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烏魚耳石微量元素組成的地理差異

　　本研究利用了耦合電漿質譜儀 (ICP-MS)，分析採自台灣五個不同河口的烏魚苗 (*Mugil cephalus*) 耳石，以了解其微量元素組成的地理差距。烏魚苗標本是在 2001 年 2-3 月間分別收集自淡水、台中大安、台南鹿耳門、枋寮以及東北部的福隆。在所分析的十二種元素中，其中五種元素濃度河口間有顯著差異，分別是錳、鎳、鋅、鍶以及鋇 (ANOVA, *p* <0.01) 。進一步的利用多變量分析法中的判別分析，發現錳、鎳、鋅三種元素與鈣的比值在第一個判別函數上扮演比較重要的角色 (49.8 %)，而鋇與總鈣比則在第二個判別函數上較為重要 (32.9 %)。結果發現，根據耳石上十個元素值所產生的此判別模式，有 84 % 的烏魚苗可以正確的回朔其來源，表示利用分析耳石微量元素組成這樣的自然標誌，可以用來判別其來源河口的方法之一。

Geographic variation of otolith elemental composition in the juvenile grey mullet (*Mugil cephaus*) collected from Taiwanese estuaries

　　The otolith chemical composition of juvenile grey mullet (*Mugil cephalus*) was analysed by solution-based inductively coupled plasma-mass spectrometry (ICP-MS). The juveniles were collected from 5 different Taiwanese estuaries, including Tanshui (TS), Daan (DA), Tainan (TN), Fonliao (FO) and Fulong (FL) during February to March 2001. Out of twelve elemental concentrations (Li, Na, Mg, K, Mn, Fe, Ni, Cu, Zn, Sr, Ba, and Pb) to calcium molar ratios analyzed, five elements (Mn, Ni, Zn, Sr, and Ba) were found to be significant difference between estuaries (ANOVA, *p* <0.01). The analysis of forward canonical discriminant function indicated that 10 of 12 elemental ratios (except Li/Ca and Cu/Ca) played significant role on discrimination. Among them, Mn/Ca, Ni/Ca and Zn/Ca contributed approximately 49.8 % in the 1st canonical function of the discrimination, while Ba/Ca and Sr/Ca contributed 32.9 % in the 2nd. As a result, 84 % of the mullet larvae could be assigned to their recruited estuaries with their otolith chemical signatures. These findings indicated that elemental composition in otolith of the fish can be used as a natural tag to trace their nursery areas.

Introduction

The grey mullet, *Mugil cephalus* Linnaeus, 1758, is an economically important species for commercial fisheries and aquaculture worldwide (Nash and Shehadeh 1980). The migration of mullet in waters adjacent to Taiwan follows seasonal changes in coastal currents. Mullet of 3-4 yr of age migrate southward with the NE monsoon driven coastal current from the coastal waters of mainland China to the waters off southwestern Taiwan to spawn during the winter (Tung 1981, Chen and Su 1986, Huang and Su 1989). Their eggs and larvae drift with the coastal current from the spawning grounds to estuaries on the western coast of Taiwan where they become juveniles at the age of 1-2 mo post-hatching (Tung 1981, Lee and Kuo 1990, Chang et al. 2000, Chang and Tzeng 2000, Chang et al. 2004a,b). Present study is aim to examine the differences of the otolith elemental fingerprinting on grey mullet larvae collected from different estuaries, and to test the possibility using the otolith fingerprints as an indicator to distinguish the recruiting estuaries of the mullet larvae.

Materials and Methods

Otolith preparation and analysis. The juveniles of mullet were collected from 5 different Taiwanese estuaries, including Tanshui (TS), Daan (DA), Tainan (TN), Fonliao (FO) and Fulong (FL) during February to March 2001 (Table 1). After measuring the standard length (SL), the sagittal otoliths were removed, cleaned of adhering tissue, and air-dried. Otoliths were then ultrasonically cleaned in double distilled water; triple rinsed and placed in acid-washed Eppendorf microcentrifuge tubes to dry. After drying, otoliths were individually weighed on a microbalance to the nearest 10 μg and transferred to acid-washed 1.5 mL high-density polyethylene vials and dissolved in 0.3 N ultrapure nitric acid. A lab standard, consisting of otolith matrix-matched chemical composition with interested elements were analyzed during each session of analysis. Lab standard solution was prepared at a serious concentration with Ca at 0.5, 1, 2.5, 5 and 10 ppm for calibration. Extra amount of 2.5 ppm Ca were prepared and that were analysed every 10 samples to monitor instrument drift. All analyses were run on a Finnigan[TM] Element 2 high-resolution ICP-MS. Preliminary analyses suggested that 13 elements, including Li, Na, Mg, K, Ca, Mn, Fe, Ni, Cu, Zn, Sr, Ba, Pb, were detectable in otoliths using ICP-MS and these elements were chosen for analyses.

Data analysis. Elemental data were normalized to Ca and expressed as part per million. Uni-variance ANOVAs were used to test the single elemental ratios between each sampling locations and Tukey's test were consequently used for the homogeneity group test if difference existed. A multi-variant ANOVA were followed to test the

differences of the otolith elemental signature among sites. We also used discriminant function analyses to determine how accurately we could classify elvers to their recruit estuaries. A stepwise discriminant function analysis was performed, and a jackknife cross-validation was calculated consequently to determine how accurately otolith elemental signature could distinguish mullet larvae from the different recruit estuaries. All statistic analyses were preformed using STATISTICA$^{©}$ (Version 6.0, StatSoft Inc.) software programs.

Results

Twelve elemental concentrations (Li, Na, Mg, K, Mn, Fe, Ni, Cu, Zn, Sr, Ba, and Pb) to calcium molar ratios in otolith of the mullet were analyzed and five elements (Mn, Ni, Zn, Sr, and Ba) were found to be significant difference among estuaries (ANOVA, $p < 0.01$) (Table 2). The analysis of forward canonical discriminant function indicated that 10 of 12 elemental ratios (except Li/Ca and Cu/Ca) played significant role on the discrimination of the mullet among estuaries (Table 3. and Fig. 1). Among them, Mn/Ca, Ni/Ca and Zn/Ca contributed approximately 49.8 % in the 1st canonical function of the discrimination, while Ba/Ca and Sr/Ca contributed 32.9 % in the 2nd function (Table 3). The result shows that 84 % of the mullet larvae could be assigned to their recruited estuaries by the otolith chemical signatures (Table 4).

Conclusion

Elemental composition in otolith of the fish is a useful natural tag for trace their nursery areas of mullet. Future work will analysis the otolith composition of adult migratory mullet and combine current result for estimating the contribution of each Taiwan nursery estuaries to the migratory mullet population.

研究成果自評

本計劃執行至今,與原計畫預定進度相符合,另外我們也已經收集了去年冬天迴游群的烏魚成魚標本,下一年度將進行這些耳石的分析,以期進一步了解烏魚迴游生態及不同河口對其產卵族群的貢獻量。今年度的研究結果以經於 2005 年 5 月在台北舉行的印度太平洋國際魚類研討會中發表,預計近期內會投至國際期刊審查。

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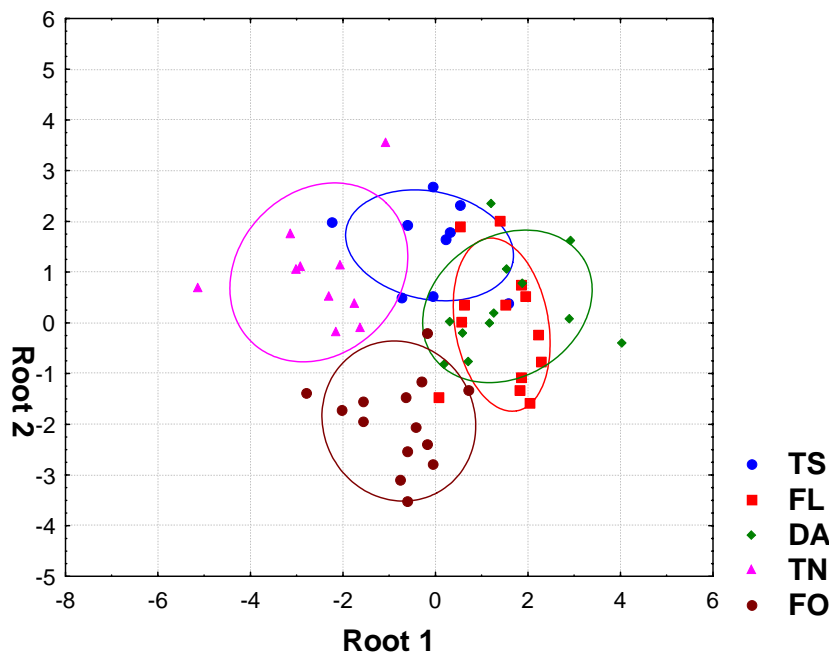
Fig. 1. Canonical discriminant plot (functions 1 & 2) of 10 elements:Ca concentration ratios in the otoliths of mullet larvae by collecting estuaries.

Table 1. Sampling details with sampling river sites, collecting dates, and the average standard length of the larvae (mean ± SD, in mm).

Group	Code	Site	Date	n	SL (mm, mean ± SD)
A	TS	Tanshui	01.02.07	20	29.1 ± 2.7
B	FL	Fulong	01.02.03	20	29.6 ± 1.9
C	DA	Daan	01.02.23	20	32.4 ± 2.0
D	TN	Tainan	01.03.02	20	23.9 ± 2.5
E	FO	Fanliao	01.02.28	20	28.8 ± 2.7

Table 2. Mean concentration to Ca ratio on mullet larvae otolith, and the results of ANOVA and homogenous group test comparing the difference between sampling estuaries.

mmol/mol	N	Mean	SE	F	p	HG (Tukey Test)
Li/Ca	77	8.0×10^{-6}	2.9×10^{-7}	0.562	0.691	
Na/Ca	75	1.4×10^{-2}	1.1×10^{-4}	3.098	0.021	
Mg/Ca	73	5.9×10^{-4}	2.2×10^{-5}	2.161	0.083	
K/Ca	74	1.6×10^{-3}	3.1×10^{-5}	1.609	0.182	
Mn/Ca	77	5.0×10^{-6}	1.9×10^{-7}	6.438	<0.001	TN=TS=FO≦DA=FL
Fe/Ca	75	1.2×10^{-5}	6.1×10^{-7}	0.993	0.417	
Ni/Ca	76	5.0×10^{-6}	4.1×10^{-7}	6.473	<0.001	DA=FL=TS≦FO≦TN
Cu/Ca	75	7.0×10^{-6}	5.8×10^{-7}	2.717	0.037	
Zn/Ca	72	3.8×10^{-5}	4.6×10^{-6}	6.978	<0.001	DA=TS=FO=FL≦TN
Sr/Ca	77	2.9×10^{-3}	2.2×10^{-5}	4.389	0.003	DA=FO≦FL=TN=TS
Ba/Ca	74	4.0×10^{-6}	1.9×10^{-7}	15.160	<0.001	TN=DA<TS=FL=FO
Pb/Ca	75	1.0×10^{-6}	9.8×10^{-8}	0.923	0.455	

Table 3. Standardized canonical discriminant function coefficients from the microelement analysis of otoliths from mullet larvae.

	Root 1	Root 2	Root 3	Root 4
Ba/Ca	0.172	-0.760	0.638	-0.126
Mn/Ca	0.641	-0.398	-0.393	-0.368
Ni/Ca	-0.789	-0.350	-0.380	0.077
K/Ca	1.563	0.467	0.180	-0.380
Na/Ca	-1.234	0.349	0.398	0.535
Sr/Ca	-0.353	0.468	0.497	0.018
Fe/Ca	0.482	0.595	0.426	-0.277
Mg/Ca	-0.383	-0.341	0.002	-0.226
Zn/Ca	-0.372	-0.156	-0.324	-1.103
Pb/Ca	-0.035	-0.382	0.109	0.865
Eigenval	2.418	1.597	0.625	0.216
Cum.Prop	0.498	0.827	0.956	1

Table 4. Classification matrix results of the multiple elements :Ca ratios in otoliths of mullet larvae.

	Correct %	TS	FL	DA	TN	FO
TS	77.8	**7**	1	1	0	0
FL	69.2	1	**9**	2	0	1
DA	83.3	0	2	**10**	0	0
TN	100.0	0	0	0	**10**	0
FO	92.9	0	1	0	0	**13**
Total	**84.5**	8	13	13	10	14