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Using otolith trace elements as biological tracer for tracking larval dispersal of black porgy, *Acanthopagrus schlegeli* and yellowfin seabream, *A. latus* among estuaries of western Taiwan

Mei-Yu Chang · Wann-Nian Tzeng · Chen-Fong You

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Abstract To understand if the trace elements in the otoliths can be used as a biological tracer for tracking natal origin and dispersal of larval black porgy (*Acanthopagrus schlegeli)* and yellowfin seabream (*A. latus*) among estuaries, the fish larvae in 1997, 1998, and 2005 and water samples in 2005 were collected from 3 estuaries on the western coast of Taiwan. The elemental composition in both otoliths of the larvae and water samples were analyzed by a solution-based inductively coupled plasma mass spectrometer (ICP-MS). Temporal and spatial differences were found in some of the measured 12 element/Ca ratios in the larval otoliths. The water elemental composition was also significantly different between estuaries and between flood and ebb tides. 87.5–100 % of the larvae of both species could be successfully assigned to their sampled estuaries. However, only 20 % of the black porgy collected from TT in 1998 could be successfully assigned to TT and the rest to GST and TK. The low assignment might be due to the mixing by the tidal current because the flood tide comes from the direction of TK in the south and GST in the north and merged in the middle of Taiwan Strait nearby TT. This study demonstrated that the trace elements in the otoliths of the fish have the potential to detect the temporal and spatial variation of environmental conditions in the estuaries and subsequently can be used for tracking the origin of the larvae from different estuaries and their dispersal rate.

Keywords Larvae · Otolith elemental composition · Natal origin · Dispersal · Estuary

M.-Y. Chang
Biodiversity Center, Academia Sinica,
Taipei, Taiwan, Republic of China
e-mail: meiyuchang0402@gmail.com

W.-N. Tzeng (✉)
Institute of Fisheries Sciences, College of Life Science,
National Taiwan University,
Taipei, Taiwan, Republic of China
e-mail: wnt@ntu.edu.tw

W.-N. Tzeng
Department of Environmental Biology and Fisheries
Science, National Taiwan Ocean University,
Keelung, Taiwan, Republic of China

C.-F. You
Department of Earth Sciences,
National Cheng Kung University,
Tainan, Taiwan, Republic of China

Introduction

Black porgy, *A. schlegeli*, and yellowfin seabream, *A. latus*, which are estuarine-dependent marine fishes belonging to the subfamily Sparinae (Jean et al. 1992) are commercially important for both aquaculture and capture fisheries and for recreational angling in Taiwan. The larvae of both species are dominant in the larval fish community in the coastal waters of Taiwan (Tzeng et al. 2002). Recently, the populations dramatically decreased because of the habitat degradation and overfishing. To sustain the fishery resources for demand, aquaculture

and stock enhancement by releasing seedling were elaborated for the two species in Taiwan (Liao 1997). However, the fundamental studies on the early life history, distribution and the metapopulation connectivity among estuaries, which are essential for the stock enhancement and fishery management, are limited.

Many studies on reproduction, growth and artificial propagation have been conducted for the *Acanthopagrus* species such as *A. australis* (Pollock et al. 1983), *A. schlegeli* (Fukuhara 1987), and *A. latus* (Akazaki and Takito 1982; Abu-Hakima 1984) and the related species *Sparus sarba* (Leu 1994; Mihelakakis and Kitajima 1995), but few studies focused on the wild populations such as recruitment and population connectivities (Pollock et al. 1983). The larval *A. schlegeli* in the coastal waters of western Taiwan showed a geographic gradient distribution in abundance according to the daily age and growth rate (Chang et al. 2002). This implies that the population of black porgy on the western coast of Taiwan may not be independent among estuaries. However, the larval origin, dispersal, and the population connectivity of these two estuarine-dependent marine fish species are not clear although the information is important for stock enhancement and fisheries management.

Tagging can give the most direct evidence of fish population movement. However, the fish larvae are too small to use the traditional tag tracking their movement and dispersal (Volk et al. 1999). Otoliths are located in the inner ear and functions as hearing and balance of the fish. It is a biomineralized aragonite crystalline structure mainly composed of $CaCO_3$ with minor organic substrates and trace elements. The deposition of the elements in otolith was a complicated biomineralization process, which is regulated by fish physiological process, environmental factors, particularly the water chemistry (Dove et al. 1996; Brown et al. 2001; Gillanders and Kinsford 2003; Swearer et al. 2003; Chang et al. 2006) and crystalline structure of the otoliths (Tzeng et al. 2007). There are at least 31 elements found in the otoliths and co-precipitated in the otolith growth increments when fish grows (Campana 1999). The elemental composition in otoliths of the fish can record the environmental information because once a trace element is deposited in the otolith, it presents a permanent record of the environmental conditions experienced by the fish at a particular time (Ruttenburg et al. 2005). The age determination of the larvae coupled with the measurement of the trace elements in the otoliths have been used as a biological tracer for tracking down their population origin, dispersal process and metapopulation connectivity among estuaries (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; Zenitani and Kimura 2007).

The present study used the trace elemental concentration in the otoliths of larval black porgies and yellow sea breams as a biological tracer for tracking the origin of the larvae from different estuaries. Then the metapopulation connectivity among estuaries was analyzed, and the possible dispersal mechanism of the larvae among possible spawning populations recruiting to different estuaries was also addressed.

Materials and methods

Sampling design

The larvae of both black porgy, *A. schlegeli*, and yellowfin seabream, *A. latus*, were collected from the estuaries of Gongshytyan Creek (GST), Tatu River (TT), and Tongkang Creek (TK) on the western coast of Taiwan at the night-time flood tides during the spring tide by a fyke net in 1997, 1998, and 2005 (Fig. 1). The distance between two successive estuaries was approximately 150 km. The net was set against the tidal current at the estuary mouth before the tide

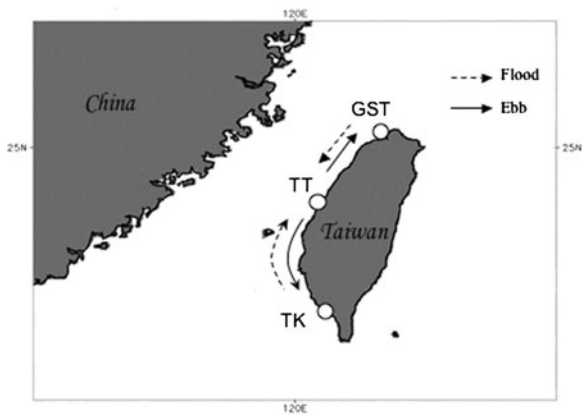

Fig. 1 The sampling sites of larval black porgies and yellowfin seabreams (GST: Gongshytyan Creek, TT: Tatu River, and TK: Tongkang Creek). Arrows indicate the direction of currents during flood and ebb tides (Chu 1963)

started flooding. The larvae drifted with the tide were collected during the tide flooding for approximately an hour. After collection, the larvae were preserved in 95 % ethanol. For black porgy, 16 and 67 individuals were collected from TT and TK in December 1997, 154, 81 and 134 individuals from GST, TT and TK in April 1998 and 95 and 66 individuals from GST and TT in April 2005 (Table 1). Yellowfin seabream larvae were only found in GST and TT and both in January. In 1998, 109 and 17 individuals were collected from GST and TT while in 2005, 38 and 62 individuals were collected from the two estuaries (Table 1). The water salinity was also measured during sampling in the estuaries of GST and TT in 2005. A total of 20 and 23 water samples were collected from each of the two estuaries. The water samples were not available in TK due to the difficulty of collection. The water samples collected in March and May in GST and in March in TT were not available because of failing to catch the timing of the flood tides.

Daily ring counting and growth rate estimation

Sagittae, the largest ones of the 3 pairs of the otoliths of the fish were extracted with glass probes under a stereo microscope. After removing the adhering organic tissue by washing with deionised water (DIW), one of the sagittal otolith was prepared for the daily age estimation and the other one for the otolith elemental composition analysis. At least 10 individuals from each location and year were used for the age determination except the black porgies from TT and TK in 1997 and the yellowfin seabreams from TT in 1998 (Table 1). The otoliths for age determination were embedded with Epofix (Stuers), polished with sandpaper and aluminum oxide (Al_2O_3) powder with frontal section until the core was exposed, and then etched with 5 % ethylenediamine tetraacetic acid (EDTA) for about 2 min to enhance the daily growth increments (DGIs). The age of the larvae was determined by counting the DGIs.

Table 1 The total numbers of larval black porgy, *A. schegeli* and yellowfin seabream, *A. latus* collected from GST (Gongshytyan Creak), TT (Tatu River), TK (Tongkang River) in 1997, 1998, and 2005 and those used for age determination using otoliths (the 1st numerals in the parenthesis) and otolith elemental composition analysis by ICPMS (the 2nd numerals in the parenthesis)

	NO. of fish		
	GST (Mixed rocky and sandy bottom)	TT (Muddy botom)	TK (Sandy bottom)
A. schlegeli			
Dec. 1997	–	16 (2, 16)	67
Apr. 1998	154 (29, 20)	81 (11, 20)	134 (24, 22)
Apr. 2005	95 (20, 17)	66 (20, 16)	–
A. latus			
Jan. 1998	109 (24, 16)	17 (–, 10)	–
Jan. 2005	38 (20, 18)	62 (20, 15)	–

Measurement of elemental concentration in the otolith

The otolith elemental concentrations of the larvae were measured by solution-based high resolution inductively-coupled plasma mass spectrometry (HR-ICPMS, Thermo Finnigan Element 2) with a procedure similar to the previous study (Chang et al. 2006). First, the otoliths were soaked with H_2O_2 in the vials to dissolve the organic tissues under ultrasonic bath for 5 min, then triple-rinsed with DIW and were dried overnight in an oven at 50 °C. The dried otoliths were soaked in 0.00075 N double distilled ultrapure HNO_3 for 30 s to remove possible contaminations, rinsed in mili-Q water for 3 times, then dried in the fume hood and weighed to 0.001 mg. The decontamination and weighing procedures were processed in the class 100 clean room. Two ml of 0.3 N double distilled ultrapure HNO_3 were added to the vial to dissolve the otolith and the vial was weighed to 0.01 g before put to ultrasonic bath for at least 2 h to ensure complete dissolving and solution homogeneity. A home-made standard solution containing 13 elements (Li, Na, Mg, K, Mn, Fe, Ca, Ni, Cu, Zn, Sr, Ba and Pb) was used to calibrate the elemental concentration in the otoliths of the larvae. For the calibration, the standard was diluted with 0.3 N HNO_3 into 4 concentrations according to the concentration of Ca, i.e. 0.5, 1, 2.5, and 5 ppm to set the calibration curves for each element for each runs of ICPMS analyses. The detection limit of each element was 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. 0.3 N HNO_3 was used as a blank, which was checked every 5

samples during the progress of the analysis as well as at the beginning and at the end of the analysis. The 2.5 ppm standard solution was analyzed after every 10 otolith samples for detecting the machine drift in intensity.

Measurement of elemental concentration in the water sample

The concentration of the elements in the water samples was also measured with ICPMS. First, the water samples were filtered with 0.45 μm filter paper to exclude the biological contents. 2 N HNO_3 was then added to acidify the water samples under pH 2, and the samples were preserved in acid-washed PP bottles. Two kinds of pre-treatment were conducted for the water samples based on the analyzed elements. Each of the water samples was separated into two parts. The first one was diluted to 1/800 to analyze the concentrations of Ca, Sr, and Ba. The second one was processed with ion-exchange resin (chelex-100) excluding the elements Na, K, Mg, and Ca to prevent matrix effect for analyzing the concentrations of other trace elements.

Similarly, two kinds of standard solution were prepared, one containing 3 elements (Ca, Sr, and Ba) and the other containing 8 elements (Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb) to be used to calibrate the unknown elemental concentration of the water samples measured by ICPMS. The standard solutions were diluted with double distilled ultra pure 0.3 N HNO_3 into 4 concentrations to set the calibration curves for each element in each runs of ICPMS analyses. The detection limit of each element was calculated from the concentration of the signal equivalent to 12 times the mean of the blank signal. The double distilled ultra pure 0.3 N HNO_3 was used as a blank to check the machine stability for every 5 samples during the progress of analysis and at the beginning and at the end of the analyses.

Data analysis

The 13 elements (Li, Na, Mg, K, Mn, Fe, Ni, Cu, Ca, Zn, Sr, Ba, and Pb) measured from the otolith were all statistically analyzed whether they were metabolically-related or environmentally-related elements (Campana 1999; Thresher 1999). The concentrations of all the 13 elements were over the detection limits. The elements were chosen because of their different environmental implications, like salinity (Sr and Ba), nutrient (Mg) and pollutants (Ni, Cu, Zn and Pb). All elements were standardized to relate to the concentration of Ca as element/Ca ratios and then the ratios were natural-log transformed to fit the normal distribution hypothesis. The homogeneity of variances of the element/Ca ratios was then tested before further analyses. The element/Ca ratios were excluded if the variance of the ratios was different among estuaries or years. Then the temporal and spatial differences in elemental composition of both otoliths and water samples were tested with MANOVA (Everitt 1978), respectively. If the MANOVA was significant, the differences of single element/Ca ratios in the otoliths of the larvae and in the water samples among estuaries and between years were further tested with one-way ANOVA or multiple range tests. Classification success percentage of the larvae to their sampled estuaries was calculated with Jackknife classification of discrimination function analysis (Williams and Titus 1988). The discriminant model was constructed based on the measured otolith elemental concentrations, and the geographical assignment of each larva was according to how closely each individual could fit to the group means. Backward stepwise canonical discrimination analysis was used to estimate the relative contributions of the otolith element/Ca ratios to the grouping of the larvae among estuaries (Williams and Titus 1988). All the statistical analyses were conducted with the software STATISTICA 6.0.

Results

The daily ages of larval black porgy and yellowfin seabream

The mean (± SD) ages of larval black porgies did not differ significantly between GST (27.6±7.2 days) and TT (26.9±5.5 days) ($p>0.05$), but both were significantly older than TK (17.9±3.6 days) in 1998 ($p<0.001$) (raw data from Chang et al. 2002). Similarly, the mean ages were not significantly different between GST (24.4±3.7 days) and TT (23±3.5 days) in 2005 ($p>0.05$).

The mean ages of yellowfin seabreams also did not differ significantly between GST (34.9±3.4 days) and TT (36.1±4.4 days) in 2005 ($p>0.05$).

Salinity, and elemental composition of water samples in the estuaries

The monthly variation of salinity in the estuaries of GST and TT was shown in Figs. 2 and 3. The salinity was higher in winter than in summer and higher in flood than ebb tides except in July and August in TT. This is probably due to the relatively larger amount of river discharge.

Eleven elements (Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, Ca, Sr, and Ba) were detected in the water samples (Table 2). The elemental composition of the water samples was significantly different between GST and TT, and also between flood and ebb tides (MANOVA, $p<0.001$). Two-way ANOVA indicated that the concentrations of Ba, Fe, Ni, Cu, and Zn were significantly different between the two estuaries ($p=0.0004\sim0.0225$) while Mn, Cu, and Zn were significantly higher in ebb than flood tides ($p=0.004\sim0.0245$). This indicated that the elemental composition of the water was estuarine-specific, and influenced by the tidal current.

Variability of the differences in elemental composition of otoliths among estuaries and years

Black porgy - A total of 13 elements (Li, Na, Mg, K, Mn, Fe, Ni, Cu, Ca, Zn, Sr, Ba, and Pb) were detected in otoliths of the larvae of both species. Li/Ca, Na/Ca, Zn/Ca, and Ba/Ca ratios in GST and Mg/Ca ratio in TT were excluded after the homogeneity test ($p>0.05$). MANOVA analysis indicated that the composition of element/Ca ratios of the black porgy in GST, TT, and TK were all

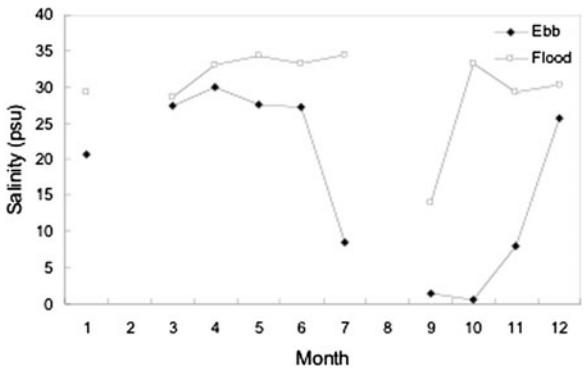

Fig. 2 The monthly change of salinity during flood and ebb tides in the estuary of GST in 2005

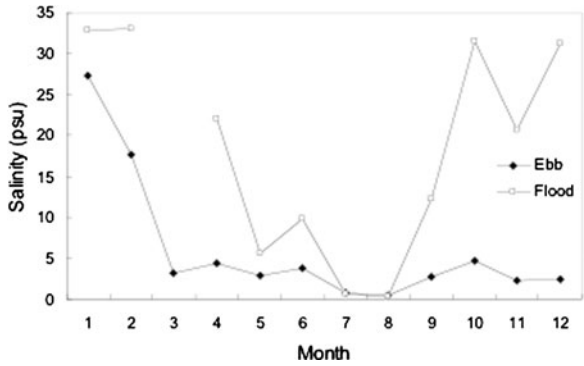

Fig. 3 The monthly change of salinity during flood and ebb tides in the estuary of TT in 2005

significantly different among years ($p<0.001$). In addition, one-way ANOVA indicated that the 6 element/Ca ratios in otoliths of black porgies in GST were significantly different between 1998 and 2005; 11 element/Ca ratios except Mg/Ca were found significantly different in TT among 3 years; and 10 element/Ca ratios except Ni/Ca and Sr/Ca were significantly different in TK between 1997 and 1998 (Table 3a).

Mg/Ca, K/Ca, Mn/Ca, Cu/Ca, and Pb/Ca ratios in the otoliths collected from GST, TT, and TK in 1998 were excluded after the homogeneity test ($p>0.05$). MANOVA analysis indicated that the composition of element/Ca ratios were significantly different among estuaries

Table 2 The concentrations (ppm) of elements in the water samples collected during flood and ebb tides in GST and TT, 2005

	Mean (± SD) concentration in ppm			
	GST ($n=20$)		TT ($n=23$)	
	Flood tide	Ebb tide	Flood tide	Ebb tide
Ca	260±17	800±79	170±200	160±210
Sr	30±22	93±84	22±31	20±33
Ba	0.23±0.54	0.75±0.18	0.19±0.20	0.19±0.21
Mn	0.094±0.053	0.065±0.066	0.15±0.064	0.071±0.054
Fe	29±26	13±0.96	0.71±0.28	0.96±0.89
Co	0.22±0.10	0.18±0.048	0.17±0.0093	0.17±0.012
Ni	10±78	42±51	0.37±0.073	0.42±0.097
Cu	0.68±0.55	0.25±0.16	0.25±0.092	0.27±0.15
Zn	21±17	0.78±0.43	0.49±0.22	0.45±0.15
Cd	0.17±0.036	0.15±0.021	0.16±0.014	0.16±0.019
Pb	0.44±0.33	0.23±0.10	0.31±0.34	0.24±0.14

n = sample size

Table 3 (a) Inter-estuaries and (b) inter-years comparison of mean (± SD) element/Ca ratios in the otoliths of larval black porgies collected from GST, TT, and TK in 1997, 1998, and 2005. The significant differences in each element/Ca ratio among different years were further analyzed in TT and in the year 1998 (analyzed with Tukey HSD test), and discriminated by a, b, and c. The same letter shared between years indicates no significant difference. The symbol "–" means the element/Ca ratios were excluded because of the significant differences in the homogeneity of variances

(a)

	GST		TT			TK	
	1998 (n=20)	2005 (n=17)	1997 (n=16)	1998 (n=20)	2005 (n=16)	1997 (n=10)	1998 (n=22)
Li	–	–	$2.66\times10^{-4}\pm3.43\times10^{-5}$ ab	$8.36\times10^{-4}\pm1.04\times10^{-4}$ a	$1.19\times10^{-4}\pm1.42\times10^{-5}$ b	$5.95\times10^{-5}\pm8.3\times10^{-6}$	$4.81\times10^{-4}\pm4.8\times10^{-5}$*
Na	–	–	$2.09\times10^{-3}\pm1.32\times10^{-4}$ a	$6.37\times10^{-3}\pm5.64\times10^{-4}$ ab	$2.09\times10^{-2}\pm2.25\times10^{-4}$ b	$1.44\times10^{-3}\pm2.2\times10^{-4}$	$5.56\times10^{-3}\pm4.79\times10^{-4}$*
Mg	$5.51\times10^{-3}\pm7.42\times10^{-4}$	$2.5\times10^{-3}\pm5.32\times10^{-4}$*	–	–	–	$4.81\times10^{-2}\pm6.97\times10^{-3}$	$4.36\times10^{-3}\pm4.51\times10^{-4}$*
K	$1.74\times10^{-4}\pm1.59\times10^{-5}$	$1.27\times10^{-3}\pm2.82\times10^{-4}$*	$1.28\times10^{-3}\pm8\times10^{-4}$ a	$8.64\times10^{-5}\pm1.08\times10^{-5}$ b	$9.85\times10^{-5}\pm9.86\times10^{-6}$ b	$3.54\times10^{-4}\pm5.38\times10^{-5}$	$5.03\times10^{-5}\pm4.65\times10^{-6}$*
Mn	$3.03\times10^{-4}\pm2.49\times10^{-5}$	$2.61\times10^{-6}\pm5.06\times10^{-7}$*	$6.73\times10^{-5}\pm2.98\times10^{-6}$ a	$1.3\times10^{-4}\pm1.83\times10^{-5}$ a	$4.91\times10^{-7}\pm5.76\times10^{-8}$ b	$2.27\times10^{-4}\pm3.09\times10^{-5}$	$8.12\times10^{-4}\pm6.61\times10^{-5}$*
Fe	$7.63\times10^{-4}\pm7.53\times10^{-5}$	$1.41\times10^{-4}\pm2.9\times10^{-5}$*	$2.89\times10^{-5}\pm1.99\times10^{-6}$ a	$1.63\times10^{-4}\pm3.78\times10^{-5}$ a	$2.8\times10^{-6}\pm2.86\times10^{-7}$ b	$1.64\times10^{-6}\pm2.27\times10^{-7}$	$2.13\times10^{-4}\pm2.19\times10^{-5}$*
Ni	$6.77\times10^{-3}\pm1.39\times10^{-3}$	$1.85\times10^{-5}\pm2.8\times10^{-6}$*	$4.82\times10^{-4}\pm2.31\times10^{-5}$ a	$3.77\times10^{-4}\pm3.6\times10^{-5}$ a	$6.81\times10^{-7}\pm8.71\times10^{-8}$ b	$6.9\times10^{-4}\pm9.91\times10^{-5}$	$9.19\times10^{-4}\pm1.97\times10^{-4}$*
Cu	$2.1\times10^{-5}\pm6.29\times10^{-6}$	$4.3\times10^{-6}\pm1.11\times10^{-6}$*	$3.83\times10^{-5}\pm4.97\times10^{-6}$ ab	$4.51\times10^{-5}\pm3.51\times10^{-6}$ a	$2.44\times10^{-5}\pm3.71\times10^{-6}$ b	$1.08\times10^{-7}\pm1.4\times10^{-8}$	$5.64\times10^{-5}\pm7.77\times10^{-6}$*
Zn	–	–	$2.09\times10^{-3}\pm1.32\times10^{-4}$ a	$6.4\times10^{-3}\pm5.64\times10^{-4}$ ab	$2.09\times10^{-2}\pm2.25\times10^{-3}$ b	$8.54\times10^{-5}\pm1.18\times10^{-5}$	$1.76\times10^{-3}\pm5.63\times10^{-4}$*
Sr	$1.19\times10^{-2}\pm2.50\times10^{-3}$	$9.77\times10^{-3}\pm2.56\times10^{-3}$	$2.61\times10^{-3}\pm1.35\times10^{-4}$ a	$1.43\times10^{-2}\pm7.28\times10^{-3}$b	$9.49\times10^{-3}\pm3.07\times10^{-3}$ b	$4.53\times10^{-3}\pm1.72\times10^{-3}$	$9.82\times10^{-3}\pm7.1\times10^{-4}$
Ba	–	–	$1.3\times10^{-5}\pm1.58\times10^{-6}$ a	$1.35\times10^{-4}\pm1.39\times10^{-5}$ b	$1.89\times10^{-6}\pm2.9\times10^{-7}$ c	$1.45\times10^{-6}\pm1.79\times10^{-7}$	$6.08\times10^{-5}\pm5.6\times10^{-6}$*
Pb	$1.37\times10^{-3}\pm3.29\times10^{-4}$	$9.51\times10^{-4}\pm2.06\times10^{-4}$	$2.81\times10^{-4}\pm6.75\times10^{-5}$ a	$6\times10^{-5}\pm1.59\times10^{-5}$ b	$8.43\times10^{-7}\pm1.29\times10^{-7}$ c	$1\times10^{-4}\pm1.77\times10^{-5}$	$2.81\times10^{-4}\pm2.91\times10^{-5}$*

(b)

	1997		1998			2005	
	TT (n=16)	TK (n=10)	GST (n=20)	TT (n=20)	TK (n=22)	GST (n=17)	TT (n=16)
Li	$2.66\times10^{-4}\pm3.43\times10^{-5}$	$5.95\times10^{-5}\pm8.3\times10^{-6}$*	–	–	–	$2.78\times10^{-5}\pm1.03\times10^{-6}$	$1.19\times10^{-4}\pm1.42\times10^{-5}$*
Na	$2.09\times10^{-3}\pm1.32\times10^{-4}$	$1.44\times10^{-3}\pm2.2\times10^{-4}$	–	–	–	$1.85\times10^{-3}\pm5.11\times10^{-4}$	$2.09\times10^{-2}\pm2.25\times10^{-3}$*
Mg	$3.24\times10^{-3}\pm1.9\times10^{-4}$	$4.81\times10^{-2}\pm6.97\times10^{-3}$	$5.51\times10^{-3}\pm7.42\times10^{-4}$ a	$1.9\times10^{-3}\pm2.22\times10^{-4}$ b	$4.36\times10^{-3}\pm4.51\times10^{-4}$ b	$2.5\times10^{-3}\pm5.32\times10^{-4}$	$1.24\times10^{-2}\pm1.15\times10^{-3}$*
K	$1.28\times10^{-3}\pm8\times10^{-4}$	$3.54\times10^{-4}\pm5.38\times10^{-5}$*	$1.74\times10^{-4}\pm1.59\times10^{-5}$ a	$8.64\times10^{-5}\pm1.08\times10^{-5}$ b	$5.03\times10^{-5}\pm4.65\times10^{-6}$ b	$1.27\times10^{-3}\pm2.82\times10^{-4}$	$9.85\times10^{-5}\pm9.86\times10^{-6}$*
Mn	$6.73\times10^{-5}\pm2.98\times10^{-6}$	$2.27\times10^{-4}\pm3.09\times10^{-5}$*	$3.03\times10^{-4}\pm2.49\times10^{-5}$ a	$1.3\times10^{-4}\pm1.83\times10^{-5}$ b	$8.12\times10^{-4}\pm6.61\times10^{-5}$ b	$2.61\times10^{-6}\pm5.06\times10^{-7}$	$4.91\times10^{-7}\pm5.76\times10^{-8}$*
Fe	$2.89\times10^{-5}\pm1.99\times10^{-6}$	$1.64\times10^{-6}\pm2.27\times10^{-7}$*	–	–	–	$1.41\times10^{-4}\pm2.9\times10^{-5}$	$2.8\times10^{-6}\pm2.86\times10^{-7}$*
Ni	$4.82\times10^{-4}\pm2.31\times10^{-5}$	$6.9\times10^{-4}\pm9.91\times10^{-5}$*	–	–	–	$1.85\times10^{-5}\pm2.8\times10^{-6}$	$6.81\times10^{-7}\pm8.71\times10^{-8}$*
Cu	$3.83\times10^{-5}\pm4.97\times10^{-6}$	$1.08\times10^{-7}\pm1.4\times10^{-8}$*	$2.1\times10^{-5}\pm6.29\times10^{-6}$ ab	$4.51\times10^{-5}\pm3.51\times10^{-6}$ a	$5.64\times10^{-5}\pm7.77\times10^{-6}$ b	$4.3\times10^{-6}\pm1.11\times10^{-6}$	$2.44\times10^{-5}\pm3.71\times10^{-6}$
Zn	$2.09\times10^{-3}\pm1.32\times10^{-4}$	$8.54\times10^{-5}\pm1.18\times10^{-5}$*	–	–	–	$5.21\times10^{-4}\pm1.75\times10^{-4}$	$2.09\times10^{-2}\pm2.25\times10^{-3}$*
Sr	$2.61\times10^{-3}\pm1.35\times10^{-4}$	$4.53\times10^{-3}\pm1.72\times10^{-4}$*	–	–	–	$9.77\times10^{-3}\pm2.56\times10^{-3}$	$9.49\times10^{-3}\pm3.07\times10^{-3}$
Ba	$1.3\times10^{-5}\pm1.58\times10^{-6}$	$1.45\times10^{-6}\pm1.79\times10^{-7}$*	–	–	–	$1.4\times10^{-6}\pm4.62\times10^{-7}$	$1.89\times10^{-6}\pm2.9\times10^{-7}$*
Pb	$2.81\times10^{-4}\pm6.75\times10^{-5}$	1×10^{-4} 1.77×10^{-5}*	$1.37\times10^{-3}\pm3.29\times10^{-4}$ a	$6\times10^{-5}\pm1.59\times10^{-5}$ ab	$2.81\times10^{-4}\pm2.91\times10^{-5}$ b	$9.51\times10^{-4}\pm2.06\times10^{-4}$	$8.43\times10^{-7}\pm1.29\times10^{-7}$*

in all 3 years ($p<0.001$). In addition, one-way ANOVA indicated that the 10 element/Ca ratios except Na/Ca and Mg/Ca in the otoliths of black porgy were significantly different between TT and TK in 1997; Mg/Ca, K/Ca, Mn/Ca, Cu/Ca, and Pb/Ca ratios were found to be significantly different among GST, TT, and TK in 1998; and 10 element/Ca ratios except Cu/Ca and Sr/Ca were significantly different between GST and TT (Table 3b) in 2005.

These indicated that the elemental signature in the otoliths of larval black porgies was different both among estuaries and years.

Yellowfin seabream - The variances of Mg/Ca, K/Ca, Mn/Ca, Fe/Ca, and Sr/Ca ratios of yellowfin seabream were not significantly different between 1998 and 2005 in TT (Homogeneity test, $p>0.05$). MANOVA analysis indicated that the composition of element/Ca ratios of the yellowfin seabream in GST and TT were all significantly different between years ($p<0.001$). In addition, one-way ANOVA indicated that the 10 element/Ca ratios except Mn/Ca and Sr/Ca in the otoliths of yellowfin seabreams in GST were significantly different between 1998 and 2005, and Mg/Ca, K/Ca, and Fe/Ca ratios were found significantly different in TT between the 2 years (Table 4a).

The variances of 9 element/Ca ratios except Li/Ca, Ni/Ca, and Pb/Ca were not significantly different between GST and TT in 1998 (Homogeneity test, $p>0.05$). MANOVA analysis indicated that the composition of element/Ca ratios were all significantly different between the two estuaries in 1998 and 2005 ($p<0.001$). In addition, one-way ANOVA indicated that Na/Ca and Zn/Ca ratios in the otoliths of yellowfin seabreams were significantly different between GST and TT in 1998, and K/Ca and Mn/Ca ratios were found significantly different between GST and TT in 2005 (Table 4b).

These also indicated that the elemental signature in the otoliths of larval yellowfin seabreams was significantly different between estuaries and years.

Classification success of sampled estuaries of the larvae

The discriminant function analysis indicated that the larval black porgies from the 3 estuaries GST, TT, and TK in 1998 can be clearly classified into 2 groups by the otolith Mg/Ca and Cu/Ca ratios (Fig. 4), however the larvae from TT were scattered to GST and TK.

Jackknife classification of discriminant function analysis indicated that 87.5 % and 90 % of the larval black porgies could be successfully assigned to their sampled estuaries for TT and TK in 1997. Around 81.82 % and 100 % of the larvae could be successfully assigned to their sampled estuaries for GST and TK, but only 20 % for TT in 1998. And 100 % of the larvae could be successfully assigned to their sampled estuaries for both GST and TT in 2005 (Table 5a).

Similarly, Jackknife classification of discriminant function analysis also indicated that 100 % and 90 % of larval yellowfin seabreams could be successfully assigned to their sampled estuaries for GST and TT in 1998, and 100 % and 93.33 % of the larvae could be successfully assigned back to their sampled estuaries for GST and TT in 2005 (Table 5b). These also suggested that the larval yellowfin seabreams in GST and TT might be independent populations.

Discussion

The otolith elemental composition may reflect the differences of water chemistry. The ototlih elemental composition of the two larval Sapridae species should contain the signal of the water masses that they have been through, which including the coastal and estuarine waters. This characteristic gave us the chance to track back the larval origin in different estuaries.

Factors affecting the elemental composition of water samples and fish otolith among estuaries

Estuary is a tidal mixing area which receives chemicals from both offshore seawater and freshwater from river discharge. The current system in the western coast of Taiwan changes with season. In winter, the coastal water of Taiwan Strait in the north is influenced by the China Coastal current, which is driven by the northeastern monsoon while those in the south Taiwan Strait is influenced by the branch of Kuroshio Current. During summer, the current system is all influenced by the South China Sea current driven by the southeastern monsoon (Tzeng et al. 2002). These seasonal changed currents carried different chemical contents. In addition, the water chemistry of the three estuaries in this study is influenced by the substratum and pollutants from its corresponding rivers. Ba, Cd, and Zn in estuarine waters mainly come from the sediment of the river and pollutant (Bruland 1983; Bath et al. 2000; Alibert et al. 2003; Elsdon and Gillanders 2003, 2005; Wells et al. 2003). Gongshytyan Creek (GST) was slightly polluted in 1998; however the

Table 4 (a) Inter-estuaries and (b) inter-years comparison of mean (± SD) element/Ca ratios in the otoliths of larval yellowfin seabreams collected from GST and TT in 1998 and 2005. The symbol "–" means the element/Ca ratios were excluded because of the significant differences in the homogeneity of variances

(a)

	GST		TT	
	1998	2005	1998	2005
Li	$7.97\times10^{-7}\pm2.16\times10^{-7}$	$1.98\times10^{-4}\pm3.39\times10^{-5}$*	–	–
Na	$2.39\times10^{-6}\pm3.62\times10^{-7}$	$2.09\times10^{-5}\pm3.39\times10^{-6}$*	–	–
Mg	$1.7\times10^{-3}\pm4.21\times10^{-4}$	$7.79\times10^{-3}\pm1.22\times10^{-4}$*	$7.26\times10^{-2}\pm4.22\times10^{-4}$*	$2.66\times10^{-3}\pm3.06\times10^{-4}$
K	$1.07\times10^{-4}\pm3.43\times10^{-5}$	$1.92\times10^{-3}\pm2.8\times10^{-4}$*	$2.18\times10^{-5}\pm1.18\times10^{-5}$*	$7.74\times10^{-4}\pm1.08\times10^{-5}$
Mn	$9.09\times10^{-4}\pm2.56\times10^{-4}$	$3.39\times10^{-4}\pm4.19\times10^{-5}$	$4.31\times10^{-4}\pm2.33\times10^{-4}$	$4.41\times10^{-4}\pm4.79\times10^{-5}$
Fe	$8.5\times10^{-5}\pm0.2.23\times10^{-5}$	$2.74\times10^{-4}\pm5.35\times10^{-5}$*	$5.48\times10^{-4}\pm3.31\times10^{-5}$*	$2.54\times10^{-3}\pm3.45\times10^{-4}$
Ni	$6.87\times10^{-4}\pm2.21\times10^{-4}$	$5.05\times10^{-5}\pm9.41\times10^{-6}$*	–	–
Cu	$4.14\times10^{-7}\pm1.11\times10^{-7}$	$1.13\times10^{-4}\pm3.17\times10^{-5}$*	–	–
Zn	$1.74\times10^{-4}\pm2.86\times10^{-5}$	$3.41\times10^{-3}\pm7.69\times10^{-4}$*	–	–
Sr	$3.53\times10^{-3}\pm7.82\times10^{-4}$	$4.12\times10^{-3}\pm1.75\times10^{-3}$	$3.61\times10^{-3}\pm1.62\times10^{-4}$	$4.37\times10^{-3}\pm6.92\times10^{-4}$
Ba	$6.49\times10^{-5}\pm1.6\times10^{-5}$	$4.43\times10^{-4}\pm8.28\times10^{-5}$*	–	–
Pb	$3\times10^{-4}\pm5.05\times10^{-5}$	$7.47\times10^{-5}\pm1.82\times10^{-5}$*	–	–

(b)

	1998		2005	
	GST	TT	GST	TT
Li	–	–	$1.98\times10^{-4}\pm3.39\times10^{-5}$	$2.31\times10^{-5}\pm5.91\times10^{-6}$
Na	$2.39\times10^{-6}\pm3.62\times10^{-7}$	$6.02\times10^{-6}\pm2.91\times10^{-6}$*	$2.09\times10^{-5}\pm3.39\times10^{-6}$	$1.81\times10^{-3}\pm3.38\times10^{-4}$
Mg	$1.7\times10^{-3}\pm4.21\times10^{-4}$	$7.26\times10^{-2}\pm4.22\times10^{-4}$	$7.79\times10^{-3}\pm1.22\times10^{-3}$	$2.66\times10^{-3}\pm3.06\times10^{-4}$
K	$1.07\times10^{-4}\pm3.43\times10^{-5}$	$2.18\times10^{-5}\pm1.18\times10^{-6}$	$1.92\times10^{-3}\pm2.8\times10^{-4}$	$7.74\times10^{-4}\pm1.08\times10^{-5}$*
Mn	$9.09\times10^{-4}\pm2.56\times10^{-4}$	$4.31\times10^{-4}\pm2.33\times10^{-4}$	$3.39\times10^{-4}\pm4.19\times10^{-5}$	$4.41\times10^{-4}\pm4.79\times10^{-5}$*
Fe	$8.5\times10^{-5}\pm0.2.23\times10^{-5}$	$5.48\times10^{-4}\pm3.31\times10^{-5}$	$2.74\times10^{-4}\pm5.35\times10^{-5}$	$2.54\times10^{-3}\pm3.45\times10^{-4}$
Ni	–	–	$5.05\times10^{-5}\pm9.41\times10^{-6}$	$4.2\times10^{-3}\pm6.73\times10^{-4}$
Cu	$4.14\times10^{-7}\pm1.11\times10^{-7}$	$1.72\times10^{-8}\pm8.95\times10^{-9}$	$1.13\times10^{-4}\pm3.17\times10^{-5}$	$2.21\times10^{-5}\pm2.88\times10^{-6}$
Zn	$1.74\times10^{-4}\pm2.86\times10^{-5}$	$2.17\times10^{-4}\pm1.23\times10^{-5}$*	$3.41\times10^{-3}\pm7.69\times10^{-4}$	$4.36\times10^{-3}\pm8.01\times10^{-4}$
Sr	$3.53\times10^{-3}\pm7.82\times10^{-4}$	$3.61\times10^{-3}\pm1.62\times10^{-4}$	$4.12\times10^{-3}\pm1.75\times10^{-3}$	$4.37\times10^{-3}\pm6.92\times10^{-4}$
Ba	$6.49\times10^{-5}\pm1.6\times10^{-5}$	$3.67\times10^{-6}\pm1.86\times10^{-6}$	$4.43\times10^{-4}\pm8.28\times10^{-5}$	$1.56\times10^{-4}\pm2.73\times10^{-5}$
Pb	–	–	$7.47\times10^{-5}\pm1.82\times10^{-5}$	$4.42\times10^{-4}\pm6.18\times10^{-5}$

Tatu River (TT) and Tongkong Creek (TK) were moderately polluted. Thus, the elemental composition of the water samples collected in this study was different between estuaries. For example, the concentrations of Zn, Cu, Cr, Pb, and Cd of the water samples investigated by Environmental Protection Administration of Taiwan in 1998 nearby estuary in TT were all higher than those in GST. The elemental composition of the water samples we collected in 2005 also differed between GST and TT. The concentrations of Ba in the water were higher in TT than GST, whereas those of Fe, Ni, Cu, and Zn were higher in GST than TT. These indicated that the elemental signature is river-independent. If the estuarine-dependent marine fish larvae, such as the black porgy and yellowfin seabream, dispersed from the offshore spawning ground to the estuaries, their otolith elemental

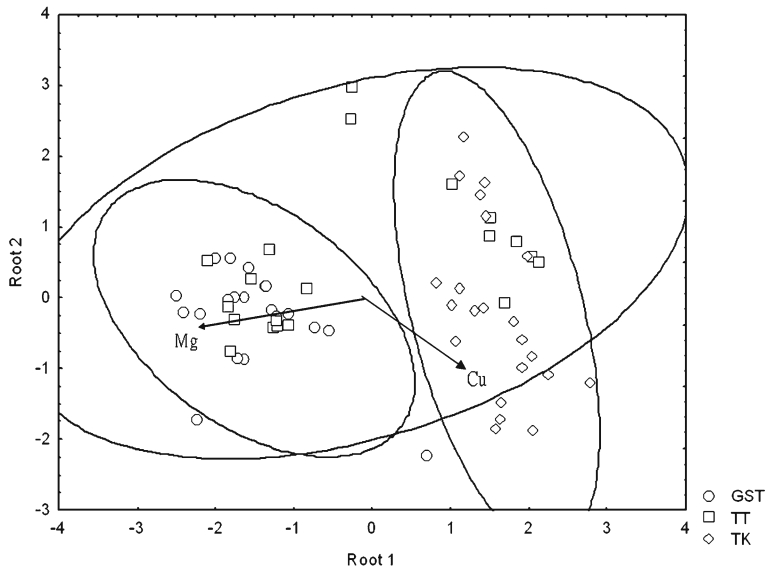

Fig. 4 The clustering of larval black porgies from three estuaries in 1998 (GST, TT, and TK)

composition should be influenced by both offshore water and freshwater of the river discharge. Since the larvae were only approximately 30 days old, the otoliths might contain more signals from the offshore. Accordingly, the elemental signature in the otoliths of the larvae can be used as tracer for tracking their natal origin.

The ANOVA tests indicated that most of the 12 element/Ca ratios in the otoliths of the larval black porgies differed significantly among 3 estuaries and years, however only four element/Ca ratios (Na/Ca, K/Ca, Mn/Ca, and Zn/Ca) were significantly different in the otoliths of larval yellowfin seabreams between GST and TT in both years. Among the investigated elements, Mn and K are physiologically related, while the elements, such as Cu, Zn, Pb, Fe, and Ni, are pollutant-related; Sr and Ba are salinity and freshwater related. The concentrations of some elements such as Sr, Mg, and Ba in the fish otoliths are positively related to their presence in the ambient water (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). The concentration of Mn in fish otoliths may reflect the metabolic requirement of fish physiology or ontogeny more than environmental influence (Elsdon and Gillanders 2003; Brophy et al. 2004). The larvae investigated in the previous study (Chang et al. 2002) and in this study were all in the same development stage with similar age; therefore age-related ontogenetic and physiological effects on the otolith elemental composition were negligible among estuaries. This may be due to the dispersal range of larval black porgies among GST, TT, and TK covered by the two current systems, the China Coastal current from north Taiwan Strait and Kuroshio branch from south Taiwan Strait as mentioned above, while larval yellowfin seabream from GST and TT distributed in north Taiwan Strait only.

Accordingly, the differences in elemental composition in otoliths of the larvae among estuaries could be used as a tracer to track their natal origin and dispersal range because the otolith elemental composition reflected environmental factors more than fish physiology in this study.

Table 5 The successful classification rates (%) of estuarine origin of larval (a) black porgies and (b) yellowfin seabreams in 1997, 1998 and 2005 by otolith elemental signature using discriminant function analysis

(a)

	1997		1998			2005	
	TT	TK	GST	TT	TK	GST	TT
GST	–	–	95	0	5	100	100
TT	87.5	12.5	50	20	30	0	0
TK	10	90	0	9.1	90.9	–	–

(b)

	1998		2005	
	GST	TT	GST	TT
GST	100	0	100	0
TT	10	90	6.7	93.3

Classification of larval natal origin by otolith elemental composition

Jackknife classification indicated that 87.5–100 % of the larval black porgies (except TT in 1998) and 90–100 % of the larval yellowfin seabreams were correctly assigned to their original estuary by otolith elemental signature (Tables 5). This indicated that the otolith trace elements were very sensitive in the discrimination of the larval origin of these two species in the estuaries of western Taiwan. However, only 20 % of larval black porgies were correctly classified to TT in 1998. This suggested that the larvae in TT may be coming from two spawning populations, which mainly supply the larval populations to GST and TK, because the larvae were collected when the larvae drifted with the tidal current during night-time flood tide of spring tide, and the flood tide in the Taiwan Strait originated from both north and south of Taiwan Strait (Fig. 1) and accumulated in the middle of the Strait nearby the sampling site TT. Accordingly, the black porgies of GST and TK may belong to two different populations and those of TT are the mixture of those two populations. On the other hand, most of the rivers in Taiwan were polluted and the substrates of the three estuaries in this study also differed: TK is sandy bottom, TT has muddy bottom, and GST has a mixture of rocky and sandy bottom. Natural differences in sediment and anthropogenic pollutants among estuaries lead to the differences in water chemistry among estuaries and subsequently reflected in the otolith trace elements of the larvae. Differences in the otolith elemental composition of fish have also been found among estuaries in other studies with similar analysis (Hoff and Fuiman 1995; Kafemann et al. 2000; Thorrold et al. 2001; Ruttenberg and Warner 2006; Chang et al. 2008). In this study, the otolith elemental composition can act as an environmental cue for tracking the natal origin of these two species of larvae among estuaries. The different successful classification rates among estuaries implied that the spawning populations recruiting larval black porgy and yellowfin seabream to each estuary were different. In 1998, some individuals of larval black porgy in TT might come from different spawning populations. However, the spawning populations of larval yellowfin seabream recruiting to the two estuaries might be independent. It suggested that the yellowfin seabream in GST and TT might be independently self-sustaining rather than dispersed to other estuaries. In addition, the mixing rate of larvae varied with years and the distance between estuaries. This also implied why the recruitment of fish larvae greatly fluctuated among years and estuaries.

In conclusion, the elemental composition in otoliths of the larval black porgy and yellowfin seabream in the western coast of Taiwan was different among estuaries and years, which indicated that the environmental condition was different among estuaries and changed between years. Although the elemental composition in the otoliths of the larvae is influenced by both fish physiology and water chemistry, the difference in fish physiology of the larvae at recruitment to the estuary among sampling sites seems negligible in this study, the difference in elemental signatures in the otoliths of the larvae among estuaries mainly reflected that of ambient water more than physiology. Therefore, the elemental signatures in the otoliths of the larvae can be used as natural tags for tracking their natal origin and dispersal. Moreover, the otolith elemental signature also indicated that the larvae among estuaries may come from different populations. This can help us to delineate the management unit of the larvae among estuaries.

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