

Drastic changes in otolith microstructure and microchemistry accompanying the onset of metamorphosis in the Japanese eel *Anguilla japonica*

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ABSTRACT: Otolith microstructure and microchemistry were examined in *Anguilla japonica* final stage leptocephali and glass eels. Otolith increment width, relatively constant and narrow in the leptocephalus, increased sharply from age 80 to 160 d. Sr:Ca ratios in the otolith, which increased during the leptocephalus stage, thereafter showed a rapid drop, coinciding with the increase in increment width. A comparison of the ontogenic change patterns in otolith increment width and Sr:Ca ratios between leptocephali and glass eels strongly suggested that these coincidental changes between 80 and 160 d indicate the onset of metamorphosis in the leptocephali. Metamorphosis appeared to have been completed before the increment width had reached its maximum, suggesting a duration of the former of some 20 to 40 d.

KEY WORDS: Eel · *Anguilla japonica* · Metamorphosis · Otolith · Daily growth increments · Sr:Ca ratios

INTRODUCTION

The spawning area of the Japanese eel *Anguilla japonica* has been determined as being in the North Equatorial Current west of the Mariana Islands (Tsukamoto 1992). Leptocephali drift from the spawning area toward the coastal waters of eastern Asia via the North Equatorial and Kuroshio Currents. It has been suggested that leptocephali metamorphose to glass eels while they drift along the Kuroshio Current, changing their life style from pelagic to demersal, and that they thereafter begin inshore migration (Tsukamoto & Otake 1995). Knowledge of the timing and duration of metamorphosis, therefore, seems to be essential for understanding the migration mechanism, geographical distribution and, consequently, life-history strategy.

Many reports have shown relationships between otolith characteristics, such as growth pattern (Tabeta et al. 1987, Tsukamoto & Umezawa 1990, Tzeng 1990, Umezawa & Tsukamoto 1990, Lecomte-Finiger 1992, Tzeng & Tsai 1992) and Sr:Ca ratios (Otake et al. 1994, Tzeng & Tsai 1994, Cheng & Tzeng 1996, Tzeng 1996), and the timing of metamorphosis in *Anguilla* spp. However, these relationships have not been validated, and the timing and duration of metamorphosis is still uncertain. This is due to the fact that the above studies have been based only on analysis of glass eels; very few metamorphosing leptocephali (only 4 specimens) having been sampled. Lee & Byun (1996) and Otake et al. (1997) examined metamorphosing conger eel *Conger myriaster* leptocephali and defined microstructural and microchemical fluctuations during metamorphosis.

We collected fully grown *Anguilla japonica* leptocephali (i.e. just prior to metamorphosis) during the cruise of RV 'Tansei-maru', Ocean Research Institute, The University of Tokyo, conducted in November

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1996. Minute examination of otoliths from these specimens, compared with glass eels, provided important information for determining the relationships between otolith characteristics and metamorphosis. The objectives of this study were to describe the ontogenic changes in otolith microstructure and microchemistry and to determine the relationship between these variations and the timing of metamorphosis in *A. japonica*.

MATERIALS AND METHODS

A total of 5 leptocephali and 12 glass eels were used in this study. The leptocephali were collected by oblique tows of 3 m Isaacs-Kidd Midwater Trawl (IKMT; mesh aperture: 1.0 mm) between the surface and 300 m depth in the North Pacific east of Taiwan, between 20 and 28 November 1996. Water temperature and salinity of the sampling area ranged from 25.9 to 27.5°C and from 34.0 to 34.5‰, respectively.

The total length of the leptocephali examined ranged from 49.8 to 58.3 mm (mean \pm SD: 54.3 \pm 3.4 mm) (Table 1), with total myomeres (TM) and preanal myomeres (PAM) ranging from 113 to 116 and 77 to 80 (PAM/TM: 0.68 to 0.70), respectively. The anal position, which moves anteriorly during metamorphosis and is used to define each metamorphosis stage (Tanaka et al. 1987), ranged from 75 to 83 in fully grown *Anguilla japonica* leptocephali (Mochioka 1996). Since the only metamorphosing leptocephali previously collected were 52.0 to 63.0 mm in TL (Tabeta & Takai 1975a, b) and a leptocephalus in the early stages of metamorphosis was caught in the sampling net together with the above leptocephali, classification of the latter as fully grown (just prior to metamorphosis) appeared to be justified. Twelve glass eels were collected from the beach of Tanegashima Island, Japan, on 27 December 1996, the total length ranging from 55.7 to 60.7 mm (mean \pm SD: 57.2 \pm 1.5 mm). All belonged to the VB stage (metamorphosis recently completed) according to their pigmentation characteristics (Bertin 1956).

Sagittal otoliths extracted from each individual were embedded in epoxy resin (Strues, Epofix) and mounted on glass slides. These samples were then ground to expose the core and further polished with 6 μ m and 1 μ m diamond paste on a polishing wheel (Strues, Planopol-V). Subsequently, they were cleaned in an ultrasonic bath, rinsed with deionized water and given a carbon coating by high vacuum evaporation.

Electron microprobe analyses were carried out on all leptocephalus otoliths and those of 5 glass eels (TL range: 55.9 to 59.1 mm, mean \pm SD: 57.6 \pm 1.3 mm). Sr and Ca concentrations (% dry weight) were measured along the longest axis of the otolith using a wavelength dispersive X-ray electron microprobe (JEOL

Table 1. *Anguilla japonica*. Total length (TL), total myomeres (TM), preanal myomeres (PAM), otolith radius and age of leptocephali and glass eels

Specimen	TL (mm)	TM	PAM	Otolith radius (μ m)	Age (d)
Leptocephali					
a ^a	49.8	116	80	82	94
b ^a	52.1	114	77	96	134
c ^a	54.8	115	80	93	122
d ^a	56.4	114	78	101	102
e ^a	58.3	113	79	94	117
Glass eel					
f ^a	59.1			151	165
g ^a	58.8			159	201
h ^a	55.9			156	186
i ^a	56.8			149	148
j ^a	57.5			149	203
k	55.7			151	206
l	56.2			142	143
m	56.2			150	156
n	56.3			156	182
o	56.4			144	181
p	57.0			150	170
q	60.7			153	159

^aSpecimens used for electron microprobe analysis

JXA-733). Calcite (CaCO₃) and strontianite (SrCO₃) were used as standards. Accelerating voltage and beam current were 15 kV and 7 nA, respectively. The electron beam was focused on a point about 1 μ m in diameter, spacing measurements at 1 μ m intervals. Each data point represents the average of 3 measurements (each counting time: 4.0 s). Microprobe measurement points, seen as burn depressions, were assigned to otolith growth increments which were examined as described below. The averages of successive data for Sr and Ca concentrations pooled for every 10 successive growth increments were used for the life-history transect analysis.

Following the electron microprobe analysis, the otoliths were repolished to remove the coating, etched with 0.05 M HCl and vacuum coated with Pt-Pd in an ion-sputterer for observation by scanning electron microscope (SEM, Hitachi S-4500). Otoliths of the remaining 7 glass eels were also etched and prepared for SEM observation in the same way. SEM photographs at magnifications between 1000 \times and 2000 \times were used for counting growth increments and measuring their widths. The longest axis of the ground otolith surface was regarded as the radius along which increment widths were measured. The averages of every 10 successive increment widths from the hatch check to the edge were used for otolith growth analysis. Since Umezawa et al. (1989), Tsukamoto (1989) and Umezawa & Tsukamoto (1991) clearly showed that

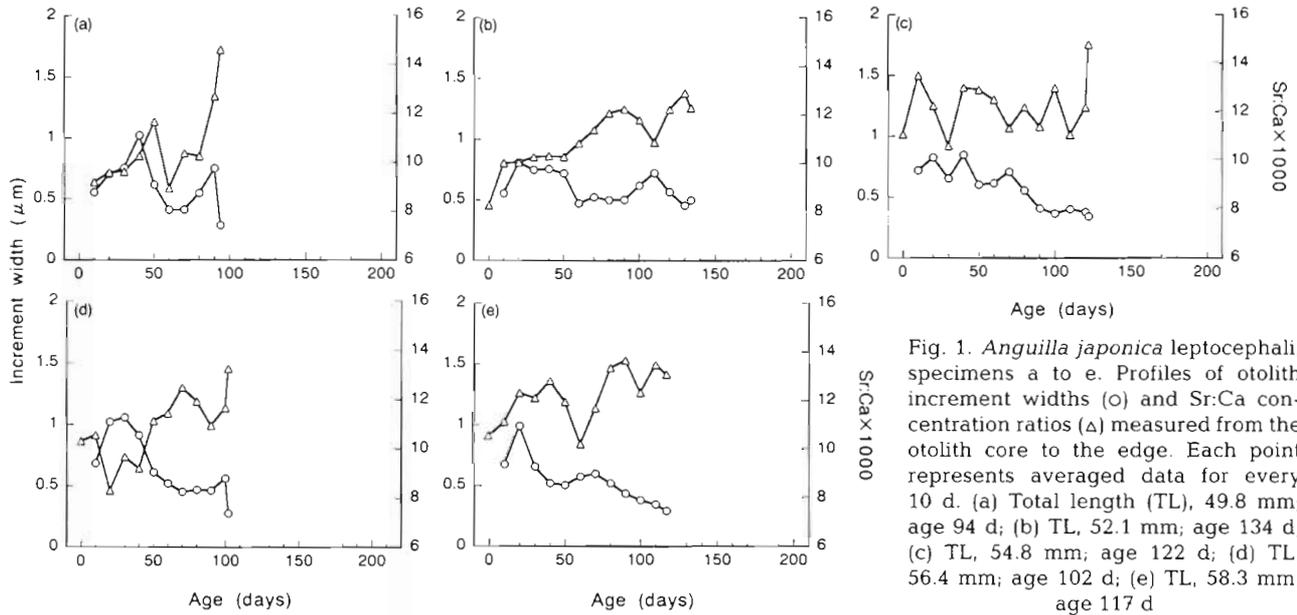


Fig. 1. *Anguilla japonica* leptocephali, specimens a to e. Profiles of otolith increment widths (○) and Sr:Ca concentration ratios (△) measured from the otolith core to the edge. Each point represents averaged data for every 10 d. (a) Total length (TL), 49.8 mm; age 94 d; (b) TL, 52.1 mm; age 134 d; (c) TL, 54.8 mm; age 122 d; (d) TL, 56.4 mm; age 102 d; (e) TL, 58.3 mm; age 117 d

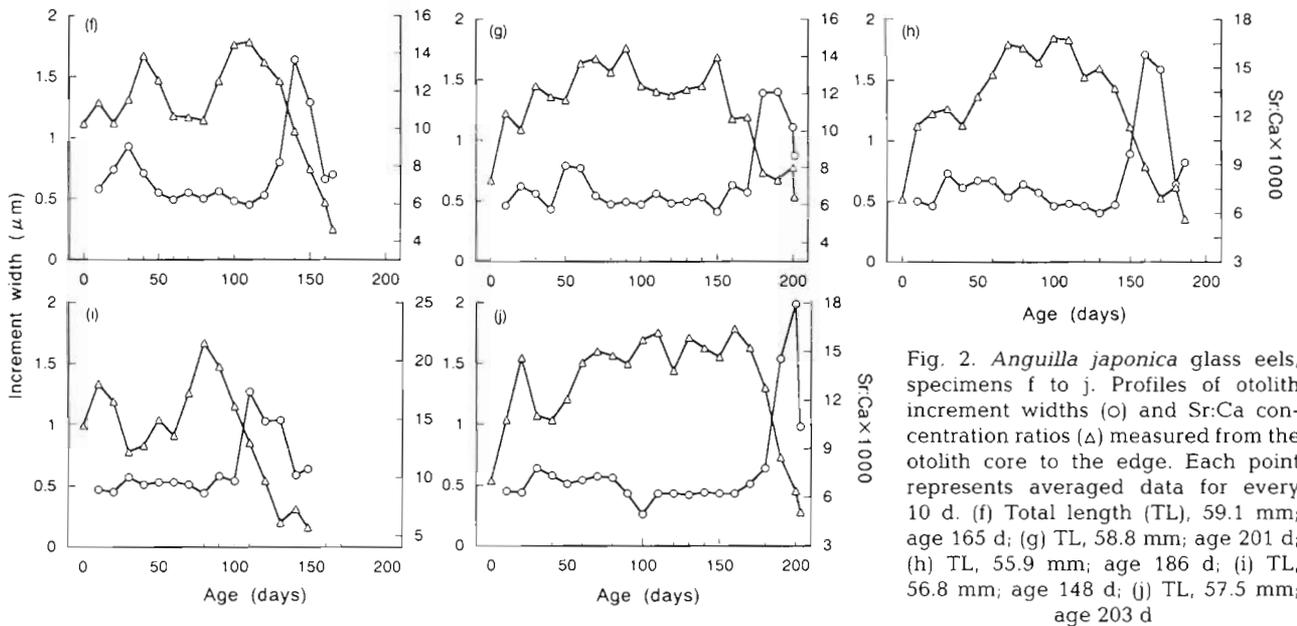


Fig. 2. *Anguilla japonica* glass eels, specimens f to j. Profiles of otolith increment widths (○) and Sr:Ca concentration ratios (△) measured from the otolith core to the edge. Each point represents averaged data for every 10 d. (f) Total length (TL), 59.1 mm; age 165 d; (g) TL, 58.8 mm; age 201 d; (h) TL, 55.9 mm; age 186 d; (i) TL, 56.8 mm; age 148 d; (j) TL, 57.5 mm; age 203 d

otolith increments in *Anguilla japonica* were deposited daily, we considered the increment number as directly representing specimen age.

RESULTS

The ages of 5 fully grown leptocephali and 12 glass eels ranged from 94 to 134 d and 143 to 206 d, respectively (Table 1). Patterns of change in the otolith increment widths and Sr:Ca ratios in the leptocephali along the life-history transect from the core to the edge are

shown in Fig. 1. Otolith increment widths increased between the hatch check and age 20 to 40 d, thereafter becoming constant or gradually decreasing toward the edge. The average increment width was $0.59 \pm 0.19 \mu\text{m}$ (mean \pm SD) in the leptocephalus stage. Otolith Sr:Ca ratios tended to rise from the core toward the edge. A slight drop in the ratio was found from age 20 to 60 d. The minimum ratio was recorded in the core, and averaged 10.0×10^{-3} , with the maximum levels, averaging 13.6×10^{-3} , occurring in the outermost regions.

Fig. 2 shows patterns of change in otolith increment width and Sr:Ca ratios along the life-history transect of

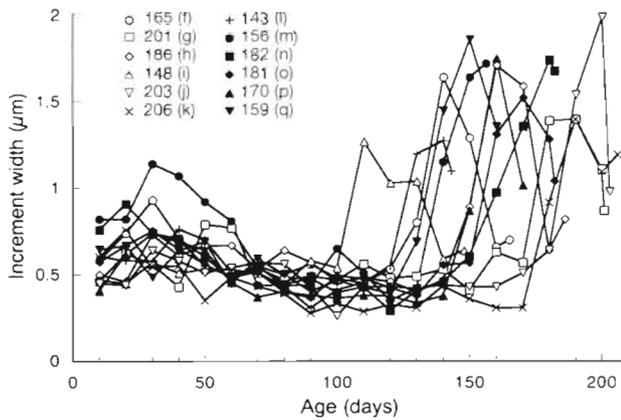


Fig. 3. *Anguilla japonica* glass eels, specimens f to q. Profiles of otolith incremental width from the otolith core to the edge. Each point represents averaged data for every 10 d

the glass eels. The patterns were characterized by drastic changes in both increment widths and Sr:Ca ratios in the outer region of the otoliths. It was found that younger specimens, i.e. younger migrants, tended to undergo such drastic changes at an earlier age (Figs. 2 & 3). Otolith increment width sharply increased during the period 100 to 160 d, followed by a rapid decrease. The duration between the onset of width increase and maximum peak was 20 to 40 d. Sr:Ca ratios reached a maximum level between 80 to 160 d, the marked decrease found thereafter coinciding with the increase in growth increment width. The maximal level of the ratio in glass eels averaged 16.8×10^{-3} , with the minimum value recorded in the outermost regions of the otoliths, being 5.5×10^{-3} .

DISCUSSION

The study showed that the timing of metamorphosis can be defined by a comparison of otolith microstructure and microchemistry between fully grown leptocephali and glass eels. The pattern of change in otolith growth increments in the glass eels was divided into 2 phases, with a shift from the first to the second between 80 and 160 d. The first phase was characterized by a relatively constant or gradually decreasing increment width, which on the whole overlapped with those of the leptocephali. In the second phase, the increment width in glass eels changed dramatically, such a fluctuation not being found in the otoliths of fully grown leptocephali, in which increment widths remained at a low level throughout. Furthermore, Sr:Ca ratios in the otolith showed a drastic decrease in the outer region in glass eel otoliths, whereas the ratio did not drop in the fully grown lep-

tocephali. These results suggest that coincidental changes in the otolith increment width and Sr:Ca ratios found in glass eel otoliths occurred in the period immediately following the fully grown leptocephalus stage. In the conger eel *Conger myriaster*, it has been reported that otolith increment widths increased at the beginning of metamorphosis (Lee & Byun 1996), which coincided with dramatic decreases in otolith Sr:Ca ratios (Otake et al. 1997). Campana (1984) found that otolith growth increment widths widened during the metamorphic period in starry flounder. These considerations all lead to the conclusion that a marked increase in otolith increment widths, coinciding with a dramatic decrease in Sr:Ca ratios, heralds the onset of metamorphosis.

Anguilla japonica may complete metamorphosis in the Kuroshio Current (Tsukamoto & Umezawa 1990) or in marginal waters on the continental shelf (Tabeta & Takai 1973, Tabeta & Konishi 1986) before beginning inshore migration. Individual glass eels have been estimated to cover up to 7 km d^{-1} (Umezawa 1991). Since the distance between the flow axis of the Kuroshio Current and the coast of Tanegashima Island (where the glass eels examined here were collected) is 56 km (Umezawa 1991), direct migration to the coast following metamorphosis in the Kuroshio Current would require some 8 d. Because the minimum number of otolith increments (days) after the maximum peak was only 2 (specimen n) and the increment width did not lessen in specimen m, it can be confidently suggested that metamorphosis was completed before the age at which the increment widths reach their maximum level. Therefore, the duration of metamorphosis is likely to be at most 20 to 40 d, which agrees with the estimations made by Tabeta et al. (1987), Tsukamoto (1990) and Tzeng & Tsai (1992).

Changes in otolith Sr:Ca ratios have been considered related to environmental factors such as water temperature (Radke 1989, Townsend et al. 1989, 1992, 1995, Radke et al. 1990) and salinity (Casselmann 1982, Radke et al. 1988, 1996, Kalish 1990, Secor 1992, Secor et al. 1995). In the eel leptocephali, however, the ratios did not seem to be significantly affected by environmental factors. *Anguilla japonica* leptocephali drift with the North Equatorial Current from the spawning area to the origin of the Kuroshio Current (Kimura et al. 1994). The sampling site of the leptocephali examined was in the transition area between the 2 currents. Because the leptocephali apparently spend their lives in the North Equatorial Current water mass, undergoing only mild variations in environmental conditions, we suggest that the increase in otolith Sr:Ca ratios during the leptocephalus stage was the result of some endogenous factor(s) rather than environmental factors. As suggested by Otake et al. (1997) for conger eels, variations

in otolith Sr:Ca ratios in the leptocephali possibly reflected the synthesis and accumulation of body glycosaminoglycan (GAG) during its ontogeny. Furthermore, Otake et al. (1994, 1997) ascertained that drastic changes in otolith Sr:Ca ratios in metamorphosing leptocephali were associated with decreasing body Sr content, caused by catabolism of GAG in the body during metamorphosis. The same phenomenon would probably occur in metamorphosing *A. japonica* leptocephali, as suggested by Otake et al. (1994).

The patterns of change of otolith increment widths and Sr:Ca ratios along the life-history transect are summarized in Fig. 4, with special reference to the timing of metamorphosis, and oceanic and inshore migration. Although the otoliths of metamorphosing leptocephali could not be examined in this study, future examination of such otoliths should validate the relationships between otolith characteristics and metamorphosis.

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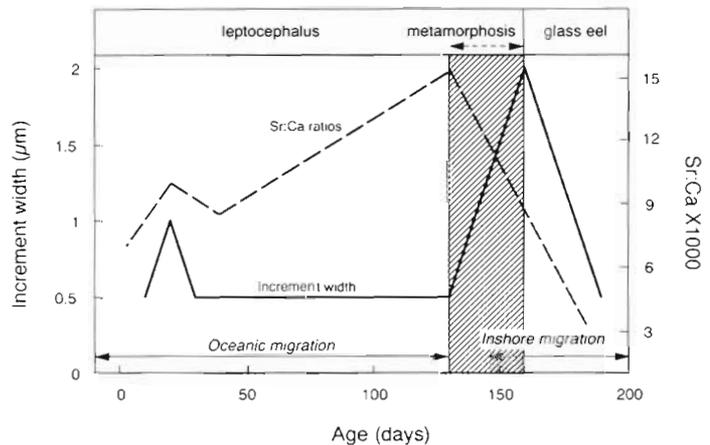


Fig. 4 *Anguilla japonica*. Patterns of change of otolith increment widths and Sr:Ca ratios in the early life-history of the Japanese eel, with special reference to the timing of metamorphosis and migration. Solid and broken lines represent increment widths and Sr:Ca ratios, respectively. The age at onset of metamorphosis represents the average of 12 glass eels examined

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