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Elemental composition of otoliths as a discriminator of life stage and growth habitat of the European eel, *Anguilla anguilla*

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Abstract. The hypothesis that elemental composition of otoliths of the eel (*Anguilla* spp.) changes with life stage and growth habitat was tested in the present study. The minor elements Cl, Na, K, Mg, Ca, Sr and P in otoliths of European eels (*Anguilla anguilla*) were examined by using an Electron Probe Microanalyser (EPMA) equipped with wavelength dispersive spectrometers (Cameca SX-50). Yellow-stage eels were collected from coastal waters and lakes of Sweden in 1987, 1988, 1991, and 1994, with ages ranging from 5 to 18 years old. Strontium maps and profiles of Sr : Ca ratio, as well as the elver check in otoliths, were used to classify life history stages of the eels as leptocephalus, and freshwater- and seawater-resident yellow eels. Canonical score plots of the otolith elemental compositions of the freshwater-resident yellow eel were completely separated from those of leptocephalus and seawater-resident yellow eel, but the latter two partially overlapped. Strontium is the primary component in determining the discrimination, but the nutrient-related (S and P), and the physiologically controlled elements (Na and Cl), may also play an important role in the discrimination. These results indicate that multiple-elemental information can provide additional insight into the migratory environmental history of diadromous fishes.

Extra keywords: electron probe microanalysis, microchemistry.

Introduction

The European eel, *Anguilla anguilla* (L.), is a diadromous fish, widely distributed in freshwater and marine littoral areas of North Africa, the Mediterranean Sea, the British Isles, Iceland, and the western and northern European continent (Tesch 2003). After spawning in the Sargasso Sea, the leptocephali are transported by the North Equatorial Current, Gulf Stream and North Atlantic Current to the continental shelf of northern European countries (Bertin 1956). The larvae metamorphose into glass eels in coastal waters. Glass eels become pigmented elvers when they enter estuaries. Their migration from the Sargasso Sea to the estuaries requires 6–9 months (Lecomte-Finiger 1992), or 14–16 months (Wang and Tzeng 2000). Male eels grow in rivers for 3–7 years, whereas female eels grow from 4 to 15 years in rivers (Vollestad and Jonsson 1986). In late autumn, they transform from the yellow eel into silver eel stage and start the downstream migration back to the Sargasso Sea. Recent studies on Sr (strontium) : Ca (calcium) ratios in otoliths indicate that a part of the eel population may skip the freshwater life of the yellow eel phase and can complete their entire life history in the seawater (Tzeng *et al*. 1997, 2000; Tsukamoto *et al*. 1998; Tsukamoto and Arai 2001).

Ratios of Sr : Ca in the otolith of anguillid eels dramatically change at metamorphosis from leptocephalus to glass eel during their migration from the ocean to the river (Otake *et al*. 1994; Tzeng and Tsai 1994; Arai *et al*. 1997). This drastic change in Sr/Ca ratios was proposed to be related to metamorphosis rather than the transition of habitat from seawater to fresh water (Tzeng 1996). In contrast, the Sr : Ca ratios in otoliths of yellow eels changed significantly when the eel migrated between fresh water and seawater (Tzeng *et al*. 1997, 2002, 2003*a*, 2003*b*; Jessop *et al*. 2002, 2004; Kraus and Secor 2003; Limburg *et al*. 2003; Shiao *et al*. 2003; Cairns *et al*. 2004). These indicated that the changes in otolith microchemistry throughout their life history of the eel were more complicated than our current understanding. Therefore, it is important to examine how elemental composition of eel otoliths is influenced by both ontogenetic developments and habitat shift before using the elemental signature to reconstruct their migratory environmental history.

In the present study, elements in addition to Ca and Sr were measured to further understand the change in elemental composition of otoliths in European eels during metamorphosis from leptocephalus to glass eel and during migration of yellow-phase eels between fresh water and seawater.

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Materials and methods

Sampling designs

Ten otoliths of European eels collected from three freshwater lakes and two brackish estuaries in Sweden were used for microchemical analysis. These samples were classified into four groups according to sampling sites (Tzeng *et al*. 1997). The origin of groups 1 through 3 was clear: these eels were captured from areas where the restocking programme is well known. The individuals of group 4 were collected from a site some kilometres away from Eastern Lake Mälaren in 1994, and the origin of this group was unknown. They were derived either from a natural population that had migrated from brackish Baltic Sea, or from a stocked population caught from brackish waters on the west coast of Sweden and released at the yellow eel stage at ~40 cm in total length, or from a stocked population released at the elver stage imported from France or the British Isles. The stocking and sampling dates and biological characteristics of the four groups of eels (including sampling date, mean (± s.d.) salinity of sampling sites, stage, age, total length, bodyweight, and the stage and year at stocking) are given in Table 1.

Microchemical analysis

After removal from the eel, the otoliths were cleaned with distilled water, dried in air, embedded in thermo-epoxy (Petropoxy 154; Palouse Petro Products, Pullman, WA) and cured for 1 h at 135°C. Embedded otoliths were ground from the proximal side of the sagittal plane of the fish until the primordium of the otolith was revealed. For microprobe analysis, the polished otoliths were coated under vacuum with a 30 nm layer of carbon to increase electron conductance.

The elemental composition of the eel otolith was measured with an electron probe microanalyser (EPMA) equipped with wavelength dispersive X-ray spectrometer (Cameca SX-50, Paris, France). Measurements were made at ~18 μm intervals along a transect from the primordium to the edge of the otolith for each of the 10 yellow eels in Table 1. The beam condition of the EPMA used in the measurement of these elements was 15 kV, 4 nA, and a 15 μm diameter beam. The EPMA has minimum detection limits of a few hundred ppm (Statham 1981, 1982) and is not sensitive enough to detect trace elements. As a result of the detection limit of EPMA, only calcium and seven minor elements (Na, Mg, Cl, P, S, K and Sr) were selected for analysis. The counting time, standard for calibration, detection limit and analytical error for each of the eight elements are listed in Appendix 1.

Data analysis

The element : Ca concentration ratios on each sampling spot of the otoliths were calculated by weight (%) and grouped by stage and habitat use, which determined the life-history stages of leptocephalus of both freshwater- and seawater-resident yellow eels. Leptocephalus and yellow eel were separated by the elver check on the otolith (Shiao *et al*. 2003) and yellow eels were further divided into freshwater and seawater residents according to the Sr concentration on the Sr map and the Sr : Ca ratio profile in their otoliths (Tzeng *et al*. 1997, 2002, 2003*a*, 2003*b*). Freshwater-resident yellow eel referred to the individuals with a Sr : Ca ratio less than 4×10^{-3} in between the elver check and the otolith edge. Seawater-resident yellow eel had Sr : Ca ratios higher than 4×10^{-3} indicating that the individuals did not migrate upstream and lived in high salinity seawater during the yellow eel phase. The estuarine-resident yellow eel migrated between fresh water and seawater.

The significant difference of the mean values of the Ca element concentration ratios among stages of the eel (leptocephalus, freshwater- and seawater-resident yellow eels) were tested by non-parametric Tukey-type multiple comparisons. The significance of the correlation among different Ca element ratios on each sampling spots was tested for each life history stages by Pearson product-moment correlation coefficient (Zar 1984). The contribution of the elemental composition to the grouping of the above mentioned life history stages was analysed by using stepwise Canonical Discriminant Analysis. The classification success of the life history stages by the elemental composition was calculated with a discriminant function analysis. All the statistics were preformed by using the software SPSS (SPSS Inc., Chicago, IL).

Results

Otolith elemental composition changed with life history stage and habitat use

The mean value of the seven elements : Ca ratios within otoliths of the yellow eel were ranked in descending order by life history stages and habitat use (Table 2). The Sr : Ca ratios constituted the first ranking in the elemental composition in the leptocephalus stage, but dropped to second rank in those of seawater-resident yellow eel stage and to the sixth rank in freshwater-resident yellow eel stage. On the contrary, Na : Ca ratios rose to the first rank in both seawater- and freshwater-resident yellow eels. This indicated that many elements other than Sr in otoliths of the eel changed with developmental stage and habitat use.

Each of the mean (± s.e.) element : Ca ratios in otoliths were also compared among life history stages and habitat use (Table 2). Tukey's honestly significantly different (HSD) tests indicated that Sr : Ca ratios in otoliths of the

Table 1. Life history of the four groups of yellow stage, female European eels used in the present study

Group	Specimen no.	Sampling date	Sampling site	Salinity (psu)	Age (years)	Total length (mm)	Bodyweight (g)	Stage and year at stocking
1	11538	9 Jul 1987	Strömstad (West Coast)	25.54 ± 3.38	5+	393	96	Natural
	11550	9 Jul 1987	Strömstad (West Coast)	25.54 ± 3.38	7+	410	90	Natural
	11906	20 Aug 1987	Björkö (West Coast)	23.12 ± 3.71	9+	403	96	Natural
2	13743	31 May 1988	Eastern Lake Mälaren	Fresh water	8+	562	442	Elver, 1980
	18589	7 Jun 1991	Eastern Lake Mälaren	Fresh water	11+	717	566	Elver, 1980
3	18793	1 Jul 1991	Lake Ången	Fresh water	≥14+	760	820	Yellow eel, 1979
	19315	16 Jul 1991	Lake Ången	Fresh water	≥18+	728	668	Yellow eel, 1979
4	25106	30 Aug 1994	Lake Mälaren	Fresh water	13+	723	597	Unknown origin
	25108	30 Aug 1994	Lake Mälaren	Fresh water	18+	721	535	Unknown origin
	25110	30 Aug 1994	Lake Mälaren	Fresh water	14+	680	461	Unknown origin

eel were the highest at marine leptocephalus stage ($1.16 \times 10^{-2} \pm 5.56 \times 10^{-4}$), followed by seawater-resident yellow eel ($4.75 \times 10^{-3} \pm 1.04 \times 10^{-4}$), and it was the lowest in freshwater-resident yellow eels ($3.36 \times 10^{-4} \pm 3.32 \times 10^{-5}$) (Table 2). Similarly, the ratios of K : Ca, Cl : Ca, P : Ca, and S : Ca were higher in the leptocephalus stage compared with the freshwater- and seawater-resident yellow eels (Table 2). The ratios of Na : Ca and Mg : Ca were higher in leptocephalus than seawater-resident yellow eels. The ratio of Na : Ca was higher in freshwater than seawater-resident yellow eels (Table 2). These indicated that except for Sr : Ca ratios, the other six element : Ca ratios also changed with life history stages and habitat use of the yellow eels.

Stage/habitat use mediated correlation among element : Ca ratios in otoliths

The correlations matrix of element : Ca ratios in otoliths of the eel were different in different life history stages and habitat use (Table 3), for example, Sr : Ca ratios were positively correlated with K : Ca, Cl : Ca and Na : Ca, and negatively with S : Ca in seawater-resident yellow eel stage, but positively correlated with P : Ca and S : Ca in the leptocephalus stage, and only slightly positively correlated with K : Ca in freshwater-resident yellow eels. Similarly, the other element : Ca ratios also have different combinations of cross correlation. This may indicate that uptake of elements was discriminated among different life history stages of the eel.

Table 2. Ranking of the seven otolith elements to calcium concentration ratios by life history stages of the eel
Ratios were compared among stages

Me/Ca	Leptocephalus ($n = 99$)			Seawater-resident Yellow eel ($n = 392$)			Freshwater-resident Yellow eel ($n = 304$)			Comparison by Tukey's HSD test
	Mean	s.e.	Rank	Mean	s.e.	Rank	Mean	s.e.	Rank	
Sr/Ca	1.16×10^{-2}	5.56×10^{-4}	1	4.75×10^{-3}	1.04×10^{-4}	2	3.36×10^{-4}	3.32×10^{-5}	6	a > b > c
Na/Ca	8.94×10^{-3}	3.82×10^{-4}	2	7.85×10^{-3}	1.1×10^{-4}	1	8.39×10^{-3}	9.57×10^{-5}	1	a = c > b
Cl/Ca	1.48×10^{-3}	1.70×10^{-4}	3	1.05×10^{-3}	4.13×10^{-5}	3	9.27×10^{-4}	4.46×10^{-5}	2	a > b = c
S/Ca	1.07×10^{-3}	4.49×10^{-5}	4	7.97×10^{-4}	1.74×10^{-5}	4	7.92×10^{-4}	2.07×10^{-5}	3	a > b = c
P/Ca	8.98×10^{-4}	6.07×10^{-5}	5	5.52×10^{-4}	2.28×10^{-5}	6	5.95×10^{-4}	2.76×10^{-5}	5	a > b = c
K/Ca	8.22×10^{-4}	5.80×10^{-5}	6	6.9×10^{-4}	1.59×10^{-5}	5	6.39×10^{-4}	1.48×10^{-5}	4	a > b = c
Mg/Ca	2.76×10^{-4}	2.78×10^{-5}	7	2.02×10^{-4}	1.03×10^{-5}	7	2.14×10^{-4}	1.8×0^{-5}	7	a > b; c = a, b

HSD, Honestly significantly different.

Table 3. Pearson correlation coefficient among seven elements : Ca ratios in weight % within otoliths of the eel by life history stage/habitat use

	Sr : Ca	K : Ca	Cl : Ca	P : Ca	S : Ca	Na : Ca	Mg : Ca
Leptocephalus							
Sr : Ca	1						
K : Ca	0.15	1					
Cl : Ca	0.19	0.62***	1				
P : Ca	0.38***	0.19	0.05	1			
S : Ca	0.31**	0.27**	0.27**	0.16	1		
Na : Ca	0.03	0.39***	0.86***	−0.07	0.14	1	
Mg : Ca	0.09	0.21*	0.24*	−0.01	0.13	0.22*	1
Seawater-resident yellow eel							
Sr : Ca	1						
K : Ca	0.24***	1					
Cl : Ca	0.35***	0.14**	1				
P : Ca	0.03	0.05	−0.12*	1			
S : Ca	−0.11*	0.01	0.01	−0.02	1		
Na : Ca	0.32***	0.14**	0.72***	−0.12*	0.03	1	
Mg : Ca	−0.04	0.04	0.01	0.06	0.05	–0.03	1
Freshwater-resident yellow eel							
Sr : Ca	1						
K : Ca	0.12*	1					
Cl : Ca	0.03	0.02	1				
P : Ca	−0.04	0.02	0.13**	1			
S : Ca	0.02	0.09	0.20***	0.07	1		
Na : Ca	0.01	−0.04	0.62***	0.17**	0.25***	1	
Mg : Ca	−0.07	0.07	0.06	0.07	0.06	0.02	1

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Classification of the life history stage and habitat use of the eel by otolith multiple elements

Forward stepwise discriminant function analysis indicated that the other five element : Ca ratios (except K : Ca and Mg : Ca) contributed significantly to the discrimination of the life history stage and habitat use of the eel (Table 4). The relative contribution of the element : Ca ratio to the groupings are listed in decreasing order as Sr : Ca, Na : Ca, P : Ca, S : Ca and Cl : Ca (Table 4).

The plot of the first and second components of the canonical discriminant functions indicated that freshwater-resident yellow eels can be clearly separated from the leptocephali and seawater-resident yellow eels by the five selected element : Ca ratios in otoliths of the eel, but the latter two partially overlapped (Fig. 1). The first component of the canonical discriminant function contributed 96.3% of the variance for the grouping of the eels whereas the second component only contributed 3.7% of the variance. These two components significantly contributed in the discrimination (χ^2-square test, $P < 0.001$). In the first component, the Sr : Ca ratios contributed the greatest, followed by Na : Ca and S : Ca. In the second component of the canonical discriminant function, the Na : Ca ratios were the most important, followed by Cl : Ca, P : Ca and S : Ca (Table 4). The cross-validated correct classification percentage in the prediction of the three different eel groups by the five element : Ca ratios was the highest in freshwater-resident yellow eels (98.0%), followed by seawater-resident yellow eel (84.4%), with leptocephalus being the lowest at 69.7% (Table 5).

Discussion

In earlier studies of the elemental composition of fish otoliths, it was believed that elemental composition reflects the elemental concentration of ambient water that the fish lives in. Thus, the Sr : Ca ratio in otoliths was widely used to study the eel migration between freshwater and marine environments (e.g. Tzeng *et al.* 1997, 2000, 2002, 2003*a*, 2003*b*; Tsukamoto and Arai 2001; Jessop *et al.* 2002, 2004; Shiao *et al.* 2003).

Table 4. Standardised canonical discriminant function coefficients of element : Ca ratios for discriminating the eel among different stage/habitat use as leptocephalus and freshwater- and seawater-resident yellow eels in Fig. 1

Elemental ratio	Function	
	1	2
Sr : Ca	1.011	−0.077
Cl : Ca	−0.047	−0.607
P : Ca	−0.041	0.594
S : Ca	0.092	0.426
Na : Ca	−0.093	0.997
Eigenvalue	2.148	0.082
% of variance	96.3	3.7
Cumulative %	96.3	100.0

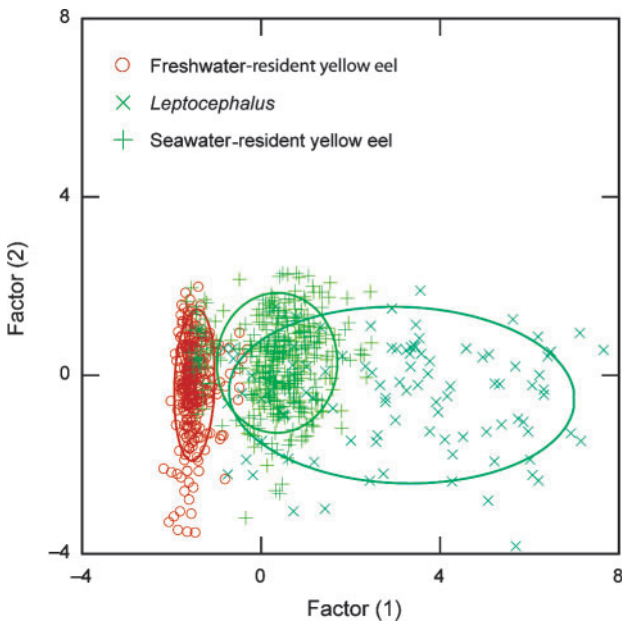

Fig. 1. *Anguilla anguilla*. Canonical discriminant scores plot of otolith element : Ca ratios for the European eel. The different stages assayed were those present in the adult eel otoliths, and classified by life stage and habitat use as leptocephalus, freshwater- and seawater-resident yellow eels. The elliptical circles indicate 95% confidence limit of each group.

Table 5. Cross-validated classification of the eel of different stage/habitat use by leave-one-out method using otolith elemental signature (Sr : Ca, Cl : Ca, P : Ca, S : Ca, and Na : Ca)

Correctly classified percentages in bold

Stage/habitat use	n^A	Correct classification (%)		
		Leptocephalus	Yellow eel	
			Seawater-resident	Freshwater-resident
Leptocephalus	99	**69.7**	24.2	6.1
Yellow stage eel				
Seawater-resident	392	2.3	**84.4**	13.3
Freshwater-resident	304	0.0	2.0	**98.0**

[A]The number of sampling spots for the measurement of elemental composition on the otoliths of the 10 yellow eels in Table 1.

In the present study, we explored elements other than Sr and found that they also changed with life stage and habitat. The Sr : Ca ratios ranked first in the leptocephalus stage, but the top rank changed to Na : Ca in freshwater- and seawater-resident yellow stage eels. In other words, the ranking of the relative importance of the element : Ca ratios were different between leptocephalus and both freshwater- and seawater-resident yellow stage eels (Table 2). This demonstrated that the elemental composition in otoliths of the eel significantly changed with life stage and habitat transition. The mechanism of the change of elemental composition of the otolith with life stage is not clear in most fishes, but may relate to the elemental concentration of ambient water and osmotic pressure regulation of the diadromous eel when they change from osmoconformer in the leptocephalus stage to osmoregulation in the yellow stage eel. The body fluid of a leptocephalus is isotonic to seawater and its body tissue is known to contain extensive amounts of a gelatinous extracellular matrix composed of sulphated glycosaminoglycans (GAGs), which have an affinity for alkali elements, particularly Sr^{2+} (Comper and Laurent 1978; Nishizawa 1978; Hascall and Hascall 1981; Toole 1981). Sulphated glycosaminoglycans breakdown during metamorphosis from leptocephalus to glass eel may possibly reduce the absorption of Sr^{2+} and other elements. Thus, the Sr : Ca ratios in otoliths of leptocephali significantly decreased when they metamorphose into glass eels (Otake *et al*. 1994; Arai *et al*. 1997). However, the effect of ambient environment on the otolith Sr : Ca ratios of the eel cannot be excluded because the growth habitat shifted from high to low saline water when the eel metamorphoses from leptocephalus to glass eel and Sr concentration is ~100 fold higher in seawater than in fresh water (Campana 1999). On the contrary, the Na : Ca ratios in otoliths of the eel were higher in freshwater- compared with seawater-resident yellow stage eels, although Na concentration is higher in seawater than fresh water. This may indicate that sodium uptake is physiologically controlled by the eel. Accordingly, except with the exception of Sr, the other elements in fish otoliths are also very important in the linking study between otolith microchemistry and the life and migratory environmental history of the fish.

This is the first paper to use multiple elements in addition to Sr to study the life history and migratory environment of the eel. The eight elements selected in the present study were reported to have a positive correlation in abundance between otoliths and ambient water (Goldberg 1965; Thresher 1999). With the exception of the Sr : Ca and Na : Ca ratios, no significant difference was found among leptocephalus and both freshwater- and seawater-resident yellow eels (Table 2). This indicates that it is difficult to use a single element : Ca ratios to discriminate the eel stage and habitat use. However, the cross correlation matrix of the seven element : Ca ratios in otoliths was found to be different among stage and habitat use of the eel (Table 3). This may indicate that the elements incorporated into otoliths of the eel were conditionally selected. The canonical plot and stepwise canonical discriminant analysis also indicated that along with Sr : Ca, the other four element : Ca ratios (Cl : Ca, P : Ca, S : Ca and Na : Ca) have obviously contributed to the grouping of the eels (Fig. 1). The classification success of the different stage and habitat use of the eel reached 70.7–98.0% (Table 5). This suggested that multiple-elemental information could provide additional insight into environmental life history traits of the facultative catadromous eels. A total of 31 elements have been detected in fish otoliths to date (Campana 1999). Future studies are needed to assess the use of elements that are below the detection limits of EPMA for more detailed fingerprints regarding eel otoliths.

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Appendix 1. Electron probe microanalyser counting times (peak and background), standards, detection limits, and analytical errors

Detection limits (wt %) and typical analytical errors (wt %, 1 σ) calculated after Scott *et al*. (1995)

Element	Counting time (sec)	Standard	Detection limit	Typical analytical error
Na	60	Halite (CM Taylor)	0.029	0.023
Mg	60	Osumilite (USNM 143967)	0.019	0.022
P	60	Apatite (Wilberforce)	0.036	0.027
S	60	Gypsum (CM Taylor)	0.023	0.017
Cl	46	Halite (CM Taylor)	0.027	0.015
K	46	Osumilite (USNM 143967)	0.019	0.012
Ca	20	Calcite (NMNH 136321)	NA	0.245
Sr	120	Strontianite (Smithsonian R-10065)	0.036	0.019

NA, Not applicable.