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EFFECTS OF SALINITY GRADIENT AND ONTOGENETIC SHIFT ON STRONTIUM : CALCIUM RATIO IN OTOLITH OF THE JAPANESE EEL, *Anguilla japonica* TEMMINCK & SCHLEGEL

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To understand the Sr incorporation mechanism in otoliths of the Japanese eel, *Anguilla japonica*, a total of 100 elvers collected from estuary was reared in the laboratory at salinity of 0, 10, 25 and 35‰ for approximately 7 months. The elvers grew from 56 mm TL to 100-300 mm TL. Twenty elvers were randomly selected and the Ca and Sr contents from primordium to the edge in the otoliths were analyzed, using an electron microprobe equipped with 4 channels of wavelength-dispersive spectrometer. The mean Sr/Ca ratios in the new increments of the otoliths of the eel during the rearing period were highly correlated with salinity(S), $[Sr/Ca]\times 10^3=3.797+0.14S$ ($n=20$, $r=0.97$). However, Sr/Ca ratios in otolith of the eel reared in various salinity were much lower than 15×10^{-3} observed in the otoliths of the elvers about one month before collected. The irreversibility of Sr/Ca ratios at 35 ‰ salinity in this experiment indicated that the drastic change of the Sr/Ca ratios in otoliths of elvers was not due to the reduction of salinity in the coastal waters, but more likely to the transition of development stages from leptocephalus to glass eel.

Key words: Japanese eel, otolith, migration, strontium, calcium, salinity, development stage.

INTRODUCTION

Like other teleost fishes, the eel otoliths are composed of calcium carbonate in the aragonite crystal form (Carlstrom, 1963; Degens *et al.*, 1969; Morales-Nin, 1987; Lecomte-Finiger, 1992), which is deposited daily in alternating protein-rich and carbonate-rich layers as fish grow (Pannella, 1971; Campana & Neilson, 1985; Lee & Lee, 1989; Tsukamoto, 1989). This enables us to determine the daily age of the fish and to study their growth history (Tabeta et al, 1987; Tsukamoto, 1989; 1990; Tzeng, 1990; Jones, 1992; Tzeng & Tsai, 1992). In the aragonite crystal formation, a few trace elements were found to co-precipitate with calcium carbonate (Gunn et al., 1992; Ophel, 1967; Rosenthal, 1956; 1963; Smith, 1979; Toole & Nielsen, 1992). Sr is one of the most common elements to replace Ca , because it has a similar ionic radius and valence to those of Ca (Amiel *et al.*, 1973). Recently, Sr/Ca ratio in otolith in relation to ambient temperature along with daily age was used to reconstruct the past environmental history of the fish (Radtke *et al.*,1990; Townsend et al., 1992). Seawater contains more Sr than does freshwater (Tzeng & Tsai, 1994). The change of the Sr/Ca ratios in otoliths was also used to study the migratory history of diadromous fishes between seawater and freshwater environments for American and European eels (Casselman, 1982), Hawaiian gobies (Radtke *et al.*, 1988), salmonids (Kalish et al., 1990) and striped bass (Secor, 1992). These studies indicated that Sr/Ca ratios in otoliths have a potential to retrieve the past environmental history of the fish. However, the incorporation of Sr in otolith is a complicated biogeochemical process influenced by environmental factors and physiological conditions, and the Sr/Ca ratios as environmental predictors in the fish have scarecely been experimentally validated (Kalish, 1989; 1991; Gallahar & Kingsford, 1992; Radtke & Shafer, 1992; Sadovy & Severin, 1992).

The Japanese eel, *Anguilla japonica*, Temminck & Schlegel, is a catadromous fish. Their life cycle is similar to the European eel, *Anguilla anguilla*, including five stages: leptocephalus, glass eel, elvers, yellow eel and silver eel (Bertin, 1956). The Japanese eel spawned in the waters west of Mariana Island (Tsukamoto, 1992). The leptocephalus larvae drift with North Equatorial Current and Kuroshio Current (Kimura et al., 1994). As close to continental shelf, leptocephali metamorphose to glass eels to leave the Kuroshio for coastal waters (Tsukamoto & Umezawa, 1994). The glass eels become elvers in the estuary (Bertin, 1956). During this long journey, they experience a salinity gradient from oceanic to coastal waters and a developmental-stage transition from leptocephali to glass eels. The otolith microchemistry analysis indicates that the Sr/Ca ratios in otolith of elver drastically changed during its migration from spawning ground to the river estuary (Otake et al., 1994; Tzeng & Tsai, 1994). The drastic change of Sr/Ca ratio in otoliths of elvers has been suggested to be resulted probably from the metamorphosis of leptocephalus to glass eel or induced by the reduction of salinity in coastal waters (Otake et al., 1994; Tzeng and Tsai, 1994).

This study attempted to elucidate the factors aflecting the Sr/Ca ratios in otoliths of the Japanese eel during its migration from ocean to the river by examination of Sr and Ca contents in the otoliths of elvers reared with various salinity and to determine the reliability of using Sr/Ca ratio as an environmental indicator for wild eels.

MATERIALS AND METHODS

Due to difficulty in collection of leptocephali and keeping them alive, elvers collected from the Tanshui River estuary in the northern Taiwan on 8th March 1992 were used for the study. The elvers were at the pigmentation stage VA with no pigment except caudal spots (Strubberg, 1913; Bertin, 1956). Their total lengths (TL) ranged from 52.2mm to 58.9mm with a mean of 55.3mm. After the acclimation of two days in brackish water at salinity of 11.57‰ under natural photoperiod, 100 randomly selected elvers were divided into 10 treatment groups, each containing 10 eels and reared in growth chambers at two temperature regimes of 22-23°C and 27-28°C and salinity of 0, 10, 25 and 35‰, two of the 10 treatments with repeated temperature and salinity at low density of two-fold water volume more than others. The temperature and salinity were set by referring to those where leptocephali and glass eels were sampled (Kimura et al., 1994; Otake et al., 1994; Tzeng & Tsai, 1994). The salinity of the rearing water was prepared from an approximately 32‰ natural seawater adjusted with tap water and crude salt (dried natural sea salt obtained from Tainan table-salt processing plant). The fish were fed to satiation once a day with formulated larval eel food supplemented with live tubifex worms. Feces were siphoned daily. Rearing water was changed at intervals of approximately two weeks. The water level was maintained constant by adding water periodically. The Sr and Ca contents and salinity of the rearing water (Table 1) were measured with a atomic absorption spectrophotometry (Perkin-Elmer 703) and salinometer (WTW microprocessor conductivity meter LF196), respectively.

The eels were reared approximately 7 months, and the experiment ended on 12 October 1992. The survived eels were sacrificed, their total lengths were measured to nearest 0.01 with caliper, and their weights were taken to 0.01g with electronic balance. Sagittal otoliths were removed one week after fixation in 95% alcohol.

The maximum diameter of each of the otoliths was measured with computer-aid image processing system (VIPRO 512) and then embbed in resin. The procedures of embedding, polishing and etching of the otolith for Sr and Ca contents measurment followed those described by Tzeng (1990) and Tzeng & Tsai (1992,1994). The Sr and Ca contents in otoliths of the 20 randomly selected eels were analyzed using an electron microprobe equipped with 4 channels of wavelength dispersive spectrometer (WDS; Shimadzu-ARL EMX-SM). In the analyses of Sr and Ca, $SrTiO_3$ and $CaSO_4$ were used as standards. The weight percentage composition of $CaSO_4$ equals 41.19% CaO plus 58.81% SO_3, and $SrTiO_3$ equals 56.46% SrO plus 43.54% TiO_2. The electron beam was focused on an area approximately 5 μm in diameter at intervals of approximately 20μm along the frontal section of the otolith maximum radius from primordium to otolith edge. The accelerating voltage of electron was 15 kV with specimen current of 0.01μA. The counts for spectrometers of the elements were evaluated using five 4s integration periods and at the wavelengths of 3.3685Å (Ca-Ka) and 6.8628Å (Sr-La), respectively. In the ZAF correction procedures (Chen & Tung, 1984), the weight percentages of SrO and CaO in otolith were calculated using a constant of CO_2 at 43.97%. Then, the relative contents of Sr and Ca in % weight in otolith of the fish were estimated.

For understanding the positions of primordium, elver checks and microprobe spots on the otoliths, before and after the WDS analysis each of the otoliths was photographed using light microscope (LM) with transmitted and reflected visible light, respectively (Fig. 2a, b). Then, the otolith was etched with 5% EDTA (ethylene diamine tetra-acetate; pH adjusted with NaOH to 7.4) and photographed with transmitted LM to reconfirm the primordium and elver checks (Fig. 2c) and to discriminate leptocephalus metamorphosis check from the transition of otolith microstructures and Sr/Ca ratios (Otake et al., 1994; Tzeng & Tsai, 1994). With the aid of the microphotographs, the distance from primodium to each of the microprobe spots and the checks was precisely measured. Then, the data of Sr/Ca ratios in the new increment of otolith during rearing period were examined.

The significant difference between treatments for both fish growths and otolith Sr/Ca ratios was examined with two-way analysis of variance (ANOVA). A forward stepwise regression analysis was used to test the relation of otolith Sr/Ca ratios to temperature, salinity, ambient Sr/Ca ratios, fish length and weight and otolith diameter. The significant difference of otolith Sr/Ca ratios between treatments was further tested by Scheffe's multiple range analysis (Sokal & Rohlf, 1981).

RESULTS

GROWTH

After 7 months of rearing, 91 eels survived. The elvers grew from approximately 56 mm to 100-300 mmTL. Mortality rates of elvers did not differ among treatment groups ($P > 0.05$). The somatic and otolith growths were respectively significantly different between treatments of temperature ($p < 0.01$), salinity ($p < 0.05$) and rearing density ($P < 0.05$), and no interaction among these three treatments ($P > 0.05$). Both somatic and otolith growths grew faster at 27°-28°C than at 22°-23°C, at lower salinity than at higher salinity, and at low than at higher rearing density. The relationships between body weights and total lengths (TL) and between otolith diameters and total lengths were calculated from the pooled data, irrespective of different temperature, salinity and rearing density (Fig. 1).

ONTOGENETIC CHANGES IN SR AND CA CONTENTS AND SR/CA RATIOS IN OTOLITHS

The otolith of a 162 mm TL eel reared from elvers of 56 mm TL at the salinity 10‰ and the temperature 27-28°C shows leptocephalus metamorphosis and elver checks at approximately 89 µm and 145 µm from the primordium, respectively, which were discernible from the change of microstructural pattern of their growth increments (Fig. 2). The elver check provides the convenience to distinguish the otolith deposited before and during experimental period. It was deposited when the glass eel arrived at estuary (Tzeng & Tsai, 1994; Tzeng et al., 1994). Both Sr contents and Sr/Ca ratios in otoliths of 4 selected eels before experimental period show great fluctuation; but the level of Ca contents in otolith was stable, irrespective of before and during experimental period (Fig, 3). The Sr contents and Sr/Ca ratios in otolith of the 4 eels were respectively approximately 0.3 and 8×10^{-3} at primordium, reached the highest values of 0.6 and 15×10^{-3} at the metamorphosis check and then dramatically decreased to the low levels of 0.3 and 8×10^{-3} at the elver check (Fig. 3). This indicated that the drastic change of Sr contents and Sr/Ca ratios in otolith of elver occurred before the fish arriving at estuary. The highest Sr/Ca ratios in the otoliths were suggested to correspond most likely to the period when the leptocephalus metamorphosed into glass eel before moving into the coastal waters (Otake et al., 1994; Tzeng & Tsai, 1994).

EFFECTS OF SALINITY ON OTOLITH SR/CA RATIOS

Sr/Ca ratios in otolith deposited during experimental period varied with the salinity of the rearing water. Sr/Ca ratios in new increments of otoliths of the 20 romdomly selected eels were apparently higher in seawater than in freshwater, irrespective of high and low temperature (Fig. 4). Mean Sr/Ca ratios significantly increased, from 4.20×10^{-3} in the freshwater to 9.27×10^{-3} at 35‰ salinity (Table 2). ANOVA indicated that Sr/Ca ratios were not significantly different between temperature ($P>0.5$) but significantly different between salinity ($P>0.01$) and no interactions between temperature and salinity ($P>0.9$). Forward stepwise regression analysis also indicated that salinity was the only factor to influence the Sr/Ca ratios in otolith among the 6 variables, temperature, salinity, Sr/Ca ratio, fish length and weight and otolith diameter. The relationship between otolith Sr/Ca ratio and salinity (Fig. 4) was estimated to be $[Sr/Ca]\times10^{3}=3.797+0.14S$ ($n=20$, $r=0.77$). This indicated that Sr/Ca ratio in otolith could be used as an environmental indicator to study the migration of the eel between freshwater and sea water. However, Sr/Ca ratios in otoliths of the eels at the various salinity and both 22-23°C and 27-28°C were much lower than the highest value of 15×10^{-3} in the otoliths of the elvers (Fig. 4, Table 2). The irreversibility of Sr/Ca ratios in otolith of the eel at the salinity of 35‰ in the rearing experiment indicated that the drastic change of the Sr/Ca ratios in otoliths of elvers was not due to the reduction of salinity in the coastal waters, but more likely to the transition of development stages from leptocephalus to glass eel.

DISCUSSION AND CONCLUSIONS

Incorporation of Sr into otolith is a complicated biogeochemical process influenced by physical factors such as temperature, salinity and water chemistry, as well as by biological factors such as genetic, developmental stage, growth rate, food and physiological status of the fish (Dodd, 1967; Gallahar & Kingsford, 1992; Kalish, 1989; Radtke & Shafer, 1992; Sadory & Severin, 1992; Yamada *et al.*, 1979). The Sr/Ca ratios in otoliths of elvers drastically decreased from a highest value of 15×10^{-3} to a low level of approximately 8×10^{-3} about one month before the fish were collected in the estuary (Fig. 3). This suggested that leptocephali metamorphoses to glass eel and leave the warm Kurshio Current for cold coastal waters (Otake et al., 1994; Tzeng & Tsai, 1994). Accordingly, salinity, temperature and the change of developmental stage may influence the Sr/Ca ratios in the otoliths.

The relationship between Sr/Ca ratio in otolith of the reared eel and the ambient salinity (Fig. 4) is consistent with the change of Sr/Ca ratio in otolith of the wild eel associated with the salinity gradient during its migration from estuary to the river (Tzeng & Tsai, 1994). This validated that Sr/Ca ratios in otolith of the Japanese eel can be used as an environmental indicator to reconstruct the migratory history of the yellow eel between freshwater and seawater. The change in Sr/Ca ratio in otolith was also found in the other diadromous fishes, e.g. American and European eels (Casselman, 1982), salmonids (Kalish et al., 1990), Hawaiian gobies (Radtke et al., 1988) and striped bass (Secor, 1992) during their migration between freshwater and seawater. These indicated the relationship between Sr/Ca ratio in fish's otolith and the ambient salinity is a universal rule in the diadromous fishes.

The experimental eels were exposed to the temperature of 27-28°C and salinity of 35‰ which were very close to 27.0-29.0°C and 34.5-34.7‰ of the natural waters where the leptocephali were collected (Otake et al. 1994). However, the mean Sr/Ca ratios in the new increments of otoliths of the eels reared in 35‰ seawater were much lower than the highest value of 15×10^{-3} observed in otoliths of elvers before experimental period (Fig. 3). The temperature and salinity in the natural environment are gradually decreased during its migration from Kuroshio area to coastal waters. If the change of Sr/Ca ratios in otoliths of elvers is resulted from salinity gradient, when the salinity of the rearing water was elevated to the salinity of 35‰, the Sr/Ca ratios in otolith of the eel during the experimental period should increase to the highest value as observed in the otoliths of elvers before experiment period. But, it is not the case found in this study. In the other hand, if the change of Sr/Ca ratios is due to temperature, the Sr/Ca ratios in otoliths from metamorphosis to elver checks should increase but not decrease because during this period the fish migrate toward a low temperature and the Sr/Ca ratio in otolith is inversely related to ambient temperature (Radtke et al., 1990; Townsend, 1992; Tzeng, 1994). Therefore, salinity and temperature are probably not the primary factors determining the drastic change of Sr/Ca ratios in otoliths of elvers during its migration from open ocean to the river. An alternative possibility is the saltatory change of the developmental stage from leptocephalus to glass eel.

Until now, the leptocephali of Japanese eel or other species of Genus *Anguilla* were scarcely collected, no experiment has been conducted to study the Sr incorporation mechanism in otolith at the time of metamorphosis. The metabolism of salt in glass eel was considered to be different from leptocephalus (Hulet, 1978; Keys & Willmer, 1932; Ophel & Judd, 1967; Utida & Hirano, 1973). Pfeiler (1986) indicated that physiological status of the leptocephalous larvae of Elopiforms changed from salt loading to salt unloading from the premetamorphosis phase (Phase I) to metamorphosis period (Phase II), which was related to the contents of sulfated glycosaminoglycans (GAG) in the tissues. GAG contents increased as the Phase I larvae grew and with this anionic charge also increased, but decreased by about 87% in the Phase II larvae (Pfeiler, 1984; 1986). GAG was known to affect tissue microion distribution and to have affinity to Sr^{2+} (Comper & Laurent, 1978; Nishizawa, 1978; Hascall & Hascall, 1981; Toole, 1981). In this study, Sr/Ca ratios in otoliths of elvers increased gradually from promordium, reached a peak prior to metamorphosis check and then dropped suddely thereafter (Fig. 3). Apparently, the drastic change of Sr/Ca ratios in otoliths of elvers are closely corresponded to the metamorphosis from leptocephalus to glass eel.

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Figure legends

Fig.1 Relationships between body weights (a) and otolith maximum diameters (b) and total lengths of the eels.

Fig.2 Microphotograghs of otolith from a 162 mmTL eel showing P, primodium; M, leptocephalus metamorphosis check; E, elver check(a,c); and 17 microprobe spots(b). Scale bar=150 μm. Photos a,b& c were taken for different purpose as described in the text.

Fig.3 Changes in Sr (a), Ca (b) contents and Sr/Ca ratios (c) in otoliths of 4 selected eels reared from elvers at 10‰ salinity and at the temperature of 22-23°C and 27-28°C (E, the mean position of elver check; M, leptocephalus metamorphosis check and P, primordium). Total lengths of the 4 eels are 143, 162, 165 and 173 mm, respectively.

Fig. 4 Relationship between otolith Sr/Ca ratios and salinity.

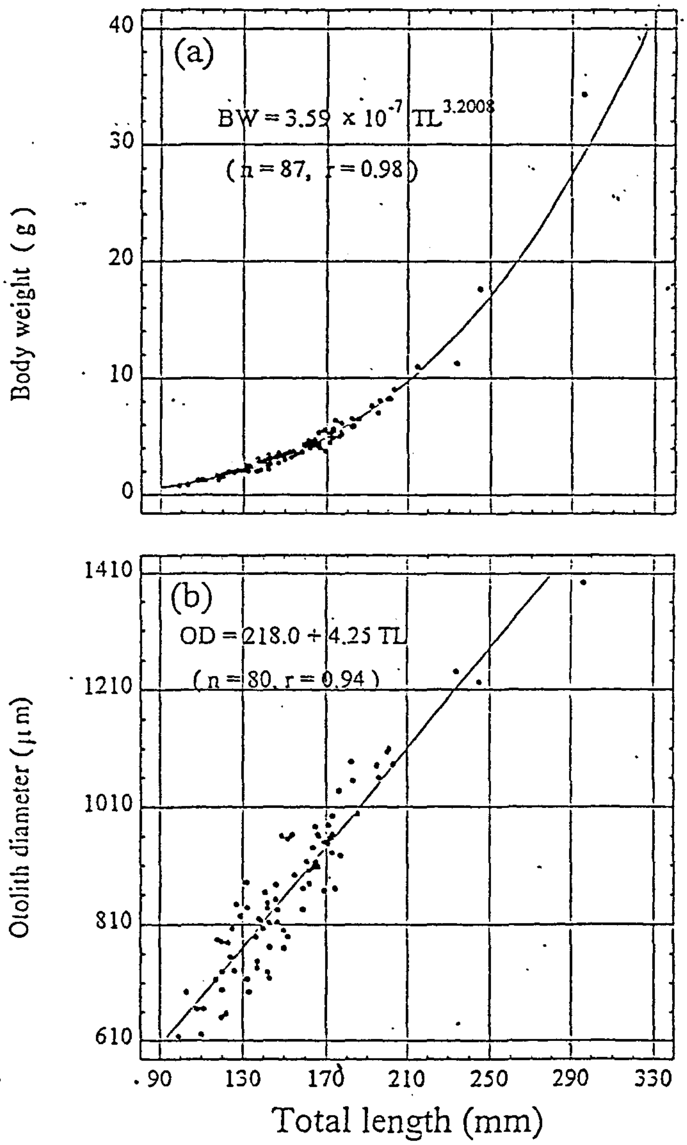
(a)
BW = 3.59 × 10^-7 TL^3.2008
(n = 87, r = 0.98)
Body weight (g)
40
30
20
10
0
(b)
OD = 218.0 + 4.25 TL
(n = 80, r = 0.94)
Otolith diameter (μm)
1410
1210
1010
810
610
90
130
170
210
250
290
330
Total length (mm)

Fig. 1

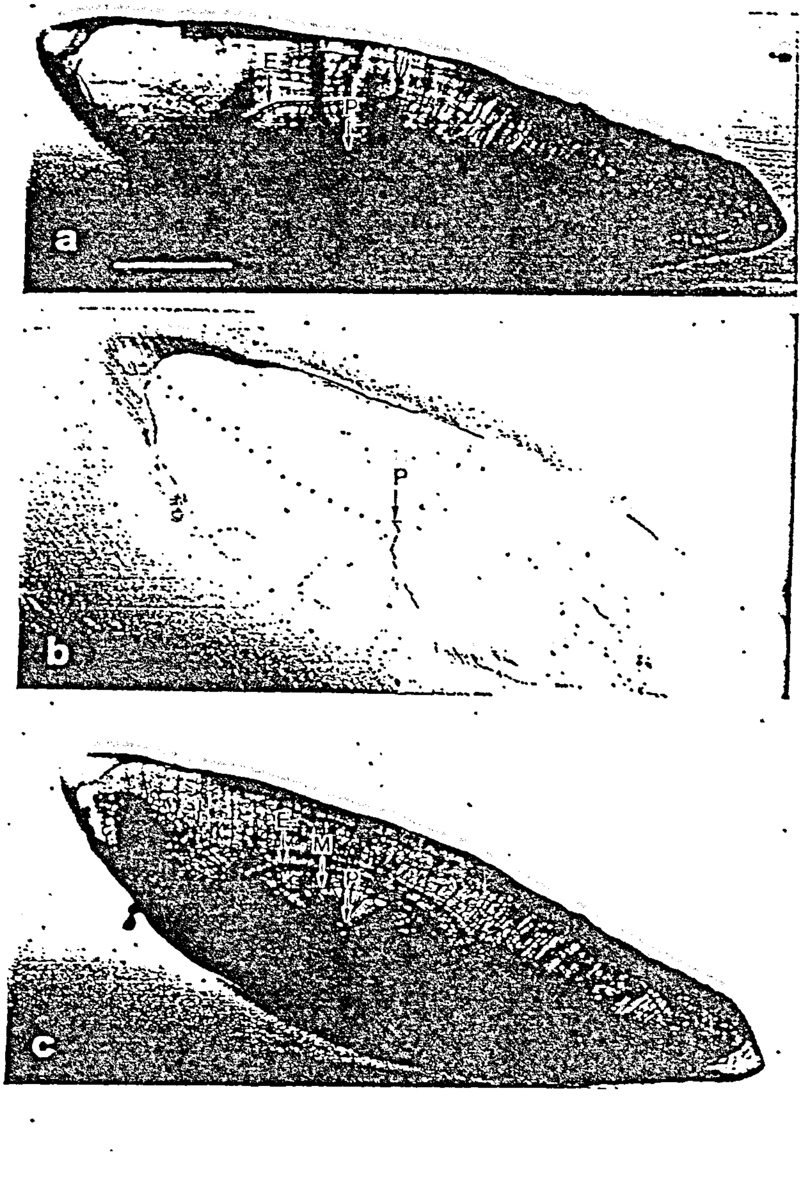

Fig. 2

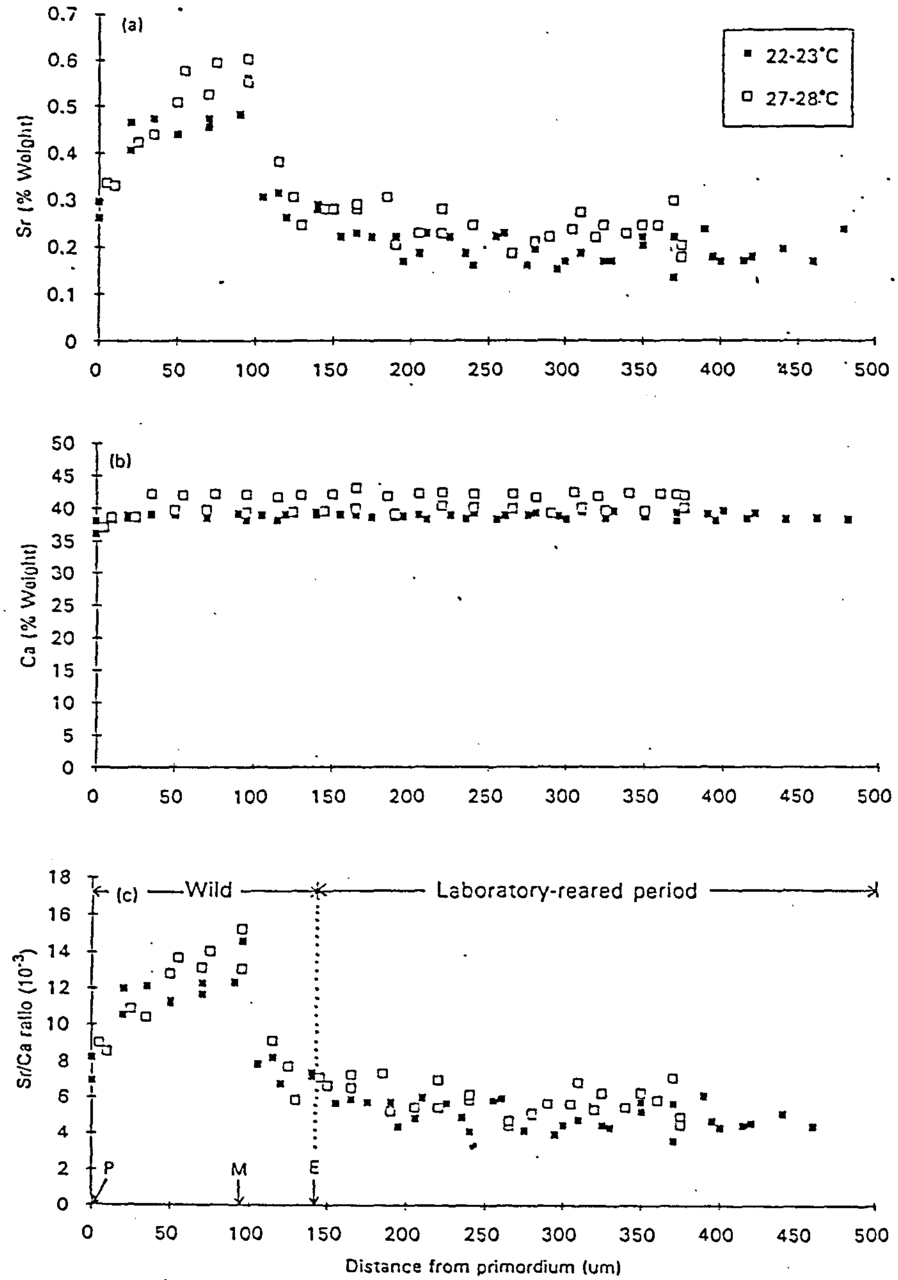
(a)
22-23°C
27-28°C
Sr (% Weight)
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
0 50 100 150 200 250 300 350 400 450 500
(b)
Ca (% Weight)
0 5 10 15 20 25 30 35 40 45 50
0 50 100 150 200 250 300 350 400 450 500
(c)
Wild
Laboratory-reared period
Sr/Ca ratio (10^-3)
0 2 4 6 8 10 12 14 16 18
P
M
E
0 50 100 150 200 250 300 350 400 450 500
Distance from primordium (um)

Fig. 3

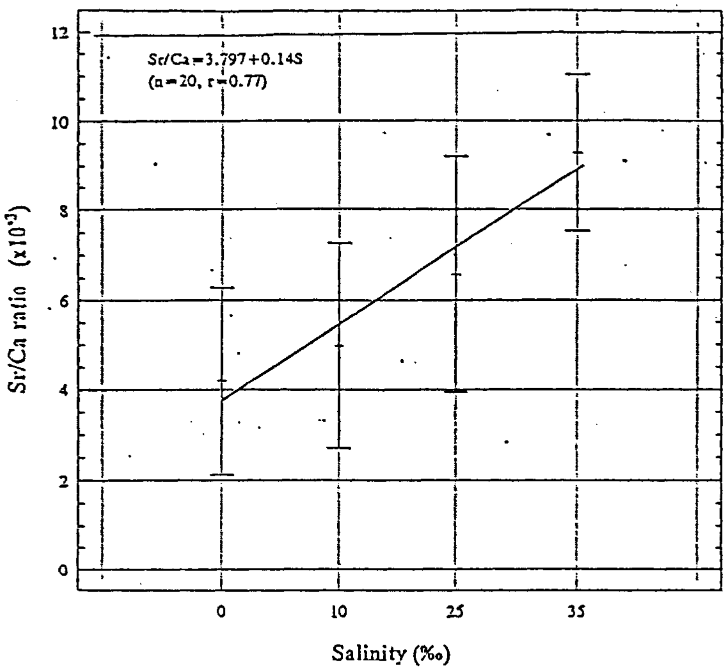

Fig. 4

Table 1. Relationship between Strontium (Sr) and Calcium (Ca) contents and Sr/Ca ratios and salinity of the rearing water.

Salinity	Sr (ppm)	Ca (ppm)	Sr/Ca ($\times 10^{-3}$)
0	0.17	27.67	6.14
10	1.9	181.0	10.50
25	3.95	314.5	12.56
35	5.4	471.67	11.45

Table 2. Homogeneous test of mean Sr/Ca ratios, by Scheffe's multiple range analysis, in new increments of otolith of the Japanese eel reared in different salinity.

Salinity (‰)	Sample size	Sr/Ca ratio ($\times 10^{-3}$)	Homogeneous group
0	5	4.20	*
10	4	4.99	*
25	4	6.57	**
35	7	9.27	*