

## Otolith microstructure and daily age of *Anguilla japonica*, Temminck & Schlegel elvers from the estuaries of Taiwan with reference to unit stock and larval migration

W.-N. TZENG AND Y.-C. TSAI

Department of Zoology, College of Science, National Taiwan University, Taipei,  
Taiwan 10617, Republic of China

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To test the hypothesis that the Japanese eel, *Anguilla japonica*, elvers in the eastern and western coasts of Taiwan are recruited from two different spawning grounds and to increase the knowledge of the early life history of the eel, the otolith microstructure and daily age of elvers collected from five estuaries in the coast of Taiwan during December 1989 through February 1990 were examined by scanning electron microscopy. The total length of elvers at arrival in the estuaries was similar among estuaries, averaging c. 56.0 mm and showed a seasonal decrease. The maximum radius of the sagittal otolith of elvers ranged from 124.46 to 181.82  $\mu\text{m}$  with a mean of  $143.15 \pm 12.72 \mu\text{m}$ . The otolith from centre to edge included an organic-rich primordium ( $9.20 \pm 2.02 \mu\text{m}$  in diameter), a diffusively calcified core ( $20.94 \pm 1.99 \mu\text{m}$ ), and the daily growth increments; these three layers were probably deposited during the embryonic, yolk sac and feeding period respectively. The growth rate of the otolith was higher at the beginning of early life ( $0.5\text{--}1.0 \mu\text{m day}^{-1}$ ), lowest at approximately 100-days old ( $<0.5 \mu\text{m day}^{-1}$ ), and highest 1 month before arrival at the estuary ( $>1.0 \mu\text{m day}^{-1}$ ). The mean age for elvers arriving in the estuaries of the coasts of Taiwan was approximately  $170.4 \pm 21.02$  days. Neither the growth pattern of the otolith nor the age of elvers arriving in the estuary were significantly different among estuaries, indicating that the elvers in both eastern and western Taiwan were probably recruited from the same spawning ground. The growth pattern of the otolith in relation to larval migration was analysed.

Key words: *Anguilla japonica*; elver; otolith growth increment; daily age; early life history; spawning ground; larval migration.

### I. INTRODUCTION

The Japanese eel, *Anguilla japonica*, Temminck & Schlegel, is one of the most important cultured species in Taiwan. For cultivation purposes, large numbers of elvers are caught from the estuary during upstream migration in the period November to March (Chen, 1975; Tzeng, 1983a, 1985). Aquaculture of eels has been commercially successful in Taiwan since 1963 (Tzeng, 1983b), however the knowledge of the early life history of this species, e.g. spawning areas and season, distribution and migration of leptocephali and elvers, is limited (Tanaka, 1975; Tabeta *et al.*, 1987; Kajihara, 1988; Ozawa *et al.*, 1989; Tsukamoto, 1990). The spawning ground of the Japanese eel was first presumed to be in the waters south of Okinawa and east of Taiwan (Matsui, 1957). However, based on the distribution of elvers along the coast of Taiwan and their transportation by the Kuroshio current, a local spawning ground in the waters southwest of Taiwan was proposed (Kuo, 1971). Recent investigations on the distribution of leptocephali and ageing studies on leptocephali and elvers indicate that the exact spawning ground is located in the waters east of the Philippines between the North Equatorial Current

at about 10° N and the Subtropical Countercurrent at about 20° N (Kajihara, 1988; Ozawa *et al.*, 1989; Tsukamoto *et al.*, 1989; Tsukamoto, 1990).

Since Pannella (1971) detected primary growth increments in otoliths of fish, larval ageing techniques based on examination of otolith microstructure have been widely used to study the early life history of fish (Campana & Nielson, 1985). The growth increments in otoliths of both newly hatched larvae and elvers were found to be deposited on a daily schedule (Umezawa *et al.*, 1989; Lee & Lee, 1989; Tsukamoto, 1989). The daily age of anguillid leptocephali and elvers can thus be examined from otolith growth increments (Tabeta *et al.*, 1987; Tsukamoto *et al.*, 1989; Umezawa & Tsukamoto, 1990). This permitted the time required for migration of the eel larvae from the spawning ground to the coasts, and the spawning season of the eel, to be estimated (Tsukamoto, 1990; Tzeng, 1990). Changes in the growth pattern of the otolith have been related to environmental conditions in juvenile chinook salmon (*Oncorhynchus tshawytscha* Walbaum) (Neilson *et al.*, 1985) and in the European eel, *Anguilla anguilla* (Linnaeus), elvers (Lecomte-Finiger & Yahyaoui, 1989).

This paper compares the daily age of elvers among the estuaries of Taiwan based on examination of the otolith microstructure and then tests the hypothesis that the elvers in the estuaries of Taiwan are recruited from two different spawning grounds. The migratory history of the eel larvae as indicated from the otolith microstructure was also analysed.

## II. MATERIALS AND METHODS

A total of 1108 *Anguilla japonica* elvers was collected from the estuaries of Su-Ao (SA), Shuang-Hsi (SH), Sha-Lung (SL), Li-Shui (LS) and Tung-Kang (TK) on the eastern and western coasts of Taiwan during the period from December 1989 to February 1990 (Fig. 1). Elvers collected were immediately fixed with 95% alcohol, and total length was measured to the nearest 0.1 mm 1 week post-fixation. The pigmentation stage of the elvers was determined according to the distribution of pigments on the body surface (Strubberg, 1913; Bertin, 1956). Twenty-five elvers at the early pigmentation stage from the samples of SA on 22 December 1989 and from SL, LS, TK on 22 and 23 January 1990 were used to examine their otolith microstructure. Because the fishing season in SA was closed earlier than in other places, the elvers from different sampling sites were not available on the same date.

The microstructure of the sagittal otolith of elvers sectioned in the frontal plane was examined by scanning electron microscopy (SEM) photographs (Hitachi S-520) at 2000×. Preparation of the otoliths for SEM were described previously (Tzeng, 1990). The otolith radius and increment width were measured along the maximum otolith radius. The daily age of elvers after hatching was estimated by the number of otolith growth increments with an additional 5-day yolk sac larval period (Tzeng, 1990).

The tests of difference of total length–frequency distribution of the elvers between month and estuaries were conducted both by two-way analysis of variance (ANOVA) and by Scheffe's multiple range analysis (Sokal & Rohlf, 1981). The test of difference of daily age of the elvers among estuaries was conducted using Kruskal–Wallis one-way ANOVA by rank (Siegel, 1956).

## III. RESULTS

### PIGMENTATION STAGE AND TOTAL LENGTH

The pigmentation stages of elvers collected from five estuaries during the period from December 1989 to February 1990 were dominated by stages VA and VB, with other advanced stages being relatively few (Table I). The elvers of stages VA and

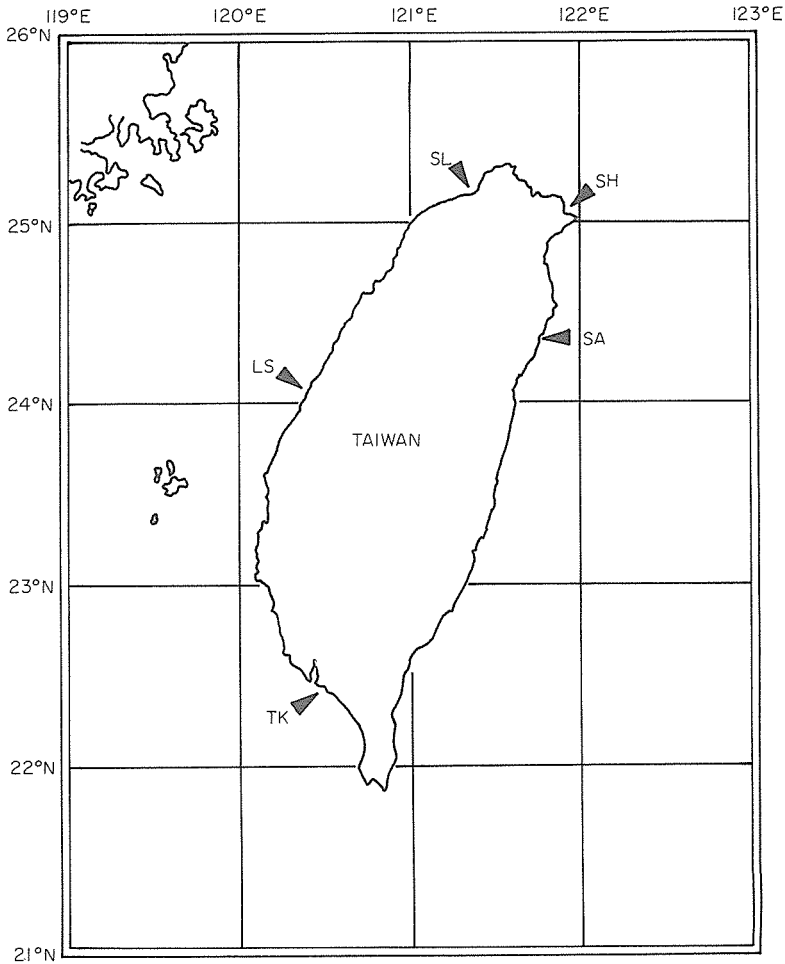


FIG. 1. Map showing sampling locations of *Anguilla japonica* elvers from five estuaries in the coast of Taiwan. LS, Li-Shui; SA, Su-Ao; SH, Shuang-Hsi; SL, Sha-Lung; TK, Tung-Kang.

VB were believed to be the new recruits in the estuary. The proportion of elvers with advanced pigmentation stages increased slightly in the later fishing season.

Individual total lengths of the elvers ranged from 49.0 to 64.5 mm, with means ranging from 53.88 to 57.20 mm, and an overall mean of 56.0 mm (Fig. 2). Two-way ANOVA indicated that the mean total length of elvers was significantly different between sampling periods and locations ( $P < 0.001$ ). Scheffe's multiple range comparison indicated that mean total length of the elvers among different sampling locations in the early fishing season (December and January) were more homogeneous than those in the later fishing season (February) (Table II). The mean total length of the elvers from SL, LS and TK appeared to be smaller in the later fishing season (Fig. 2).

#### DIMENSION AND MICROSTRUCTURE OF OTOLITHS

Sagittae are the largest of the three pairs of elver otoliths. The maximum radius of the sagittal otoliths in the frontal section of 17 elvers ranged from 124.46 to

TABLE I. Pigmentation stages of *Anguilla japonica* elvers collected from five different estuaries in the coasts of Taiwan on 22 December 1989 and 22 and 23 January 1990. Sampling sites SA, SH, SL, LS and TK as Fig. 1, pigmentation stages after Strubberg (1913)

Sampling site	Date	Sample size	No. fish in pigmentation stage					
			Va	Vb	VIa1	VIa2	VIa3	VIa4
SA	Dec. 1989	98	98					
SH	Dec. 1989	40	40					
SL	Jan. 1990	88	65	16	6	1		
	Dec. 1989	93	93					
	Jan. 1990	99		79	16	4		
LS	Feb. 1990	99	72	18	2	3	3	1
	Dec. 1989	100	61	38	1			
	Jan. 1990	99	68	31				
TK	Feb. 1990	100	46	46	8			
	Dec. 1989	93	35	58				
	Jan. 1990	100	100					
Total	Feb. 1990	99	15	70	13	1		
		1108	693	356	46	9	3	1

181.82  $\mu\text{m}$  with a mean of  $143.15 \pm 12.72 \mu\text{m}$ . The otolith is elliptical and the shape changes with growth. The growth of otoliths in the anterior–posterior direction is faster than in other directions. The growth increment is thus best discernible in an anterior–posterior direction (Fig. 3).

The primordium of the otolith was substantially composed of an organic material (probably fibroprotein otolin), and appeared deep after EDTA-etching [Figs 3, 4(a)]. The primordium measured from four well-sectioned otoliths was approximately  $9.20 \pm 2.02 \mu\text{m}$  and  $7.60 \pm 1.99 \mu\text{m}$  in diameter along their long and short axes, respectively. The core was constituted by well-calcified crystal substances arranged in radial form [Figs 4(a, b)]. The core was approximately  $20.94 \pm 1.99 \mu\text{m}$  and  $17.31 \pm 1.47 \mu\text{m}$  in diameter along the long and short axes, respectively. The daily growth increments began outside these structures (Figs 3, 4). A daily growth increment was a bipartite structure, composed of one carbonate-rich incremental zone (light band) and one organic-rich discontinuous zone (dark band), deposited on a daily schedule (Degens *et al.*, 1969; Tanaka *et al.*, 1981; Campana & Neilson, 1985). The number of daily growth increments was used to estimate the daily age of the elvers. A deeply-etched ring separated the first growth increment and the core [Fig. 4(b)]. This indicated that there was a distinctive transition of growth history of the otolith during this period. The width of the daily growth increments changed with time: they became wide, indicating fast-growing periods [FGI; Figs 4(b, d)]; narrow, indicating slow-growing periods [SGI; Figs 4(c, d)]; there was also a deeply-etched growth check during the slow-growing period [Fig. 4(c)]. Sometimes, no increments were discernible in the distal side of the otolith during a slow-growing period [Fig. 4(d)]. The daily growth increments widened distinctively again near the edges of the otolith both on the anterior–posterior and distal sides [Figs 3, 4(d)].

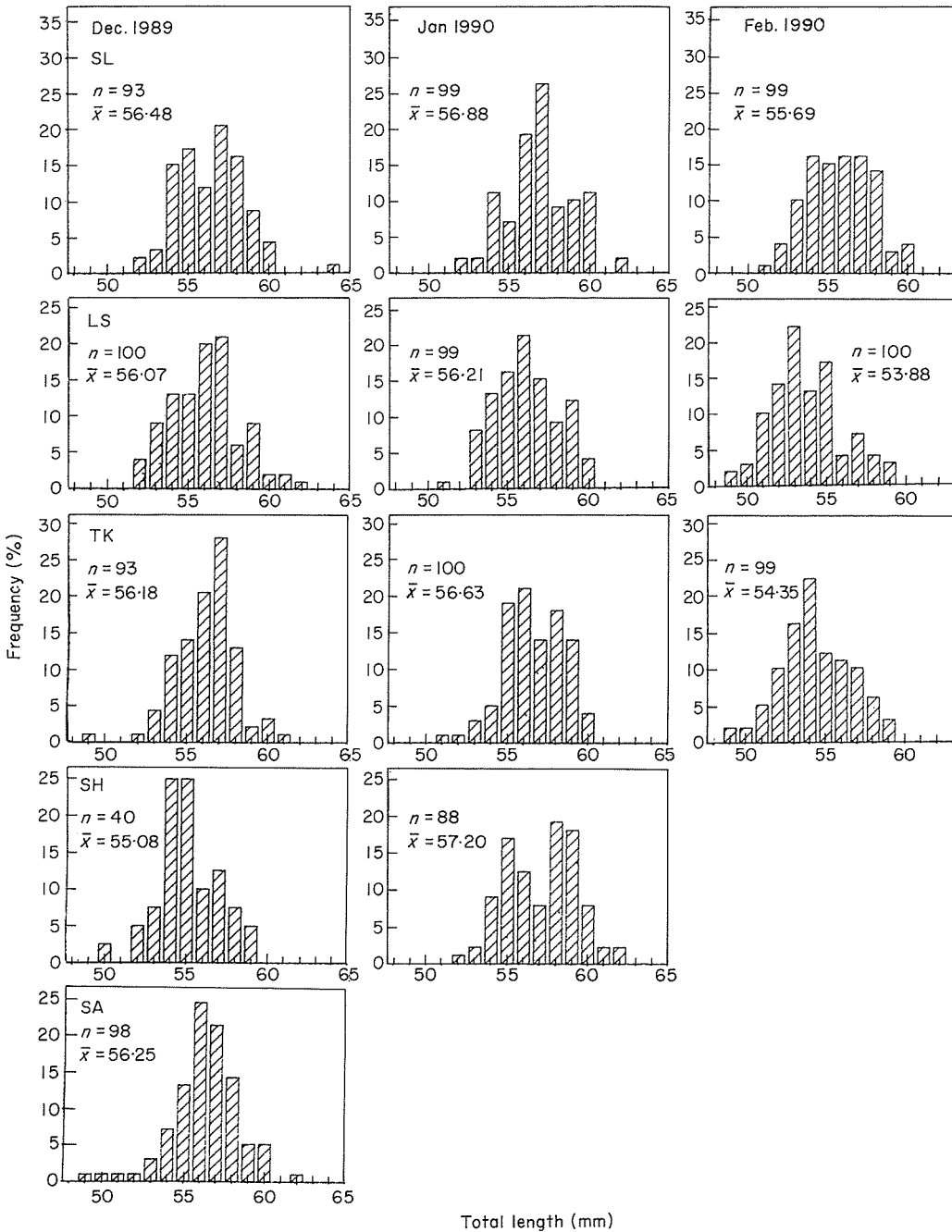


FIG. 2. Frequency distribution of total length of elvers collected from SL, LS, TK, SH and SA, December 1989–February 1990. The sampling sites and dates as in Table I.

GROWTH HISTORY AS INDICATED FROM OTOLITHS

The growth history of the elver may have influenced the change of the width of daily growth increments in otoliths. The first 60 daily growth increments were

TABLE II. Homogeneous test of mean total length, by Scheffe's multiple range analysis, of *Anguilla japonica* elvers collected from five different estuaries on 22 December 1989, and 22 and 23 January 1990. Sampling sites SA, SH, SL, LS and TK as in Fig. 1

Rank	Sampling site	Sampling date	Sample size	Mean total length (mm)	Homogeneous group
1	LS	Feb. 1990	100	53.88	*
2	TK	Feb. 1990	99	54.35	*
3	SH	Dec. 1989	40	55.08	*
4	SL	Feb. 1990	99	55.69	*
5	LS	Dec. 1989	100	56.07	**
6	TK	Dec. 1989	93	56.18	*
7	LS	Jan. 1990	99	56.21	*
8	SA	Dec. 1989	98	56.25	**
9	SL	Dec. 1989	93	56.48	***
10	TK	Jan. 1990	100	56.63	**
11	SL	Jan. 1990	99	56.88	**
12	SH	Jan. 1990	88	57.20	*

wider in a particular elver collected from Sha-Lung, north Taiwan on 22 January 1990 (Fig. 3). If otolith growth was proportional to body growth, this implied that the growth rate of the fish was higher during the first 2 months of the early life history, and then growth increments became narrow and thus the growth rate decreased gradually to a minimum at approximately 100 days old. In this slow-growing period, the growth of the otolith was interrupted, as indicated by a deeply etched growth check in Figs 3, 4(c). This growth check was observed frequently in the otoliths of elvers, and its formation period considered to correspond to the period of metamorphosis from leptocephalus to elver (Lecomte-Finiger & Yahyaoui, 1989). The increments then widened, implying that growth became extremely fast 2 months before arrival at the estuary (Fig. 3). In conclusion, the growth rate of the otolith was higher,  $0.5\text{--}1.0\ \mu\text{m day}^{-1}$  at the beginning of early life, lowest (usually  $<0.5\ \mu\text{m day}^{-1}$ ) at approximately 100 days old, and highest ( $>1.0\ \mu\text{m day}^{-1}$ ) 1 month before arrival at the estuary. However, it is emphasized that this relationship between otolith growth and body growth has not yet been validated, and that in two other species otolith growth and body growth had been shown to be uncoupled (Mosegaard *et al.*, 1988; Wright *et al.*, 1990).

#### DAILY AGE AND BIRTH DATE

The mean daily ages of elvers collected from estuaries SA, SL, LS and TK on 22 December 1989 and 22 and 23 January 1990 were estimated to be  $169.3 \pm 14.9$ ,  $175.6 \pm 27.8$ ,  $165.6 \pm 22.6$  and  $171.7 \pm 26.2$  days, respectively (Table III). Kruskal-Wallis analysis indicated that these mean daily ages were not significantly different among estuaries ( $P > 0.5$ ).

The birth date of the elvers back-calculated from the daily ages was from mid-June to late July for those collected from SA on 22 December 1989, from late June to early September for those from SL and LS collected on 22 January 1990 and from early July to late September for those collected from TK on 23 January

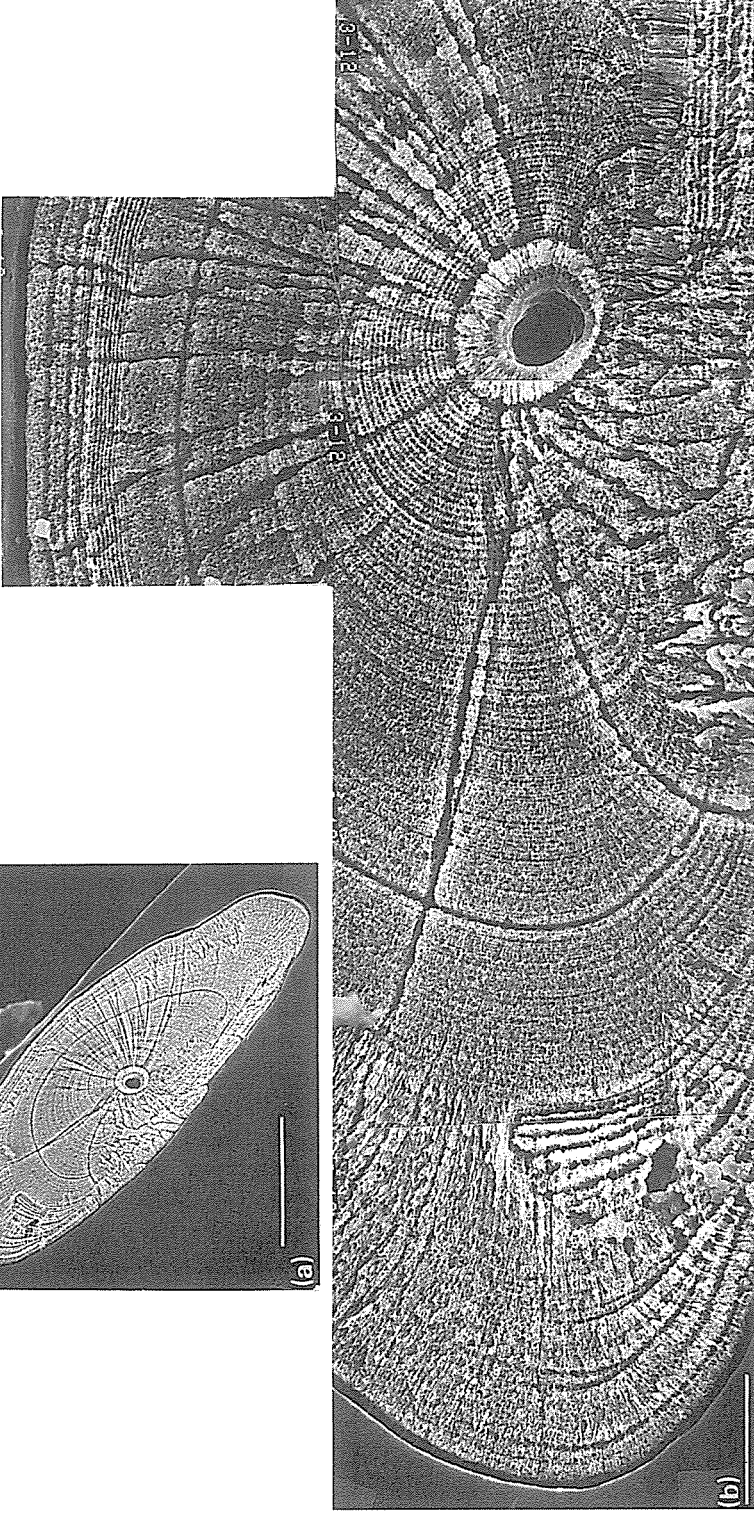
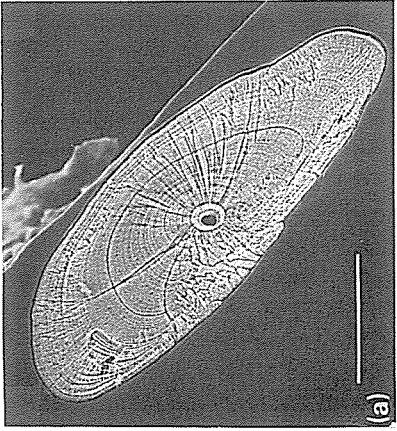


FIG. 3. Scanning electron micrographs illustrating microstructural growth pattern of sagittal otolith of a 54.31 mm T.L. elver collected from Sha-Lung estuary, north Taiwan on 22 January 1990 (a). Daily growth increments in the distal and posterior sides of the otolith were enlarged (b). Scale bar = 75  $\mu\text{m}$  (a), 15  $\mu\text{m}$  (b).