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Changes in otolith microchemistry of the Japanese eel, *Anguilla japonica*, during its migration from the ocean to the rivers of Taiwan

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The newly recruited Japanese eel, *Anguilla japonica*, elvers and 1-year-old eels collected in estuaries and in rivers, respectively, were studied. The microstructure and chemical composition of the sagittal otolith of these eels were examined by SEM and wavelength-dispersive spectrometer (WDS). A transition zone or ' elver mark ' was observed in the otolith of the young eels. A comparison of the otoliths of elvers with those from the 1-year-old eels suggests that this transition zone was deposited during upstream migration, a change from a marine to freshwater environment. Strontium (Sr) content in the primordium of the otolith of both elvers and young eels was low, probably due to the maternal or freshwater origin of the oocyte. The concentration of Sr in the otolith increased gradually during marine life and reached a peak approximately 1 month before upstream migration. As the elvers entered the estuary, the Sr concentration dramatically decreased and remained at a low level thereafter. These findings indicate that the history of the migratory environment of the eel can be reconstructed from a combined study of otolith microstructure and microchemistry analysis.

Key words: *Anguilla japonica*; elver; eel; otolith; microstructure; microchemistry; strontium; SEM; WDS; migratory environment.

INTRODUCTION

The Japanese eel, *Anguilla japonica* (Temminck & Schlegel), is one of the most important culture species in Taiwan and Japan. Large numbers of elvers are caught for cultivation from November to March during their upstream migration in the estuary (Tzeng, 1985). The eel is a catadromous fish. The spawning area of the eel was recently found to be in the North Equatorial Current west of the Mariana Islands, at a salinity front near 15° N, 140° E (Tsukamoto, 1992). Leptocephali drift with the current from their oceanic spawning grounds, and metamorphose into transparent glass eels, or elvers, before entering the estuary. After upstream migration, the elvers become yellow eels and live in rivers for 5–20 years. During late autumn, the maturing eel migrates downstream to the ocean to spawn (Tesch, 1977). An ageing technique examining daily growth increments in otoliths has been applied widely to study the age, growth, and birth date of the leptocephalus and elver (Tabeta *et al.*, 1987; Tsukamoto, 1990; Tzeng, 1990). However, knowledge concerning the migratory history from oceanic spawning grounds to the river is still limited (Tsukamoto, 1990; Tzeng & Tsai, 1992).

Recent studies have indicated that past environmental history of the fish can be reconstructed from analysis of the ratio of trace elements, especially strontium

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(Sr) incorporated during the process of otolith growth (Radtke, 1989; Radtke *et al.*, 1990; Townsend *et al.*, 1992). Strontium can interchange with calcium (Ca) in otoliths during the depositional process because Sr has the same valence as Ca, as well as a similar ionic radius (Amiel *et al.*, 1973). Strontium content in the otolith of diadromous fish was found to differ between their freshwater and seawater phases (Bagenal *et al.*, 1973; Casselman, 1982; Radtke *et al.*, 1988; Kalish, 1990). The incorporation of Sr into the otolith is a physiological process controlled by many interactive factors including temperature, salinity, ontogeny and migration (Dodd, 1967; Kalish, 1989, 1991; Kinsman & Holland, 1969; Weber, 1973; Smith *et al.*, 1979; Schneider & Smith 1982; Sadovy & Severin, 1992; Radtke & Shafer, 1992; Townsend *et al.*, 1992).

The purpose of this paper is to clarify the migratory history of the eel, during its migration from spawning grounds to the rivers of Taiwan, by examining the Sr content and microstructure of the otoliths of the elvers and young eels, using SEM and WDS. The Sr/Ca changes in otolith in relation to Sr content of ambient water and ontogenetic changes of the fish are discussed.

MATERIALS AND METHODS

SAMPLING DESIGN

The microstructure and microchemistry of the otolith of the Japanese eel, during its migration from marine to fresh water, were examined using six newly recruited elvers and six 1-year-old eels. The elvers were caught using a modified Fyke net (Tesch, 1977) at night-time flood tide in the estuary of the Gong-Shy-Tyan stream on 22 January 1990. The 1-year-old eels were caught using electric fishing apparatus during the daytime, from the Gong-Shy-Tyan stream on 9 September and 4 October 1991 and from a tributary of the Tanshui River on 24 April 1992 (Fig. 1). The elvers were fixed with 95% alcohol, and their total lengths (T.L.) were measured to the nearest 0·1 mm with vernier calipers 1 week after fixation. The developmental stage of the elver was determined according to the distribution of pigments on the body surface (Strubberg, 1913; Bertin, 1956). The young eels were preserved by freezing immediately after collection, and total length and body weight of the eels were measured after thawing. The six elvers averaged 57·78 mm T.L. (ranging from 56·2 mm T.L. to 59·0 mm T.L.) at developmental stage VA and the six 1-year-old eels 132·55 mm T.L. (92·7–169·8 mm T.L.). The elvers at stage VA have no external pigment except the caudal spot and are called glass eels (Strubberg, 1913).

PREPARATION OF OTOLITH SPECIMENS FOR ELECTRON MICROPROBE X-RAY ANALYSIS

The procedures for grinding and polishing the otolith specimens were similar to Tzeng (1990), except for the embedding. Sagittae, the largest of the three pairs of eel otoliths, were dissected from the optic vesicle, cleaned, washed with distilled water, dried in air and finally embedded in Petro poxy 154 (Palouse Petro Products, U.S.A.), which was cured for 1 h at 135° C. Embedded otoliths were ground along the anterior–posterior direction of the frontal plane of the fish and through the primordium of the otolith. The polished surface of the otolith must be extremely smooth for electron microprobe analysis, otherwise a large diffraction of X-rays occurs, resulting in analytical errors (Radtke *et al.*, 1990). The polished otoliths were coated, under vacuum, with a thin layer of carbon to reduce further X-ray diffraction and increase electron conductance.

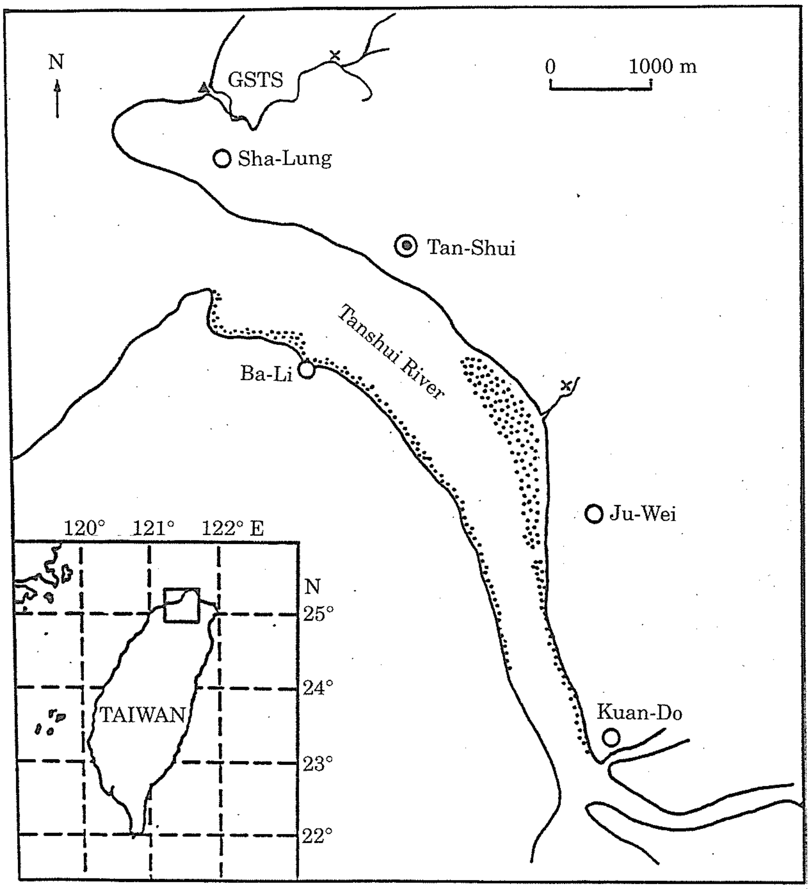

FIG. 1. Location of the sampling stations for elvers (▲) and young eels (×) from the Gong-Shy-Tyan stream (GSTS), and from a tributary of the Tanshui River. Dotted areas indicate the distribution of mangrove swamps.

MEASUREMENT OF Sr AND Ca CONTENTS IN THE OTOLITH

Quantitative measurements of the concentration of strontium (Sr) in otoliths relative to that of calcium (Ca), were made using a wavelength-dispersive spectrometer (Shimadzu-ARL 1MX-SMG). In the analysis of Sr and Ca, $CaSO_4$ and $SrTiO_3$ were used as standards. The acceleration voltage of electrons was 15 kV; specimen current 0·01 μA. The electron beam was focused on an area approximately 5 μm in diameter, at intervals of approximately 10 μm along the anterior–posterior axis of the otolith. The energy dispersive strength of the elements was evaluated using five 4 s scanning periods. After ZAF correction, the weight and molecular number of SrO and CaO were calculated using a constant of CO_2 at 43·97%. Then, the relative concentrations of Sr/Ca were estimated in weight, and the molecular number in percentages. Intentional bombardment by an electron beam with increased absorbed current voltages created a slight burn impression at the sampled location, serving as a convenient marker; however, the burn marks disappeared when lower beam power densities were used (Gunn *et al.*, 1992; Toole & Nielsen, 1992). Thus, the position on the otolith surface where the relative concentration of Sr and Ca is measured could be recognized from the otolith microphotographs.

After microprobe analysis, the otoliths were repolished and prepared for SEM (Scanning electron microscope, Hitachi s-520) examination (Tzeng, 1990). The migratory

TABLE I. The strontium (Sr) and calcium (Ca) contents, and Sr/Ca concentration ratio in water samples from oceanic waters, Gony-Shy-Tyan stream (GSTS) and the estuary of the stream

Area	Sampling location	Sampling date	Salinity (‰)	Sr (ppm)	Ca (ppm)	Sr/Ca (10^{-3})
Oceanic waters						
S1	21°54.3′N 120°53.4′E	13 Sep. 1991	34·0	6·5	472	13·77
S7	21°47.7′N 121°39.4′E	12 Sep. 1991	33·9	6·4	500	12·80
S10	21°46.0′N 122°13.0′E	12 Sep. 1991	34·1	6·4	500	12·80
S33	23°09.1′N 124°00.0′E	8 Sep. 1991	34·6	6·4	528	12·12
GSTS estuary						
E1	High water	11 Sep. 1992	31·1	4·4	388	11·34
E2	High water	11 Sep. 1992	30·5	4·5	384	11·72
E3	Low water	11 Sep. 1992	5·7	0·9	67	13·43
Stream waters						
G11-1	downstream	4 Nov. 1991	0·0	0·1	6	16·66
G11-2	midstream	4 Nov. 1991	0·0	0·1	6	16·66
G11-3	upstream	4 Nov. 1991	0·0	n.d.	—	

history of the eel was determined from both the microstructure and microchemistry of the otolith.

Sr AND Ca CONCENTRATION OF THE AMBIENT WATER

In order to determine the various concentrations of Sr and Ca experienced by eels in the ambient water during their migration from oceanic waters to rivers, water samples collected from the oceanic waters off east Taiwan and from the Gong-Shy-Tyan stream in northern Taiwan were analysed by an Atomic Absorption Spectrophotometer (Perken-Elmer 703). Then, Sr and Ca concentrations in the otoliths of the eels were compared with those of the ambient water.

RESULTS

Sr AND Ca CONCENTRATION IN AMBIENT WATER

The Sr and Ca contents from the ocean to the stream clearly decreased: Sr from 6·5 to 0·1 mg l^{-1}, Ca from 528 to 6 mg l^{-1}. Salinity in the oceanic waters ranged from 33·9 to 34·6‰, fluctuated from 5·7 to 31·1‰ between low and high water in the estuary and was zero in the stream. The Sr and Ca contents in these waters were proportional to the salinity. The Sr/Ca concentration ratio was higher in stream water compared to estuarine or oceanic waters (Table I).

Sr AND Ca CONTENTS AND THE CORRESPONDING MICROSTRUCTURE IN OTOLITHS OF ELVERS

The burn spots of the electron beam on the sectioned surface of the otolith are discernible (Fig. 2). The incremental width indicated that the otolith grew rapidly during the early stages, then slowed down at approximately spots 5 and 18, followed by another fast-growing period.

Strontium and Ca contents along the maximum edge–primordium–edge axis of the sectioned surface of the otolith of two selected elvers were shown in

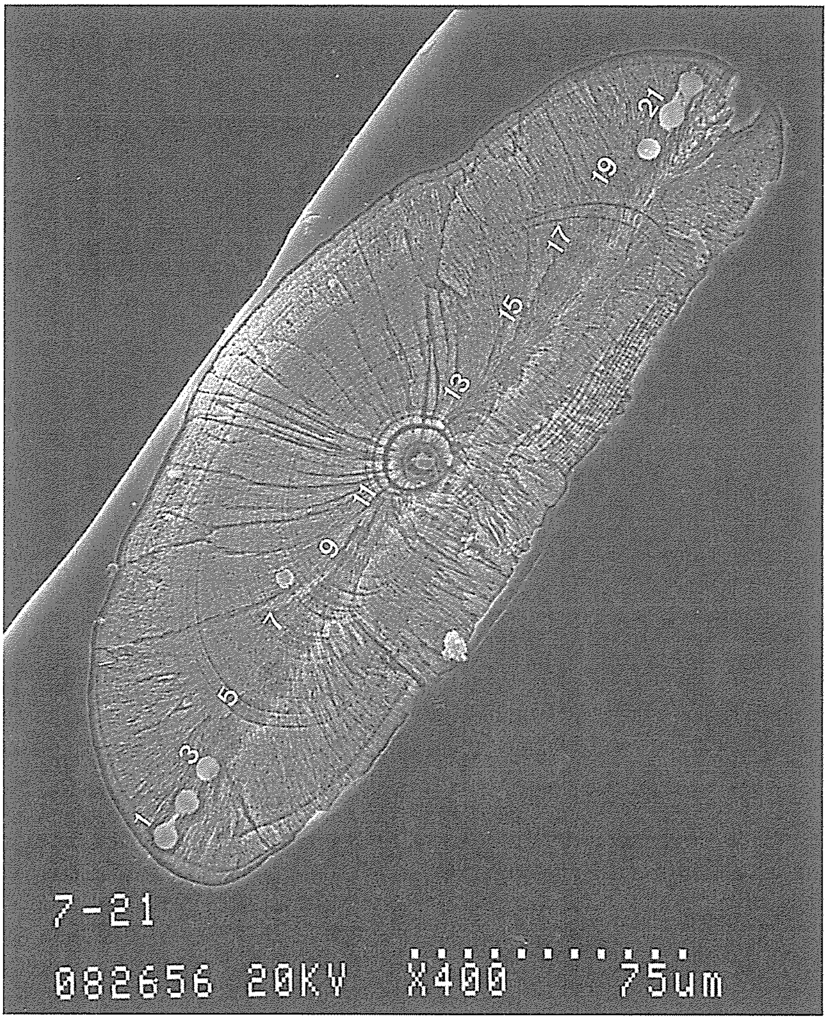

FIG. 2. Scanning electron micrographs illustrating the microstructure on the surface of a frontal section of the otolith from a 58·8 mm T.L. elver, with a maximum otolith diameter of 137·95 μm, collected in the estuary of the Gong-Shy-Tyan Stream on 22 January 1990. The spots (1–22) indicate the burn impression by electron beam bombardment.

Fig. 3(a, b). The tendency of the changes in microstructure and Sr and Ca contents of the otolith was consistent between the two elvers. The Ca content in otoliths of both elvers was lowest in the primordium, but Sr content and Sr/Ca concentration ratio were lowest both in the primordium and on the edge of the otoliths [Fig 3(a, b)]. The Sr/Ca concentration ratio was approximately 9×10^{-3} in the primordium [Fig. 3(a, spot 12; b, spot 9)], then increased gradually, reaching a maximum of $16–17 \times 10^{-3}$ at an area approximately two-thirds of the otolith radius from the primordium [Fig. 3(a, spots 5 and 18; b, spots 3 and 16)], and dramatically decreased to a low level ($8–9 \times 10^{-3}$) at the otolith edge.