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Determination of the age of young American eels, *Anguilla rostrata*, in fresh water, based on otolith surface area and microstructure

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The time elvers of the American eel, *Anguilla rostrata*, spend in an estuary prior to their migration into fresh water was assessed. A distinct mark was formed on elvers' otoliths during their first 2 to 3 weeks in the river estuary. This mark was used to distinguish between growth in fresh water and in salt water. Migrating eels collected at a falls 4 km from the estuary exhibited bimodal length and weight distributions. Frequency distributions showed that eels collected in the estuary were smaller and had smaller otoliths than eels collected at the falls, indicating that elvers do not reach the falls in the same year as they enter the estuary. The three modal groups most probably represent three age-classes. However, the otoliths of elvers collected in the estuary had only the mark of transition whereas eels in the first and second mode at the falls usually had two rings (1–4) and four rings (3–6) per otolith, respectively, in addition to the mark of transition, as viewed under SEM. The possibility that ring formation is not annual means that the use of otoliths for the age determination of eels in this study has not been validated.

I. INTRODUCTION

The American eel, *Anguilla rostrata*, grows in lakes and rivers until the onset of sexual maturation and then migrates to the Sargasso Sea where mating and spawning occur (Tesch, 1977). The pelagic eggs soon hatch into leptocephali that are carried by currents along the western Atlantic coast. One year later the leptocephali transform into glass eels and become progressively pigmented (elvers) as they enter fresh water. Generally elvers are considered to remain in estuarine environments for variable periods of time in order to acclimate to lower levels of salinity before continuing their upstream migration (Deelder, 1958; Jellyman, 1977). Measurements of the amounts of time taken for eels to penetrate river systems are required in order to obtain reliable estimates of eel recruitment. However, reliable estimates of the amount of time elvers spend in river estuaries depend on the ability of investigators to determine the age of individuals.

In eels, age determination is hindered by the inability to distinguish between otolith growth that takes place in salt water from that which takes place in fresh water (Boëtius & Boëtius, 1967; Liew, 1974; Berg, 1985). The methods used to determine age have also resulted in a great variability in the reported relationship between age and length. Nevertheless, only one (Liew, 1974) of several (Boëtius & Boëtius, 1967; Ogden, 1970; Vladykov, 1967; Gray & Andrews, 1971; Dolan, 1975) methods used to determine the age of eels has been validated. However, Liew's

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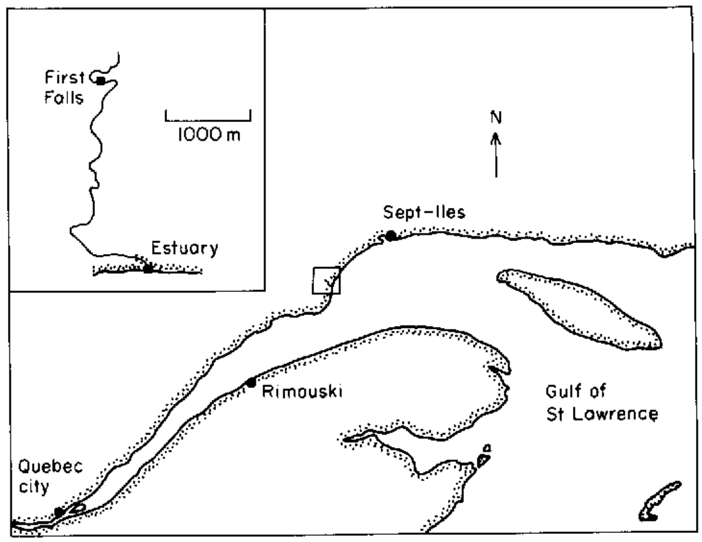

FIG. 1. Location of the study area and sampling stations (■) in the Petite Trinité River, Quebec.

(1974) method is based on a subjective criterion, i. e. the ability of the observer to distinguish between 'real' and 'false' rings on the otolith.

In this study, the period of time that elvers of the American eel spend in an estuary before continuing their migration was assessed. This was done by distinguishing between the growth of otoliths in fresh and salt water, using a novel technique to measure microstructure. The validity of age determination based on otolith readings was also assessed.

II. MATERIALS AND METHODS

Elvers and eels were collected in the period from early June to late August, from the Petite Trinité River, a stream located on the north shore of the Gulf of St. Lawrence, 100 km south-west of the city of Sept-Iles, Quebec (Fig. 1). The stream drains a 200-km^2 basin of boreal vegetation and flows 35 km over a mainly gravel, cobble and boulder substrate on Precambrian bedrock. The first waterfalls are located 4 km from the stream mouth. The stream forms a 10-m wide isolated shallow pool (maximum depth 1·5 m) at its mouth and spreads over a 500-m sandy tidal zone at low tide. The pool is invaded by salt water at high tide. Fish movements between the stream and the Gulf of St Lawrence can only occur at high tide. The tidal regime is mixed, mainly semi-diurnal.

Sampling was initiated in mid-June in the estuary and in late-June at the falls, as observations made in previous years indicated that elvers entered the estuary in mid-June and that young eels ascended to waterfalls in early July (J.-D. Dutil, in prep.). Elvers and eels in the estuary were collected daily with a hoop net and every 2 to 3 days with a scoop net. Eels climbing the rocks alongside the falls were hand-collected nightly every 4 days. The length and fresh weight of all individuals collected were measured to the nearest 0·1 mm and 1·0 mg, respectively. Their otoliths were removed, cleaned and stored dry. Developmental stages of elvers were determined using a modified form of the method established for the European eel, *A. anguilla*, (Elie *et al.*, (1982) which classifies elvers according to their pigmentation. Elie *et al.* (1982) sub-divided the two elver stages, V and VI, described in 1906 by Schmidt (cited in Elie *et al.*, 1982), into eight groups (VA and VB, and VIA_0, VIA_1, VIA_2, VIA_3, VIA_4 and VIB). From his examination of a few elvers of the American eel, Elie (pers. comm.) concluded that the same divisions might be valid for both species. However, due to their smaller size, American eels are not so readily classifiable. Thus, the eight groups were paired, reducing their number to four: VA-VB, VIA_0-VIA_1, VIA_2-VIA_3 and VIA_4-VIB. In this study, specimens which had reached stage VII of pigmentation are referred to as eels whereas others are referred to as elvers.

The otoliths were placed on glass microscope slides and embedded in a two-component epoxy resin (Hi Sol, Buehler Co.) with their convex surface exposed. The embedded otoliths were initially ground using a wet grinding machine (Buehler Co.) with a grindstone rotary disc (800 grift) until the nucleus was exposed and then ground and polished on metallurgical lapping film (5 µm and 3µm). A subsample of otoliths was chosen randomly, examined under an optical microscope and photographed. The mounted negatives were projected onto a digitizing pad (Houston Instruments HIPAD) and measurements were stored in a micro-computer (IBM-PC). Photographs of a micrometric ruler, taken at the same magnifications used to view otoliths, were projected onto the digitizing pad to calibrate the measuring device. The total surface area, the surface area inside the first distinct mark (referred to as the internal surface area) and the number of marks in addition to the first one were recorded for each otolith.

Due to their non-normal distributions, the internal and total surface areas of the otoliths as well as their growth in fresh water (calculated as total minus internal surface area) were correlated using a Spearman rank correlation (r_s) to length and weight. Their relationship to stage of pigmentation and to date (month they were collected) was tested using a Kruskal–Wallis analysis of variance.

The hypothesis that eels migrating over the falls were not in their first year of residence in fresh water was tested by comparing the length, weight and total and internal surface areas of the otolith of a random subsample of eels collected at the falls to those of eels collected in the estuary in the same period (July–August) (that summer's elvers that reached stage VII).

III. RESULTS

Elvers were present in the estuary of the Petite Trinité River from 16 June to 30 July 1983. In June, 9·1% of the elvers were in stage VA-VB, 10·4% in stage VIA_0-VIA_1, 48% in stage VIA_2-VIA_3 and 32% in stage VIA_4-VIB ($n = 154$). Elvers were classified as to the presence or absence of a ring just inside the outer edge of the otolith when examined using transmitted light (Fig. 2). In reflected light on a black background this ring is translucent. The percentage of individuals possessing this ring increased with the stage of pigmentation and sampling date (Tables I, II). Otoliths usually had attained a mean total area of $57{\cdot}3 \pm 6{\cdot}9$ µm^2 (Fig. 3) when the ring was formed. The internal surface area did not vary with the stage of pigmentation of the elvers ($\chi^2 = 2{\cdot}55$, $n = 119$, $P = 0{\cdot}28$) or with the date they were collected (June *v*. July) (Wilcoxon's $Z = 1{\cdot}40$, $n = 74$, $P = 0{\cdot}16$). The internal surface area was not significantly correlated to the length ($r_s = 0{\cdot}13$, $n = 119$, $P = 0{\cdot}15$) or weight ($r_s = 0{\cdot}06$, $n = 119$, $P = 0{\cdot}52$) of the elvers. These observations lead us to conclude that the first ring represents the transition from salt to fresh water and that the inner surface area represents sea growth.

Total surface area and growth of the otoliths after the formation of the ring increased as a function of the elver's stage of pigmentation ($\chi^2 = 50{\cdot}53$ and $\chi^2 = 61{\cdot}07$, respectively, $n = 119$, $P = 0{\cdot}0001$, Fig. 4) and the month they were collected ($\chi^2 = 41{\cdot}98$ and $\chi^2 = 52.28$ respectively, $n = 119$, $P = 0{\cdot}0001$, Fig. 4). These variables were also correlated with the length ($r_s = 0{\cdot}37$ and $r_s = 0{\cdot}33$, respectively, $n = 119$, $P < 0{\cdot}0002$) and the weight ($r_s = 0{\cdot}67$ and $r_s = 0{\cdot}65$, respectively, $n = 119$, $P < 0{\cdot}0001$) of the elvers.

Throughout the summer, the lengths and weights of eels climbing the rocks beside the falls were distributed bimodally (Fig. 5) as was the total surface area of their otoliths (Fig. 6). Eels started to ascend the falls of the Petite Trinité River in

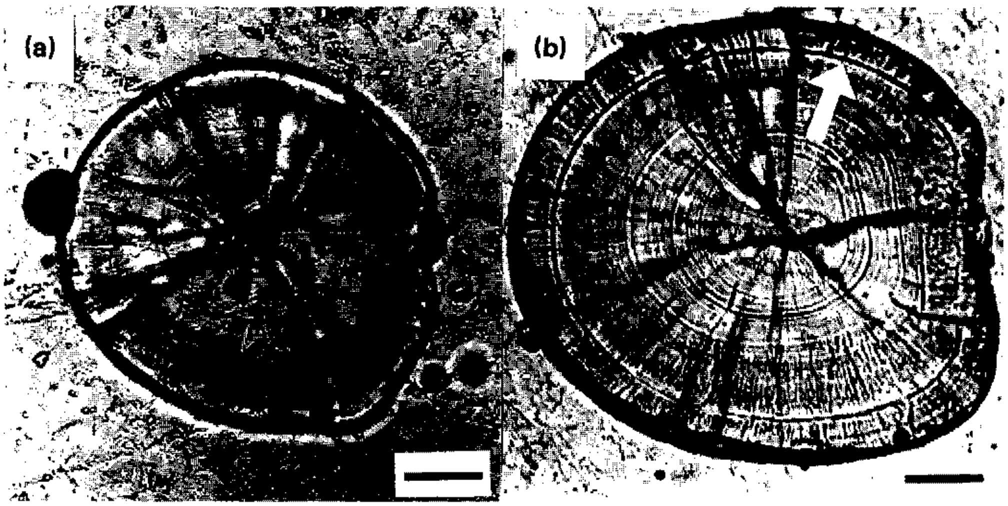

FIG. 2. Otoliths of elvers in pigmentation stage VIA_2–VIA_3 collected from the estuary of the Petite Trinité River in June and July, showing (a) the absence of a ring and (b) the formation of a ring near the edge, viewed under transmitted light. The arrow indicates the transition zone. The scale bars represent 50 μm. The specimen in (a) was caught 27 June 1983 (62 mm, 129 mg), that in (b) 13 July 1983 (62 mm, 166 mg).

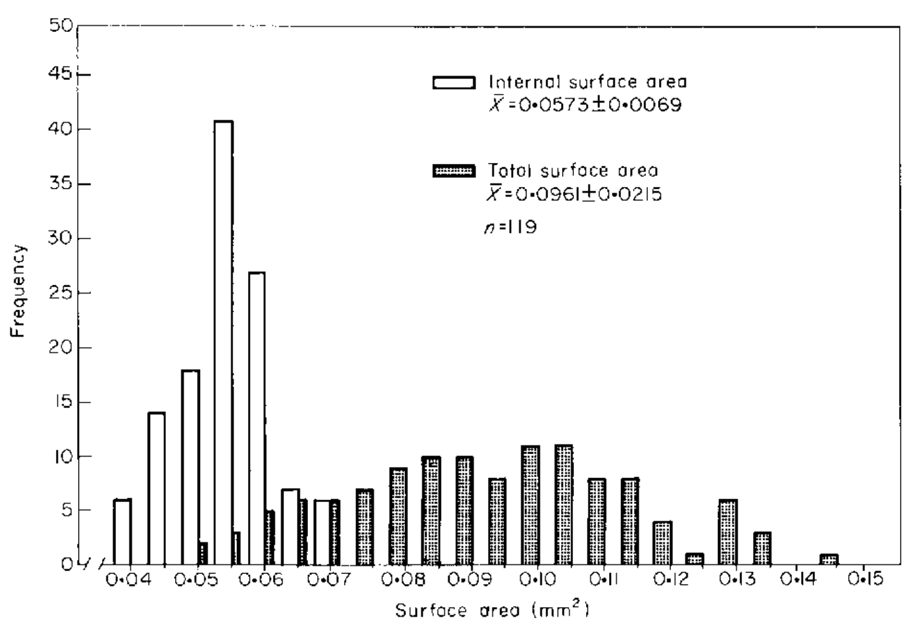

FIG. 3. Total and internal surface areas of otoliths of elvers and eels from the estuary of the Petite Trinité River ($\bar{X}$±S.D.).

TABLE I. Relationship between the percentage of elvers which possessed a ring near the margin of their otoliths and their stage of pigmentation

Stage of pigmentation	n	% of individuals with ring
VA–VB	6	0
VIA_0–VIA_1	5	20
VIA_2–VIA_3	31	64
VIA_4–VIB	50	96
VII	46	100

TABLE II. Relationship between the percentage of elvers which possessed a ring near the margin of their otoliths and sampling date

Sampling date	n	% of individuals with ring
15–30 June	43	55
1–15 July	49	89
16–31 July	32	96
1–15 August	14	100

July, but no elvers were ever observed or collected at the falls. The average length, weight and total surface area of the otolith of eels in the estuary (that summer's elvers that reached stage VII) and those of eels in the first mode at the falls were, respectively: $67{\cdot}0 \pm 0{\cdot}7$ mm and $89{\cdot}6 \pm 1{\cdot}0$ mm, $0{\cdot}30 \pm 0{\cdot}09$ g and $0{\cdot}95 \pm 0{\cdot}04$ g, and $0{\cdot}0961 \pm 0{\cdot}0158$ mm^2 and $0{\cdot}2860 \pm 0{\cdot}0060$ mm^2 ($\bar{X} \pm$ S.D.). There was no overlap in total surface area of otoliths between eels from the estuary and eels from the falls, indicating that elvers do not reach the falls (4 km upstream) in the same year as they enter the estuary.

There was no difference in mean internal surface area between individuals collected in the estuary (both elvers and eels) and eels collected at the falls (Wilcoxon's $Z = 1{\cdot}61$, $n = 191$, $P = 0{\cdot}11$). However, the otoliths of elvers collected in the estuary contained only the internal ring previously mentioned (Fig. 2) and the otoliths of eels collected at the falls showed up to 13 additional rings (Table III). Individuals in the first mode and in the second mode at the falls usually possessed two rings (1–4) and four rings (3–6) per otolith respectively, in addition to the ring formed during the transition to fresh water (Table III).

The number of rings (beyond the mark of transition) per otolith of individuals in the first mode at the falls was not correlated to the length of the eels ($r_s = 0{\cdot}23$, $n = 65$, $P = 0{\cdot}06$) but was significantly correlated to the surface area of their otoliths ($r_s = 0{\cdot}31$, $n = 65$, $P = 0{\cdot}01$). For individuals in the second mode, the number of

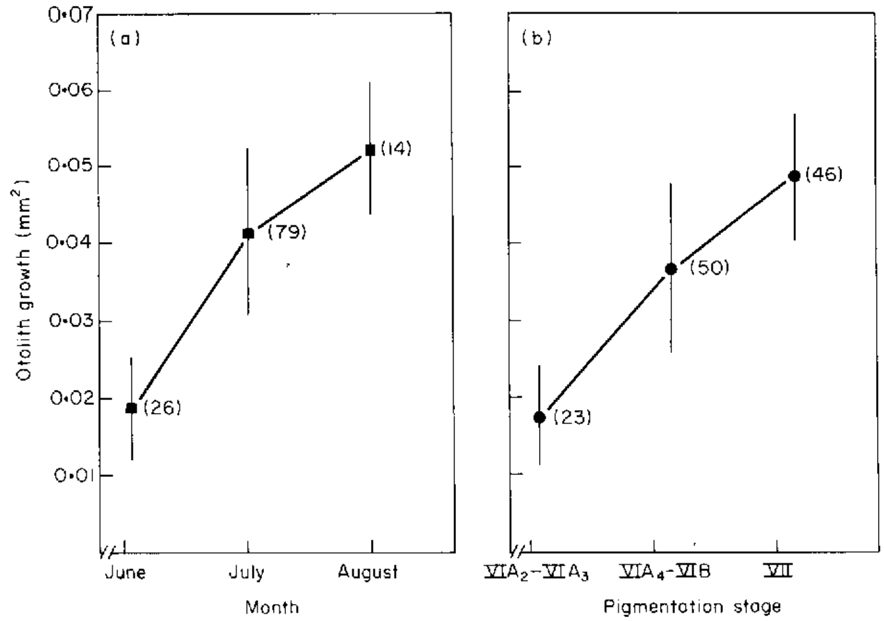

FIG. 4. Relation between the mean growth of otoliths and (a) the month of sampling, and (b) the stage of pigmentation. Numbers in brackets represent sample sizes and vertical lines represent standard deviations.

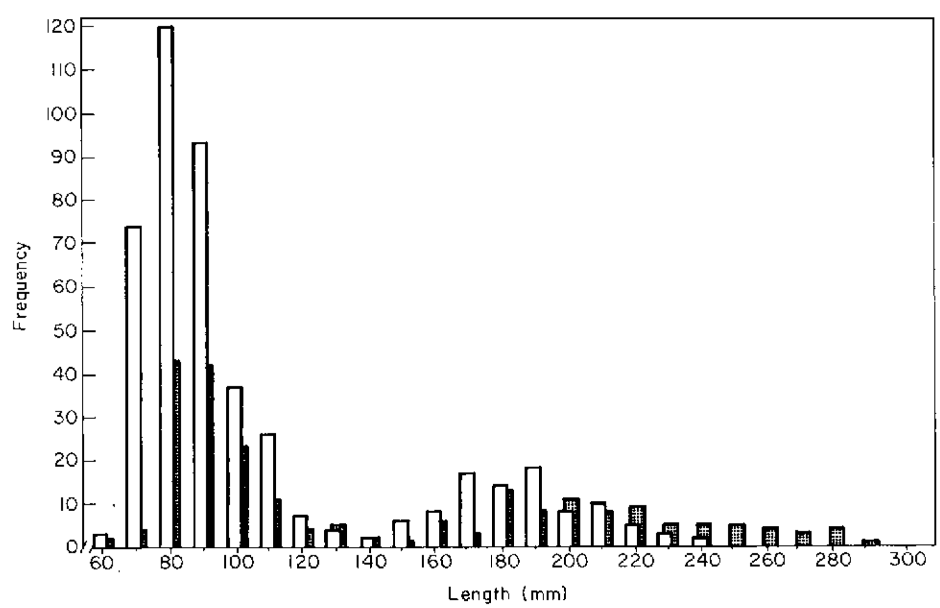

FIG. 5. Length–frequency distribution of eels ascending the first falls in the Petite Trinité River in summer 1983. □, July; □, August; $n=728$.

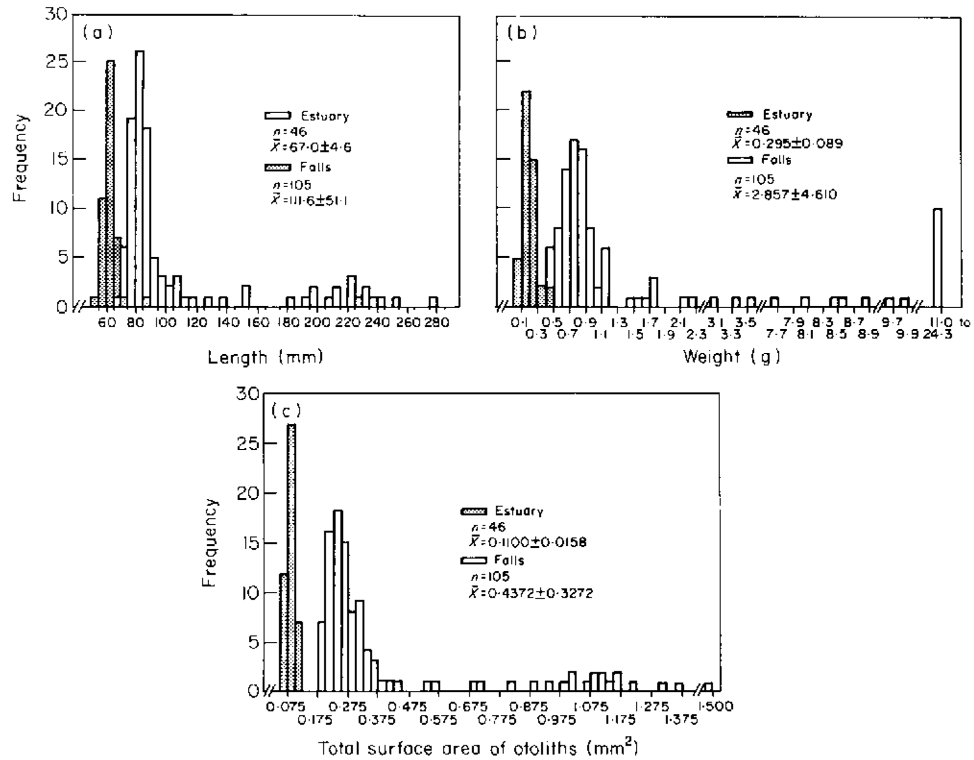

FIG. 6. Frequency distribution of (a) lengths, (b) weights, and (c) total surface areas of the otoliths of eels collected from the estuary and at the first falls in the Petite Trinité River.

rings formed in fresh water was not significantly correlated to the length of the eels ($r_s = -0{\cdot}20$, $n = 17$, $P = 0{\cdot}45$) or to the surface area of their otoliths ($r_s = 0{\cdot}46$, $n = 17$, $P = 0{\cdot}061$).

IV. DISCUSSION

This study demonstrated that a distinct mark was formed on the otoliths of elvers of the American eel during the first 3 to 4 weeks they spent in a river estuary. This mark, which appears as a translucent ring when viewed on a black background using reflected light, may be used to distinguish growth in salt water from that in fresh water. The results obtained in this study contradict those obtained in previous studies. The sea ring (i.e. the centre portion of the otolith produced when the fish is in salt water) of *A. rostrata* as described by Gray & Andrews (1971) and Dolan (1975) contains one more opaque zone than was described in this study. This study indicates that the last opaque zone of the sea ring described by these authors represents the first growth ring formed in fresh water. Gray & Andrews (1971) and Dolan (1975) described the otolith of elvers of the American eel as similar to that of the European eel (Sinha & Jones, 1967), even though the latter apparently spends much more time in salt water. Liew (1974) described what we

TABLE III. Number of rings appearing after first ring formed in estuary in otoliths of individuals at waterfalls

Length (mm)	Number of rings									
	1	2	3	4	5	6	7	8	9	13
70·0–79·9	3	2								
80·0–89·9	7	18	7	1						
90·0–99·9	2	11	4	1						
100·0–109·9	2	2	0	1						
110·0–119·9		1	2			1				
120·0–129·9		1								
130·0–139·9					1					
140·0–149·9										
150·0–159·9				2	1					
160·0–169·9										
170·0–179·9										
180·0–189·9				1						
190·0–199·9					1					
200·0–209·9					1					
210·0–219·9					1	1				
220·0–229·9					2	1				
230·0–239·9			1		1					
240·0–249·9										
250·0–259·9										
260·0–269·9										
270·0–279·9										
280·0–289·9				1						
290·0–299·9										1
Total	14	35	14	7	8	3				1

have called the transition zone in the present study but interpreted it differently, claiming that it was formed in the preceding winter.

Discrepancies in descriptions of the sea ring of American eels in estuaries are mainly due to variations in the schedule of sampling. Previous authors collected elvers irregularly (Gray & Andrews, 1971; Dolan, 1975) or over a 4-day period (Liew, 1974). Since the transition zone is formed during the first 2 to 3 weeks after the elvers enter an estuary, otoliths from samples taken at the entry of elvers in the estuary do not contain a transition zone while those obtained 4 to 5 weeks later contain both a transition zone and another opaque zone, which increases in width as the summer progresses.

Though it has now been established that the passage from salt to fresh water for American eels, or from fresh to salt water for chinook salmon, *Oncorhynchus tshawytscha*, (Neilson *et al.*, 1985), is associated with the formation of a distinct ring on the otolith, the mechanism resulting in such a transition zone is not known. The changes in salinity and/or temperature encountered by elvers entering the estuary, and the related osmoregulatory changes are potential causes for the formation of such a transition zone.

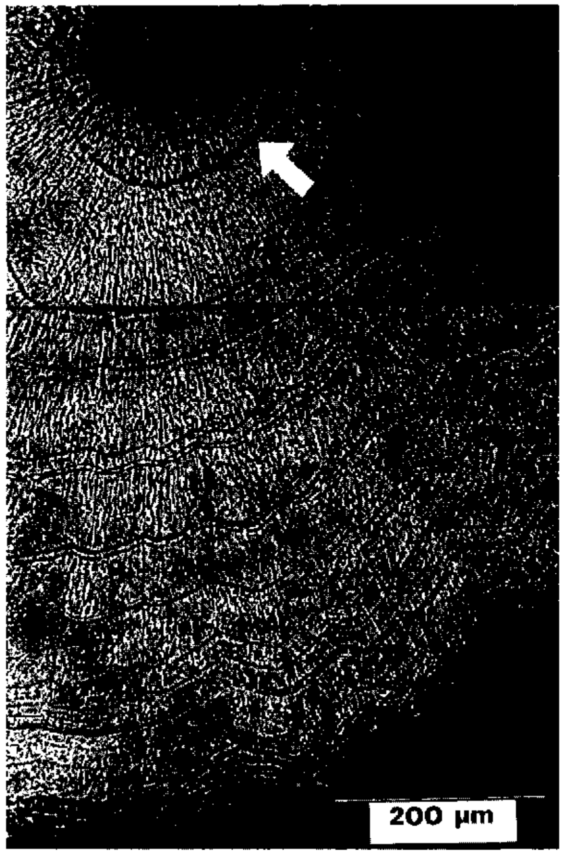

FIG. 7. Otolith of a 26-cm eel collected on 1 August 1983 in the falls of the Petite Trinité River, showing the formation of several rings (viewed under SEM). The arrow indicates the transition zone.

Studies of the microstructure of otoliths and lengths and weights of individuals in the two segments of the 'population' confirmed that the young eels at the waterfalls were at least one year older than those entering the estuary. The elvers entered the Petite Trinité River estuary at the same time as young eels started to ascend a waterfall farther upstream. Their length–frequency distributions showed virtually no overlap, suggesting a trimodal age distribution, and this was confirmed by otolith surface area distribution. However, the examination of otolith microstructure indicated that more rings were formed than would be expected from length-distributions. The conclusion is reached that the incapacity to distinguish 'false' rings from those produced annually invalidates the use of otoliths to determine the age of eels in this study (Fig. 7). The poor relationship reported in many studies between age and length might have been caused in part by this problem.

False annuli (Liew, 1974; Deelder, 1976) and 'multiple bands' (Boëtius & Boëtius, 1967; Deelder, 1976, 1981) result from fluctuations in ambient temperature or in the kind or quantity of food eaten (Liew, 1974). Certainly, the eels in the Petite Trinité River were exposed to fluctuating environmental conditions, as the mean water temperature fluctuated between 12 and 22° C during the summer season and the eels climbing the rocks beside the waterfalls were exposed to air temperatures ranging from 8 to 30° C during the same period. The amount of food ingested by eels in the Petite Trinité River must also have varied over time, as Dutil (unpubl. data) found that most of the eels climbing the waterfalls had empty stomachs and Walsh *et al.* (1983) confirmed that American eels do not eat when the ambient temperature declines below 10° C. However, although the otoliths of the eels sampled in our study undoubtedly contained false annuli, we were unable to distinguish them from annually produced rings when viewing them either with an optical or with an electron microscope (Fig. 7).

We conclude that it is possible to distinguish otolith growth in fresh and salt water as well as to distinguish three modal groups based on distributions of otolith surface areas or eel lengths that may represent three age classes. However, the observation that ring formation is not annual means that the use of otoliths for the age determination of eels in this study has not been validated.

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