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Age Determination of Eels in the French Mediterranean Lagoons Using Classical Methods and an Image Analysis System

key words: eel, age determination, otolith, image analysis, mediterranean lagoons

Abstract

Several methods are used for age determination of eels in the French mediterranean lagoons (observation of the whole otolith after clearing, grinding and polishing, dyeing, SEM and image analysis). Two types of otoliths are observed depending on the width of the growth rings which is probably related to the environmental conditions. Furthermore, the growth checks are located nearby or inside the opaque rings. A computer assisted method is developed, with reference to the other methods, with the aim of improving the efficiency of collecting and processing age and growth data. With further developments and increasing knowledge of the life-history of elvers and juvenile eels, it seems possible to use an image analysis system for ageing eels in lagoons. Nevertheless, the most important point is to validate the results before using them for growth populations studies.

1. Introduction

Despite many studies that have been carried out on the age determination of eels, some questions remain unsolved due to the diversity of the methods and to the difficulties involved in analysing the growth discontinuities observed on the otoliths (MORIARTY & STEINMETZ, 1979; BOETIUS, 1985; LECOMTE-FINIGER, 1985; VOLLESTAD, 1985; VOLLESTAD *et al.*, 1988).

This paper presents and compares some methodological approaches to the examination of eels otoliths in ageing studies. Among these, a microcomputer-based system is used to count and measure automatically the zonations in video displayed otolith structures.

2. Materials

Biological samples were collected in the frame of a program between Cemagref/University of Montpellier II/University of Perpignan. Otoliths (sagittae) are removed from eels fished in the Etang du Vaccarès (Camargue) and in the Etang de Mauguio and Vic (Languedoc) (XIMENES, 1986). They are prepared on their convex side before examination.

3. Comparison of Otolith Reading Methods and Results

a) Observation of whole otolith

The whole otolith is immersed in methyl benzoic acid (used here), cedar oil or alcohol (VOLLESTAD, 1985) before examination with a conventional microscope, using reflected light against a dark background (Fig. 1.a). Usually, the growth rings observed are hyaline (winter rings) or opaque (summer rings).

The eel otoliths sampled in the lagoons of Camargue and Languedoc show wide opaque and hyaline rings. But, some of those coming from the Etang of Mauguio have narrow or lightly marked rings; these structures are similar to those obtained with otoliths from freshwater eels (XIMÉNÈS, pers. comm., 1988).

b) Observation with conventional microscope after grinding and polishing

Otoliths prepared for ageing are embedded in a synthetic resin (polyester) then ground on the convex side (with grinding paper from 300 to 1200 grit) and polished (Fig. 1.b). The grinding plane is important as the crystalline structures of the otoliths are examined from sagittal sections taken through the nucleus and the edge. This procedure is often used (VOLLESTAD *et al.*, 1988) and some improvements are also known (FROST, 1945; SINHA & JONES, 1967; PENAZ & TESCH, 1970; ROSSI & COLOMBO, 1976; ROSSI & VILLANI, 1980; HANSEN & EVERSOLE, 1984; BOETIUS, 1985; PAULOVITS & BIRO, 1986; VERO *et al.*, 1986). The purpose of the method proposed by the ICES/EIFAC Working Group on Eels in 1979 (DEELDER, 1981) is to make 0.2 mm sections in the otoliths which gives similar results to grinding and polishing. Nevertheless, it has not been used here because the sectioning longitudinal angle of the skeletal piece with a convex shape does not provide a proper plane which must include both the nucleus and the greatest diameter.

The observations made with the entire otolith or after grinding show little differences but the latter technique includes additional opaque rings in contrast with hyaline ones.

c) Observation with conventional microscope after grinding, polishing and dyeing

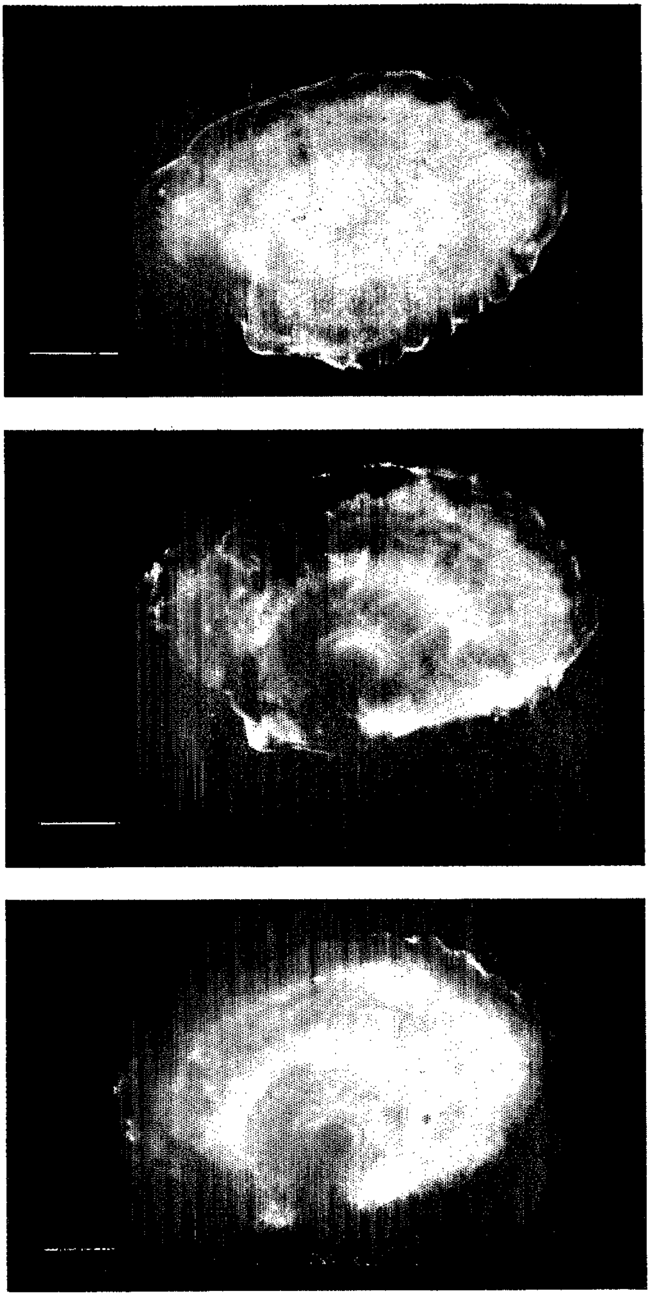
In addition, the protein matrix can be stained by 1 % toluidine blue after grinding, polishing and etching the otoliths with EDTA (Ethylenediaminetetraacetic acid 5 %). The duration of staining and acid-etching does not exceed a few seconds (from ALBRECHTSEN, 1968; BENECH, 1975; CHARLON, 1975; *inter alia*; modified).

Colored rings with high protein concentration appear (Fig. 1.c) on stained otoliths. Usually, thin and highly stained rings are considered as the principal growth checks and they are located in the winter hyaline zones (VOLLESTAD *et al.*, 1988). Staining also reveals supernumerary rings which are, however, less colored.

Our observations are made on sedentary eels caught in brackish lagoons. They show that two types of otoliths can be considered according to the width of the rings and that the narrow stained rings are easily recognizable before staining, on the whole or ground otolith.

aa) Wide ringed otoliths ("brackishwater type")

These otoliths are the most numerous. The colored ring is located between the wide hyaline and opaque zone or slightly inside the latter. The polishing plane is critical in revealing the location of the colored ring (Fig. 1.b & 1.c). A more or less



a

b

c

Figure 1. Different methods applied to the same otolith. Observations are made on the convex side, under reflected light against a dark background.

a. Whole otolith in methyl benzoic acid.

b. Otolith after embedding, grinding and polishing.

c. Otolith after grinding, polishing, etching with EDTA and staining with toluidine blue. One can observe the wide opaque and hyaline rings. (Scale = 0.5 mm).

pronounced polishing angle with regard to the otoliths' median longitudinal plane will displace the colored ring from the boundary of the hyaline and opaque zones. Figure 1.c shows that the colored ring is in the hyaline zone on the right. In this case, error measurements could happen, particularly when back-calculation is used. To avoid this, one should observe the nucleus: the light ring around the nucleus is always present and recognizable without staining, and corresponds to the transition from sea to continental water (MICHAUD *et al.*, 1988); it is distorted in the direction of the polishing axis; the constancy of this ring diameter shows that the polishing technique is appropriate.

bb) Narrow ringed otoliths ("freshwater type")

As above, the otoliths must be prepared carefully. Many rings are observed and the colored ones are located at the boundary of hyaline and opaque zones (Fig. 2). The colored rings are often divided into two, rather inside the hyaline zones than in the

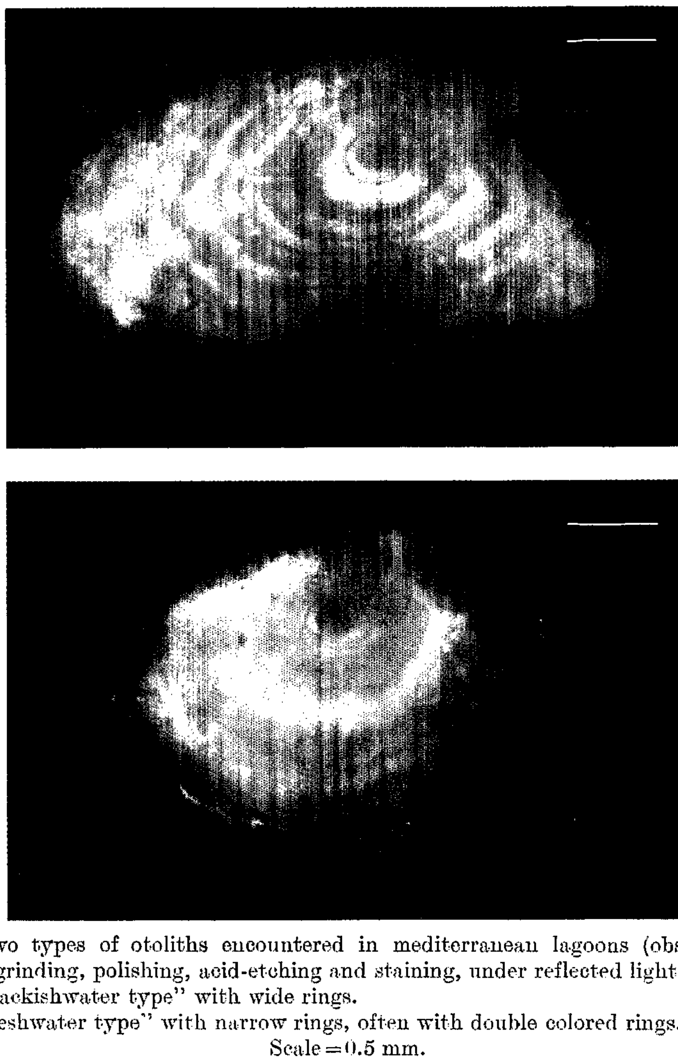


Figure 2. Two types of otoliths encountered in mediterranean lagoons (observations after grinding, polishing, acid-etching and staining, under reflected light):
a. "Brackishwater type" with wide rings.
b. "Freshwater type" with narrow rings, often with double colored rings.
Scale = 0.5 mm.

opaque ones, but one cannot know if these rings are supernumerary or not, as they are mentioned by several authors (Dahl, 1967; Wiedemann-Smith, 1968; Liew, 1974; Moriarty, 1975; Deelder, 1976; Berg, 1985). Michaud *et al.* (1988) met the same situation with American eels. However, narrow hyaline rings (additional rings?) which are slightly colored can be observed inside the opaque zones.

In these two types of otoliths, the opaque zone corresponding to the end of the elver stage, which occurs at the beginning of the continental life (Sinha & Jones, 1967) has a very variable thickness. A wide opaque zone always includes a narrow hyaline one which cannot be stained. So, what is the significance of such structures in relation with the life history of the elvers?

The location of colored rings in the otoliths suggests that the growth rate is high after the winter decrease of growth. The relative width of the hyaline zones could correspond to an important growth period during the setting up of these zones.

d) Observation with scanning electron microscope (SEM)

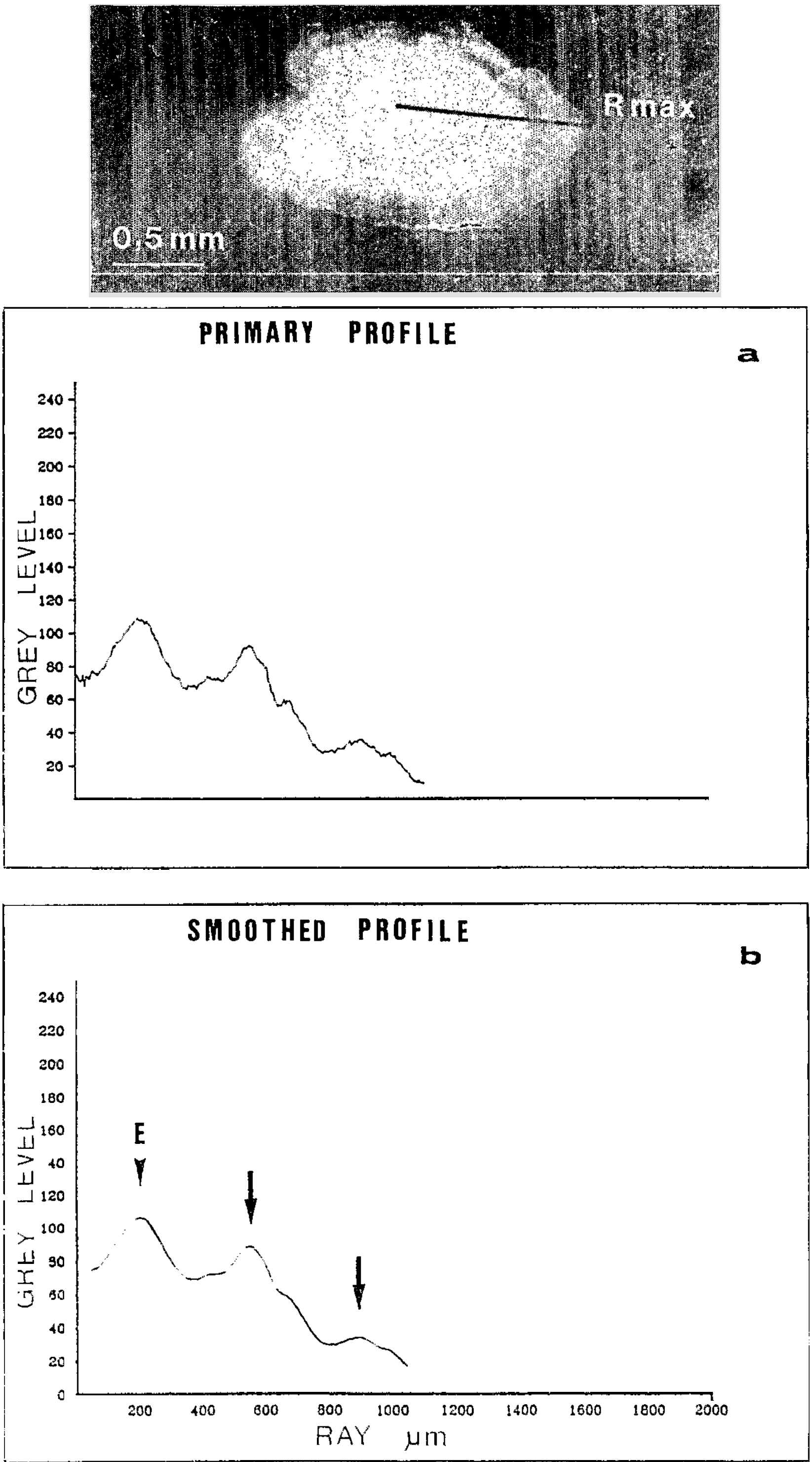
Ground, polished and acid-etched otoliths are coated with gold or carbon for observation with a SEM (Liew, 1974; Lee, 1979; Berg, 1985).

Our treatment showed that carbon-coated otoliths provided better results. The crystalline structures revealed are comparable to the zonations defined by the dyeing technique (Albrechtsen, 1968). Growth check rings are recognizable by the lack of aragonite prism structures and are used for age determination. The comparison of images of ground and polished otoliths with those obtained with scanning electron microscope show that the principal marks of slow growth are located at the boundary of the two zones or in the opaque ones. These conclusions are similar to those obtained with dyeing techniques but they are not in agreement with Liew (1974) and Lee (1979) who noted that slow growth rings are observed in hyaline zones, even if supernumerary rings are also present.

e) Computer-assisted age determination

The recent use of microcomputer and image analysis systems in ageing studies allows one to improve the efficiency of collecting and processing age and growth data (Troadec, 1987; Mc.Gowan *et al.*, 1987; Small & Hirschhorn, 1987; Panfili, 1988; *inter alia*).

A personal computer and a digitizing board were combined with a video camera fitted to a microscope for analyzing zonations in otoliths of eels. The system allows rapid and precise counts and measurements of zonations displayed on a video monitor. The digitizing program in use at the CEMAGREF (Montpellier) was designed for use on a microcomputer with the IBAS software (Kontron Bildanalyse). It follows one: a) to observe under reflected light a whole otolith against a dark background; b) to analyse the brightness levels of light and dark zonations along a transect of the video displayed otolith; c) to identify growth discontinuities. Numerical processing of the data is used to smooth the curves, to remove "noise" and to reveal more clearly the alternating light and dark zonations, in this case, opaque and hyaline zones (Fig. 3). The curves obtained with the entire otolith and then ground and polished ones are similar (Fig. 4). This allows one to use the whole otoliths with microcomputer-based systems although some preparation gives a better isolation of the peaks of darkness or lightness. The comparisons made between the curves and the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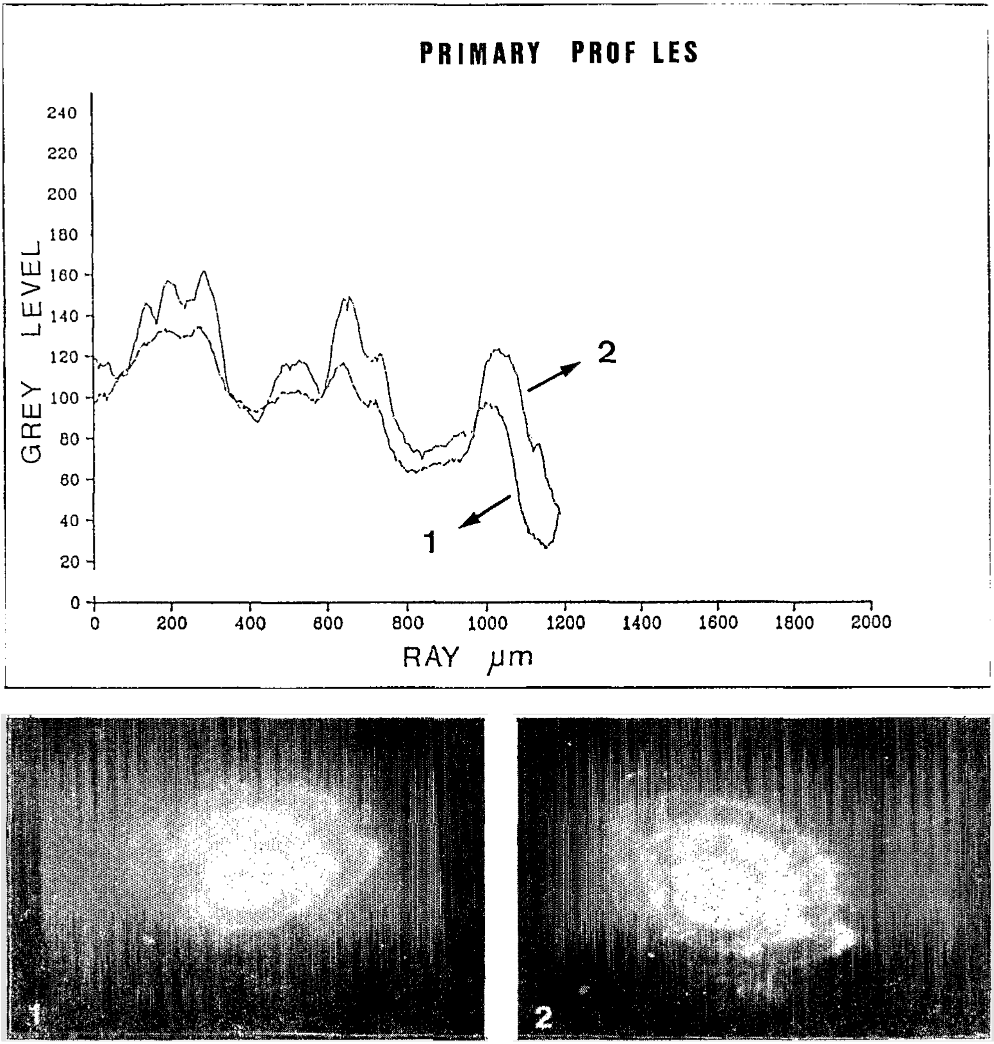

Figure 4. Grey level curves (primary profiles) obtained with a whole otolith (*1*) and after grinding and polishing (*2*). The similarity of profiles *1* and *2* shows that an entire otolith can be used for ageing with an image analysis system.

Figure 3. Computer assisted age determination. The image is recorded from a whole otolith observed under reflected light (against a dark background) in methyl benzoic acid. Using a video enhanced image and a digitizing board, grey levels are obtained along the otolith ray (maximum ray of reading = *R*max) which is the distance between the nucleus and the edge (primary profile *a*). After treatment, the profile is smoothed (*b*). The grey levels correspond to the brightness of the light (opaque) and dark (hyaline) zonations. The age determination is based on counting the peaks observed after the elver stage (*E*). The secondary peaks are supposed to be supernumerary.

observations with a conventional microscope method or scanning electron microscope show that counts and measurements of zonations (peaks of lightness) in video displayed otoliths can be used for ageing eels and eliminate probable "supernumerary peaks" (DEELDER, 1976). The shape of the curves reflects a gradual calcification process of the otolith.

The colored rings or protein matrix observed do not correspond to the hyaline zones formed in winter (lower part of the curve) but are close to the peaks of the curve, which raises the question of how to distinguish between these zonations during an annual cycle.

4. Conclusion

With otoliths of known age, many methods lead to different results (MORIARTY & STEINMETZ, 1979; VOLLESTAD, 1985; VOLLESTAD & NAESJE, 1988). Observation of the entire otolith with classical methods using a light microscope is easy but its reliability depends on the importance of organic and inorganic components of the structures. The identification of the discontinuities is often subjective (MINA, 1968) and the opaque zone is obvious in comparison with the hyaline and contiguous zone. Grinding and polishing or staining are useful to reveal the structures but it seems important to have a grinding angle which allows one to reveal an undistorted view of increments for an entire otolith. In comparison, the scanning electron microscope has the advantage of providing much higher magnification and resolution power. Preparation is in this case more involved and requires grinding or sectioning, polishing and etching. After that, the sample should be coated with a layer of conductive film such as gold. The scanning electron microscope is an expensive and time-consuming method and it requires a skilled operator to give good results.

The burning technique (CHRISTENSEN, 1964) was not applied. The fractured plan obtained with otoliths is hazardous and does not allow an accurate comparison of the results issued from different methods.

A microcomputer-based system provides the capability of making rapid and precise counts and measurements of zonations displayed on a video monitor; total processing time including compiling and editing data sets is shorter using the computer system compared to classical procedures. The digitizer reads brightness as well as position of cursor, so the system has potential for automatic reading of calcified structures and for population studies.

Nevertheless, validation of automatic ageing systems is as critical as it is for the other classical methods. An improvement of the image analysis method is expected in the future as well as the validation of age determination (BROTHERS, 1987; VOLLESTAD *et al.*, 1988). Application of an *in vivo* tetracycline marker (DEKKER, 1986; MEUNIER, 1988; *inter alia*) to eels caught in lagoons should allow determination of the sequential development of the zonations during an annual cycle and validate the methods used in ageing studies.

5. Acknowledgement

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6. Summary

Several methods are used for age determination of eels in the French mediterranean lagoons: observation of the whole otolith after clearing, observation of the otolith after grinding and polishing, after dyeing, under a scanning electron microscope and with an image analysis system. Two types of otoliths are observed depending on the width of the growth rings which is probably related to the environmental conditions: otoliths with wide rings called "brackishwater type" and otoliths with narrow rings called "freshwater type".

With reference to the other methods, a computer assisted method is developed with the aim of improving the efficiency of collecting and processing age and growth data, and eliminate the probable subjectivity of the reader. Nevertheless, further developments are necessary, with an increasing knowledge of life-history of eels, to use this computer method.

The most important point will be to validate the age determination method, for example with the application of an *in vivo* otolith marker (such as tetracycline calceine . . .) on captured eels, and determine the sequential apparition of the otolith, zonations.

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