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Temperature effects on the incorporation of strontium in otolith of Japanese eel *Anguilla japonica*

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The effects of temperature on somatic and otolith growth and the incorporation of strontium in otolith of the Japanese eel, were studied in laboratory-reared and field-caught eels. The somatic and otolith growth rates of the eel increased significantly with temperature and were estimated as approximately 0·096 mm T.L. ($P<0·01$) and 0·36 μm in otolith diameter per degree-day ($0·01<P<0·05$), respectively. In contrast, the Sr/Ca ratio in the otoliths was negatively correlated with water temperature. The feasibility of using the otolith Sr/Ca ratio to reconstruct environmental temperature history of the eel is discussed.

Key words: *Anguilla japonica*; eel; otolith; strontium; electron microprobe analysis; temperature.

INTRODUCTION

Otoliths of teleost fish are composed of calcium carbonate in the aragonite crystal form (Degens *et al.*, 1969), and are deposited rhythmically in alternating protein-rich and carbonate-rich layers as fish grow (Pannella, 1971). These two layers are often formed daily, and this pattern is used to determine the daily age of the fish (e.g. Campana & Neilson, 1985; Jones, 1992). Chemical analyses of otoliths could provide new insights on migrations, growth and other ecological parameters (Radtke & Shafer, 1992). To realize these potentials, we need to understand how the chemical constituents of fish otoliths are incorporated and what factors influence this incorporation.

Strontium (Sr), a trace element, is known to co-precipitate with $CaCO_3$ as otolith deposition progresses. The rate of substitution of calcium by strontium in aragonite coral skeletons is inversely correlated with temperature (Smith *et al.*, 1979; Schneider & Smith, 1982). As fish are poikilotherms, it would be expected that strontium would be substituted for calcium in otolith aragonite with a similar temperature dependence. Such a relationship has been used in attempts to reconstruct the environmental temperature history of larval herring, *Clupea harengus* L. (Radtke *et al.*, 1990; Townsend *et al.*, 1992). However, this phenomenon is not an universal rule (Kalish, 1989). Several abiotic (e.g. temperature, salinity, water chemistry and pollution) as well as biotic factors (e.g. ontogeny, phylogeny, population, growth, diet, reproduction, physiology and migration) may all have potential to influence strontium incorporation into the otolith (Radtke & Shafer, 1992). Accordingly, an understanding of the relationship between strontium incorporation in otoliths and corresponding ambient environmental factors is necessary before applying this technique.

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The Sr/Ca concentration ratio in otolith of the eel, *Anguilla japonica* Temminck & Schlegel, changed dramatically during its migration from the ocean into rivers of Taiwan (Tzeng & Tsai, 1994). This phenomenon was also found in the American eel *Anguilla rostrata* LeSueur and European eel *Anguilla anguilla* (L.) (Casselman, 1982). However, factors affecting the incorporation of Sr in eel otoliths are still not completely understood. Salinity is probably a key factor in affecting Sr/Ca ratios in otoliths of anadromous and catadromous fishes (Kalish, 1990). On the other hand, temperature plays an important role in regulating strontium incorporation in fish otoliths (Gauldie & Nathan, 1977; Gauldie *et al.*, 1978, 1980, 1986; Radtke & Targett, 1984; Radtke, 1989; Radtke & Morales-Nin, 1989; Radtke *et al.*, 1990). This paper attempts to clarify the temperature sensitivity of strontium incorporation in otoliths of both reared and wild-caught eels and to understand the reliability of its implications in the wild eel population.

MATERIALS AND METHODS

EXPERIMENTAL FISH

Four groups of eels were used to test the sensitivity of the incorporation of strontium into otoliths in response to temperature. The eels of Groups 1 and 2 were reared from elvers collected from the Tanshui River estuary, northern Taiwan, on 6 January 1991. The elvers were at approximately developmental stage Va with no external pigments except caudal spots (Strubberg, 1913). Their total length (T.L.) averaged approximately 56·0 mm. Elvers were acclimated first under ambient light for 3 days at a stocking density of approximately 1 fish per litre in natural brackish water (salinity 11·57‰), and fed with artificial formulated larval eel food. The salinity of rearing water was regulated to simulate changes in ambient water experienced by elvers during upstream migration, and diluted to fresh water in the first 40 days (Tzeng, 1982, 1985). After adapting to the artificial environment, the elvers were classified into two groups: one was held under natural conditions of photoperiod and temperature (Group 1), and the other was provided with 5° C additional heat (Group 2). The experiment was conducted until 23 April 1991. The daily change of the rearing temperature was recorded with a maximum and minimum thermometer. At the end of the experiment the fish were killed and fixed in 95% alcohol. Total lengths and body weights were measured to the nearest 0·1 mm and 0·1 g. Sagittal otoliths were removed 1 week post-fixation. The diameter of the otolith was measured with the aid of microscope–TV camera–IBM/PC image processing system (Vipro 512).

In addition, to understand the sensitivity of the incorporation of Sr into eel otoliths in response to annually cyclic changes of temperature, a third group of elvers, collected in the Tanshui River estuary on 30 December 1989, was held under natural conditions of photoperiod and temperature over 1 year. Meanwhile, the strontium content in otoliths of wild eels (Group 4) was compared to that of reared ones. The wild eels, approximately 1-year-old, were collected in Gong-Shy-Tyan Stream on 12 December 1992 and 9 January 1993.

OTOLITH Sr/Ca MEASUREMENT

Sr and Ca contents were measured along the maximum radius from the primordium to the otolith edge, using a wavelength dispersive X-ray electron microprobe (Shimadzu-ARL EMX-SMG). The detailed procedures of otolith preparation and Sr and Ca measurements were similar to a previous study (Tzeng & Tsai, 1994). Before and after microprobe analysis, the otolith was photographed by light microscope with both reflected and transmitted light sources to reveal the positions of the primordium, elver mark and microprobe sampling sites. The microstructure of the otoliths was examined

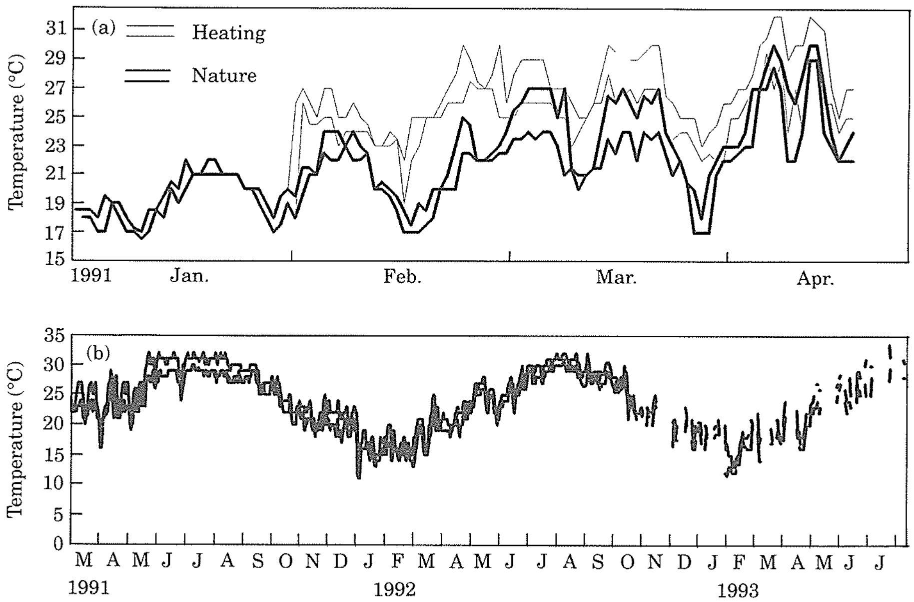

FIG. 1. Time series changes of daily maximal and minimal temperatures in eel rearing tanks. (a) 6 January–23 April 1991. Light and bold lines indicate heating and natural temperature; (b) March 1991–July 1993, natural temperature only.

using a scanning electron microscope (SEM, Hitachi S-520) at 20 kV. The Sr/Ca data in the area from the elver mark to the otolith edge, which was deposited during experimental rearing periods, was used for temperature sensitivity analysis.

DATA ANALYSIS

An allometric growth equation, $Y=aX^b$, was fitted to the length–weight and fish length–otolith diameter data of the eels. The effect of temperature on somatic (or otolith) growth rates was calculated as length increment (K) per degree-day as follows:

$$K=(G_2-G_1)/(T\,.\,t) \quad (1)$$

where, G_1 and G_2 are mean total length (mm) [or otolith diameter (μm)] of the fish reared in the natural condition (Group 1) and in the heated system (Group 2). T is the rearing duration (days). t is the difference of the temperature between the natural and heated condition, approximately 5° C.

The relationship between otolith strontium incorporation and temperature changes was fitted best with a simple linear equation for the eels of Groups 1 and 2, and a quadratic equation for the year-round rearing eels and the field-caught 1-year-old eels (Groups 3 and 4), respectively. The equations were selected according to the seasonal warming tendency and the yearly cyclic change of the rearing temperature.

RESULTS

CHANGES IN WATER TEMPERATURE

Due to a cold spell in the experimental period, the daily water temperature fluctuated greatly in the tanks for both control and heated groups of eels [Fig. 1(a)]. The temperature decreased dramatically approximately 10° C when the cold spell came. Then, the temperature increased rapidly because of the short

TABLE I. Comparison of fish and otolith growth of the eel reared under natural photoperiod and temperature conditions (group 1 and 3) and with 5° C additional heat (group 2)

Group	No. of fish		Temperature	Rearing period	Fish length (mm)		Otolith diameter (μm)	
	Initial	Final			Range	μ ± S.D.	Range	μ ± S.D.
1	20	13	Nature	6 Jan.–23 Apr.	68·4–122·0	91·6 ± 16·8	477·2–715·5	577·0 ± 75·4
2	19	7	Heating	6 Jan.–23 Apr.	84·6–175·5	130·2 ± 29·2	536·0–776·3	695·3 ± 101·6
3	47	23	Nature	30 Dec.–10 Jan.	87·0–377·5	254·2 ± 62·5		
Growth rate					+0·096 mm/°C · day		+0·36 μm/°C · day	
Significance level					$P<0{\cdot}01$		$0{\cdot}01<P<0{\cdot}05$	

The effect of temperature on fish and otolith growth rates was expressed in ° C days. Initial rearing size of the eel was approximately 56·0 mm T.L.

duration of the cold spell. However, overall the temperature showed a seasonal tendency to warm, increasing from approximately 17–19° C in early January to 29–31° C at maximum in late April. The diel maximum and minimum temperature difference ranged between 0 and 5° C.

On the other hand, the seasonal water temperature showed a yearly cyclic change [Fig. 1(b)]. The highest temperature was approximately 32° C in July–August and the lowest approximately 13° C in January–February. The range of diel temperature variation was greater in both increasing and decreasing temperature seasons than in both high and low temperature seasons.

EFFECT OF WATER TEMPERATURE ON FISH AND OTOLITH GROWTH

A total of 86 elvers was reared under three different temperature regimes (Table I). Mean sizes of the eels increased from approximately 56·0 mm T.L. at the elver stage on 6 January 1991, to 130·2 mm T.L. ± 29·2 (with heating) and 91·6 mm T.L. ± 16·8 (natural temperature), respectively, at the end of the experiment on 23 April 1991. Thus the growth rate of the elvers reared with heating was significantly greater than under natural temperatures ($P<0{\cdot}01$). By contrast, mortality rate of the elvers was higher with heating (63·1%) than without heating (35·0%). The mortality rate of the elvers was positively correlated with growth rate. Meanwhile, the fish and otolith growth rates in relation to accumulated water temperature were estimated to be 0·096 mm T.L./° C day and 0·36 μm/° C day in otolith diameter, respectively (Table I).

In addition, the relationship between fish length (T.L. in mm) and body weight (B.W. in g) as well as the relationship between otolith diameter (O.D. in μm) and fish length were plotted and fitted with the allometric growth equation, $Y=aX^b$ (Fig. 2). The experimental data from the three different temperature regimes were pooled, because of small sample size.

$$\text{B.W.}=8{\cdot}05 \times \text{T.L.}^{3{\cdot}04905} \times 10^{-7} \quad (n=43,\ r^2=0{\cdot}99) \tag{2}$$

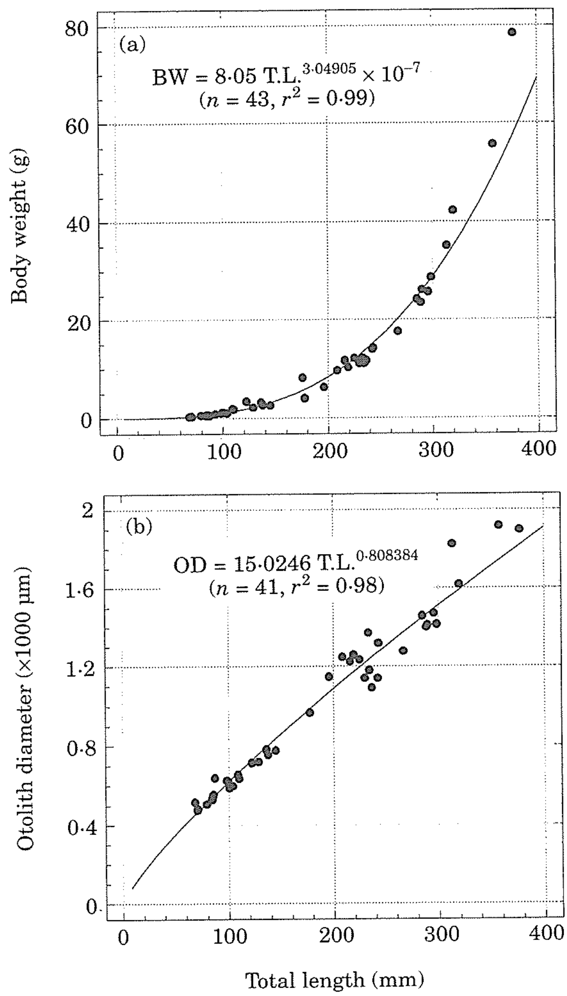

FIG. 2. Relationships between (a) body weight and (b) otolith diameter and total length of the Japanese eel.

$$\text{O.D.} = 15{\cdot}0246 \times \text{T.L.}^{0{\cdot}80838} \qquad (3)$$
$$(n=41,\ r^2=0{\cdot}98).$$

DISCRIMINATION OF WILD AND LABORATORY-REARED ZONES IN EEL OTOLITHS

The microprobe sampling sites on the otolith of a selected eel were shown by reflected light microscope [Fig. 3(a)]. Further the same otolith etched with EDTA was photographed by transmitted light to reveal the elver mark [Fig. 3(b)]. The microstructure of the otolith was examined by SEM [Fig. 3(c)]. The maximum radius of the elver mark on otoliths was 134·9 μm [Fig. 3(c)], which was similar to the maximum otolith radius of elvers in previous studies (Tzeng, 1990; Tzeng & Tsai, 1992, 1994). This eel was reared from a 56·0 mm T.L. elver from 30 December 1989 to 10 January 1991 and grew up to approximately 320 mm, 42·23 g and its otolith diameter became 1618·3 μm. The elver mark was

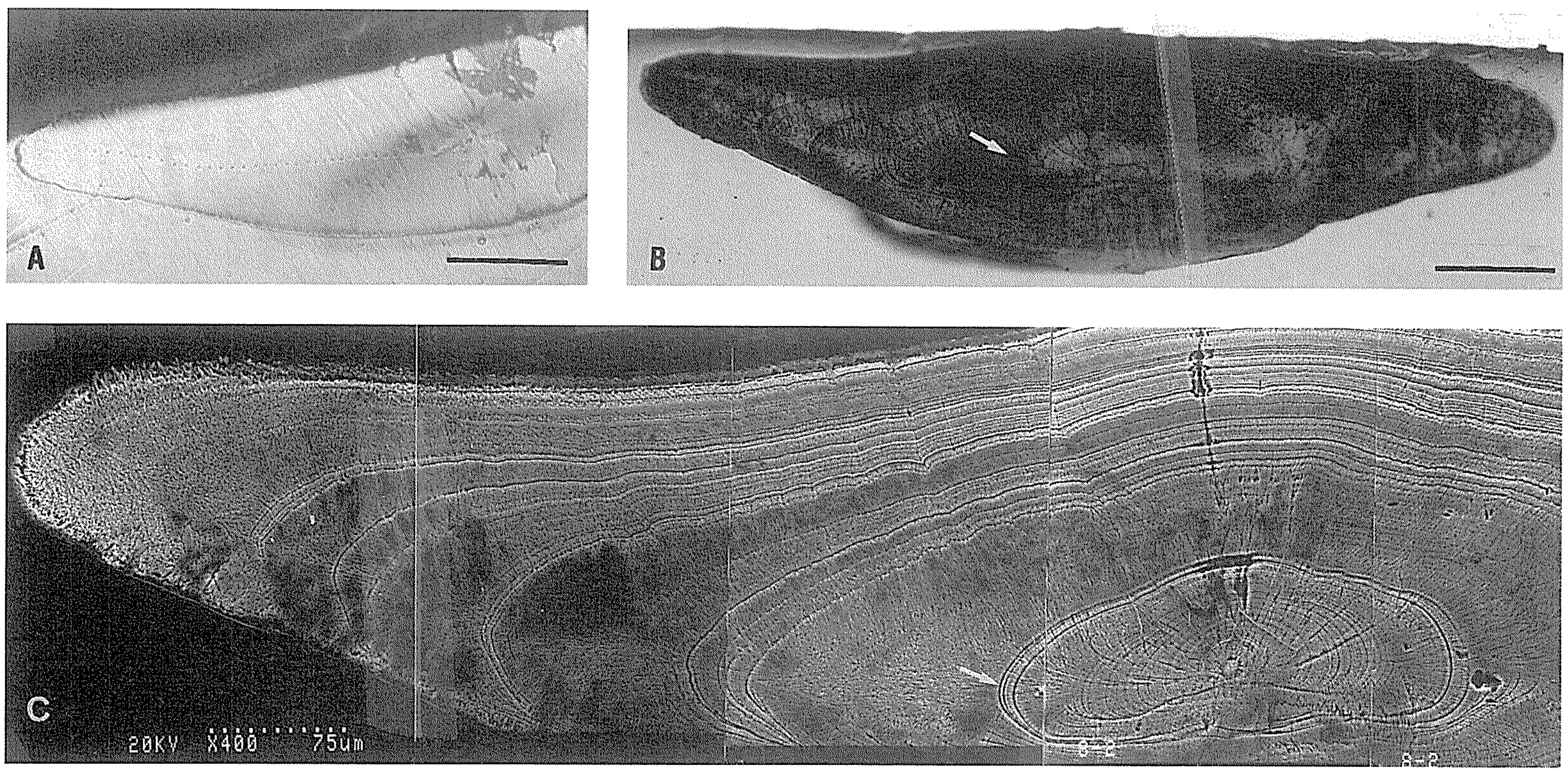

FIG. 3. Photographs showing otolith from a 320 mm T.L. and 42·23 g eel reared from an elver during the period from 30 December 1989 to 10 January 1991. (A) Electron microprobe sampling sites on the otolith photographed by light microscope with reflected light and (B) with transmitted light to show the position of the elver mark (arrow), scale bar=200 μm. (C) SEM micrograph showing microstructure of otolith deposited in wild and laboratory-reared periods.

a unique structure which enabled distinction of the parts of the otolith deposited during wild and laboratory-reared periods. Several growth checks were observed in the middle part of the otolith deposited during the laboratory-reared period, indicating that the growth of the otolith may have halted in summer. The otolith beyond the elver mark was deposited during the laboratory-controlled experimental period, in which the Sr/Ca data was adopted for the following temperature sensitivity analysis.

OTOLITH Sr/Ca RATIO IN RELATION TO WATER TEMPERATURE

Sr/Ca concentration ratios from the elver mark to the edge of the otoliths of the eels reared with and without heating during increasing temperature season seemed to decrease with time [Fig. 4(a)], which was indicated by the linear regression of Sr/Ca ratios on spot numbers [Fig. 5(a)]. Because temperature increased with date during experimental period [Fig. 1(a)], the relative quantity of Sr incorporated into the otolith could be considered to be inversely correlated with environmental temperature.

In addition, the Sr/Ca ratios in the otoliths of both laboratory-reared and field-caught 1-year-old eels were also examined in relation to temperature [Fig. 5(b)–(d)]. Because the laboratory eels were reared from December 1989 to January 1991 [Fig. 5(b) and (c)], and the field-caught 1-year-old eel was collected on 12 December 1992 [Fig. 5(d)], they all have experienced a yearly cyclic low–high–low temperature history. The levels of Sr/Ca ratio in the otolith after the elver mark seemed to fluctuate in an opposite high–low–high tendency over the corresponding period, which could be observed clearly in the quadratic equation of Sr/Ca ratios on spot numbers in otoliths of those eels [Fig. 5(b)–(d)]. This fact indicated that the Sr/Ca ratio in eel otoliths was inversely correlated with temperature whether they were laboratory-reared or wild.

DISCUSSION

Sr incorporation in fish otoliths is of special interest because of its potential utility as an indicator of past environmental temperatures. Our results indicated that the Sr/Ca concentration ratio in eel otoliths was inversely correlated with water temperature for both laboratory-reared and field-caught eels. The inverse relationship between Sr/Ca ratios and temperature coupled with assignment of age to each microprobe sampling site, has been used to imply the relative hydrographic histories of individual fish (Radtke, 1984, 1987, 1989; Radtke & Targett, 1984; Radtke & Morales-Nin, 1989; Townsend *et al.*, 1989, 1992; Radtke *et al.*, 1990), and to compare the temperature histories of different subpopulations of larval herring in the Gulf of Maine (Townsend *et al.*, 1989). On the other hand, Kalish (1989) found that there was only a slight correlation between temperature and otolith Sr/Ca ratios, and that there was no significant linear relationship in his temperature-controlled studies of juvenile Australian salmon *Arripus trutta* Forster. Kalish (1989) suggested that Sr concentration in the saccular endolymph, and hence in the otoliths, may vary seasonally and with age for a particular species, but not directly with temperature, and he urged caution in relating otolith chemistry to environmental variables.

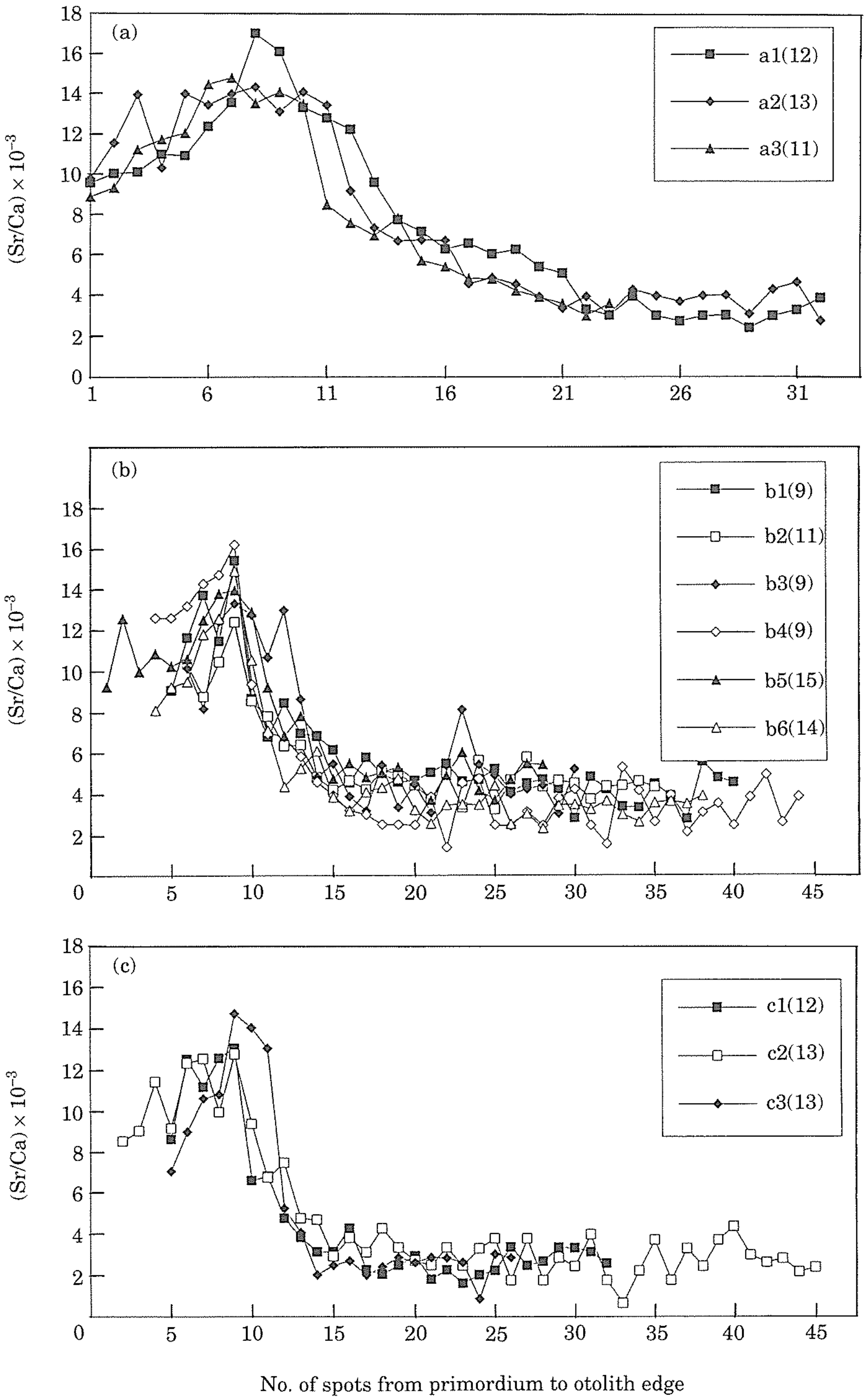
(a)
(b)
(c)
a1(12)
a2(13)
a3(11)
b1(9)
b2(11)
b3(9)
b4(9)
b5(15)
b6(14)
c1(12)
c2(13)
c3(13)
(Sr/Ca) × 10⁻³
No. of spots from primordium to otolith edge

FIG. 4.

Although our results support the hypotheses that the Sr/Ca concentration ratio in the otolith was inversely correlated with water temperature, we didn't attempt to establish an equation for the relationship between otolith Sr/Ca ratio and temperature history of the wild eel, because: (1) the daily difference in temperature was large in the reared system, and we believe that this is similar in the habitat of the eel; and (2) the average daily increment width in the eel otolith was narrow, approximately 0·80–1·4 μm (Tzeng, 1990). Each microprobe sampling site for Sr and Ca measurements was approximately 5 μm^2, which covered several daily growth increments. In other words, a Sr/Ca ratio in a spot on the otolith represents an average temperature of several days. Even if the daily age of the eel was counted by daily growth increment (Tzeng, 1990), we could only reconstruct a temperature history of the eel by average status rather than daily level.

In addition, the life history of the eel included several different life history stages, namely the leptocephalus in the ocean, glass eel in coastal waters, elver in the estuary, and yellow eel in the freshwater stream. The fish experienced different water chemistry during its migration from the ocean into the river. Physiological condition and water chemistry both have the potential to influence the change of the Sr/Ca ratio in the otolith. The 1-year-reared and 1-year-old field-caught eels (Groups 3 and 4) have experienced the seasonal highest and lowest temperature of the year [Fig. 1(b)]. However, the maximum Sr/Ca ratio in the part of the otolith deposited during freshwater life was only approximately $4{\cdot}0 \times 10^{-3}$, which was much lower than the peak Sr/Ca (approximately 16·0–$17{\cdot}0 \times 10^{-3}$) inside the elver mark (Fig. 4). The peak Sr/Ca probably corresponded to the metamorphosis stage of leptocephalus in marine life (Tzeng & Tsai, 1994). This indicated that temperature alone could not explain completely the fluctuation of the Sr/Ca in the eel otolith. Accordingly, a Sr/Ca ratio–temperature curve derived for reared eels as done for other marine species (e.g. Radtke *et al.*, 1990; Townsend *et al.*, 1992) is not feasible for back-calculating the environmental temperature history of the diadromous eel.

The deposition of the otolith is a physiological and chemical process. The incorporation of Sr into the otolith is regulated by various factors, including biochemical fractionation, environmental parameters, mineralogy, water chemistry, ontogeny and phyletic position (Dodd, 1967; Milliman, 1974; Townsend *et al.*, 1989). The eel leptocephalus is a very primitive form, whose nutrition is achieved by direct absorption through the epidermis (Hulet, 1978). The Sr/Ca ratio in the otolith at this stage was greater than or equal to that in sea water as in other primitive organisms. After metamorphosis, the Sr/Ca ratio decreased

FIG. 4. Time series changes of Sr/Ca concentration ratio measured from primordium to edge of the otolith of the Japanese eel. (a) Reared from elvers with heating (a_1) and natural temperature (a_2 and a_3) during the period from 6 January to 23 April 1991. (b) Reared from elvers for 1 year from 30 December 1989 to 10 January 1991. (c) One-year-old wild eels collected from Gong-Shy-Tyan Stream, north Taiwan on 12 December 1992 (c_1: 236 mm T.L.) and 9 January 1993 (c_2: 244 mm T.L.; c_3: 165 mm T.L.). Numerals in parentheses in the legend indicate the position of the elver mark which enables discrimination of the otolith deposited in marine and fresh water or laboratory-reared periods. Total lengths and otolith diameters: a_1, 136 mm T.L. and 781·2 μm; a_2, 110 mm and 633·2 μm; a_3, 99·3 mm T.L. and 636·5 μm; b_1, 242·5 mm T.L. and 1205·1 μm; b_2: 216·0 mm and 1248·4 μm; b_3, 177·5 mm T.L. and 965·6 μm; b_4, 320 mm T.L. and 1618·3 μm; b_5, 87·0 mm T.L. and 655·3 μm; b_6: 289·0 mm T.L. and 1378·9 μm.

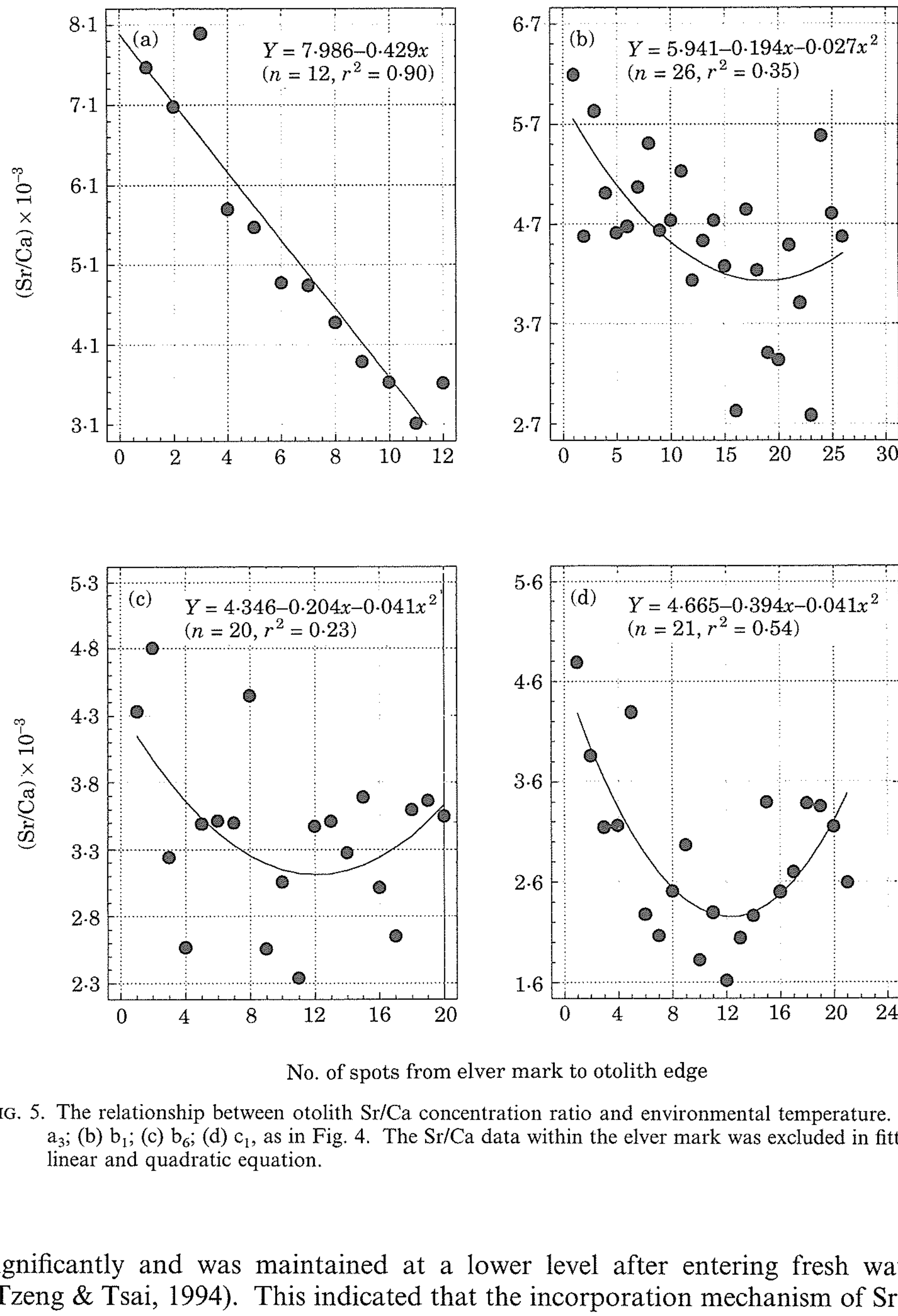

FIG. 5. The relationship between otolith Sr/Ca concentration ratio and environmental temperature. (a) a_3; (b) b_1; (c) b_6; (d) c_1, as in Fig. 4. The Sr/Ca data within the elver mark was excluded in fitting linear and quadratic equation.

significantly and was maintained at a lower level after entering fresh water (Tzeng & Tsai, 1994). This indicated that the incorporation mechanism of Sr in the eel otolith differed at different life stages. Accordingly, unless the relationship between the otolith Sr/Ca ratio and temperature was defined according to life history stage, it would be impossible to reconstruct the environmental temperature history of the eel.

In conclusion, the incorporation of Sr into otoliths of the eel is an integrated process. Besides temperature, other environmental factors such as salinity, as well as the ontogenic development of the fish itself, should be considered together.

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