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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.

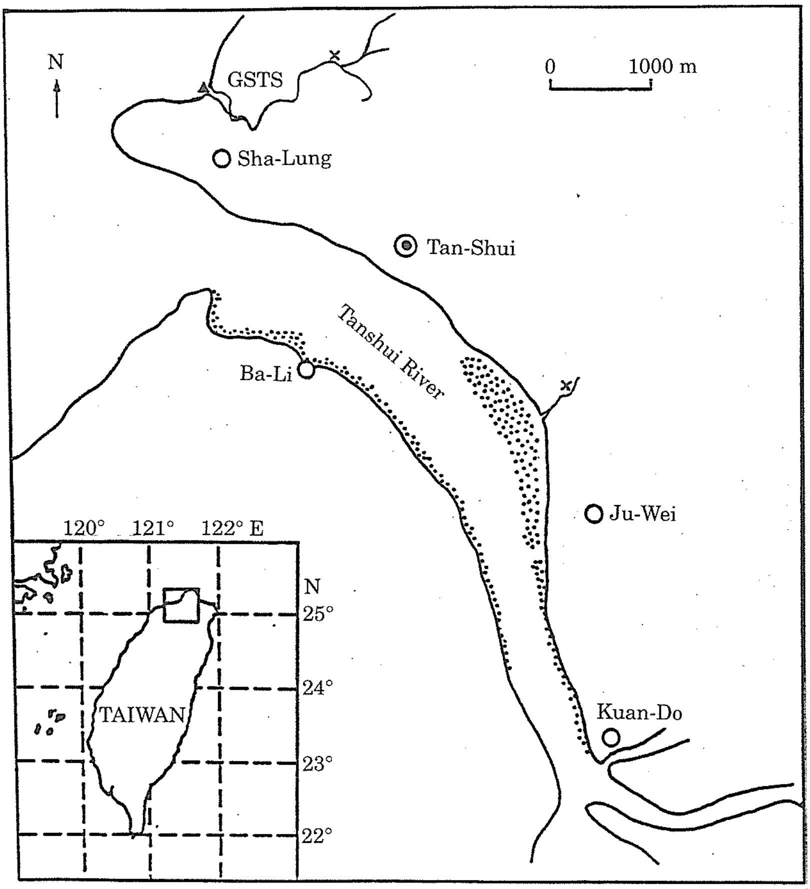

FIG. 1. Location of the sampling stations for elvers (▲) and young eels (×) from the Gong-Shy-Tyan stream (GSTS), and from a tributary of the Tanshui River. Dotted areas indicate the distribution of mangrove swamps.

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

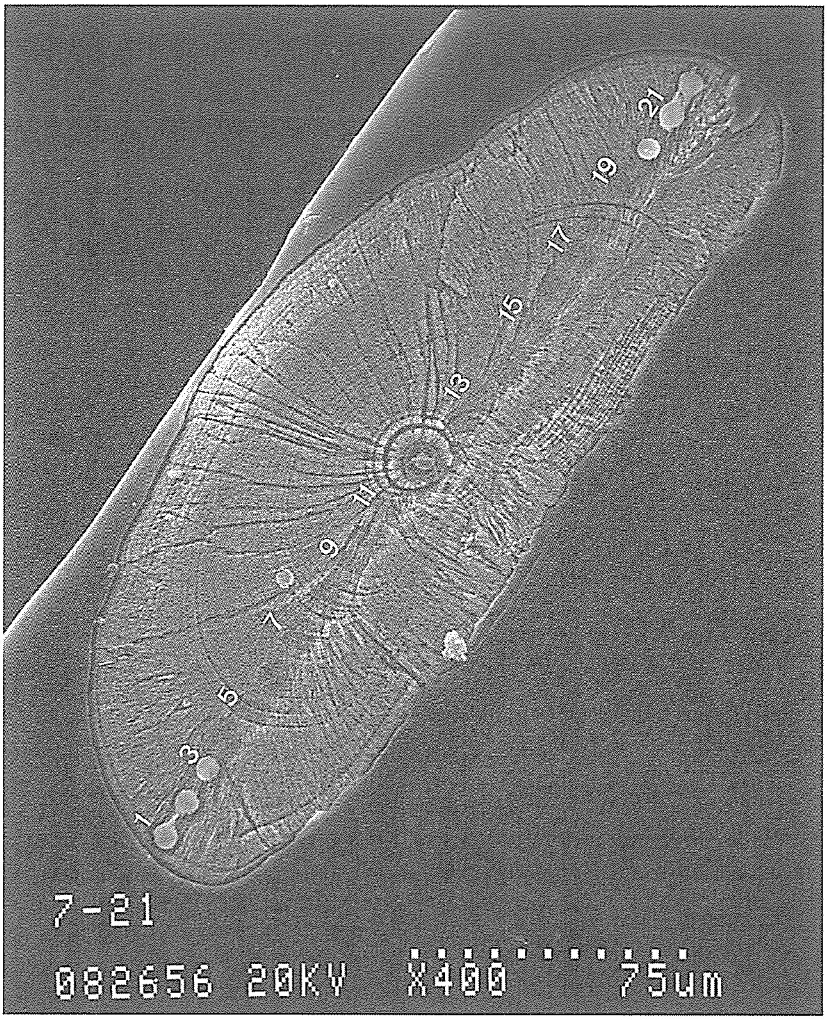

FIG. 2. Scanning electron micrographs illustrating the microstructure on the surface of a frontal section of the otolith from a 58·8 mm T.L. elver, with a maximum otolith diameter of 137·95 μm, collected in the estuary of the Gong-Shy-Tyan Stream on 22 January 1990. The spots (1–22) indicate the burn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

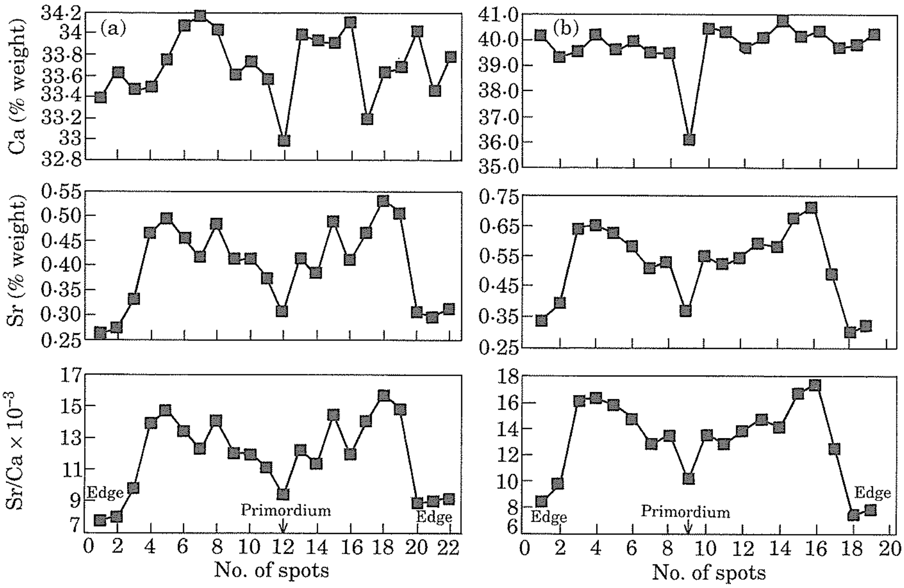

FIG. 3. Calcium and strontium percent dry weights and Sr/Ca concentration ratio measured through the maximum edge–primordium–edge axis on the surface of a frontal section of the otoliths of two selected elvers collected in the estuary of the Gong-Shy-Tyan Stream on 22 January 1991. (a) The same elver as in Fig. 2; (b) 56·2 mm T.L. with a maximum otolith diameter of 142·1 μm. Numerals (1–22) in (a) indicate the spots as in Fig. 2.

The Sr/Ca concentration ratio in the otolith increased with decreasing otolith growth rate from the primordium to the area of peak Sr/Ca concentration ratio which coincided with the slow growth zone of the elver otolith where the daily growth increment was most narrow. The appearance of a peak Sr/Ca ratio in the elver otolith is believed to correspond to the timing during which eel leptocephali migrate from oceanic waters into coastal waters, or when they metamorphose to elvers, as postulated from otolith microstructure by Tzeng & Tsai (1992).

ELVER MARK IN THE OTOLITH OF YOUNG EEL

A dramatic change in otolith microstructure of the eel during its migration from the ocean to the river was described from a selected group of young eels, 150 mm T.L., collected from Gong-Shy-Tyan Stream on 9 September 1991 (Fig. 4). A transition zone made up of approximately four deep-etched discontinuous rings was located at approximately 145·0 μm from the primordium along the maximum radius of the otolith. The radius from the primordium to this transition zone was close to the above-mentioned elver's maximum otolith radius (137·95 and 142·1 μm). The microstructure of the otolith from the primordium to the transition zone for the young eel was also similar to that of the otoliths of elvers in the estuary (Fig. 2). Accordingly, this transition zone in the otolith of the young eel seemed to be deposited during upstream migration, as the elver moved from coastal waters into the river. This transition zone can be used as a time mark in the growth history of the eel. For convenience, this transition zone is called the elver mark (Fig. 4).

FIG. 4. Scanning electron micrographs illustrating the microstructure and elver mark (EM) in otolith of a young eel, 150 mm T.L. and 3·78 g, collected from the Gong-Shy-Tyan Stream on 9 September 1991. (b) Enlarged portion of (a). Scale bar=75 μm.

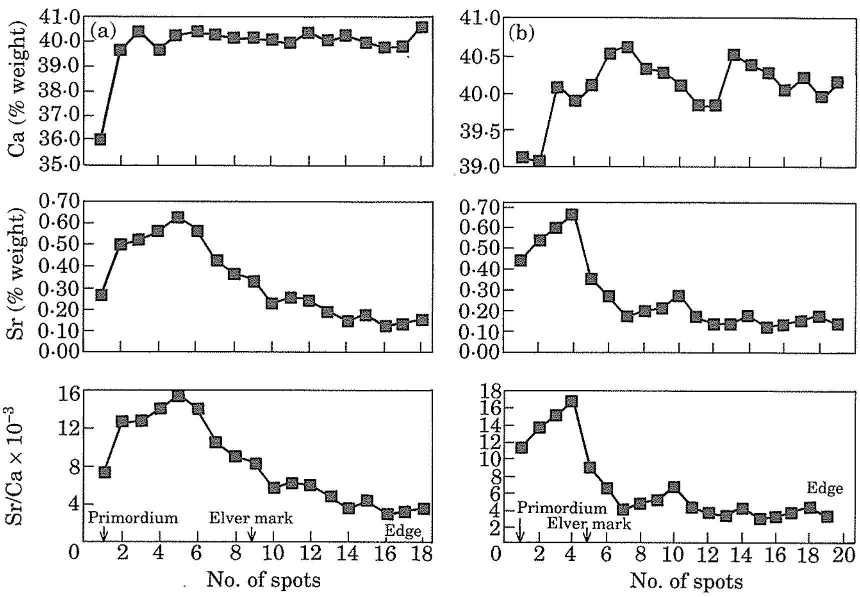

FIG. 5. Calcium and strontium percent dry weights and Sr/Ca concentration ratio measured along the maximum axis from the primordium to the edge on the surface of a frontal section of the otoliths of two selected young eels. (a) 98 mm T.L. and 1·02 g; (b) the same eel as in Fig. 4.

THE CHANGES OF Sr AND Ca CONTENTS IN THE OTOLITHS OF YOUNG EELS

Calcium and Sr contents along the maximum radius from the primordium to the edge on the surface of a frontal section of the otoliths of two selected young eels, are shown in Fig. 4(a, b). The Ca content was lowest in the primordium in both eels, approximately 36·0 and 39·0. The Sr/Ca concentration ratio in the primordium of the otolith of the first eel was approximately 7×10^{-3} [Fig. 5(a)], which was similar to that in the primordium of the otoliths of the elvers (Fig. 3). However, the Sr/Ca concentration ratio at the first spot on the otolith of the second eel was slightly higher because it was not scanned through the exact primordium of the otolith [Fig. 5(b)]. The tendency of the Sr/Ca concentration ratio within the elver mark in the otoliths of the two eels, first nine and six spots [Fig. 5(a, b)] was similar to that of the elvers [Fig. 3(a, b)]. The Sr/Ca ratio reached a maximum, 15×10^{-3} and $16–17 \times 10^{-3}$, at the fifth and fourth spots in the otoliths of these two eels, then decreased to approximately 8×10^{-3} around the elver mark (Fig. 5). Thereafter, the ratio further decreased to a low level, approximately 4×10^{-3}, for both eels.

The microstructure of the otolith from the primordium to the elver mark for the young eel was similar to that of the elver. Accordingly, the migratory history of the elver could be traced from the boundary of the two different habitats through which the eel migrated, which was further substituted by the change of Sr/Ca concentration ratio in the otolith of the young eel. The elver mark on the otolith was particularly clearly delineated. The Sr/Ca concentration ratio in the otolith beyond the elver mark for the young eels was approximately $3–4 \times 10^{-3}$ [Fig. 5(a, spots 10–18; b, spots 7–20)], which was only about one-quarter of the maximum Sr/Ca ratio (15 and $16–17 \times 10^{-3}$) within the mark. This phenomenon indicates that the strontium incorporated into the otolith of the young eel during its stay in fresh water was significantly lower than that incorporated while in a marine habitat.

DISCUSSION

The reasons for differences in Sr contents in the otoliths of fish from different areas are not known, but presumably result from temperature, seawater chemistry, ontogeny, physiological and dietary differences etc. (Radtke & Shafer 1992). Japanese eels, migrating from spawning sites in the open ocean into the rivers of Taiwan, experience different water temperatures as well as different water chemistry (i.e. sea water, brackish water and fresh water), in which the Sr content is quite different (Table I). They also experience a series of ontogenetic changes, passing through the yolk sac, leptocephalus, elver and yellow eel stages. These environmental and physiological changes may all affect the incorporation of Sr into the otolith of the eel. Studies on other species also indicated that variation in otolith chemistry was due to the interaction of numerous factors (Kalish, 1991).

The incorporation of Sr into the otoliths of fish has been reported to be negatively correlated with water temperature and this, in turn, was used to determine the temperature history of the fish (Radtke *et al.*, 1990; Townsend *et al.*, 1992). Previous studies indicate that the elvers hatched during the summer (Tsukamoto, 1990, 1992; Tzeng, 1990) arrived in the estuary during the lowest temperature winter period (Tzeng, 1985, 1990). The temperature was approximately 28° C in the open ocean during hatching, and 15° C in the estuary (Tzeng, 1985; Kajihara *et al.*, 1988). The elvers used for otolith examination were collected on 22 January 1990 in winter. If the incorporation of Sr into the otoliths is controlled mainly by water temperature, the maximum Sr/Ca ratio in the otolith of elvers should be at the outer edge of the otolith, where the fish is exposed to the lowest temperature period during its migration from the ocean to the estuary, not at the 5th and 18th spots where the daily growth increment of the otolith was most narrow and the otolith growth rate was most slow [Figs 2 and 3(a)]. This indicates that the effect of water temperature on the Sr incorporation in the eel otolith is not as important as that of other above-mentioned factors.

As the change of Sr content in the otoliths of eels and salinity of the ambient water was consistent (Table I, Fig. 5), we believe that the dramatic changes in the Sr/Ca concentration ratio in the otoliths may reflect the sudden changes in salinity of the migratory environment of the eel. A similar phenomenon was also reported in other fishes, e.g. the Sr content in otoliths of deep-water fishes has previously been correlated with that of the ambient water (Gauldie *et al.*, 1991). The otoliths of freshwater fishes typically have lower Sr levels than those of fish from sea water (Odum, 1957). The difference in Sr concentration in scales has been used to distinguish the freshwater brown trout (*Salmo trutta* L.) from the sea trout (Bagenal *et al.*, 1973). Similarly, the difference in Sr concentration in otolith has also been used to distinguish the progeny of sympatric anadromous and non-anadromous salmonids (Kalish, 1990). These phenomena further support the theory that Sr content in the otoliths of eels is related to the Sr content in ambient water, because Sr content in ambient water was positively correlated with salinity (Table I). Accordingly, salinity was probably one of the most important factors influencing otolith Sr incorporation in the eel.

In addition, the Sr incorporation into the otolith was also related to physiological condition during their developmental stage. Adult eels accumulate the required nutrition in fresh water before migration to the spawning ground (Boëtius & Boëtius, 1985). The primordium of the otoliths of eels was deposited during the yolk-sac larval stage, and the sources of deposition were of maternal origin. The Sr contents in ambient water were lower in fresh water than in sea water (Table I). Probably due to this reason, the Sr contents in the primordium of the otoliths of eels were lower (Figs 3 and 5). On the other hand, the Sr/Ca concentration ratio in elver otolith increased with decreasing otolith growth rate during the period when they changed from internal yolk-sac nutrition to external feeding until metamorphosis in a marine habitat (Figs 2 and 3). These phenomena indicated that ontogenetic development and growth rate probably influenced the Sr incorporation into the otolith.

The Sr/Ca concentration ratio in the otolith of elvers reached a maximum value of approximately $16–17 \times 10^{-3}$ at an area where the width of the otolith growth increment was most narrow (Figs 2 and 3). This area most likely corresponds both to the period when the leptocephalus metamorphosed into an elver, as well as to the period when the fish was going to leave the strong Kuroshio current to move into the coastal waters (Tzeng & Tsai, 1992). The Kuroshio current is warmer, with a higher salinity than neighbouring waters. The Sr concentration in sea water has previously been positively correlated with salinity (Angino *et al.*, 1966; Broecker, 1974; Chen & Jeng, 1972), while this study, and others, have positively correlated Sr concentration in otoliths with that of the ambient environment (Figs 3, 5 and Table I) (Gauldie *et al.*, 1991). Accordingly, the peak Sr/Ca concentration ratio in the otolith seemed to indicate the period when the fish left oceanic waters for coastal waters. After the elvers entered the coastal waters, the Sr/Ca concentration ratio at the otolith edge decreased dramatically to a low level of 8×10^{-3} (Fig. 3). This change of Sr/Ca ratio seems to reflect its migratory environmental history and ontogenetic changes.

A transition zone, or elver mark, was found in the otoliths of the 1-year-old eel (Fig. 4), which was probably deposited when the elvers moved from estuarine waters into the river. The Sr/Ca concentration ratio was approximately 8×10^{-3} at this mark. After this point, the ratio decreased to a low level, $3–4 \times 10^{-3}$, which corresponded to the freshwater yellow eel stage (Fig. 5). As stated before, the Sr concentration in ambient water was much lower in the river than in sea water (Table I). Probably for this reason, the Sr/Ca concentration ratio in the otoliths was lower in eels from fresh water than in those from marine habitats. This phenomenon was also found in the American eel *Anguilla rostrata* (LeSueur) and European eel *Anguilla anguilla* (L.) (Casselman, 1982). On the other hand, the eels at elver stage had been in the estuary, where salinity changed dramatically from 30 to 5‰ between high and low water, and where Sr content changed along with salinity (Table I). The dramatic decrease of the Sr content or Sr/Ca concentration ratio in the otolith edge of the elver was probably because the fish, upon arriving in the estuary, migrated rapidly in a salinity-decreasing upstream direction with selective tidal-stream transport (Greutzberg, 1961; McCleave & Kleckner, 1982; Tzeng, 1984, 1985). Otherwise, the Sr content or Sr/Ca concentration ratio in the elver otolith would fluctuate along with the tidal period.

In conclusion, it is clear that, through a combined study of otolith Sr/Ca concentration ratio and microstructure analysis, it is possible to reconstruct the migratory history of the Japanese eel during its migration from the ocean to the rivers of Taiwan. However, the Sr incorporation in the eel otolith is a complex physiological process which is interactively influenced by migratory environmental factors and ontogenetic changes, and the behaviour of the fish.

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