Daily Growth Increments and Lunar Pattern in Otolith of the Eel, *Anguilla japonica*, in the Freshwater

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The eels, *Anguilla japonica*, were reared in a tank with daily feeding for up to 97 days, and otoliths were regularly collected for the observation of their microstructures. Microscopic observation of the thin-sectioned otolith under dark field provided significant information on daily growth increments as well as the difference in visual contrast shown by the increments. Clearly defined elver mark formed during the metamorphosis from leptocephalus to the elver can be considered as the origin of the age for the sedentary yellow eel in continental water. The close correspondence between the number of increments outside elver mark and chronological age in days from the beginning of feeding indicates that increment deposition on a daily basis was initiated with the start of feeding for the sedentary yellow eel. Either 7 or 14 daily growth increments were grouped together into 2 alternative units, each distinguished by prominent checks or by visual contrast. The absence of any apparent environmental variations with 7 or 14 day period in the reared tank implies that the phase of the moon could be a zeitgeber for the endogenous rhythm.

Introduction

Since Pannella's suggestion on the presence of the daily growth increments in the otolith of fishes (Pannella, 1971, 1974), many others have confirmed his findings from reared fish of known age (Brothers et al., 1976; Strusaker and Uchiyama, 1976; Taubert and Coble, 1977; Tsuji and Aoyama, 1984). Accurate interpretation of the microstructures in otolith requires knowledge on periodicity of their recurrent patterns and causative factors responsible for the deposition of rhythmic patterns. Age at first increment deposition and daily growth patterns are generally determined from known-age fish reared in the controlled environment from eggs through larval stages. Sexual maturation and cultivation of Japanese eel larvae in their early stage were succeeded in aquaria (Yamamoto and Yamauchi, 1974; Yamauchi et al., 1976). Tabeta et al. (1987) described the microstructures of the acid-etched otolith of the elvers. Detailed growth patterns in otolith of the eel, however, have yet to be investigated.

In addition to the daily growth increments, the lunar patterns in otolith growth have been reported in some marine fishes (Pannella, 1971, 1974, 1980; Brothers et al., 1976; Campana, 1984). The eel's behaviours related with phases of the moon, probably induced by some physiological changes, have been widely reported (Deelder, 1970; Tesch, 1977). One can easily expect that these changes could be recorded in otoliths. Little has been known about the records of the lunar phase in otolith of the eels.

We reared the elvers in the controlled environment and regularly collected the otoliths to examine their growth patterns. In this paper, we present the evidence for the existence of daily growth increments and lunar patterns in the otoliths of eels during their freshwater life.

Materials and Methods

The elvers collected in the Geum River mouth on April 1, 1985 were reared in a tank. They were acclimated in a dark room at an initial temperature of 15℃ to 25℃ for 4 days without feeding. Thereafter, water temperature was maintained in the range of 23－27℃ and artificial food and raw fishes were fed once per day.

About 20 eels were regualrly sampled from the beginning of the rearing (Table 1). Two sagittal otoliths were picked out from each fish, brushed free of tissue, rinsed with distilled water and dried.

Table 1. Number of growth increments in otolith of the eel after elver mark, their ranges and chronological age in days

Age in days		No. of otolith	No. of increments	
After rearing	After feeding	examined	Mean	Range
23	19	8	16.6	16-18
33	29	13	29.4	26-32
45	41	20	40.3	33-43
63	59	11	59.5	56-63
97	93	5	88.8	87-90

The otoliths were prepared for viewing by embedding them in polyester resin, grinding them to the vertical mid sagittal plane with a series of graded silicon carbide papers(600, 800 and 1200 grits), and polishing with 1μm and successive 0.25 μm alumina powders. The ground surface of the otolith was then mounted on a the glass slide employing resin (Lakeside 70). The exposed side of the mounted otolith was then ground and polished successively in the above sequence. The thin section was ground and polished until the daily growth increments were most visible. Prepared otoliths were examined and photographed at 200X or 500X magnification under bright or dark field. Dark field metallurgical microscope was particulary helpful when both transmitted and reflected lights were used at the same time with regulating the light intensities. This technique permits to observe not only the daily growth increments but the visual contrast shown by the increments.

Thin section technique used in this study provided the clear observation of daily growth increments and the exact point of nucleus as well. With this preparation, light microscope examination was considered reliable (Campana, 1984). Moreover, metallurgical microscope with dark field condenser in helpful for the determination of the exact focal plane during the preparation of thin section of otolith.

Results

The sagitta of the elver, formed during the larval migration in the sea, was spherical and measured about 160μm in radius. Under dark field, thin sectioned sagitta showed a central hyaline part (about 100 μm in radius), surrounded by an outer opaque zone, about 60μm in width (Fig. 1A). Detailed description of the different zone fomation in otolith of the elver will be presented as a seperated paper (Lee, in preparation).

Otoliths of the elvers collected at the beginning of rearing did not show any growth increments outside the opaque margin. In the thin-sectioned otolith of the reared eels, the central part corresponding to the portion of the elver otolith was clearly distinguished from the outer part formed after rearing (Fig. 1B). Clearly defined growth stop, two in some specimens, was observed in otolith at the outer portion of the opaque margin of the elver otolith. This ring was defined as "elver ring" or "elver mark" by Ehrenbaum and Marukawa (1914). The elver ring can be considered as the origin of the continental age of the eel.

First two to four growth increments next to the elver mark were almost spherically concentric. Thereafter, they become laterally compressed with the highest growth rate being along the anterior-posterior axis and intermediate growth in the dorso-ventral direction. Growth increments were best obtained through the sagittal vertical anterior-posterior direction (Fig. 1C). In this plane, the sequence of growth increments were the thickest and had the least number of growth interruptions.

The number of increments formed outside elver mark was counted from otoliths of fish reared for up to 97 days (Table 1). A plot of the number of increments from elver mark (y) versus chronological age in days after the beginning of feeding (x) (Fig. 2) yielded a regression equation ;

$$y = 0.98x + 0.34 \quad (r = 0.93,\ n = 57)$$

The slope was not significantly different from 1 (t-test, $P > 0.05$). It is clear that there was an extremely close correspondence between the number of increments and the chronological age in days after feeding for the young eels.

The microstructures in otolith under dark field showed that a certain number of daily growth increments was sequentially grouped together into 2 alternative units (Fig. 1D). Each unit contained an opaque or a transluscent zone under dark field, distinguished from the successive unit by prominent checks under transmitted light. Checks are not often continuous around otolith, suggesting the presence of localized growth interruption.

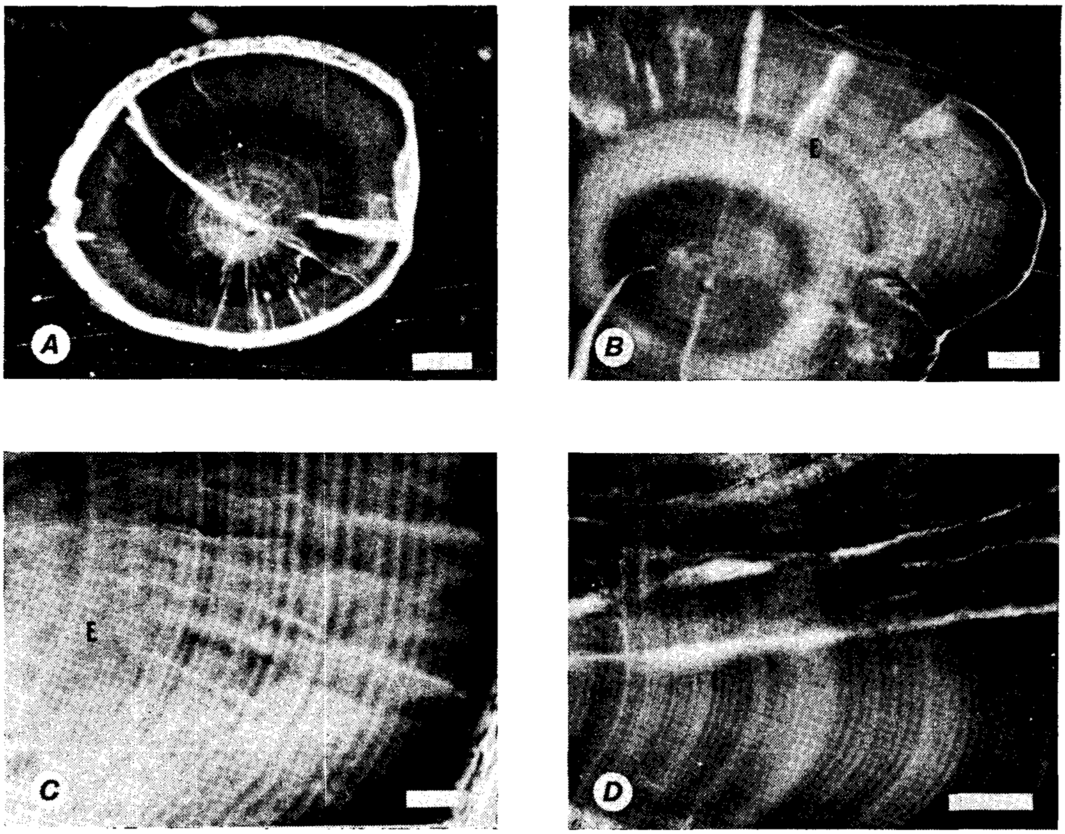

Fig. 1. Microstructural features and growth increments in otolith of fthe eel, *Anguilla japonica*, under dark field. Scale bars: A, B, D, 50 μm; C, 20 μm. (A) thin sectioned otolith of the elver. (B) The elver mark (E) in otolith of the reared eel is clearly distinguished from the outer part formed after rearing. (C) Outside elver mark, 29 growth increments are shown for 29 days of growth after feeding.
(D) Lunar patterns (t) are apparent in daily growth sequence through cyclic difference in visual contrast.

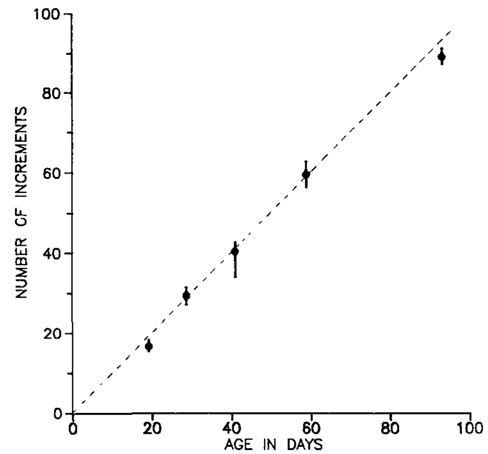

Fig. 2. Relationship between chronological age (days after feeding) and number of daily growth increments (mean and range) outside elver mark in otoliths of the reared eel, *Anguilla japonica*. The dashed line represents 1-to-1 correspondence.

The number of increments in a unit was commonly either 6 to 8 or 13 to 15. By back counting daily growth increments from the margin which was supposed to be formed at the time of collection of the otolith, the time of formation of the prominent check or sharp visual contrast corresponds to the date of the full or old moon. This result suggests that there exists a lunar pattern in the otolith of the eel, although eels were reared under the condition without apparent weekly or fortnight fluctuations.

Discussion

We observed that the daily growth increments in otolith began to be deposited outside elver mark in the freshwater. The eel do not feed during the period between the two metamorphoses of larvae turning to elvers and elvers turning to eels (Johansen, 1905). Consequently they diminish in size. This characteristics of the life history are supposed to be recorded as a discontinuity mark in the otolith of the young eel. We did not observe any growth increments outside the opaque margin of the elver otoliths collected at the beginning of rearing. Therefore, the elver mark in otolith of the young eel can be considered as the age origin of the continental water.

The relationship between chronological age and number of increments shows 1-to-1 agreement after 4th days of rearing. The elvers in our study were acclimated for the first four days without feeding. This fact indicates that the daily growth increments in otolith of the eel were initiated and regularly deposited from first feeding in the continental water. This result can be compared with the increment initiation after yolk sac absorption or the first feeding for the other fishes (Radtke and Scherer, 1982; Tsuji and Aoyama, 1984).

In this study, the lunar cycle is evident in otolith of young eels reared in the freshwater without apparent tidal influences. A cluster of 6 to 8 or 13 to 15 daily increments were delimited by prominent checks. Checks were not often continuous around the otolith, but their presence can be easily recognizable in the thin sectioned otolith through cyclic visual contrast under dark field. Highest increment contrasts occur at prominent checks. Campana (1984) suggested that a 14 day increments width cycle was entrained by the interaction of a 14 day tidal cycle with temperature and salinity. Tidally modulated environmental variables are, however, hardly applicable for the reared eels in our study.

It is well known that upstream migration of the elver or downstream migration of the silver eels showed an active behaviour correlated with the phase of the moon (Deelder, 1952; Higashi and Sakurai, 1975; Tzeng, 1985). This fact was mainly based on the fluctuations in daily catch of the eels. Silver eels even in an experimental area showed an active escape activity during the waning moon (Boetius, 1967). However, little is known about the causative factors of the relationship with lunar phase. Few data are available on the lunar rhythm of the sedentary young eel. The presence of 7 or 14 day cyclic pattern in otolith of the eels in our study suggests that there exists also lunar rhythm for the young eels in sedentary stage. The absence of 7 or 14 day periodic environmental variations in our experiment implies that the phase of the moon could be a zeitgeber for the endgenous rhythm.

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