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NOTE

Drastic changes in otolith strontium/calcium ratios in leptocephali and glass eels of Japanese eel *Anguilla japonica*

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ABSTRACT: Strontium (Sr) and calcium (Ca) contents and Sr/Ca ratios in otoliths of Japanese eel *Anguilla japonica* leptocephali and glass eels were examined. The Ca content was almost constant throughout the otoliths, except for the core, in which it was lower. Both Sr content and Sr/Ca ratios increased with age during the leptocephalus stage, but dramatically decreased at age 90 to 100 d. The otolith increment width also showed marked variation at ages 80 to 110 d, indicating the period of metamorphosis. These suggest that the changes in Sr content and Sr/Ca ratios are associated with metamorphosis. X-ray intensity maps revealed that the Sr content in glass eel otoliths increased for short periods at ages of about 20, 50 and 90 d. Such changes seem to be related to events, such as beginning of vertical migration or differing current influences, occurring during the oceanic migration of Japanese eel leptocephali.

KEY WORDS: *Anguilla japonica* · Leptocephalus · Glass eel · Otolith · Sr/Ca ratios

Fish otolith microchemistry has suggested that otolith strontium (Sr)/calcium (Ca) ratios are correlated with external environmental factors such as ambient temperature (Radtke & Targett 1984, Radtke 1989, Radtke et al. 1990, Townsend et al. 1992) and salinity (Radtke et al. 1988), and internal factors such as somatic growth rate (Sadovy & Severin 1992). It is also suggested that Sr incorporation in the otolith may be related to the total Ca-binding protein level in the blood plasma, which may vary with factors including growth, reproduction and stress, and not be directly affected by ambient water temperature (Kalish 1989, 1991).

Recent efforts have accumulated considerable information on the life history of Japanese eel *Anguilla japonica* Temminck et Schlegel, especially concerning spawning area, birth date, larval age, and growth (Tsukamoto 1989, 1990, 1992, Tsukamoto et al. 1989, Tsukamoto & Umezawa 1990, Tzen 1990, Umezawa & Tsukamoto 1990, Tzen & Tsai 1992). However, knowledge about the ecological and physiological aspects of, for example, oceanic and inshore migration, metamorphosis and feeding remain at only a rudimental level, since very few Japanese eel leptocephali, including metamorphosing individuals, have been sampled during oceanic migration and they are difficult to rear in the laboratory. This preliminary investigation, therefore, sought to extract information on their biological aspects from otolith microchemistry and to shed some light on their mysterious early life history.

Materials and methods. Three leptocephali collected during the research cruise of RV 'Hakuho-Maru', Ocean Research Institute, the University of Tokyo, conducted in the North Equatorial Current west of the Mariana Islands in June and July 1991 (Tsukamoto 1992) and 6 glass eels collected from a beach on Tanegashima Island, Japan, on 30 October 1991 were used in the study. Temperatures and salinities of the waters from which the leptocephali were sampled were 27.0 to 29.0 °C and 34.5 to 34.7‰, respectively (from Preliminary Report of the Hakuho-Maru Cruise KH-91-4). Water temperature of the glass eel sampling site was 23.2 °C. Total lengths (TL) of the leptocephali were 13.8, 17.2 and 27.6 mm. The glass eels averaged 55.5 mm TL (range: 52.3 to 59.0 mm). Otoliths (sagittae) of each fish were embedded in epoxy resin and prepared for measurements of Sr and Ca content in a wavelength dispersive X-ray electron microprobe (JEOL JXA-8900), by grinding and polishing the sample to the mid-plane with polishing papers and 1 µm diamond paste. Prior to analysis with the X-ray electron microprobe, the sample was coated with a thin layer of gold. $CaSiO_3$ and $SrTiO_3$ were used as standards.

Otoliths from all the leptocephali and from 3 of the glass eels were used for 'life-history transects' of Sr and Ca content, carried out at 0.1 to 0.5 µm intervals across the longer axis of the otolith. The accelerating voltage and beam current were 15 kV and 0.1 µA, respectively. The electron beam was focused on a point about 0.5 µm in diameter and counting time was 1.0 s. 'X-ray intensity maps' of both elements were executed on 3 glass eel samples. The beam current was 0.3 µA, counting time was 0.04 s, and other analytical conditions following those for life-history transects analyses.

Following the microprobe analyses for Sr and Ca content, the otoliths were repolished, etched with 0.1 N HCl and sputter-coated with gold in an ion-sputterer. The daily growth increments were examined with a scanning electron microscope (Hitachi S-2150). Each point at which microprobe measurements were made on the otolith was assigned an increment number which corresponded to days after hatching (Umezawa et al. 1989). When the increments were faint and hard to count, the age represented by the measurement position was estimated by using the relationship between the otolith radius (µm) and increment number in Japanese eel (Umezawa 1991). The fitted equations were:

for leptocephali:

Otolith radius = $1.09 \times$ No. of increments

for glass eels:

Otolith radius = $(0.80 \times$ No. of increments$) + 63.1$

The former equation was used for positions located less than 100 µm from the otolith core and the latter for positions greater than 100 µm from the core.

Results. Sr content in the otoliths averaged 0.34 % in the core and increased outwards to a maximum level of about 0.48 % at ages 50 to 90 d (Fig. 1). Subsequently, Sr content decreased rapidly at age 90 to 100 d to about 0.3 %. Ca content was almost constant (36.2 %) throughout the otolith, except for the core in which the content averaged 35.1 % (Fig. 1).

Sr/Ca ratios changed in a similar manner to Sr content, averaging 9.7×10^{-3} in the core and reaching a maximum level of about 13.5×10^{-3} some 50 d after hatching (Fig. 1). The high level continued until 90 d after hatching, followed by a rapid drop between 90 and 100 d. The minimum value recorded was 7.5×10^{-3} at the outermost regions.

X-ray intensity maps of Sr content in the glass eel otoliths revealed 1 distinct and often additional concentric rings showing areas of relatively high Sr content (Fig. 2). The distinct ring was located at an area corresponding to age 50 to 60 d, with the others corresponding to ages 20 to 30 and 80 to 90 d.

Otolith increment width was markedly increased at age 80 to 90 d, decreased at ages 90 to 110 d, and then

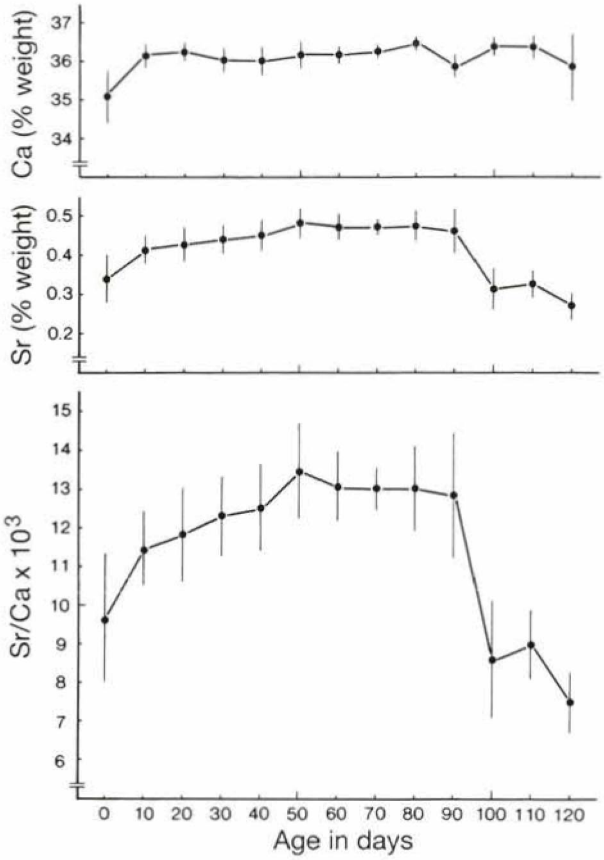

Fig. 1. *Anguilla japonica*. Profile of Ca and Sr contents and Sr/Ca ratios from the core to the edge of otoliths from Japanese eel leptocephali and glass eels, determined by wavelength dispersive electron microprobe analysis. (●) Mean value of data of every 10 d. Error bar = 1 SD. Number of samples for ages 0–10 d (n = 9); 20–30 d (8); 40–50 d (7); 60–90 d (6); 100 d (5); and 110–120 d (4)

increased again (Fig. 3). The marked variation of otolith increment width at ages 80 to 110 d coincided with the drastic changes in otolith Sr content and Sr/Ca ratios. There was no distinct correspondence between variations of the otolith increment width and Sr content at ages 20 to 30 and 50 to 60 d revealed in X-ray intensity maps.

Discussion. Japanese eel leptocephali spawned in the North Equatorial Current, west of the Mariana Islands (Tsukamoto 1992), are transported to the coastal waters of eastern Asia by the Kuroshio Current. It has been suggested that the leptocephali begin to metamorphose to glass eels, and then leave the Kuroshio Current to migrate inshore, when they experience a rapid decrease in ambient water temperature (Tabeta et al. 1987, Tsukamoto & Umezawa 1990, Tzen 1990, Tzen & Tsai 1992). The period of the eel leptocephalus metamorphosis followed by exit from the Kuroshio Current is estimated to be 80 to 110 d after hatching, when the otolith increment width showed a marked decrease

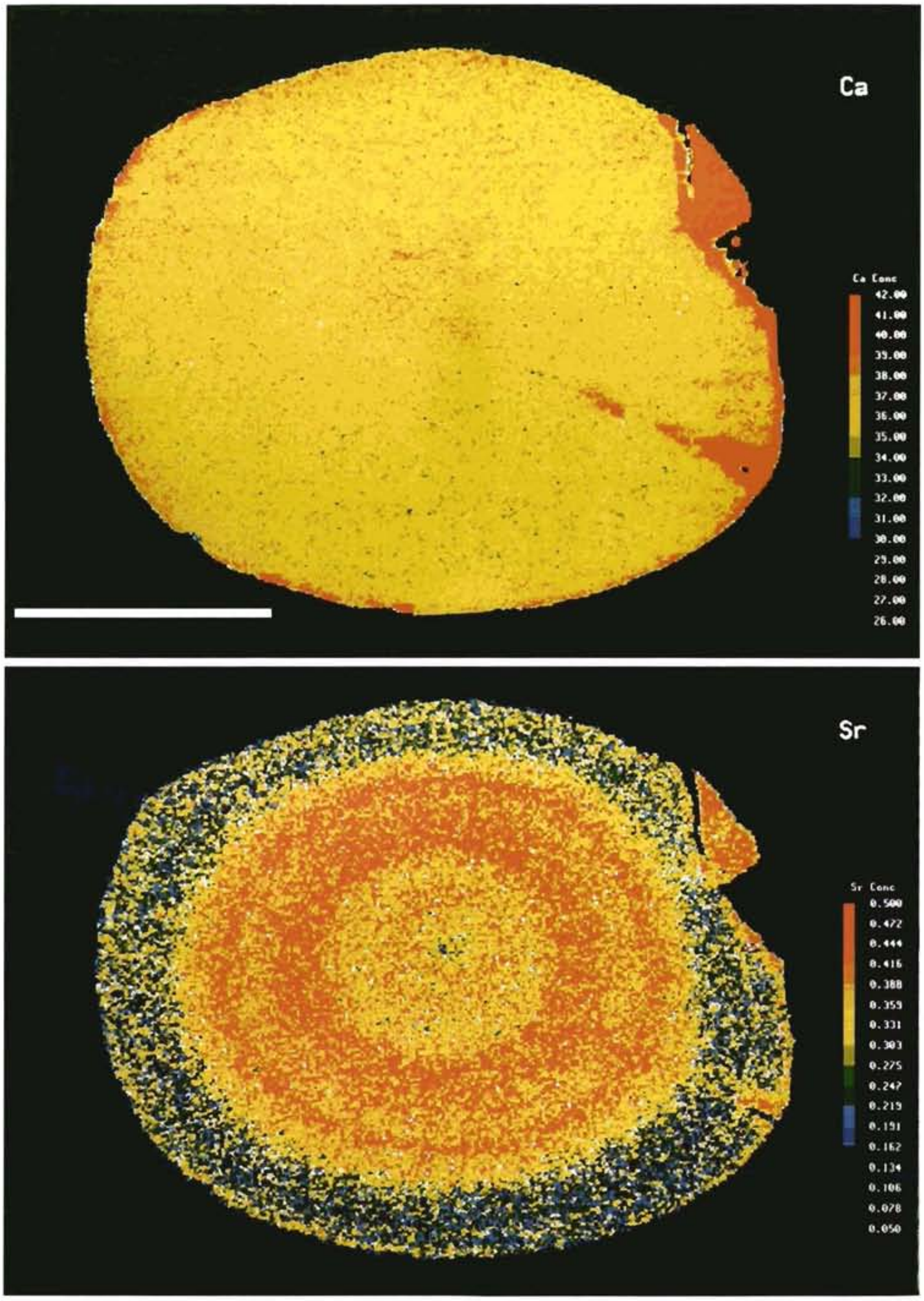

Fig. 2. *Anguilla japonica*. X-ray intensity map of Ca (upper) and Sr (lower) contents in an otolith from a 55.2 mm TL glass eel, representative of 3 glass eels examined. Sr content map shows 3 red concentric rings, assigned to ages 20 to 30, 50 to 60 and 80 to 90 d, indicating relatively high Sr content. The intensely red edge in the Ca content map resulted from grinding of the otolith and does not indicate the true element content. Scale bar = 100 µm

subsequent to the increase (Tsukamoto & Umezawa 1990). Our examination of the otolith increment width also showed the similar pattern at ages 80 to 110 d, coincident with the ages at which both Sr content and Sr/Ca ratios changed dramatically.

In various marine fishes, otolith Sr/Ca ratios have been reported to be inversely related to ambient water temperature (Radtke & Targett 1984, Radtke 1989, Townsend et al. 1989, 1992, Radtke et al. 1990). In contrast, the eel otolith Sr/Ca ratios decreased in the period when metamorphosing leptocephali possibly experienced rapid decrease in ambient water temperature. Furthermore, we have found that high Sr/Ca ratios in the otoliths of *Conger myriaster* leptocephali decreased during the progress of metamorphosis without influences of ambient water temperature and salinity (author's unpubl. data). These results suggest that ambient water temperature and salinity are not major factors in the change of the otolith Sr/Ca ratios in metamorphosing eel leptocephali.

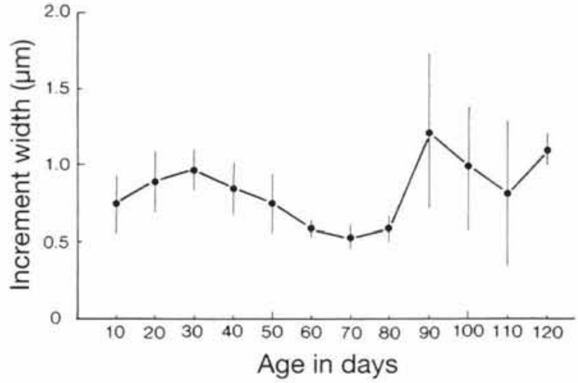

Fig. 3. *Anguilla japonica*. Profile of otolith increment width from ages 10 to 120 d. The marked variation in ages 80 to 110 d overlaps the period showing the dramatic change in Sr/Ca ratios (Fig. 1). (●) Mean value of data of every 10 d. Error bar = 1 SD. Number of samples for ages 0–10 d (n = 9); 20–30 d (8); 40–50 d (7); 60–90 d (6); 100 d (5); and 110–120 d (4)

Otake et al. (unpubl.) have also found that the otolith Sr/Ca ratios decrease proportionally with a decrease in body Sr content in metamorphosing *Conger myriaster* leptocephali. Leptocephali are known to contain extensive amounts of gelatinous extracellular matrix composed of sulfated glycosaminoglycans (GAG) which are broken down during the process of metamorphosis (Pfeiler 1984, 1986, 1991). Since such sulfated polysaccharides indicate an affinity to alkali earth elements, particularly high to Sr (Nishizawa 1978), GAG formation and breakdown may influence the body Sr content. In eel leptocephali, which have a simple circulatory system (Hulet 1978), elements contained in GAG - extracellular matrix seem to be directly taken up, under an enzymatic reaction, by the saccular epithelium, and transported into the endolymph. Although data on GAG content in eel leptocephali are not available, rapid GAG breakdown in metamorphosing leptocephali, causing a loss of Sr from the body, may result in a drastic decrease of otolith Sr content and, consequently, Sr/Ca ratios. In addition, the discrimination against Sr in saccular epithelium might be developed during metamorphosis.

The temporary increase in Sr content at ages 20 to 30, 50 to 60 and 80 to 90 d (revealed by X-ray intensity maps of Sr content in the glass eel otoliths, although not apparent in Fig. 1 where each plot represents a mean value for 4 to 9 samples) nevertheless corresponds to a small change in Sr/Ca ratios at those ages. Such changes in Sr/Ca ratios during the leptocephalus stage might reflect changes in environmental conditions related to events occurring during oceanic migration, such as the beginning of vertical migration or differing current influences. Further studies to clarify environmental and physiological effects on the otolith chemical formation in eel leptocephali are necessary to provide valid inferences for those changes in otolith Sr/Ca ratios.

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