicydium japonicus* Tanaka. Sci. Bull. Faculty Agric. Kyushu. Univ. **15:** 213-221. (in Japanese with English abstract)

Dove SG, BM Gillanders, MJ Kingsford. 1996. An investigation of chronological differences in the deposition of trace metals in the otoliths of two temperate reef fishes. J. Exp. Mar. Biol. Ecol. **205:** 15-33.

Elsdon TE, BM Gillanders. 2005. Alternative life-history patterns of estuarine fish: Barium in otoliths elucidates freshwater residency. Can. J. Fish Aquat. Sci. **62:** 1143-1152.

Elsdon TS, BM Gillanders. 2003. Relationship between water and otolith elemental concentrations in juvenile fish, Acanthopagrus butcheri. Mar. Ecol. Prog. Ser. **260:** 263-272.

Everitt B. 1978. Graphical techniques for multivariate data. London: Heinemann.

Farrell J, SE Campana. 1996. Regulation of calcium and strontium deposition on the otolith of juvenile tilapia, *Oreochromis niloticus*. Comp. Biochem. Physiol. A. **115:** 103-109.

Forrester GE. 2005. A field experiment testing for correspondence between trace elements in otoliths and the environment and for evidence of adaptation to prior habitats. Estuaries **28:** 974-981.

Fowler AJ, SE Campana, CM Jones, SR Torrold. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. Can. J. Fish. Aquat. Sci. **52:** 1421-1430.

Gallahar NK, MJ Kingsford. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: an experimental investigation. J. Fish Biol. **48:** 174-186.

Gillanders BM, KW Able, JA Brown, DB Eggleston, PF Sheeidan. 2003. Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Mar. Ecol. Prog. Ser. **247:** 281-295.

Gillanders BM, MJ Kinsford. 2003. Spatial variation in elemental composition of otoliths of three species of fish (family Sparidae). Estuar. Coast. Shelf S. **57:** 1049-1064.

Govoni JJ, DE Hoss, DR Colby. 1989. The spatial distribution of the larval fishes about the Mississippi River plume. Limnol. Oceanogr. **34:** 178-187.

Hoff GR, LA Fuiman. 1995. Environmentally induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. Bull. Mar. Sci. **56:** 578-591.

Kafemann R, S Adlerstein, R Neukamm. 2000. Variation in otolith strontium and calcium ratios as an indicator of life history strategies of freshwater species within a brackish water system. Fish. Res. **46:** 313-325.

Katoh M, N Nishida. 1994. Biochemical and egg size evolution of freshwater fishes in Katoh M, N Nishida the *Rhinogobius brunneus* complex (Pisces, Gobiidae) in Okinawa, Japan. Biol. J. Linn. Soci. **51:** 325-335.

Kawanabe H, N Mizuno. 1989. Freshwater fishes of Japan. Tokyo: Yama-Kei. (in Japanese)

Kennedy BD, CL Folt, JD Blum, CP Chamberlain. 1997. Natural isotope markers in salmon. Nature **387:** 766-767.

Kennedy BP, JD Blum, CL Folt, KH Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Can. J. Fish Aquat. Sci. **57:** 2280-2292.

Kennedy BP, A Klaue, JD Blum, CL Folt, KH Nislow. 2002. Reconstructing the lives of fish using Sr isotopes in otoliths. Can. J. Fish. Aquat. Sci. **59:** 925-929.

McDowall RM. 1988. Diadromy in fishes: migration between freshwater and marine environments. London: Croom-Helm.

McDowall RM. 1997. Is there such a thing as amphidromy? Micronesica **30:** 3-14.

Milton DA, SR Chenery. 2001. Sources and uptake of trace metal in otoliths of juvenile barramundi (*Lates calcarifer*). J. Exp. Mar. Biol. Ecol. **264:** 47-65.

Nelson JS. 1994. Fishes of the world. New York: J Wiley Press.

Okiyama M. 1988. An atlas of the early stage fishes in Japan. Takai Univ. Press.

Pannella G. 1971. Fish otoliths: daily growth layers and periodical patterns. Science **173:** 1124-1127.

Raynie BE, RF Shaw. 1994. Ichthyoplankton abundance along a recruitment corridor from offshore spawning to estuarine nursery ground. Estuar. Coast. Shelf. Sci. **39:** 421-450.

Rieman BE, DL Myers, RL Nielsen. 1994. Use of otolith microchemistry to discriminate *Oncorhynchus nerka* of resident and anadromous origin. Can. J. Fish Aquat. Sci. **51:** 68-77.

Ruttenberg BJ, SL Hamilton, MJH Hickford, GC Paradis, MS Sheehy, JD Standish, OB Tzvi, RR Warner. 2005. Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Mar. Ecol. Prog. Ser. **297:** 273-281.

Ruttenberg BJ, RW Warner. 2006. Spatial variation in the chemical composition of natal otoliths from reef fish in the Galapagos Islands. Mar. Ecol. Prog. Ser. **328:** 225-236.

Ryan PA. 1991. The success of the Gobiidae in tropical Pacific insular streams. NZ J. Mar. Freshw. Res. **18:** 25-30.

Schroder S, C Knudsen, E Volk. 1995. Marking salmon fry with strontium chloride solutions. Can. J. Fish Aquat. Sci. **52:** 1141-1149.

Secor DH, JR Rooker, E Zlokovitz, VS Zdanowicz. 2001. Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Mar. Ecol. Prog. Ser. **211:** 245-253.

Shao KT, JP Chen, SC Wang. 1997. Biogeography and database of marine fishes in Taiwan waters. *In* B. S'eret, JY. Sire, eds. Proceeding of the 5th Indo- Pacific Fish Conference. Nouméa, 3-8 Nov. 1997. pp. 673-680. Paris: Société Française d'Ichthyologie et Insitut de Recherche pour le Développement.

Shen KN. 1997. Early life history and recruitment dynamics of amphidromous goby *Sicyopterus japonicus*. MS thesis, Institute of Fishery Science, National Taiwan Univ., Taipei, Taiwan.

Shen KN, YC Lee, WN Tzeng. 1998. Use of otolith micro-chemistry to investigate the life history pattern of gobies in a Taiwanese stream. Zool. Stud. **37(Supplement 4):** 322-329.

Shiao JC. 1998. Early life history and fry resources of amphidromous gobies in Hsuikuluan River. MS thesis, Department of Life Science, National Tsing Hua Univ., Hsinchu, Taiwan.

Swearer SE, GE Forrester, MA Steele, AJ Brooks, DW Lea. 2003. Spatio-temporal and interspecific variation in otolith trace-elemental fingerprints in a temperate estuarine fish assemblages. Estuar. Coast. Shelf Sci. **56:** 1111-1123.

Tamada K. 2000. Distributions of the spawning grounds of four amphidromous gobies (*Rhinogobius* spp.) in the Aizu River, Wakayama Prefecture, Japan. Jpn. J. Ichthyol. **47:** 55-59. (in Japanese with English abstract)

Thorrold SR, C Latkoczy, PK Swart, CM Jones. 2001. Natal homing in a marine fish metapopulation. Science (Washington, DC) **291:** 297-299.

Thresher RE. 1999. Elemental composition of otoliths as a stock delineator in fishes. Fish. Res. **43:** 165-204.

Tukey JW. 1949. Comparing individual means in the analysis of variance. Biometrics **5:** 99-114.

Tzeng WN. 1996. Effects of salinity and ontogenetic movements on strontium: calcium ratios in the otoliths of the Japanese eel, *Anguilla japonica*. J. Exp. Mar. Biol. Ecol. **199:** 111-122.

Tzeng WN, CW Chang, CH Wang, JC Shiao, Y Iizuka, YJ Yang, CF You, L Ložys. 2007. Misidentification of the migratory history of anguillid eels by Sr/Ca ratios of vaterite otoliths. Mar. Ecol. Prog. Ser. **348:** 285-295.

Tzeng WN, YC Tsai. 1994. Changes in otolith microchemistry of Japanese eel, *Anguilla japonica*, during its migration from the ocean to the rivers of Taiwan. J. Fish Biol. **45:** 671-683.

Tzeng WN, YT Wang, CW Chang. 2002. Spatial and temporal variations of the estuarine larval fish community on the west coast of Taiwan. Mar. Freshwater Res. **53:** 419-430.

Wells BK, BE Rieman, JL Clayton, DL Horan, CM Jones. 2003. Relationships between water, otolith, and scale chemistries of westslope cutthroat trout from the Coeur d'Alene River, Idaho: the potential application of hard-part chemistry to describe movements in freshwater. Trans. Am. Fish. Soc. **132:** 409-424.

Williams BK, K Titus. 1988. Assessment of sampling stability in ecological applications of discriminant analysis. Ecology **69:** 1275-1285.

Zenitani H, R Kimura. 2007. Elemental analysis of otoliths of Japanese anchovy: trial to discriminate between Seto Inland Sea and Pacific stock. Fish. Sci. **73:** 1-8.