USE OF OTOLITHS FOR STOCK DISCRIMINATION AND AGE ESTIMATION IN SARDINELLAS: PROPOSAL FOR A REGIONAL RESEARCH PROJECT IN WEST AFRICA

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Abstract :

Several populations of Sardinellas (*Sardinella aurita* and *S. maderensis*) probably exist along the West African coast. At present they are discriminated mainly by their spawning grounds and nursery areas. Previous observations based on reproductive and growth characteristics have tended to show that there are five populations and perhaps some sub-populations.

Although genetic analysis of individual samples from each population could provide an absolute reference for discriminating these populations, some of the new methods for analysing calcified structures (e.g. otoliths) could also give comparable or complementary results. Two types of methods are used in stock discrimination: (1) numerical analysis of scale or otolith morphometry, or (2) microchemical analysis of trace elements in otoliths. Microchemistry of otoliths is particularly promising because their elemental composition seems to be closely related to the fish environment.

A regional research project on Sardinellas in West Africa is proposed, with the aim of characterising populations in two different ways : (1) stock discrimination, using principally otolith microchemistry and (2) age estimation for growth studies. A single sampling will be conducted at the end of 1993 on different target populations off the West African coast (Mauritania, Senegal, Guinea, Ivory Coast, Ghana and Congo) and off the South American Atlantic and Mediterranean coasts in order to compare the elemental composition of otoliths. This analysis should provide conclusions on the method's level of precision and on the possibility of discriminating populations. Age estimation and growth of individuals from the Ghana-Ivory Coast complex will be undertaken on juveniles (otolith microstructures) and adults (otoliths macrostructures) over an annual cycle, to determine: (1) the timing of formation of growth marks on otoliths, (2) the difference in growth between Ivory Coast

and Ghanaian individuals, (3) hypotheses on reproductive migrations and (4) to compare growth, genetic polymorphism and environmental conditions. At each level, the results will be compared with those of genetic studies on the same Sardinella populations conducted by ORSTOM.

Résumé :

Plusieurs populations de Sardinelles (S. aurita et S. maderensis) existent probablement le long de la côte d'Afrique de l'Ouest. A présent elles sont discriminées surtout selon les aires de ponte et de nourriceries. Les études antérieures basées sur les caractéristiques de reproduction et croissance tendent à montrer cinq populations et peut être des sous-populations.

Bien que ce soient les analyses génétiques d'échantillons individuels qui puissent fournir des discriminations dans l'absolu, certaines nouvelles méthodes d'analyse des tissu calcifiés (otolithes par exemple) pourraient fournir des résultats comparables ou complémentaires. Deux types de méthodes sont utilisées à cet effet: (1) L'analyse numérique de la morphométrie des otolithes, ou (2) l'analyse microchimique des éléments a l'état de traces dans les otolithes. La microchimie semble particulièrement prometteuse car la composition élémentaires des otolithes semble étroitement liée aux conditions de l'environnement du poisson.

Un projet régional de recherche sur les Sardinelles d'Afrique de l'Ouest est donc proposé, qui vise à caractériser les populations selon deux méthodes: (1) une discrimination des stocks en utilisant surtout la microchimie des otolithes. (2) l'estimation des ages pour des études de croissance. Un seul échantillonnage sera conduit a la fin 1993 sur différentes populations cibles d'Afrique de l'Ouest (Mauritanie, Sénégal, Guinée, Côte d'Ivoire, Ghana, Congo) et sur les côtes d'Amérique du Sud et de Méditerranée afin de comparer la composition élémentaire des otolithes. Cette analyse devrait fournir des conclusions sur le degré de précision de la méthode et les possibilités de discrimination des populations. L'estimation de l'age et de la croissance, des individus du complexe Ghana-Côte d'Ivoire sera entreprise sur les juvéniles (microstructures des otolithes) et des adultes (macrostructure des otolithes) sur un cycle annuel pour déterminer: (1) la périodicité de formations de marques sur les otolithes, (2) la différence de croissance entre individus du Ghana et Côte d'Ivoire, (3) des hypothèses sur les migrations génétiques et (4) comparer la croissance, le polymorphisme génétique et les conditions de l'environnement. selon . A chaque niveau , les études seront comparées à celles des études génétiques conduites par l'ORSTOM sur les populations de Sardinelles.

1. Introduction

Two of the main Clupeoid species fished along the West African coasts from Morocco to Angola are *Sardinella aurita* and *Sardinella maderensis*. *S. aurita* is also present along the East American coasts, from Connecticut to the North of Argentina, in the Mediterranean sea and in the Western Pacific (Japan, Indonesia, Philippines). The repartition area of this species is therefore very large but restricted to warm waters. Along the West African coasts, the spatial distribution, the resource exploitation, and the reproductive patterns of these two species are closely related with upwelling dynamic systems (Binet 1982; Cury and Roy 1987; Cury and Fontana 1988; Mendelssohn and Cury 1989; Roy et al. 1989; Binet et al. 1991; Pezennec and Bard 1992). The dynamic and the trophic level of these types of habitats are very variable. The life-history and spatio-temporal reproductive strategies of these species show great plasticity and adaptability to environmental conditions (Cury and Fontana 1988; Roy et al. 1989).

Several populations of *S. aurita* and *S. maderensis* probably exist along the West African coast referring to their fragmented repartition and reproduction areas (Cury and Roy 1991; Fig. 1). At present they are discriminated mainly by their spawning grounds and nursery areas following the hypotheses of Sinclair (1988) for the population characterisation (Fig. 1). Actual hypotheses suppose that there are 5 populations and perhaps some sub-populations (Marchal 1991a, 1991b) (Fig. 1). The *Mauritania-Senegal population* is exploited. It has two spawning grounds and migrations of its individuals along the coast are possible. It is probably composed of sub-populations. The *Guinea population* is heterogeneous with smaller individuals and it is slightly exploited. The *Ghana-Ivory population* is probably composed of sub-populations with smaller individuals off Ghana. It has been exploited since a long time with fluctuating catches in function of years. The *Cap Lopez population* has also smaller individuals and it is not exploited. The *Angola population* is exploited from Gabon to Southern Angola. Hypotheses on the identity of these populations and the possibilities of exchanges and migrations between them are finally not confirmed. Moreover, climatic changes in some areas can induce apparition of «new populations» or movement of populations (Binet and Marchal 1992).

Growth studies conducted by different authors on *S. aurita* and *S. maderensis* tend to show that there are differences in growth rates along the West African coast (Marchal 1993): for example, the growth of *S. aurita* seems higher off Senegal, than off Congo, and than off Ivory Coast (Ghéno 1975; Boely et al. 1982). Nevertheless, growth comparison between populations made with only one age estimation method does not exist. For the same species, populations of Guinea, Ghana and Gabon belong to a «sedentary type» (Marchal 1991c). They also present a dwarfism as evoked by the reduction in maximum length, average length in the catches and size at first maturity (Marchal 1991c).

Although genetic analysis from each population can provide an absolute reference for discriminating these populations, some of the new methods for analysing calcified structures (*sclerochronology*) could also give comparable or complementary results. A regional research project on Sardinellas in West Africa is proposed, with the aim of characterising populations in two different ways: (1) stock discrimination using principally otolith microchemistry and (2) age estimation for growth studies in larvae (otolith microstructures) and adults (otolith macrostructures).

2. Motivations of the study

Stock discrimination

Hypotheses on populations repartition have to be tested. In this aim, a Thesis untitled «*Identification of Sardinellas populations off Atlantic African coasts: genetic characterisation*» conducted by ORSTOM and the «Institut des Sciences de l'Evolution» (Montpellier, France) has started in 1991 (Chikhi *present symposium*). This study is based on two techniques of molecular polymorphism analysis: (1) protein electrophoresis, and (2) DNA fragment restriction length polymorphism analysis (FRLP with polymerase chain reaction, PCR) of mitochondrial DNA. It should bring conclusions on populations and exchanges between them. The first results show no electrophoretic structuration along West Africa for *S. aurita* but a structuration for *S. maderensis* (Chikhi *pers. comm.*). The genetic study on Sardinellas can constitute a reference.

Other new approaches allow to characterise populations when studying calcified structures of fish. In **sclerochronology** (i. e. estimation of time from marks recorded on hard tissues), the stock discrimination is possible using two different ways: (1) the study of morphometric parameters, such as scale or otolith shape, and (2) the study of elemental composition of otoliths (microchemistry).

The principle of **morphometrics analysis** is to measure some morphological parameters of calcified structures and to use a discriminant analysis to separate individuals. Some studies have used simple measurements as radius on otoliths (Hopkins 1986; Maceina and Murphy 1989; Messieh et al. 1989; Dawson 1991; Smith 1992). Image analysis microsystems with specially developed softwares enable outline extraction and computation of features which are not available in the conventional approach of morphometry. Those parameters (shape factors, moment invariants and elliptic Fourier coefficients) have been extracted on scales (Pontual and Prouzet 1987, 1988; Ross and Pickard 1990) or on otoliths (Castonguay et al. 1991; Smith 1992). For the scales, the shape analysis with computer-aided devices was superior to the classical technique (i.e. circuli counts) for discriminating North American and European Atlantic salmon populations (Reddin et al. 1992). Discriminant analysis on those parameters gave correct classification rates, higher than 99%, between European salmon stocks (Pontual and Prouzet 1988) and generally higher than 90% for Atlantic salmon stocks (Pontual and Prouzet 1987). The scale features were used to successfully discriminate (87-91% accuracy) between yearling hatchery and wild striped bass (Ross and Pickard 1990). Nevertheless, Castonguay et al. (1991) could demonstrate that temporal instability in otolith shapes indicates that confounding effects of age and class-year on these shapes need to be assessed carefully before drawing conclusions on stock structure.

The principle of **microchemical analysis of otoliths** is to analyse elemental composition (heavy elements) in one otolith area (Fig. 2). This type of analysis seems particularly promising (Kingsmill 1993): «otoliths are constantly recording informations about the environment and about how the fish lived; the makeup of the ear stones also includes trace elements from the water in which the fish is swimming; the idea is that of elemental fingerprinting analogous to DNA fingerprinting, except instead of using the genetic makeup of the fish, the elemental makeup of its otoliths can be used». For now, this type of analysis is limited by the technology used to detect the elements. Methods are varied and allow to analyse simultaneously several elements with a low quantitative power or to quantify finely element contents but individually (Coutant 1990). Electron microprobe spectroscopy, an analytical tool developed from scanning electron microscope (SEM) and energy-dispersive X-ray analysis, is the most common approach to *in situ* analysis at a fine scale (Coutant 1990). Advanced laser-based techniques are promising in the future for highly localised and very sensitive microchemical analysis (Coutant 1990). In the aim of stock discrimination and migration characterisation, elemental ratios coming from electron microprobe are mainly used (Townsend et al. 1989; Kalish 1990; Radtke et al. 1990). Sr/Ca ratios seem related to the environmental temperature (Radtke and Targett 1984; Radtke 1989 ; Radtke et al. 1990) and to the environmental salinity to distinguish between anadromous and non-anadromous populations (Kalish 1990). In these cases, methodology should be described with great precision to avoid interpretation errors (Toole and Nielsen 1992). Stress can also modified elemental ratios in otoliths: Sr/Ca, S/Ca, Na/Ca and K/Ca ratios according to Kalish (1992). Discriminant analyses conducted on rates of several elements (Si, Al, Cl, S, Na, K, Mn, Tc, Cr, Cu, Ra, V, Mg, P, Fe, Sr) for otoliths coming from different populations of the same species showed also possibilities of stock identification (Mulligan et al. 1987 ; Edmonds et al. 1989).

Because of their variability, otolith microchemistry data indicate the need for large sample size, validation experiments and extreme caution when investigating the relationship between otolith microchemistry and physiology, life-history stage and environment (Kalish 1989; Gallahar and Kingsford 1992). Moreover, one possible hitch in element analysis is that hormonal changes in the fish may affect the chemical composition of the otolith, leading to false element signatures. Eventhough there are still contradictions in the use of these methods, otoliths are not restructured during the life of the fish, contrary to other calcified structures. Elemental informations can then be followed on otoliths during the whole life.

3. Age and growth

The first studies on age and growth of *S. aurita* started in the years 1960. Some studies were made on Mediterranean and West African fish (Boely et al. 1982). The most descriptive studies were made by Ghéno (1975) in Congo and by Boely et al. (1982) in Senegal with scale interpretation. Marchal (1993) mentions difficulties in otolith interpretation. Ghéno and Le Guen (1968) have been able to estimate the age of *S. eba* in the Pointe Noire area (Congo) from the scales because the alternating seasons induce typical marks on these scales.

The growth curves for *S. aurita* in Congo and Senegal were established by scale reading. However, Ghéno (1975) and Boely et al. (1982) pointed out problems of interpretation of marks, specially for old individuals. In Senegal, the two annual periods of reproduction greatly complicate the growth patterns and cohorts cannot be identify (Boely et al. 1982). The period of annulus formation is not known with accuracy. A double annual growth cycle could even exist, at least for young individuals. The clearest results show a very fast growth of juveniles during the first year of life, after whom they reach about 20 cm. But we do not know if the growth is regular or if there is seasonal growth cycles during the first year.

The hypotheses on dwarf populations in Guinea, Ghana and Gabon (Marchal 1991c) should be confirmed by the study of individual growth. A double annual growth cycle could also occur for the Ghana-Ivory Coast complex because of the two periods of reproduction. There are still many deficiencies in the knowledge of growth of Sardinellas in this part of African coasts (Marchal 1993).

Some of the growth mechanisms of *S. aurita* along the West African coasts need precision. Is scale the best piece for age estimation ? The formation of seasonal growth mark should be precised during an annual cycle and for different populations. Is there more than one growth cycle during the year ? Is there growth differences between juveniles coming from two different reproduction periods (e.g. Mauritania-Senegal and Ghana-Ivory Coast complexes) ? Does the difference between dwarf populations and others occur during the first months of growth or is it more regular during the life ?

The interpretation of seasonal growth marks on calcified tissues has been used since a long time to estimate the age of fish in years. However, counting those marks was not useful in estimating the age of young fish which have not yet formed their first mark. The discovery of daily increments in otoliths (Fig. 2) by Pannella (1971, 1974) solved the previous problem by permitting the estimation of daily age and numerous applications (see Stevenson and Campana 1992, for review). Moreover, the absence of restructuration of the otolith during the fish life lead to conclude that otolith is a precious tool for age estimation and growth studies. In the Sardinellas case, examination and interpretation of otolith microstructures (daily increments) could precise the growth of juveniles in different nursery areas. The interpretation of otolith macrostructures of adults (seasonal marks) in different populations could bring informations on the ulterior growth.

4. Work proposed on Sardinellas

4. 1 Stock discrimination from calcified structures

Two types of experimentations will be undertaken from scales and otoliths of the two species ***S. Aurita*** and ***S. maderensis***: (1) morphometrical analysis from scales and otoliths, and (2) elemental composition of otoliths. The study will first be conducted on *S. aurita*.

The fish sampling will concern the same populations than the genetic study: Mauritania, Senegal, Guinea, Ivory Coast, Ghana, Congo, Cameroon, Venezuela, North America and Mediterranean (Algeria). The sample size will be of 50 individuals for each population collected at the same date (November 1993) and individual length will be comprised between 21 and 23 cm (fork length) in order to homogenise samples. For each individual, the variables retained will be the fork length, the body height, the head length, the weight and eventually the sex. Scales and otoliths will be extracted.

Morphometrical analysis from scales and otoliths. Scales and otoliths features will be extracted with an image analysis system and a special software. From the coded contours of scales or otoliths, three types of shape descriptors will be computed to describe and compare shape quantitatively: shapes factors (perimeter, area, maximum length and width, and ratios between all of them), moment invariants, and elliptic Fourier coefficients (Fourier series analysis). Then a discriminant analysis will be conducted on these features in order to classify all individuals. «Classical» morphometrical parameters (i. e. body height and head length), already used in literature on Sardinellas, will be added in the discriminant analysis.

Elemental composition of otoliths. A quantitative elemental analysis will be extracted by means of electron microprobe spectrometry along an otolith radius with a constant step of sampling along this radius. Elements analysed will be: Na, Mg, Al, P, S, Cl, K, Ca, V, Mn, Fe, Ni, Sr, Tc. Afterward, elemental ratios could be calculated (Sr/Ca, S/Ca, Na/Ca, K/Ca etc.). A discriminant analysis will be conducted on the values of elemental quantities and ratios in order to classify individuals respecting the location of the microprobe impact point on the otolith radius. At the beginning, this analysis will not able us to take into account the otolith growth rate.

4.2 Age and growth of Sardinella aurita

The aim of this study will be to use the same technique to compare age and growth between populations of *S. aurita*. The study is proposed at first on the Ghana-Ivory Coast complex and will be principally conducted on otoliths. Two development stages will be studied: larvae/juveniles (otolith microstructures) and adults (otolith macrostructures).

Larvae and juveniles (otolith microstructures). The first step is to validate the daily increment deposition on otoliths (rearing for example). Then a study of the growth of juveniles issued from two reproduction areas (Ivory Coast and Ghana) and two periods of reproduction will be undertaken for comparisons. The possibilities of experimentation (location, logistic etc.) still need precision.

Adults (otolith macrostructures). The choice of a calcified piece for age estimation is the first step of the study, but otoliths will be privileged. The age estimation validation will be undertaken both in Ivory Coast and in Ghana with a regular monthly sampling during 15 months (beginning during the end of 1993). The sample size will be of 50 individuals / month / population, measuring morphological variables such as fork length and weight, and extracting scales and otoliths. At first on a small sample, scales, otoliths and dorsal spines will be compared for the age estimations.

5. Expected results

At each level, the «sclerochronological» results will be compared with those of genetic. The duration of this study will be 3 years with a starting point at the end of 1993.

Stock discrimination

Conclusions will lead on: (1) the precision level of these methods, (2) the possibilities of stock discrimination, (3) the comparisons between genetic results and sclerochronological results and (4) the hypotheses on population locations.

Age and growth

Conclusions will lead on: (1) the timing of formation of growth marks on otoliths, (2) the difference in growth between Ivory Coast and Ghanaian individuals (juveniles and adults), (3) hypotheses on reproductive migrations and (4) the comparison of growth, genetic polymorphism and environmental conditions.

COLLABORATIONS

All the persons and research centers mentioned bellow are involved in this research project.

«Stock discrimination» study

- CNROP (Centre National de Recherches Océanographiques et des Pêches), *Mauritania*, D. Jouffre.
- CNSHB (Centre National des Sciences Halieutiques de Boussoura) *Guinea*, S.B. Camara, F. Domain.
- CRODT (Centre de Recherches Océanographiques de Dakar-Thiaroye) *Senegal*, J.J. Levenez, B. Samb, I. Sow.
- CRO (Centre de Recherches Océanographiques d'Abidjan) *Ivory*

Coast, F.-X. Bard, K. N'Da, O. Pezennec.
- FRUB (Fisheries Research & Utilization Branch de Tema) *Ghana*, K.A. Koranteng.
- ORSTOM, *Congo*, L. Maloueki.
- ORSTOM, *Cameroon*, O. Njifonjou.
- ORSTOM, *Venezuela*, P. Cardenas.
- UBO (Université de Bretagne Occidentale) for *Algeria*, F. Djabali.

«Age and growth» study

- CRO (Centre de Recherches Océanographiques d'Abidjan) *Ivory Coast*, X. Bard, K. N'Da, O. Pezennec.
- FRUB (Fisheries Research & Utilization Branch de Tema) *Ghana*, K.A. Koranteng.
- CRODT (Centre de Recherches Océanographiques de Dakar-Thiaroye) *Senegal*, J.J. Levenez, B. Samb, I. Sow.

French laboratories

- LASAA (IFREMER-ORSTOM-Brest).
- Laboratoire de Microscopie Electronique (IFREMER-Brest).
- Microsonde chimique (IFREMER-Brest, Brest, Rennes and Nantes Universities).

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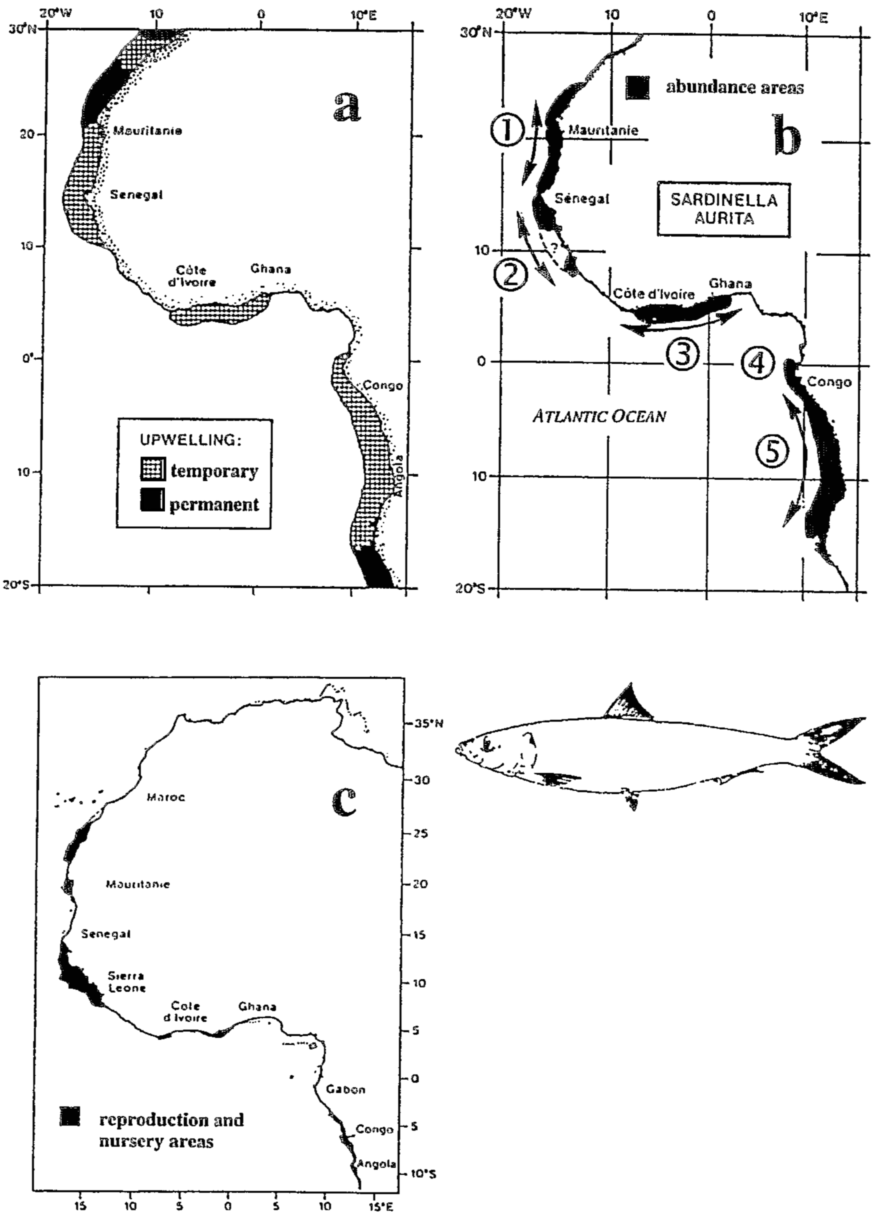
CAPTION OF FIGURES

FIGURE **1.** (**a**) Upwelling areas in West Africa. (**b**) Repartition areas of *Sardinella aurita* in West Africa with the hypotheses of population locations. (**c**) Reproduction areas of *S. aurita* in West Africa. From Cury (1991, modified).

FIGURE **2** . Scanning electron micrograph of a larval herring otolith that had previously been sampled for microchemical analysis with an electron microprobe prior to being prepared for SEM examination. The spots where the microprobe sampled are visible as a series of discrete burn depressions, made by the electron beam, running from near the center of the otolith to the outer edge. Otolith microincrements formed daily are also clearly visible and their width is reducing from the center to the outer edge. The elemental analysis is then conducted along the life and growth of the fish. Scale bar = 20 µm. From Townsend et al. (1989).



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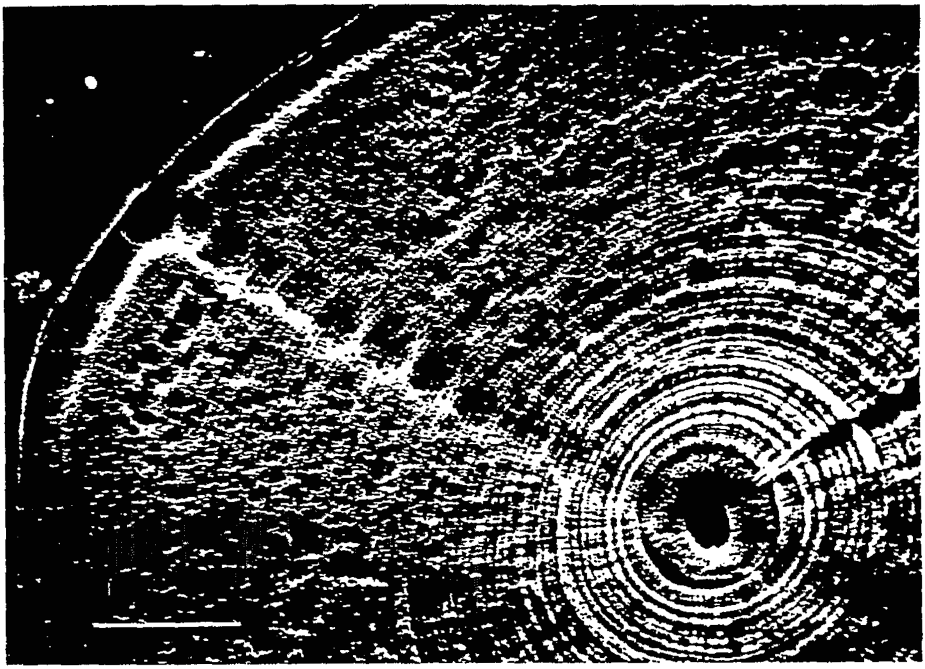

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