

Scanning electron microscopic analysis of annulus microstructure in otolith of European eel, *Anguilla anguilla*

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To validate the yearly periodicity of annulus formation in the otolith of the eel, the structure of annuli in otoliths of the European eel, *Anguilla anguilla*, stocked for 7 and 12 years in Lake Ommen on the east coast of southern Sweden, was examined. The population was stocked from elvers imported both from France (Bay of Biscay) in April 1979 and England (River Severn) in March–April 1984. The microstructure of an annulus consisted of single, double and/or composite rings depending on the location in the otolith. The counts of annuli in otoliths of these eels were approximately consistent with the expected age. However, supernumerary false annuli and/or annulus underestimation frequently occurred. The methodology for annulus discrimination with light and scanning electron microscopes is described.

Key words: *Anguilla anguilla*; eel; elver; otolith; annulus; age and growth; Sweden.

INTRODUCTION

European eel *Anguilla anguilla* L. is widely distributed in the freshwater and marine littoral areas of north Africa, the Mediterranean Sea, the British Isles, Iceland, and the western and northern European continental countries (Tesch, 1977). Due to its economic importance, the life history, resources management and aquaculture have been well studied (EIFAC/FAO, 1991).

Age and growth information is essential for complete knowledge of life history, age at recruitment, growth rates, age at first maturity, and average life span (Liew, 1974; Carlander, 1987). Age determination in European eels was first described by Ehrenbaum & Marukawa (1913) through the interpretation of otolith growth rings (annuli). Age and growth of this species have since been well discussed (e.g. Sinha & Jones, 1967; Moriarty, 1973, 1983; Deelder, 1976; Moriarty & Steinmetz, 1979; Lee, 1982; Berg, 1985; Vøllestad, 1985; Aprahamian, 1987; Lecomte-Finiger, 1992). However, due to differences in otolith preparation techniques, there have been considerable discrepancies in ageing results after successive examinations of otoliths of the same age (Moriarty & Steinmetz, 1979; Deelder, 1981; Vøllestad *et al.*, 1988). Moriarty (1983) has discussed the inaccuracies or possibilities of misinterpretation after preparing otoliths using burning techniques. Accordingly, the validation of age determination becomes very important in estimating the accurate age of the eel (Beamish & McFarlane, 1983).

This paper discusses a grinding method for preparing otoliths for microstructural examination and validation of annulus formation in the known-age European eel by using light and scanning electron microscopy.

MATERIALS AND METHODS

Ten eels used for otolith examination were selected from the 138 eels stocked in Lake Ommen. Lake Ommen is a mesotrophic lake on the SE coast of Sweden (57°95' N 16°28' E) with an area of 205 ha; fish fauna in the lake include perch *Perca fluviatilis* L., roach *Rutilus rutilus* L., bream *Abramis brama* L., rudd *Scardinius erythrophthalmus* L., tench *Tinca tinca* L., ruffe *Gymnocephalus cernuus* L., pike *Esox lucius* L. and smelt *Osmerus eperlanus* L. (Wickström, 1984). It was stocked with eels derived from elvers imported from France (Bay of Biscay) in April 1979 and England (River Severn) in March–April 1984, respectively. The average size of the elvers at stocking was approximately 70 mm in total length. The eels were recaptured during the summer of 1991.

The sagittal otoliths of the experimental eels were removed and dried at 60° C for 10 min, then each otolith, with distal side upward, was embedded in a silicone rubber mould with Petropoxy 154 (Palouse Petro Products, U.S.A.). The procedures for embedding, grinding/polishing and etching the otoliths were described previously (Tzeng, 1990; Tzeng & Tsai, 1992). The growth and morphology of the otolith and of annuli were observed with light microscopy. To discriminate the microstructure of the annuli, the otolith was vacuum-coated with gold and examined by scanning electron microscopy (SEM) (Hitachi S-520).

To validate the elver check formed in the otoliths of the stocked eels, the sagittal otolith of an elver collected from the River Severn on 17 April 1987 was also examined. The elver check was deposited at elver stage during its migration from sea water to fresh water (Tzeng & Tsai, 1994). The pigmentation stage of the elver was determined according to the distribution of pigments on the body surface (Strubberg, 1913; Bertin, 1956). The embedding, grinding and etching processes for the elver otolith were similar to those mentioned above.

The growth rate of the eel changed as life-history stage shifted. To reduce the effect of growth rate on estimation of the back-calculated total length, the total length of the eel at the time of annulus formation was estimated as follows:

$$l_n = r_n \left(\frac{L - l_e}{R - r_e} \right) \quad (1)$$

where: L = total length of the eel at capture; R = otolith radius of the eel at capture; r_e = averaged otolith radius of the elver at stocking; l_e = averaged total length of the elver at stocking; r_n = otolith radius at time of annulus formation; l_n = total length of the eel at time of annulus formation.

And, then the annual increment of total length of the eel was estimated by

$$\frac{\Delta l}{\Delta t} = l_{t+1} - l_t \quad (2)$$

RESULTS

STOCKING ENVIRONMENT OF THE EELS

Environmental data for Lake Ommen were not available for the stocking period (1979+1984). Water temperature at 3.4 m depth was recorded from the middle of April through August 1992. It increased from c. 6° C in April to 22° C at the beginning of June and remained at this high level until early August (Fig. 1). Due to the lake being frozen, there were no data for winter. Stratification of thermal structure was found in September. The temperature in the epilimnion layer from surface to 10 m depth was approximately 15.5° C. The metalimnion layer, located at approximately 11 m, revealed decreasing temperature (from 15.5

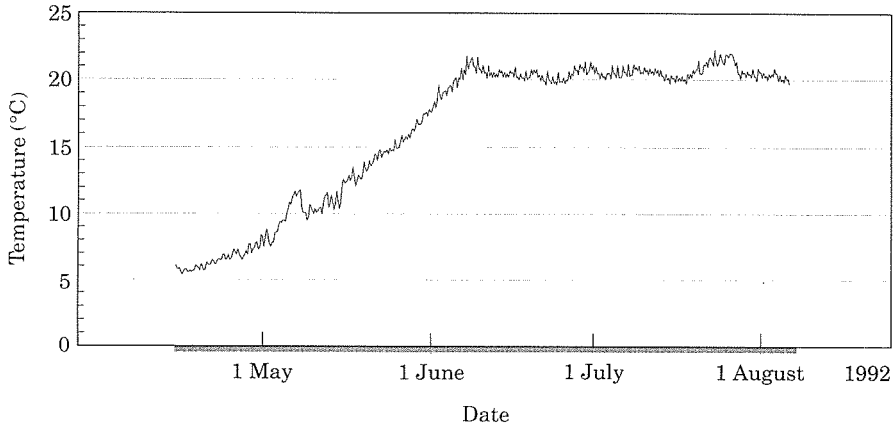


FIG. 1. Seasonal change of water temperature at 3.4 m depth of Lake Ommen off the east coast of southern Sweden, April–September 1992.

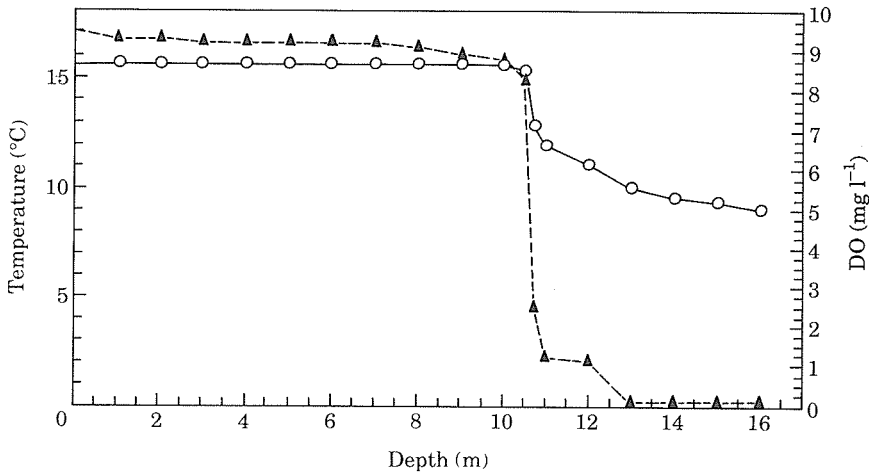


FIG. 2. The changes of temperature (○) and DO (▲) with water depth in Lake Ommen on 11 September 1991.

to 12°C), while dissolved oxygen (DO) dramatically decreased from 8.5 to 1.0 mg l⁻¹ in this layer. In the hypolimnion layer, water temperature was approximately 5°C and DO close to zero (Fig. 2).

FISH LENGTH AND WEIGHT RELATIONSHIP

Somatic growth varied greatly between individuals. The total length (T.L.) for the eels, stocked as elvers, averaged 394.7 ± 10.97 mm for the 1984 year class and 527.95 ± 19.32 mm for the 1979 year class. The logarithmic linear regression of body weight (W) on total length (L) (Fig. 3) for the 138 eels was estimated as follows:

$$\ln W = -14.53 + 3.20 \ln L (n=138, r=0.9865). \quad (3)$$

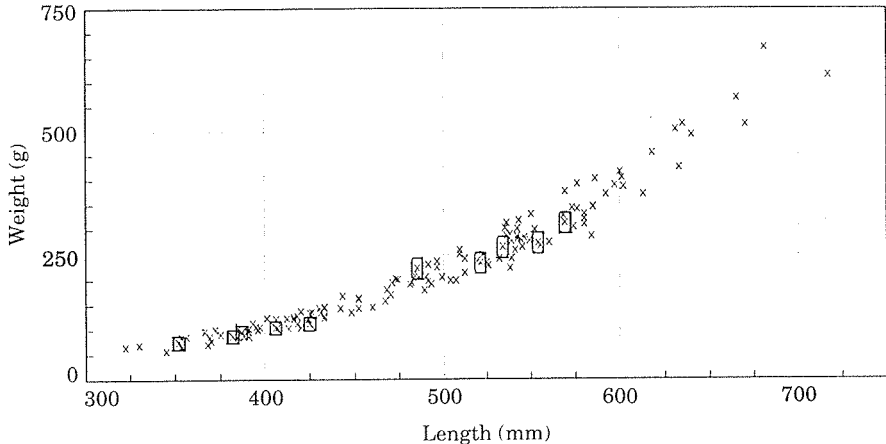


FIG. 3. Length-weight relationship for 138 yellow eels at recapture on 11 August 1991. The eels were stocked in Lake Ommen during 1979 and 1984. Data for 10 eels used for otolith examination are shown in squares.

OTOLITH GROWTH AND MORPHOLOGY

The elver's otolith is oval in shape. The elver (stage VIA₁) collected from the River Severn on 17 April 1987 was 65.4 mm T.L. and the maximum otolith radius was approximately 156.6 μm . A metamorphosing check (MC), radius 112.5 μm , located at approximately two thirds of the maximum otolith radius from the primordium, was clearly observed (Fig. 4). This check was postulated to be a metamorphosis mark from the stage of leptocephalus to glass eel, and is frequently found in the otoliths of elvers (Lecomte-Finiger, 1992; Tzeng & Tsai, 1992).

The shape of otoliths became less uniform with age, as indicated from the 7- and 12-year-old eels (Figs 5 and 7). These otoliths were concave on the distal face and convex on the proximal face, growing faster in the antero-posterior axis, specifically the rostrum and antirostrum in the anterior part and postrostrum in the posterior part, medium rate in the dorso-ventral axis, lower in the proximo-distal axis, and with a sulcus on the proximal face. Because of the thickness in the proximo-distal axis, grinding the otolith from the proximal side reduces the time necessary to reveal the primordium of the otolith and is suitable for preparing a thin otolith section for annulus structure examination with light microscopy under transmitted light. Opaque and translucent zones in otoliths of the eels were observed under transmitted light (Figs 5 and 7). Each opaque zone corresponds to a time of slow growth of the fish, and was considered to be an annulus (Schramm, 1989).

STRUCTURE OF ANNULI IN OTOLITH

The methodology on discrimination of false and true annuli as well as their microstructures were described in detail based on each one of the 10 7- and 12-year-old eels examined.

The annulus in the 7-year-old eel's otolith was more clear-cut in the antirostrum [Fig. 5(a)]. The otolith was determined to have nine annuli, plus one elver check and one leptocephalus metamorphosis check. The maximum radius

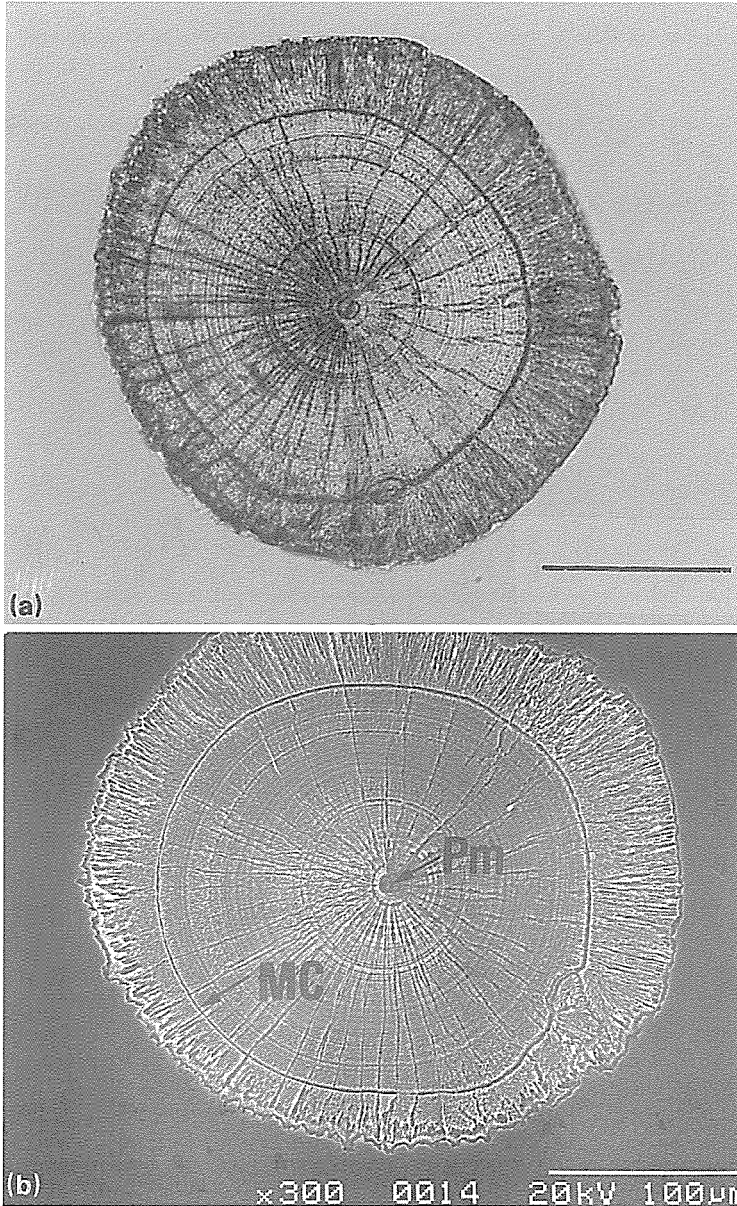
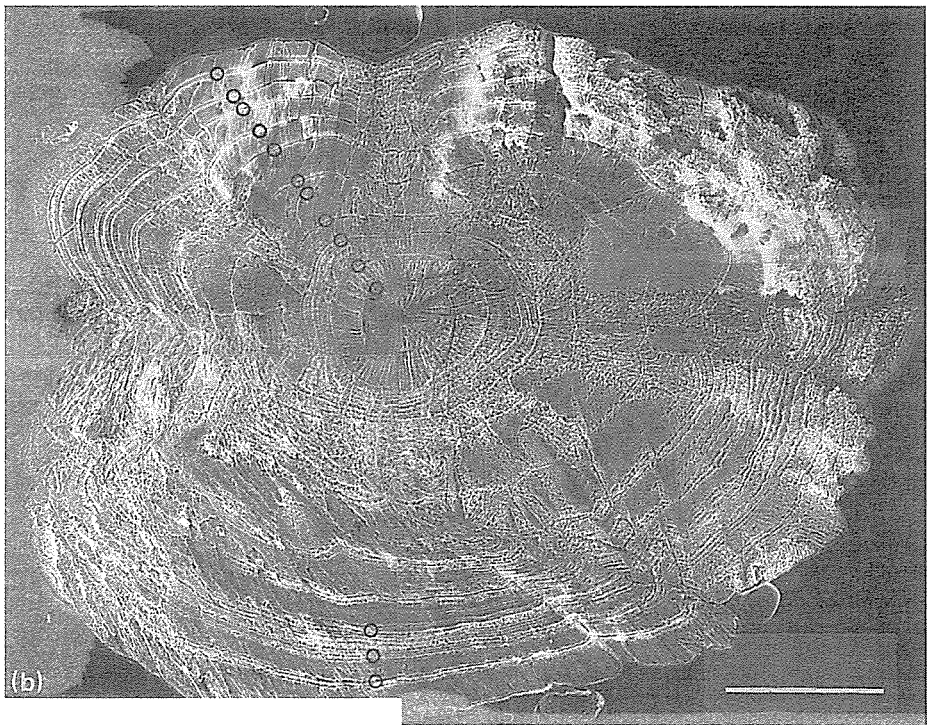
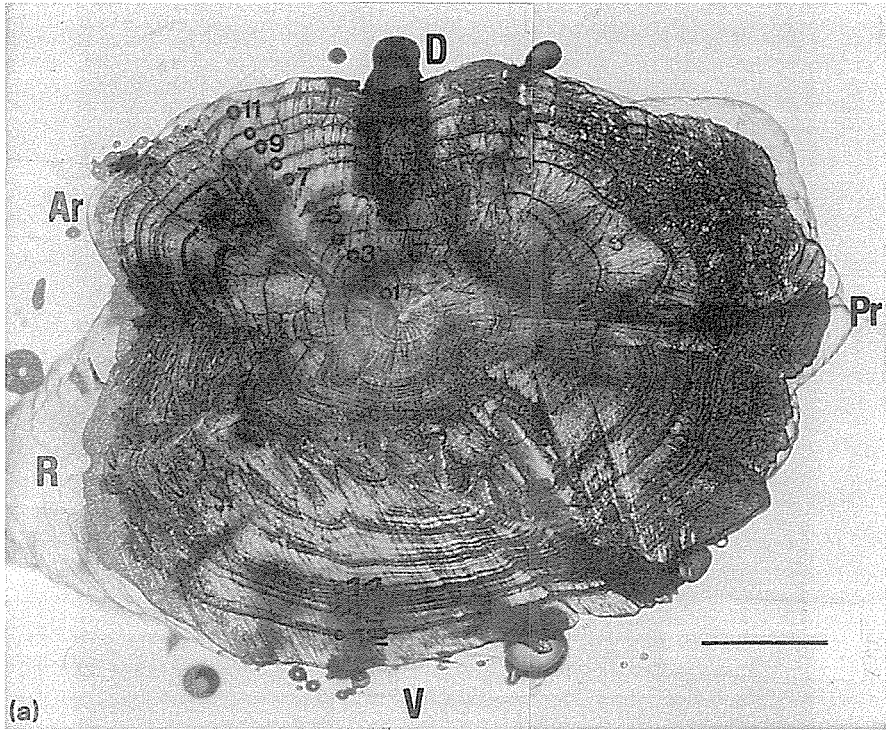


FIG. 4. Photographs taken by light microscope (a) and by scanning electron microscope (b) showing primordium (Pm) and metamorphosing check (MC) in the otolith of a European eel, *Anguilla anguilla*, elver, 65.4 mm T.L., collected from the River Severn, Gloucestershire, England on 17 April 1987. Scale bar=100 μ m.

of the elver check was approximately 171 μ m which was also similar to the maximum otolith radius of the Severn elver (Fig. 4). This elver check in the otolith of natural stock of *Anguilla japonica* Temminck & Schlegel was formed during its upstream migration from the ocean into the river (Tzeng & Tsai, 1994). It appeared that two false annuli existed among the nine counted. To



discriminate the false annulus, the otolith was etched with EDTA and examined with S.E.M. [Fig. 5(b)], the area of antirostrum of the otolith being magnified $\times 2000$ (Fig. 6). It was found that the structure of the 3rd and 5th annuli was considerably different to that of the others (Fig. 6). The structure of true annuli was observed to be a deeply etched groove, while the etched groove in the false annuli was not so deep and was made up of several discontinuous rings, especially in the third annulus (Fig. 6). Because of these discontinuous rings, it was assumed that the growth of the fish had been retarded during the period from the start of stocking to the first winter. In addition, the etching in the fifth annulus was shallow and the annulus incompletely encircled the otolith. Accordingly, the S.E.M. techniques provided a helpful supplementary tool for discriminating between real and false annuli in otoliths of the eel.

Similarly, the annulus in the otolith of a 12-year-old eel was also examined (Fig. 7). The annulus was clear-cut in the postrostrum and made up of single or double rings. The structure of the annulus became diverse in the dorsal, antirostrum and rostrum areas in which the annulus consisted of composite rings, while the ring became convergent in the excisura between antirostrum and rostrum. This otolith was determined to be 12 annuli plus one leptocephalus metamorphosis check, with one elver check. The diameter of the elver check in the otolith of the 12-year-old eel, approximately $161.6 \mu\text{m}$, was close to the maximum otolith radius of an elver as shown in Fig. 4. However, the second annulus was incomplete and very faint. The increment between the second and third annulus as well as that between the sixth and ninth was narrow, and these annuli consisted of double rings. This indicated that the slow growth period was longer in the double than single-ring annuli.

ANNUAL GROWTH RATE OF THE FISH

The annual increment in total length of the previously mentioned two and one selected 7-year-old eels was calculated using equation (2) (Fig. 8). The incremental growth of the otoliths clearly changed yearly. This indicated that the growth of the fish may have changed during the stocking period. The 7-year-old eels stocked in 1984 grew rapidly during the first year (50 to 99.1 mm yr^{-1}). The growth rate in the second year was approximately 50 mm yr^{-1} . Then it decreased gradually year-by-year, reaching the lowest rate ($<20 \text{ mm yr}^{-1}$) in the sixth year, and increased again thereafter. However, the growth rate for the 12-year-old eel stocked in 1979 changed greatly, decreasing dramatically from 50 mm yr^{-1} during the first year to 14.8 mm yr^{-1} in the third year. Growth rate ranged between 20 – 30 mm yr^{-1} during years 6–9, and then dramatically increased to approximately 45 mm yr^{-1} . This great fluctuation in growth rate, as well as the fact that growth rate did not decrease with age is an unusual growth pattern in fish.

FIG. 5. Photographs taken by light microscope (a) and by scanning electron microscope (b) showing annuli in the otolith of a 7-year-old eel, 368 mm T.L., 66 g, stocked in Lake Ommen on 25 July 1984 and recaptured on 11 August 1991 (Specimen No. 19 990). Open circles indicate metamorphosis and elver checks (1 and 2), false (3 and 5) and true annuli (4, 6–11). The annuli in the dorsal area consisted of composite rings (12–14). Ar, antirostrum; D, dorsal; Pr, postrostrum; R, rostrum; V, ventral. Scale bar = $300 \mu\text{m}$.

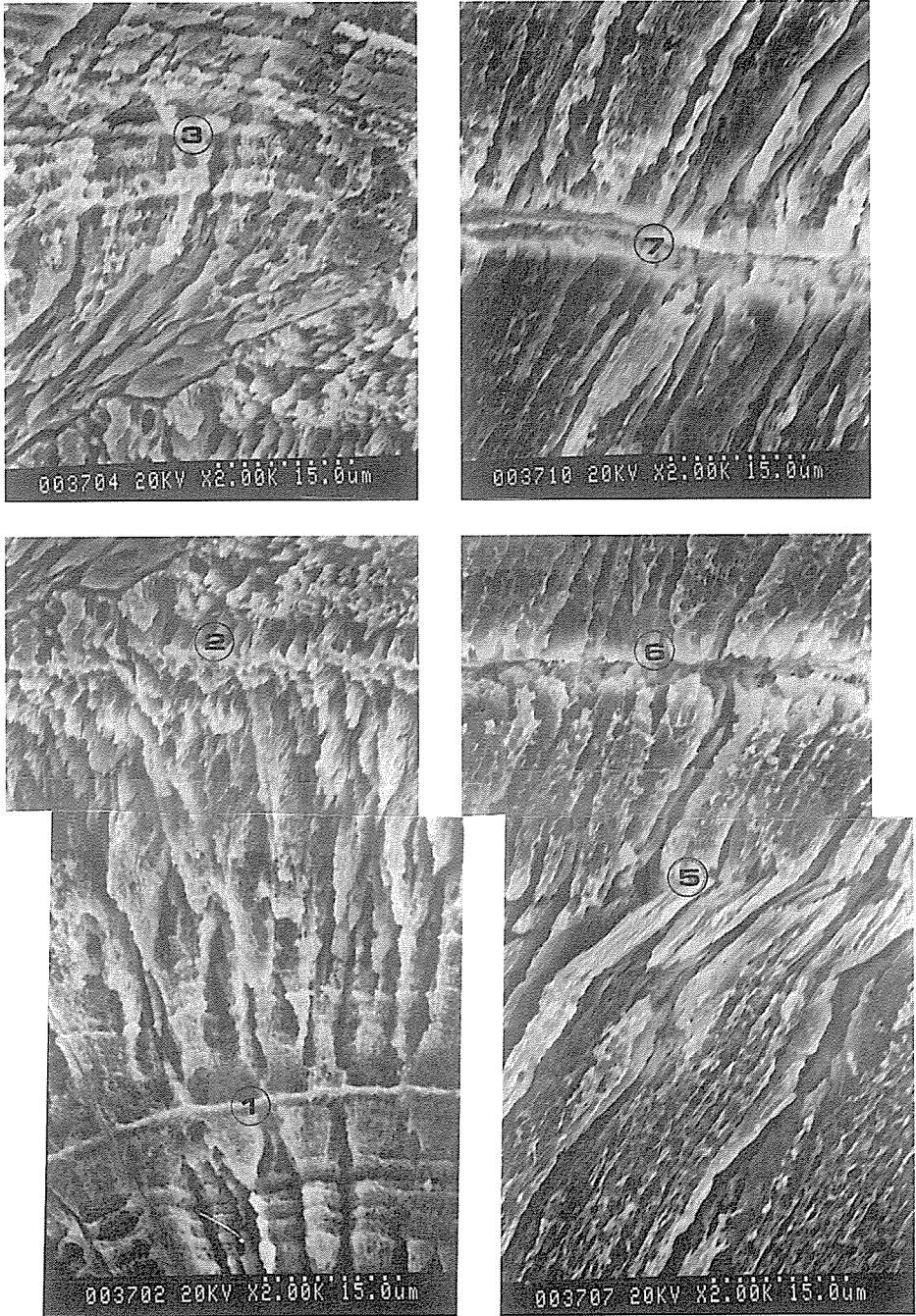


FIG. 6.

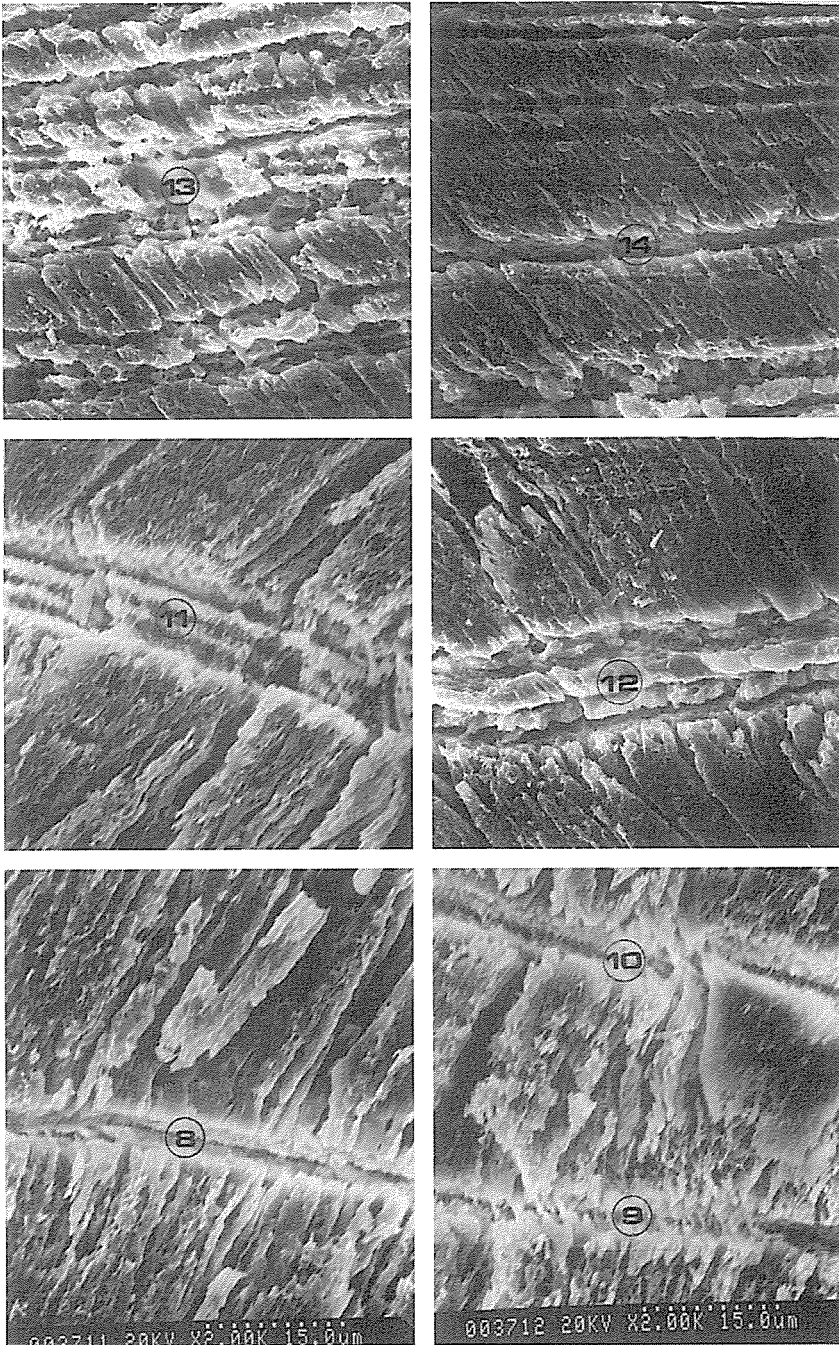


FIG. 6. *Continued.*

FIG. 6. Photographs showing the microstructural annuli magnified from the otolith of Fig. 5. Explanation of 1-14 as in caption to Fig. 5.

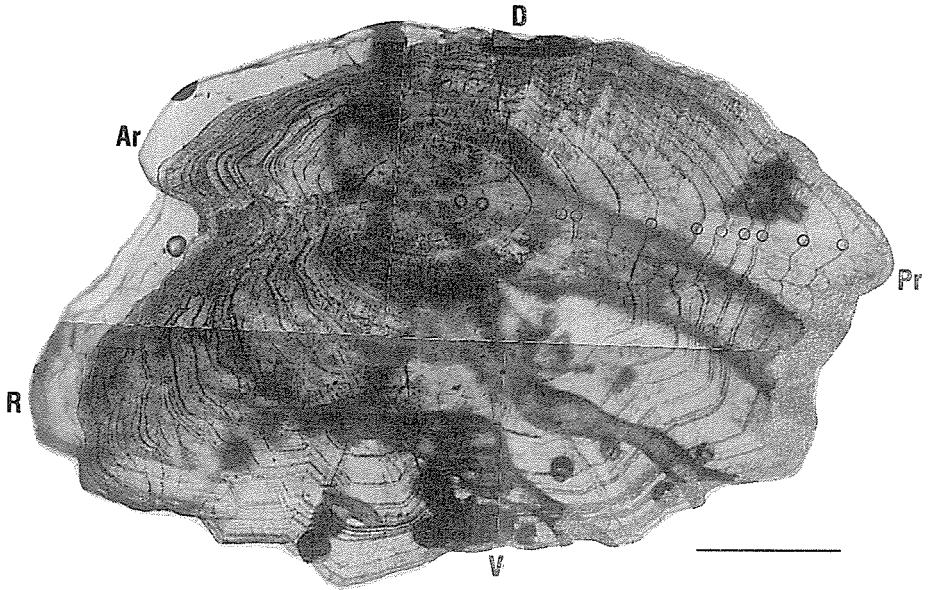


FIG. 7. Annuli in the otolith of a 12-year-old eel, 521 mm T.L., 230 g, stocked in the Ommen Lake on 27 June 1979 and recaptured on 11 August 1991 (Specimen No. 19 991). Open circles indicate metamorphosis and elver checks (Nos 1 and 2) and 12 annuli (Nos 3–14). Abbreviations as in caption to Fig. 5. Scale bar=500 μ m.

DISCUSSION

OTOLITH PREPARATION FOR ANNULUS EXAMINATION

Methods of otolith preparation for age-determination of the eel have been discussed by many researchers. The most commonly adopted method is cracking and burning (Christensen, 1964; Hu & Todd, 1981). The burning method has resulted in inaccuracies in the age determination of cultured eels of known age (Deelder, 1981) and considerable discrepancies between successive readings of the same otolith (Moriarty, 1983). These inaccuracies are probably due to the frequent formation of supernumerary zones in eel otoliths. Such supernumerary zones are formed asymmetrically in the otolith (Deelder, 1976, 1981). The cracking and burning method probably only enhances the contrast of opaque and translucent zones in the dorso-ventral and proximo-distal areas (Hu & Todd, 1981; Moriarty, 1983). Our studies indicated that an annulus was composed of single, double or composite rings in different growth areas of the otolith. The formation of these rings are related to the growth rate of the different parts of the otolith. Otoliths grew rapidly both in the dorsal part and the rostrum and thus the annuli in these parts frequently consisted of composite rings, which easily led to overestimating the number of annuli. An otolith ground from the proximal side could simultaneously exhibit different growth patterns of an annulus (Figs 5–7). One can select the appropriate area for age determination, and those with a single ring seemed to be most practical for annulus discrimination. Accordingly, although the preparation of otoliths by the grinding method is time-consuming, it provided more accurate information than that by the cracking and burning method.

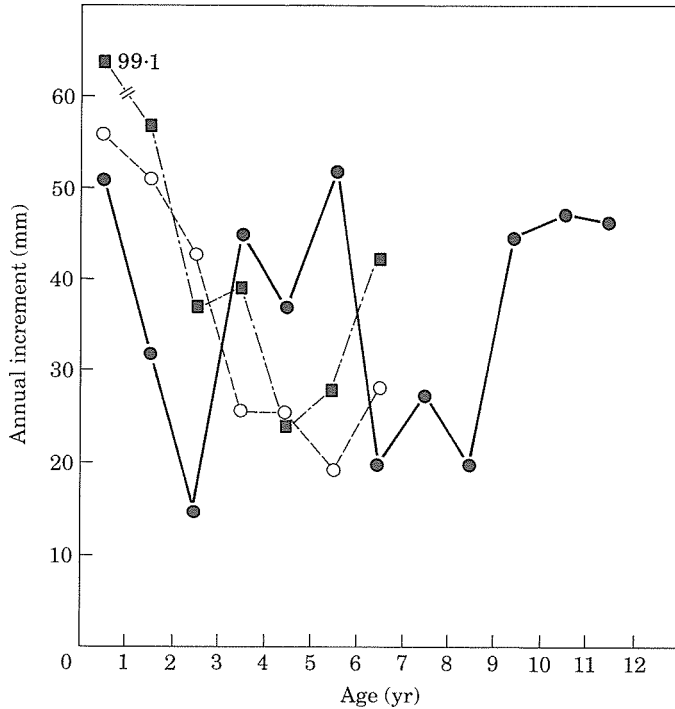


FIG. 8. Annual growth rate in total length for the three eels stocked in Lake Ommen during the Spring of 1979 and 1984 and recaptured on 11 August 1991. ●, Eel stocked in 1979 (19 991); ○, eel stocked in 1984 (19 990); ■, eel stocked in 1984 (19 989).

VARIABILITY AND DISCRIMINATION OF ANNULUS

The leptocephalus metamorphosis and elver checks, which appeared as opaque zones (Figs 5–7), have to be excluded in counting the annulus of the adult eel. The maximum otolith radius of the European eel elver varies with its pigmentation stages, as well as the time period and location of recruitment, and ranges between $154.3 \pm 14.5 \mu\text{m}$ and $171.2 \pm 26.0 \mu\text{m}$ (Lecomte-Finiger, 1992). The maximum otolith radius of the elver in our study was approximately $156.6 \mu\text{m}$ (Fig. 4). The maximum radius of the elver check in the 10 adult eel otoliths examined ranged between 157.3 – $171.0 \mu\text{m}$ (Figs 5–7). Thus, the elver check was easily determined according to its dimension.

A false annulus was found in the otolith of the 7-year-old eel in our studies (Fig. 5), similar to those seen in other investigations (Sinha & Jones, 1967; Lee, 1982; Berg, 1985). The EDTA etching revealed a shallower groove in the false annulus than in the true one, and the false annulus was usually composed of several discontinuous rings (Fig. 6). Thus, the false annulus was easily discriminated from the true one. The false annulus was probably formed when the growth of the fish was interrupted by short-term stress. On the other hand, although the number of annuli in the otolith of the 12-year-old eel coincided with the expected age, it was easily underestimated, probably one less than the expected age because of the third faint annulus in the otolith (Fig. 7). This underestimation can be corrected by using SEM microstructure examination. Even though supernumerary false annuli and the underestimation of true annuli

frequently occurred, on average the age of the eel was still able to be accurately determined from the microstructural examination of the otolith.

TEMPERATURE AND ANNULUS FORMATION

The growth rate of the eel was influenced by the seasonal change of water temperature and the otolith annulus was formed during a period of starvation induced by unfavourable environmental conditions in winter (Liew, 1974; Williams & Bedford, 1974; Bagenal & Tesch, 1978; Jearld, 1983). In Denmark eels cannot be caught by baited hooks when water temperature is lower than 10° C (Bruun, 1963). The eels eat little or nothing at low temperatures (Sinha & Jones, 1967). The fact that Japanese eels apparently lose their appetite when water temperature falls below 14° C (Matsui, 1952; Okada, 1960) suggests that artificial feeding of eels in stock ponds should be stopped at water temperatures lower than 10° C. It is also significant that American eels *A. rostrata* (Le Sueur), react similarly to low temperatures (Fowler, 1906; Bigelow & Schroeder, 1953; Smith & Saunders, 1955). Clearly, temperature plays an important part in the growth of the eel. The temperature in Lake Ommen, where the experimental eels were stocked, was between 0.5 to 4° C during the winter. Almost every winter there was ice on the lake for about 3 to 4 months. Accordingly, low temperatures in Lake Ommen during winter probably caused the growth stoppage, inducing formation of annuli.

THE CHANGE OF ANNUAL GROWTH RATE

The yearly growth rate of the eel changed greatly as indicated from the 7- and 12-year-old fish examined (Fig. 8). The annual incremental width in the otolith of the 12-year-old fish was obviously narrower during the period from 2 to 3 years, as well as from 6 to 9 years old. Meanwhile the structure of the annulus appeared to consist of double rings in this period (Fig. 7). The double-ring annulus probably indicated that growth had stopped before the normal single ring formation commenced. In addition, the size of recapture varied widely for individuals stocked in the same year. The time series change of the growth rate of each individual can be reconstructed from the otolith growth history (Fig. 8). These changes are related to environmental factors, such as temperature, feeding regime or stresses (Williams & Bedford, 1974; Bagenal & Tesch, 1978; Jearld, 1983). Unfortunately, we have no long-term environmental data to account for these interesting phenomena.

Generally, the growth rate of fish should decrease with age. Otherwise the asymptotic length could not be explained. However, it was unusual for the higher growth rate to occur in the later stages (Fig. 8). This may be due to the fact that the European eel has a long life-span, with 7- and 12-year-olds young in comparison with the recorded maximum age of over 80 years (Svärdson, 1949).

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