Baltic Committee **ICES CM 1999/H:4**

REPORT OF THE

STUDY GROUP ON BALTIC COD AGE READING

Charlottenlund, Denmark
16-20 November 1998

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 INTRODUCTION

The Study Group on Baltic Cod Age Reading (SGBCAR) met in Charlottenlund Slot, Denmark from 16–20 November 1998.

1.1 Participants

Ms. T. Baranova	Latvia
Ms. A -M. Bratt	Sweden
Mr I. Groger	Germany
Mr J. Netzel (co-Chair)	Poland
Mr H. Mosegard	Denmark
Mr D. Ustups	Latvia
Mr F. Dahl-Poulsen	Denmark
Ms. R. Sjoberg	Sweden
Ms. Y. Walther	Sweden

P. Ernst, Germany (co-Chair) did not participant in the meeting. In preparation of this meeting he compiled selected data and material from the previous meeting and presented the first draft of the Manual for the Baltic Cod Age Reading" as basic material for the study group.

1.2 Terms of Reference

At the 85th Statutory Meeting of ICES it was established (C.Res. 1997/2:42) that the Study Group on Baltic Cod Age Reading should meet to:

a) Summarise the results of otolith exchange programmed set up standards for technical methodology in age reading procedures for the manual;

b) Establish a digitalized video image collection based on reference collection of otoliths from different subdivisions, seasons and length groups of cod;

c) prepare a manual based on reference collection of otoliths, which would provide 1 guidelines for standard age reading procedure and the interpretation of cod otoliths.

1.3 Short review of the past meeting work

The ability to make an accurate age determination of fish underpin the procedure to understand fluctuations in a fish stock and hence affects the possibility to make a correct stock assessment.

Age determination by reading annual rings in otoliths is the most widely method used on gadoid fishes. In the countries exploiting the Baltic cod stock this is the exclusively used method for age determination. Although the method has been used for many years as a routine it is not considered a trivial matter to age cod in the Baltic Sea.

For age-determination purposes three stocks are important in the Baltic area, the Kattegat stock (sub-division 21) the Western Baltic stock (sub-division 22–24) and the Eastern Baltic stock (Sub-divisions 25–32) as the cod is considered to be migrating to some degree between these areas. Cod from these different stocks shows different growth patterns in the otolith growth. In general the otoliths from the Kattegat stock and the Western Baltic stock shows a more rapid growth than the otoliths from the Eastern Baltic stock. Another difficulty is that the formation of translucent zones is occurring later in the Eastern Baltic otoliths than in the other areas. The interpretation of Eastern Baltic otoliths will hence be a bit different from the other areas, which may affect the accuracy of the readers. Migration pattern of cod in the western and eastern areas might also create a mix between the two types of otoliths in the samples, which may complicate the interpretations.

Denmark and Sweden sample cod otoliths from all the areas mentioned above, while Germany sample cod otoliths in sub-divisions 22–25. Latvia, Poland and Russia concentrate sampling of cod otoliths to Sub-divisions 25–29. Hence there is a necessity to intercalibrate age reading of cod in the areas mentioned above. The need for higher accuracy between readers in different laboratories and countries has lead to establishment of an ICES Study Group on Baltic Cod Age Reading in 1995, with participants from Denmark, Estonia, Germany, Latvia, Poland, Russia and Sweden. The Study Group was supported by the EU project "Baltic Cod Stock Assessment: An International Program to Standardise

Sampling Protocol, Age Determination and Trawl Surveys" (Study Contract 94/058). The aim of the Study Group was to:

*establish a reference collection of otoliths from different sub-divisions, seasons and length-groups intercalibrate age-reading between readers in different countries and laboratories make recommendations for standardised reading procedures compile guidelines for new and established age readers promote collaboration between different institutes working with age-reading. The wish to focus on problems in age determination of fish is however not only of interest in the Baltic Sea area. Other similar, and more elaborate, networks have been formed in the last few years. One of the largest is European Fish Ageing Network (EFAN) which promotes collaborative work between laboratories working with age determination in Europe.

2 SHORT SUMMARY OF THE RESULTS OF EXCHANGE PROGRAMMED SET OF STANDARDS FOR TECHNICAL METHODOLOGY IN AGE READING PROCEDURES FOR THE MANUAL.

The included as an addendum Manual for the Baltic Cod Age Reading consist a summary of the whole otoliths exchange program and the way of its creation.. At the beginning all organizatory problems were solved.- the characteristic areas of Baltic cod were fixed and also four areas co-ordinators were chosen. It was fixed that each country should send a representative sample according to the size of fish and quarters of catch. The selected sample should be send to the area co-ordinator. The total sample consisted of 462 otoliths. The area co-ordinators have then selected 30–40 otoliths and established an otoliths exchange program. The results of ageing were kept for further analyses intake developed standard protocol.

During the Rostock meeting (the results presented in the report ICES C.M./J:1) a statistical method was used to recognise the accuracy of different readers and by which way the unknown true age could be approximated for modelling and comparison reason and also an attempt was carried out to build individual calibration models for age correction. The results of ageing by different readers and their analyses have found an existence of two different schools of cod otoliths ageing, one existing in laboratories of Western Baltic and the second in laboratories in the Eastern Baltic. This problem probably is solved during the Study Group meeting in Copenhagen due to common interpretation of the age at the video images and a presented hypothesis for the formation of optically different otoliths structure (presented on page 9 of the Manual).

3 ESTABLISHING A REFERENCE COLLECTION OF OTOLITHS AND VIDEO IMAGES

The total reference collection consist of 290 of otoliths and from were selected and from the whole number of otolith digitised video images were produced in Danish Institute for Fishery Research. During the meeting in Copenhagen only 89 digitised video images were selected out and on the images the Study Group has put down agreed comments about age, ring formation and other maters.

The purpose with the finally chosen reference collection consisting of 89 images is to use it in training situations when introducing new readers to the cod stocks of the Baltic or to refresh old readers mind on the subject.

The Danish Institute for Fisheries Research, Department of Marine Fisheries, Charlottenlund Castle agreed to storage the otoliths and digitised video images. The otoliths and the CD.-ROM with the commented images will be sent to interested readers on request.

All institutes who participated in the establishing the reference collections will receive a copy of the CD.-ROM with the 89 digitised images.

4 MANUAL FOR THE BALTIC COD AGE READING

The meetings of the Study Group resulted in a Manual for the Baltic Age Reading. The manual is included to this report as an addendum. In the manual are presented all problems in which the Group was involved and the way how they are solved.. The Group is of an opinion that the Manual would provide opportunities for age readers to harmonise their routine work towards a common interpretation of various growth structures of cod otoliths and moreover it should be used for presentation and training of new age readers.

5 RECOMMENDATION

The Study Group is of an opinion that it should meet every 6 years to reduce the variability in cod age interpretation. Further, new readers could then be introduced to the international group of cod age readers.

6 REFERENCES

Anon. Report of the Study Group on Baltic Cod Age Reading. ICES, CM 1997/J:1

MANUAL

FOR THE BALTIC COD AGE READING

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1.1 Standard method of age reading of Baltic cod

Age reading of cod has traditionally employed counting of annual zones in the Sagitta otoliths. This otolith type in cod is a large and compact calcareous body with low degree of transparency, which means that it has to be broken or cut to make the internal structures of alternating translucent and opaque growth rings visible.

1.1.1 Methods used in different countries

Denmark

The Danish readers use broken otoliths. To cut the otoliths they use a small wire cutter. If the otolith is not broken through the nucleus, they use an electric grinding machine to grind it carefully to the nucleus of the otolith. The grinding stone is partly in water and turns with a speed of 150 rounds per minute.

Each reader uses a binocular microscope with a magnification from 5 to 20, usually 6 to 12.

Most of the readers have set up two microscope lamps so that they can change between transmitted and reflected light. The direction of the microscope light is a personal matter, but the readers can manage both methods. Normally each reader uses a forceps to hold the otolith. The otolith is determined while it is submerged into water or alcohol while shading the otolith with a pen or similar object.

Sweden

The Swedish readers use broken otoliths. To cut the otoliths they use either a small wire cutter or break it by hand. The readers use binocular microscopes with transmitted light while shading the otolith with a pen or similar object. The otolith is mounted vertically in clay or held by a forceps. During the counting of annual rings the broken surface is kept wet with water. The magnifications 10–12, if the otolith is difficult to read they change the magnification.

Estonia and Latvia

The Estonian and Latvian readers generally use broken otoliths. The otoliths are broken by hand. If the otolith is difficult to read it is burned on an electric heating plate until receiving a light brown colour. But if it is still not readably the second otolith is cut to a slice (0.2–0.5 mm) by a sawing machine and embedded in pertinox. The broken otoliths are read by aid of a binocular microscope in reflected light and focused on the surface with water. The age from slices is determined in transmitted or reflected light with a dry surface.

In all cases the magnification is 16.

Poland and Germany

The Polish and German readers use broken otoliths. The otolith is cut with a scalpel and broken by hand. The age of the broken otolith is determined by aid of a binocular microscope using reflected light. The otolith is mounted in a small pot with plasticine filled with water or alcohol or in a solution of soap in water. Difficult otoliths are cut into slices (0.2–0.5 mm). These slices are read in transmitted light with a dry surface. The magnification is usually 6 -12.

1.2 General comments

It is important for all readers that the light around the determine place is as low as possible. It is also important that the surface of the cut or broken otolith is as close as possible to the nucleus. The axis of reading is dependent from the readability of the otolith and the preference of the reader, but it is preferable to read it several times along different axes.

The advantages and disadvantages using different otolith preparation methods are shown below.

Method	Advantages	Disadvantages
Slices	More efficient training	Longer preparation time
	More efficient repeated reading of otoliths	More expensive
	When mounted on slides the material is less likely to suffer during otolith exchanges	The otolith orientation within the light path cannot be manipulated
Broken	Easier to locate the nucleus if further grinding is necessary	Longer training time for new readers
	Shorter preparation time	Otoliths are easier suffering when many readers handle it and use different wet material on the surface
	Less expensive	

1.3 Equipment and techniques used

Mainly stereomicroscopes were used for viewing the otoliths. The most used magnification was 6.3 * 1.6.

Broken otoliths (untreated, burned, partly polished)

Binoculars such as from Zeiss, Olympus, Technival were set up with various filters and high quality phase objectives. The stereomicroscopes give a three-dimensional image of the otoliths.

Illumination methods were found to be very critical. Reflected light was used when examining the otoliths. Gooseneck fibre optic illuminators and free-standing-/free-hanging lamps appear to be the best light sources. The visibility of otolith growth patterns was also enhanced by applying various wetting agents, such as alcohol, water, and soap-water.

The broken otolith halves were either hand-held under the microscope or were temporarily mounted on a piece of soft black plasticine and then immersed in the wetting agent for viewing.

The ring patterns of the broken otolith surface may be further elucidated by focusing the microscope and/or simply shading the reflected light by a stick, pencil etc.

1.4 Otolith slices

The technique to produce otolith slices was demonstrated.

Slices of selected otoliths were produced by a two-diamond-blades-saw. Cutting speed, water flow (if possible) and height of the blade (blades distances from 0.5 to 0.8 mm) were adjusted before sectioning. Preparation of the otoliths before sectioning was as follows:

1. Otoliths were embedded in an epoxide casting resin (if the epoxide is coloured, for instance black, the contrasts in the slices are improved).
2. The otoliths were cut through the nucleus, giving 0.5 to 0.8 mm slices embedded in epoxide.
3. The slices were fixed between two glass plates for viewing.

When viewing the slices either reflected or transmitted light was used. Polarising filters were not used during this workshop, even if they could be used to reduce glare or to improve the contrast.

1.5 Applications of the daily increments analysis

The daily increments, found in otoliths of larval and juvenile fish allow to estimate the age of the fish. Once the age is known different parameters may be calculated: hatching time of the fish, it's growth rate etc.

In the case of adult cod age analysis there are some difficulties associated with the interpretation of the first and last hyaline zone. Thus, it would be very useful if we can estimate the time of the hyaline zone (so called „winter ring") formation. There may be some differences related to the area of sample collection (eastern-western Baltic), as well as to the time of hatching of a given specimen (spring, summer or autumn spawning).

There is information about a shift of the peak of cod spawning time towards the second half of the year (results presented at the meeting). It therefore seems to be especially important to investigate the growth of otoliths during the first six to ten months of fish life. This way also the influence of transition from a pelagic to a demersal life on the otolith structure would be investigated. This would be helpful for the interpretation of the „metamorphosis ring" and the „juvenile ring". If analysis of up to 300 - 400 daily increments on the otolith is possible, calendar date of hyaline zone formation should be back calculated. Such an analysis would provide important information improving understanding of otolith structure formation and make adult cod ageing more accurate.

1.6 Suggestion for improved quality of digitised images and otoliths in reference collections

We often find that otoliths from samples circulated among institutes are in a bad shape at the end having been handled by many readers in different ways. Some readers try to break the otolith once more or polish it and others burn it to improve readability. Every time a new reader handles the sample, there is a risk of a quality decline.

We discussed this topic and since it only is possible to get two sagittal otoliths from each fish, we have to be careful with those two. The following way to treat one of the otoliths is a step in the right direction since readers prefer to have the otoliths in their hands instead of only the images of it.

1.6.1 Procedure

The centre of an otolith is marked with a thin pencil on the side where sulcus acousticus is. The otolith is glued to a glass object slide with a thick layer of thermoplastic cement (an artificial resin that becomes liquid when heated to about 150 degrees C). Now the glass slide has to be placed in a double diamond wafer cutting machine. The distance between the wafers can be set between 0.5 and 2,0 mm. The best distance is about 1.6 mm. One of the blades is set so that it cuts right through the mark showing where the sagitta nucleus is. A slice of the middle part of the sagitta is produced by carefully sawing through the otolith using water as a wetting medium. When the glass is reached the cutting procedure is stopped and the otolith is now parted into 3 pieces. A slice with the nucleus on one of the sides, one half otolith with the nucleus on the surface of the section, and another half otolith sectioned through the outer part of the first year's formation.

The parts can easily be taken off the glass slide by again heating the mounting cement. Any remains of the glue may easily be removed in ethanol.

The thin otolith section is then mounted on a glass slide with the nucleus pointing towards the glass again using thermoplastic cement. The sanded end of the glass slide is then marked with a pencil and the text covered with heated resin making a permanent and safe identification.

The slide is now polished to a thickness giving the best image in a binocular microscope. The slide is very suitable for viewing both with reflected light and with transmitted light from underneath and also gives you the opportunity to produce digitised images of a real good quality. If you have polished it carefully to the nucleus you even might be able to count the daily increments in a microscope and get some images of that, too.

1.6.2 Equipment used

Isomer 1000, which is a two-diamond-saw-blade cutting machine, using some 76 mm diameter blades with a thickness of 0.15 mm (manufactured by Bugler).. Distance rings, thickness 0,5–2,0 mm.

Observe that these thin wafers are very delicate and easily broken.

Polishing aluminium-oxide on plastic sheets, grain sizes 30, 9, 3 microns (manufactured by 3M).

Object glass slides with sanded writing field.

Thermoplastic mounting cement (artificial resin manufactured by Bugler).

Glass ethanol flame burner for heating slides.

CCD camera mounted on a binocular microscope connected to a computer with a frame grabber.

1 or 2 free standing bench lamps or cold light lamps with gooseneck arms. (manufactured by Leica).

Programs, such as Global Lab Image or others (see list from EFAN on world wide web, http://www.efan.no: EFAN CELL 2 Information Processing Overview)

2 COD OTOLITH INTERPRETATION

2.1 Terminology

Age determination: It is the process of fish age reading to count structures (scale, sagitta) which reveal periodic changes in fish growth (annual or daily).

Age group: All the fish from a population that have a given age (e.g., the 2-year-old age-group, 4-year-old-age-group). The term is not synonymous with year-class. The change of age group is settled at the 1st of January

Annual growth zone: One opaque and one translucent zone constitute a typical annual growth zone.

Check: Narrow translucent zone that forms within the opaque zone Checks should not be included in the age estimation of Baltic cod. More than one check per year may be formed, especially within the first opaque growth zone.

Edge of otolith (Marginal zone at the edge of the otolith): Opaque or translucent (narrow or wide) zone at the edge of the otolith. The amount and structure type on the edge is region specific and related to the life stage and the annual cycle of the fish.

Hyaline (= translucent) zone: A growth zone that allows a better penetration of light than surrounding zones. On the surface of broken otoliths and slices under reflected light, the translucent zone appears dark, in transmitted light it appears bright (light).

Juvenile zone(s): One or several narrow translucent zones surrounding the otolith nucleus, it is supposed to be formed at settling but it is not found in all otoliths.

Metamorphic ring: phrase should be avoided, but usually referring to an almost circular hyaline zone surrounding the otolith centre, approximately at the initiation of the secondary primordial.

Otolith centre: The central area of the cod otolith boundered by the metamorphic ring. The centre is formed during the larval stage. It is always hyaline provided a sectioning through the nucleus.

Opaque zone: A growth zone that disperses the penetrating light. On the surface of broken otoliths and slices under reflected light, the opaque zone appears white, in transmitted light it appears dark. The opaque zones in otoliths from cod in the Eastern Baltic are formed in the period of intensive feeding.

Reflected light: Light that is directed towards and reflected from the sectioned surface of an otolith, either from above or from the side if the surface, is not shadowed.

Sagitta: In juvenile and adult cod the largest of the three pairs of otoliths found in the membranous labyrinth of the inner ear. It is elongated trapeziform with a lobed edge. It consists mainly of calcium carbonate that will dissolve in acid fluids, e.g., formalin.

Secondary primordia: Growth centres initiating the first formation of lobes, forming after the termination of the larval period.

Settling (= juvenile) ring: See juvenile zone.

Sulcus acousticus: The groove passes on the inner surface of an otolith with a bend under the otolith centre. The Sulcus acousticus is connected to the Macula area where hair cells transfer acoustical and balance signals to the nervous system.

Transmitted light: Light that passes through the otolith or from the side of a broken otolith if the surface is shadowed.

Year class: A cohort of fish defined to be born in the same calendar year (January 1 - December 31) - e.g., 1995-year-class.

Zone: Circum-central region of similar structure and optical density in the otolith (translucent, opaque).

2.2 Description of age determination procedures

The purpose of fish age reading is the determination of the age structure of a fish stock.

The method for Baltic cod is based upon area specific characteristics of the otolith zone formation.

The time of formation of a full annual zone (one opaque and one hyaline zone) in the otoliths is different for western Baltic cod (*Gadus morhua morhua* L.) and eastern Baltic cod (*Gadus morhua callarias* L.).

Rapidly growing cod with clearly defined zones in the otolith (wide opaque zones and broad sharply outlined hyaline zones) is typical for the western regions of the Baltic. The hyaline zone in the western cod otoliths is formed in autumn – winter. In eastern Baltic cod, however, otoliths the same annual structure is first completed in the spring.

2.2.1 Interpretation of cod otoliths from SD 22

An otolith from a young/small, but fast growing cod caught in the winter period until early spring often has an opaque zone on the edge of the otolith. In the winter, Nov. – Jan. it is narrow, but later it gets broader. There is no translucent/hyaline zone on the edge as expected. The opaque zone on the edge is belonging to the just started growth period. The winter-ring/translucent zone seen a little bit from the edge therefore is belonging to the winter just passed. See otolith no. 2 and 3 in the reference sample. Older fish more than 2 – 3 years old lay down broad opaque zones during the summer, Apr. – Sept. and narrower translucent zones during the winter, Oct. – Mar. Very narrow checks may be seen in the opaque area as well as narrow opaque zones in the translucent area

2.2.2 Description of age determination of cod in SD 26 and 28

The difficulty of age reading of eastern Baltic cod otoliths lies in the fact that the annual zone (one opaque and one hyaline zone) is not formed during one calendar year. In the first year of fish life (the year of hatching) before the end of December otoliths show that the first incomplete opaque zone continues to form in the next year (after January 1st). A translucent zone is formed mainly in April-August. The annual zone is fully formed mainly in the middle of the second (after birth) year of fish life. In a similar way, annual zones are formed also in adult cod otoliths. Therefore, to assign an age after the first of January until June 30th one year is added to the number of fully finalised annual zones. In the first half of the year, an opaque zone on the otolith edge is considered as an incomplete growth zone of the previous year. In the second half of the year, an opaque zone is regarded as a new increment (plus-growth) of that year.

2.2.3 The formation of zones on young cod otoliths

Settling of young cod on the bottom in the eastern Baltic occurs in late autumn or in winter. In research catches by bottom trawls young cod is found from December until April-May. The minimum length of young fish is 5 cm. The length of the modal group is 7–10 cm in December and 10–15 cm in March-April. A nucleus with a metamorphic or juvenile ring and an opaque growth zone is clearly seen in this period (March/April). A hyaline zone is formed in April-August. Schematically the formation of zones in otoliths of young cod and the corresponding age designation is the following:

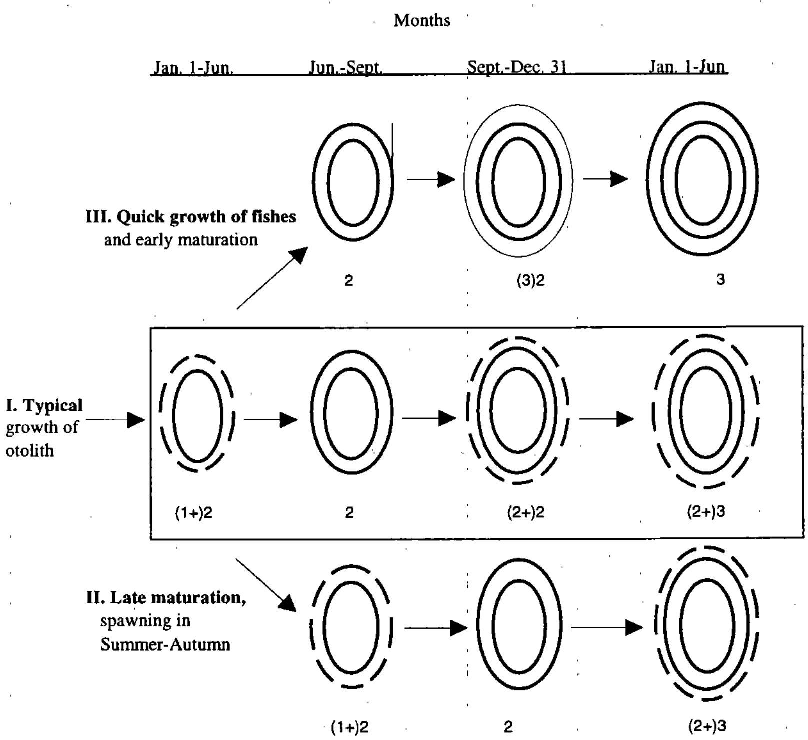
	Month	Otolith zone	Designation	Age
The first year (Year of hatching)	XII	One opaque	0+	0
The second year	I-IV	One opaque	0+)1	1
	V-VI	one opaque + one hyaline	1	1
	VII-XII	one annual zone (one opaque + one hyaline) + opaque growth zone at the edge of otolith	1+	1

In such a way, zones are formed in cod otoliths until cod becomes adult (it usually happens at age 2–3). The hyaline zone in young Eastern Baltic cod otoliths is formed earlier than it is in older fishes and is completed in a shorter time (Tokareva, 1963).

2.2.4 The formation of zones on otoliths of adult cod

An opaque zone in the otoliths of Eastern Baltic cod is formed during their intensive growth (September-March). A hyaline zone is formed during decreased somatic growth in spring-summer (April-August-September). The formation of a translucent zone in ripening fish during their spawning cycle may be related to an intensive accumulation of calcium in otoliths at tissue liquid hypercalcimia (Krivobok *et.al.*, 1976). The translucent zone in adult fish is formed during a shorter period than that of immature cod. In recent years (1992–1995), due to late spawning, there has been a shift in the formation of the translucent zone to later in the year (summer-autumn).

The scheme of zone formation in cod otoliths during the year is following:



Three variants are presented. The first variant is the most typical. The second one has been observed in recent years at late spawning when the formation of a hyaline zone is shifted to later in the year (summer-autumn). Finally, the third variant is characterised by early ripening cod where a translucent zone appears already in December, i.e., in the previous year.

These figures schematically represent otolith growth stages from January 1 to December 31. The black zones are translucent zones: the wide black zones are complete zones and the narrow black zone is the incomplete hyaline zone. The dashed lines represent an incomplete opaque growth zone. The number in the brackets indicates the number of annual zones actually seen as well as the opaque growth that is represented by a "+". The number after the brackets is the age class interpreted according to the time of the year when the fish was caught. January 1 is considered the date of fish birth.

2.2.5 A common hypothesis for the formation of optically different otolith structures

This meeting as well as prior inter-calibration meetings has revealed profound differences in the interpretation of the time of formation of the outermost translucent zone in cod otoliths from different parts of the Baltic Sea.

The differences in opinion reflects a difference between readers from the Western Baltic countries as opposed to readers from the Eastern Baltic countries.

Generally readers from Eastern Baltic countries will add an extra translucent zone (not yet formed) to get the age of mature cod caught during the first quarter of the year. This procedure is not acceptable to readers from Western Baltic countries. Here the observation of a narrow opaque zone on the otolith edge during the first quarter indicates the termination of a winter-ring and the start of a new growth season. Therefore, the number of translucent zones for these readers directly reflects the age of the cod.

At the meeting it was not possible to come to an agreement of a common interpretation of otoliths from all Baltic subdivisions. It was therefore decided that a more theoretically satisfactory approach should be taken.

A simplified hypothesis for the formation of cod otolith structure was discussed. It was thereby agreed upon that if a common hypothesis for otolith structure formation could be applied to both cod stocks the interpretation following this hypothesis would build the basis for age determination.

The hypothesis should be so specific that it would lead to a series operative decision rules for assigning age to a given specimen, given information on time of catch, geographical area and depth distribution, size, maturity and past otolith growth.

It was found that the following simplified hypothesis would satisfy the above requirements:

Otolith growth is fast during warm periods and slow during cold periods.
Otolith growth is fast when body growth is fast.
Otolith growth varies with temperature when body growth is low.
Otolith translucency is high during sub-optimal growth condition.
Otolith translucency is always high during very warm periods.

The following examples will lead to sub-optimal growth:

starvation during cold or warm periods
reduced feeding during warm periods or during spawning migration

Examples:

The first pronounced translucent zone the so-called juvenile ring is formed due to the transition from pelagic to demersal life and the corresponding changes in food acquisition.

The juvenile cod occupying the depth 30- 50 m habitats will experience changes in water temperature and feeding conditions where the hydrological year to a high degree reflects the meteorological year. The translucent zone will form during late winter due to low food availability.

After spawning mature cod in the Eastern Baltic will be move to deep cold water with high food availability from September to March leading to slow but opaque otolith growth. In April they will have attained a high energy-storage and start to move to higher temperatures leading to opaque and high otolith growth. Later migration to the spawning grounds will increase translucency. The stored energy resources will gradually be depleted during spawning from June to August leading to a high translucency and reduced otolith growth.

With this behaviour and otolith growth hypothesis an Eastern Baltic cod caught in March will have a relatively narrow opaque zone at the edge. However, in May an almost fully developed opaque zone will have formed at the edge?

The different environmental conditions and their influence on otolith structure appearance are schematically visualised in the figure below.

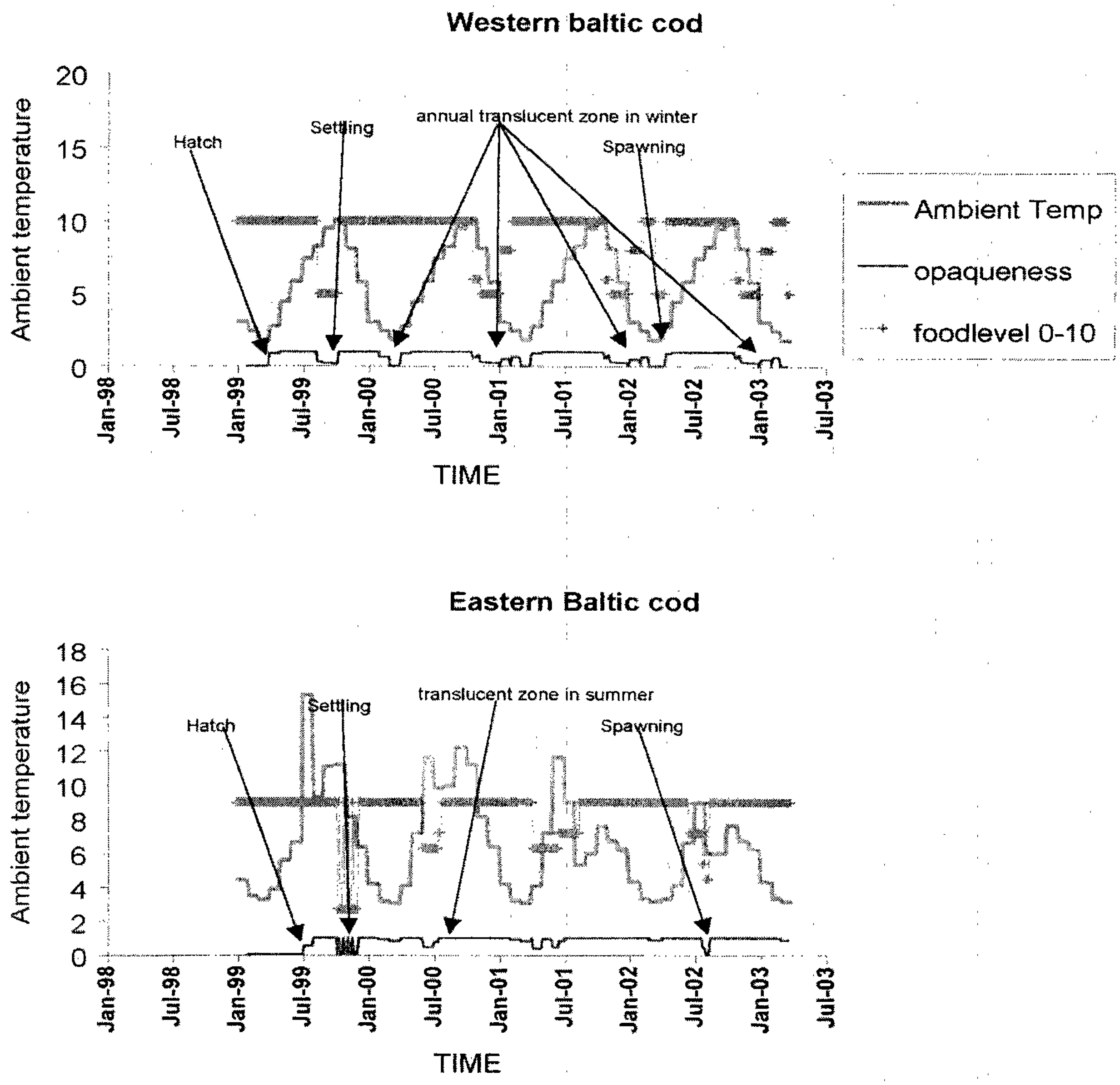


Figure 2.2.5
Illustration of how different levels of environmental factors (food and ambient temperature) in the Western (upper panel) and the Eastern Baltic (lower panel) could influence formation of translucent zones in cod otoliths.
Opaque=1 Translucent=0
The example is illustrated with two mature 4 years old cod from 1999 caught in late March 2003. Western Baltic cod = 66cm TL and an Eastern Baltic cod = 56cm.

Recommendations

More research is needed to understand the mechanisms behind the formation of optically different structures in cod otoliths. Ongoing experimental should be combined with field observations from different areas.

Extensive studies on the seasonal formation of the outer edge and the apparent otolith growth rate of optically different zones would be of great help for age validation.

Therefore, publication of such studies from all areas should be encouraged.

3 METHODOLOGY AND RESULTS OF AN OTOLITH EXCHANGE PROGRAM IN PREPARATION OF THE MANUAL

In preparation of the exchange program a group of specialists discussed the composition of the otolith collection for the exchange program and suggested the following criteria:

3.1 Area

In order to obtain a proper coverage of the cod stocks otoliths were sampled for each of the Sub-divisions 21–30. The otoliths included in the control collection were combined to cover larger areas e.g., Kattegat and Belt Seas, western and eastern Baltic depending on the outcome of studies of otolith growth patterns and characteristics. Each laboratory obtained otoliths from each of the Subdivisions with significant national fishing effort. The final collection include not more than 50 otoliths per area and year. The Sub-divisions that were covered and the countries involved per area are according to the project outline:

Sub-divisions	Main countries involved in fishing
21	Denmark, Sweden
22	Denmark, Germany (divided into north and south)
23	Sweden, Denmark
24	Denmark, Germany, Sweden
25	Denmark, Germany, Poland, Sweden,
26	Poland, Latvia, (Russia), Sweden (divided into Gotland and Gdansk)
27	Sweden
28	Sweden, Latvia
29–30+32	Sweden, Estonia, (Finland)

3.1.1 Seasons and years

The seasons of the year were covered by quarter. In each Subdivision it was important to adjust (and log) the timing of collection with the spawning time and to log the condition of the cod. Periods to be dealt with are the pre-spawning, spawning and feeding periods. Otoliths were used for this experiment from the year 1995.

3.1.2 Size of fish

Each active country (see above) collected one pair of otoliths per 5 cm length class in all about 20 pairs per sub-division per season and area. The sex and sexual maturity of the fish were registered.

3.1.3 Description of otoliths

To make the study and selection of the otoliths easier a short description of each otolith and the individual it originated from was written by the sampler. Data included information on length of fish, sex, location of catch, season, year, country and structural characteristics, etc. based on "Codes for the Age Reading Form" (see Appendix 2).

3.2 Organisation of sampling

To organise the sampling within areas and sub-divisions and to reduce the number of otoliths to represent an area four area-co-ordinators were suggested. The co-chairman co-ordinated the work among area-co-ordinators and was responsible for the results of the work in this section. The area-co-ordinators responsible for the specific Sub-divisions was suggested according to the primary national fishing areas.

Each national laboratory selected and forwarded otoliths and the descriptions to the area co-ordinator. The area co-ordinators selected 30–40 otoliths and established an otolith exchange program for each of the ICES Sub-divisions they were responsible for. The otoliths were representative for the seasons and size groups as well as for the area, but also unusual otoliths were represented. The reduced collection and the result of the exchange program were presented and are the basis of this manual.

The results of ageing were saved for further analyses in the developed standard ageing protocol "Form for Age Reading Analyses" (see Appendix 1)

3.2.1 Organisation of the exchange program

Sub-divisions 22+24

The area co-ordinator for the ICES-Sub-divisions 22+24 selected an otolith collection for the exchange program composed of basic material of Sweden and Germany (34 otoliths of Sub-divisions 22, 49 otoliths of Sub-division 24). Otoliths from the Danish institute were not available for this exchange collection.

The exchange programme started in February 1996. The following national institutes/labs were involved in the exchange programme for age reading of cod from the Sub-divisions 22+24

- Institute of Baltic Sea Fisheries Rostock, Germany
- Baltic Sea Research Station Karlskrona, Sweden
- Institute of Marine Research Lysekil, Sweden
- The Danish Institute for Fishery Research, Charlottenlund

The collections circulated among Institutes together with the protocol "Form for Age Reading Analyses", the "Codes for Age Reading", (see Appendix 1 and 2) describing how to handle the exchange material.

The results of all involved institutes/labs of Sweden, Denmark and Germany were available for further investigations (reference collection etc.)

Sub-divisions 21+25

For Sub-division 21 Otoliths for selection of a reference collection were supplied by the Swedish, the German and the Danish institutes.

60 cod otoliths covering all four quarters and all 5cm-length classes from 5 to 90 cm were selected as a reference collection from Sub-division 21 for 1995.

For Sub-division 25 otoliths were supplied by the German, the Polish, the Swedish and the Danish institutes.

48 cod otoliths covering all four quarters and all 5cm-length classes from 15 to 100 cm were selected as a reference collection from this Sub-division for 1995.

Paper copies of digitised images of each polished otolith section were sent out in duplicates to each institute involved in the specific exchange programme. The paper copy was intended, in connection with direct microscope observations, as a chart to mark out the exact positions of annual and other structures relevant for the age reading (see also App.1).

Sub-divisions 26 + 28.

The collections of cod otoliths and the age reading exchange programme for Sub-divisions 26 and 28 were organised in LatFRI (Latvia).

Fish biological parameters, i.e., length, weight, sex, maturity stages (eight-point maturity scale), as well as otolith characteristics, i.e., weight, length, width were given for each otolith of the collection. The elements of the inner structure, i.e., nucleus, edge of an otolith, the character of zones were described using the codes elaborated earlier. The codes for description of otolith zones (point 6. Zones) were supplemented with three new ones:

V - not visible but expected,
A - not sharply outlined,
M - hyaline zone consisting of several narrow rings.

Otoliths were collected from 5-cm length groups ranging from 10 to 90 cm and were distributed according to the length groups for each quarter.

In Sub-division 26, otoliths were received from Latvia (35 specimens), Poland (28 specimens), Russia (60 specimen), Sweden (42 specimens) and cover sampling in all 4 quarters. These collections were read by experts from Poland, Latvia, Russia and Sweden.

In Sub-division 28 otoliths were received from Sweden (45 specimens) and Latvia (22 specimens). Specialists from Latvia, Poland and Sweden aged these collections.

Due to high migration rates of cod between Sub-divisions 25–29 these otolith collections were read by specialists ageing cod from the total eastern Baltic stock (Sub-divisions 25–32).

Only samples from the neighbourhood of this Subdivision 28 were sent to the area co-ordinator (Latvian and Swedish samples). These samples were aged by Latvian, Polish and Russian readers.

The area co-ordinator selected the first area reference collection. The results of different readers showed some disagreements, in some cases the results varied up to 3 years.

Sub-divisions 23 + 27 + 29 + 30 + 32

In Subdivision 23, the exchange program was organised by Sweden but also involving Denmark in the compilation of otoliths.

After completion of the otolith collection the material circulated between specialists in Sweden, Denmark and Germany, and finally the readings were analysed statistically in Germany.

Sweden was the only country sampling cod in Sub-division 27 in 1995, and consequently the material on which the reference collection is based was supplied by Swedish surveys. Otoliths collected in commercial fisheries added to complete the collection, although this sampling will not provide information on the maturity stage of the cod.

Existing material consists of 33 otoliths from quarters 1, 3 and 4 in 1995 and circulated between Sweden, Poland, Latvia and Estonia for interpretation by specialists. Upon completion the age reading results were sent to Germany for statistical analysis.

In Sub-divisions 29–32 the exchange program was organised by Sweden with additional help from Finland.

Instead of using 1995 material otoliths were collected from previous year's surveys to establish a reference collection from this area. This collection was circulated to specialists in Sweden, Finland, Latvia and Estonia.

4 STATISTICAL ANALYSIS OF AGEING

4.1 Statistical methods used

In general, the whole statistical analysis was carried out by subdivision. At first, it was necessary to filter out "uncertain otoliths" from all otolith samples circulated between the different participating institutes in order to discuss the disagreement. Secondly, it was necessary to find a procedure by which the unknown true age could be approximated for modelling and comparison reasons. At third, it was necessary to build individual calibration models for age correction purposes.

4.1.1 Bias and Precision Calculations, Quality Assurance as well as Design and Use of a Reference Collection

Some general remarks

The statistical evaluation analysis of the otolith readings of Sub-divisions 22, 24, 26 and 28 showed that:

- ❸ various systematic factors can strongly bias the readings (as school effects, a fish length effect, an area or time effect, a readability effect of the nucleus etc),
- ❸ the precision between the readers is varying heavily,
- ❸ the precision is varying with age.

i.e. the individual readings are influenced in principle in the following way:

$$\textit{read age} = \textit{true age} + \textit{systematic effects} + \textit{non - systematic effects}$$

whereby the total reading variation can be decomposed into the two following principle subgroups of reading variation

$$VAR(\textit{read age}) = VAR(\textit{systematic effect}) + VAR(\textit{non - systematic effect})$$

The systematic effects are usually referred to as reading bias whereby the non-systematic effects are referred to as reading error or simply error term. The precision is assumed to be inversely proportional to the variance of the reading error i.e.,

$$\textit{precision} \approx \frac{1}{VAR(\textit{non - systematic effect})}$$

$$\underline{\textit{with}}$$

$$VAR(\textit{non - systematic}) > 0 .$$

Usually we do not know the true age. Hence, the true age has to be approximated by some suitable approach/concept. Within the framework of calibration techniques this approximation of true age is normally called calibration standard or (in this particular context) standard age. Groger (1996b) showed that a good approach for the **standard age could be the mode of the readings** of not too few age readers who would participate in some otolith rotation program meaning that highest agreement has the highest probability to meet the true age. Otherwise the median could be taken as standard age. In case of only two readers the age readings of that reader with the more precise age readings could be taken as standard age.

$$\textit{read age} = a + b \ x \ \textit{standard age} + \textit{non - systematic effect} .$$

In principle, it is possible to correct biased readings if the sources of the systematic effects can be detected quantitatively as well as the precision is high and does not vary strongly with age. Following the theory of Groger (1996a,b) as well bias as precision can be measured within the framework of a simple or (for more than one influencing systematic effects) a multiple regression approach. For the simple case this is

This regression model can be easily extended by further factors which influence the readings significantly. If the two regression parameters a and b (or more than the two in the multiple regression approach) are estimated by the least-squares method (parameter estimates will be denoted in the following with hats on top of them) and in case the reading bias is significant (meaning that $\hat{a}$ differs significantly from 0 and/or $\hat{b}$ is differing significantly from 1) then the correction (calibration) can be done as follows

$$standard\ \hat{age}_{(corr.)} = \frac{read\ age_{(uncorr.)} - \hat{a}}{\hat{b}} \quad .$$

which is similar to an inverse prediction problem with focus on interpolation (see Martens et al. 1989, Neter *et al.* 1985). This correction assumes that $\hat{b}$ is exactly estimated (see Miller 1996). To ensure this, it is necessary to calculate the following expression:

$$\hat{cv}(\hat{b}) = \frac{\sqrt{\hat{MSE}}}{\hat{b}\ x \sqrt{\sum_i (standard\ age_i - \overline{standard\ age})^2}}$$

<u>*mit*</u>

$$\hat{MSE} = \frac{1}{n-2} x \sum_i \hat{u}_i^2 = \frac{1}{n-2}\ x \sum_i (read\ read_i - read\ \hat{age}_i)^2 \ .$$

which measures the exactness of $\hat{b}$ in terms of it = s coefficient of variation. **This value should be smaller than <u>0.1</u> for any individual reader** who is doing age readings for stock assessment purposes the ICES (does not matter whether the reader is a new comer or not) in order to carry out the correction without any mistake. Furthermore, for each of the participating readers (who are reading for the purpose of ICES stock assessment) the precision in terms of the estimated coefficient of determination (which is the usual one) i.e.,

$$R^2 = 1 - \left(\frac{Var(non - systematic\ effect)}{Var(read\ age)} \right) = \frac{Var(systematic\ effect)}{Var(read\ age)}$$

should be at least <u>0.90</u>. Also the **variance should not vary strongly with standard age** otherwise a weighted regression has to be performed instead of the usual least-squares approach in order to estimate the parameters a and b under variance homogenous circumstances. Each individual calibration model has to be updated from time to time since increasing experience (personal training, effects through international otolith exchange programs, instrumental improvement, standardising effects of school oriented age interpretation) may change the characteristics of the model.

In order to be able to detect and measure the bias correctly the otolith sample scheme (i.e., the design of the otolith reference collection to be multiply rotated and comparatively interpreted) should include ideally all information/factors which could influence the readings in terms of systematic effects, does not matter whether these factors are of quantitative or qualitative nature. Such information can be: principle aspects of the preparation method, the used microscope type, the resolution of the used microscope, the rule of interpretation of the first and last rings, the information of nucleus readability, the fact whether the reader wears eye glasses, etc. The reference collection should cover all Baltic areas and quarters hoping that most of the otolith structure types will be included. Then the design of the reference collection should allow any free (randomised) creation of subsets where the single otoliths could be randomly taken from the entire reference collection. The otoliths of the reference collection should therefore be prepared that way that no one of them can get lost, unreadible or totally damaged.

Some summarising guidelines

The entire reference collection should be read, interpreted, as well as discussed in detail at least one time by each of the participating readers. The readings of each reader should be stored on CD together with the associated relevant information. This CD must be made accessible for any relevant statistical analysis. From these age readings the standard age should be calculated as modal age. This standard age should be used as calibration standard for each reader against which all individual readings should be regressed (also in later updating rotations when circulating randomised subsets of the entire reference collection). The resulting individually estimated regression models (equation 4) form the basis for the individual calibration models (equation 5) by which the individual readings can be corrected if

- the individual readings show a significant bias,
- the individual precision is larger than 0.9,
- the individual estimation error of $\hat{b}$ is smaller than 0.1 and
- the individual variance does not vary strongly with the standard age.

Any reader who does not show such a high precision in terms of the calculated R5 (equation 6) and of the coefficient of variation of $\hat{b}$ (equation 7) should be excluded from any further reading concerning stock assessment purposes within ICES. He/she should be further trained.

In case the variance per standard age is varying strongly for some reader the related calibration model must be based on a weighted regression model with the variation per standard age as weighting factor. This means especially for any newcomer that he/she must be well instructed by train them first independently of the reference collection. From time to time they should be faced with randomised subsets of the reference collection until they meet the quality criteria on a relatively stable level. Their readings must be always contrasted with the defined standard age of the more experienced readers (see above).

From time to time all individual calibration models (including those of the experienced readers) must be updated in order to detect and hence consider shifts due to some training or standardisation effect. This is particularly necessary if any of the prospective systematic factors have been changed intermediately.

5 INFORMATION CONCERNING THE FINALLY SELECTED REFERENCE COLLECTION

The reference otolith collections were all transferred to the Danish Institute for Fisheries Research, the Department for Marine Fisheries, in Charlottenlund.

Here two digitised images were produced one of the pieces of otoliths as found in the envelopes and another of the broken/polished surface chosen for reading.

The digitised pictures stored on CD-Rom created the foundation for discussions in the study group. The common image database was established to make it possible for future readers to point out the different growth zones and their interpretation of the otolith. The TIFF format of the images makes it possible use programs for image-analysis to perform specified measurements on reader identified structures. This procedure will hopefully enable standardisation of structure interpretation among readers and identify what axis gives the most reproducible results.

Sub-divisions 26 + 28

The reference collections present mainly otoliths that are typical (by their structure and width of otolith zones) for each Sub-division. Otoliths were taken from fishes with medium growth rate, as well as from slowly and rapidly growing fishes. Along with otoliths that are typical for each Subdivision the collections include both non-typical otoliths as well as ones that are more characteristic for cod from Western Baltic by the width of the zones.

<u>Collection of cod otoliths for Sub-division 26</u>

The final collection contains 38 otoliths from which 10 are from Latvia, 14 are from Poland, and 14 are from Sweden.

For quarter I: 11 otoliths were chosen representing age groups 1–10. Otoliths with an opaque zone on the otolith edge prevailed.

For quarter II: 6 otoliths of age groups 2–7 were chosen. A zone on the otolith edge varies strongly from "small hyaline zone" to "wide opaque zone".

For quarter III: 11 otoliths of age groups 2–11 were taken. In about 50% of the cases a narrow opaque zone could be seen on the otolith edge, but in the rest a narrow or wide hyaline zone could be observed.

For quarter IV: 10 otoliths of age groups 1–9 are presented. The zone on the otolith edge strongly varies. It should be noted that this part of the collection includes the otoliths of cod with maturity stages seven and eight (spawning and post spawning cod). Late spawning of cod in recent years has been common. Therefore, otoliths representing these individuals are found in the collection.

Collection of cod otoliths for Sub-division 28

The reference collection of cod otoliths for Sub-division 28 includes 42 specimens out of which 20 are from Sweden and 22 are from Latvian samples. The collection contains the otoliths collected in quarters I, III and IV of 1995.

For quarter I: 17 otoliths were chosen (8 of them were from Sweden and 9 were from Latvia). These otoliths belong to age groups 1–9. Most of them exhibit a narrow or wide opaque zone on the edge.

For quarter III: 12 otoliths represent age groups 1–10. About half of them have a hyaline zone (narrow or wide) on the edge and the other half has a narrow opaque zone on the edge.

For quarter IV: 13 otoliths represent age groups 1–7. A narrow opaque zone dominates on the otolith edge in this period. several otoliths of fishes with maturity stages seven and eight (having recently spawned) are presented here, for the same reason as for the Sub-division 26-collection.

Sub-divisions 23,27, 29–32

The reference collection in Sub-divisions 23, 27 and 29–32 are in progress and will be available after completion of the exchange programs.

6 DIGITISED VIDEO IMAGES OF OTOLITHS

The frame grabbing facilities at the Danish institute were employed to produce digitised images of otolith parts and close ups on surfaces of cut or broken otoliths.

Two types of digitised video images have been produced. 1) all existing otolith parts from each individual. 2) The magnified surface of the sectioned otolith used for ageing (otoliths were covered by water and for fish more than one year old they have also been polished).

Digitised images of all otoliths from the reference collections are stored on CD-ROM. The program used to produce the images is GLOBAL LAB Image and they are stored as Tagged Image File Format, *TIFF. Files.

The number of otoliths in each Sub-division is given below:

Sub-divisions	21	61	
	22	23	
	23	47	
	24	17	
	25	0	(will be re-established with other otoliths)
	26	38	
	27	3	
	28	21	
	29-32	50	
In total		290 images	

From these images, the Study Group has selected 89 images, covering Sub-divisions 21, 24, 26, 28 and 29–32. On the images the Study Group has put down agreed comments about age, ring formation and other matters. The information is put down on the images as 3 different layers: nucleus, translucent zones and text. The layers can be switched on and off in the original image. It is all done with the purpose of helping new readers to start reading and old readers who need to have their mind refreshed if they have been away from the work for some time.

The comments are put into the images with a computer programme called Paint Shop Pro. The programme may be downloaded from the Internet and used for free in 30 days to consider buying it or not. The program runs under

Microsoft software. The Internet address is: http/www.jasp.com where you can search for news and how to download the programme. The prices are given on this Website, too, one programme costs around 700 DKK. The images may also be displayed in the Microsoft programme Power Point.

The quality of the images is not always as good as it could have been. It all depends on how the otoliths are treated. With a digital camera, it is preferable to have a very plain surface to focus on. Therefore, it is preferable if the broken otolith is polished to the nucleus. In addition, if it is going to be burned it has to be polished first, since the surface is very delicate after the burning process and material often breaks off along the edge.

7 GUIDELINES FOR BALTIC COD AGE READERS

1. To estimate the population age distribution first of all a representative sample of otoliths should be taken. 2. To omit different obstacles in age determination the procedure of otolith preparation and conditions of readings should be as for as possible standardised. The group is of the opinion, that:

- One of the two otoliths should be broken through its centre (nucleus).
- The second otolith should not be used provided the first otolith is readable. Otherwise it could be burned or cut to a thin slice (0.2 – 0.5 mm).
- The otolith surface should be moistened with water, ethanol or water with soap and mounted in black wax or clay under the binocular microscope.
- Usually one microscope lamp should be used when reading in reflected light, but if it is necessary to read some of the otoliths in transmitted light then a second lamp could be used.
- Magnification should be in the range of X: (5 – 10) but it is not recommended to use larger magnification then 12X.

8 REFERENCE COLLECTION STORAGE AND ITS ACCESSIBILITY TO THE READERS

The reference collection of Baltic cod otoliths consists two sets of material. A physical reference collection of otoliths with an assigned age agreed to by the group. Secondly, a set digitised images on CD–ROM with 89 of the age agreed otoliths.

The reference collections from which the 89 otoliths were chosen are stored at the Danish Institute for Fisheries Research, Department of Marine Fisheries, Charlottenlund Castle, DK-2920 Charlottenlund. Fax +45 33 96 33 33.

The purpose with the finally chosen reference collection consisting of 89 otoliths is to use it in training situations when introducing new readers to the cod stocks of the region or to refresh old readers mind on the subject.

The otoliths and the CD-ROM with the commented images will be sent to interested age readers on request. Please, send the request as a fax.

All institutes who participated in establishing the reference collections will receive a copy of the CD-ROM with the 89 digitised images.

The Danish Institute for Fisheries Research agreed to store and make these two sets of data accessible to all cod readers around Baltic Sea.

To complete the collection of otolith samples all material collected during the age reading experiments should be send to Danish Institute, where it will be registered and added to the existing collection.

9 FUTURE WORK

The Study Group on the Baltic Cod Age Readers should meet every 6 years to reduce the variability in cod age interpretation. Further, new readers could then be introduced to the international group of cod age readers. The new age readers should be supervised until they reach a 90% precision.

During the six year period the reference collection should be used in different laboratories for improvement of cod age interpretation, but in the third year there should a obligatory exchange programme among the Baltic laboratories. In combination with this circulation, otolith types missing in the collection should be added.

10 CONCLUSIONS

1. A reference collection of otoliths and video images is established for Baltic Sub-divisions 21, 22, 23, 24, 26,27,28, 29 - 32.

2. The applied multiple linear regression approach together with the concept of calibration to a "true age" (mode or median)could detect the influence of external factors influencing the results of the individual readers.. In contrast to the (systematic) bias the personal precision measures the random dispersion around the observed regression line. Through inverse prediction read age could be statistically corrected towards true age. From that point of view the application of such models can be considered as a step towards a homogenisation of readings for a specific stock.

3. The existence of different national schools of reader interpretation was confirmed by the applied statistical analysis.

4. The group found it useful to mark out the position of different otolith structures (i.e.: metamorphosis ring, juvenile rings, annual hyaline zones) in two different ways – otoliths and video images.

5. Various environmental conditions in different areas of western and eastern Baltic may influence the time of formation of hyaline zones, therefore, the interpretation of them as annual markers should be cautious.

6. It was generally agreed that the expressions "summer and winter ring" might be misleading. The group found that for practical use it is better to use the expression opaque and hyaline (translucent) zone.

7. The daily increments may be used in order to obtain information about the calendar date of hyaline zones (so called winter rings) formation, occurrence of a metamorphosis ring" and of „juvenile ring/s" may by analysed as well.

8. The specialists working on otoliths from the adjacent areas should also read the samples of otoliths collected according to the agreed areas. Practically this means all specialists from Eastern Baltic.

9. It is agreed that the collection will be stored at the Danish Institute for Fisheries Research.

10. All laboratories working on Baltic cod age reading should on request have access to the reference collection.

11. If some otoliths are lost during circulation or reading they should be replaced during later meetings. Several readers should agree upon the interpretation of the replacement otoliths.

12. The reference collection should provide opportunities for age readers to harmonise their routine work towards a common interpretation of various growth structures. Moreover it should be used for presentation and training of new age readers.

13. Each institute should be supplied with a CD–ROM with video images of otoliths with an agreed age interpretation.

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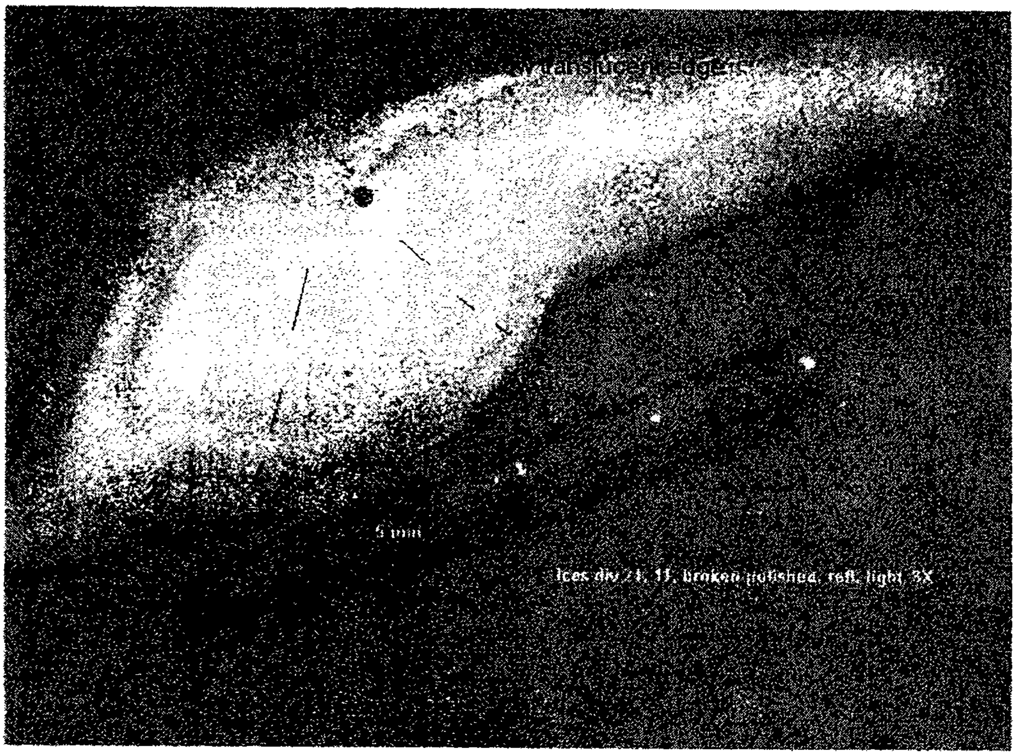
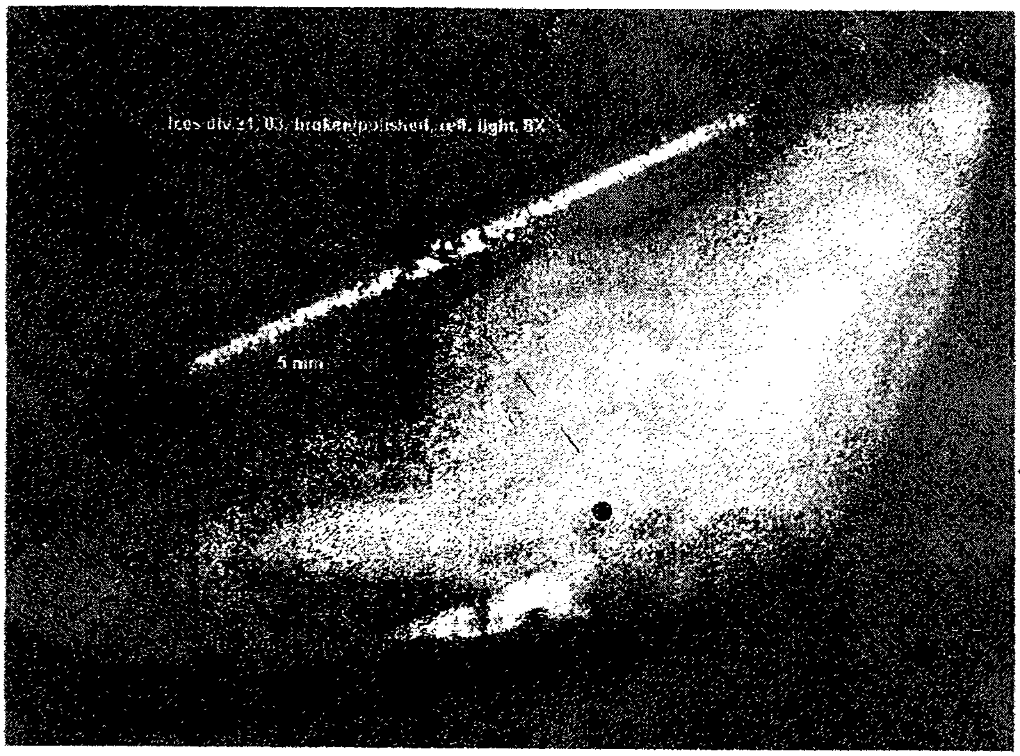
Prace morsk. inst. ryback., T.17, ser. A.


5 mm
Ices div.21, 11, broken polished, refl. light 3X


Ices div.21, 03, broken/polished, refl. light 8X
5 mm

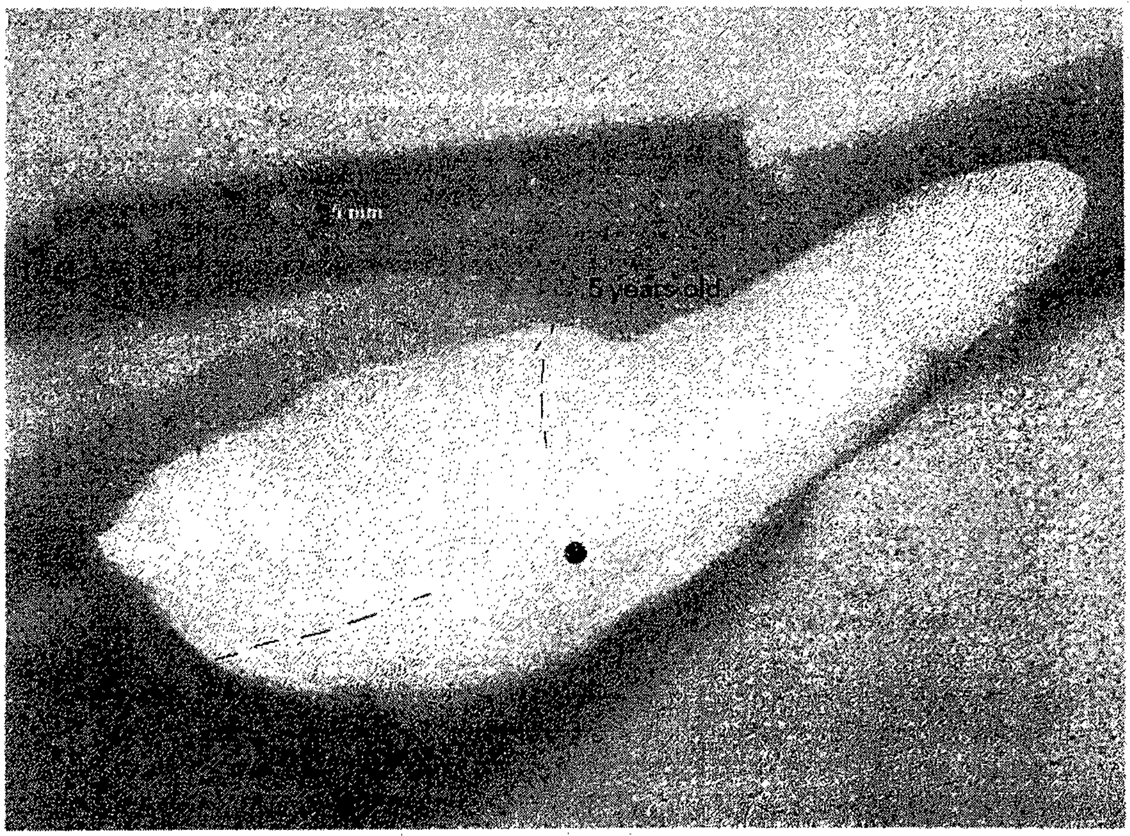
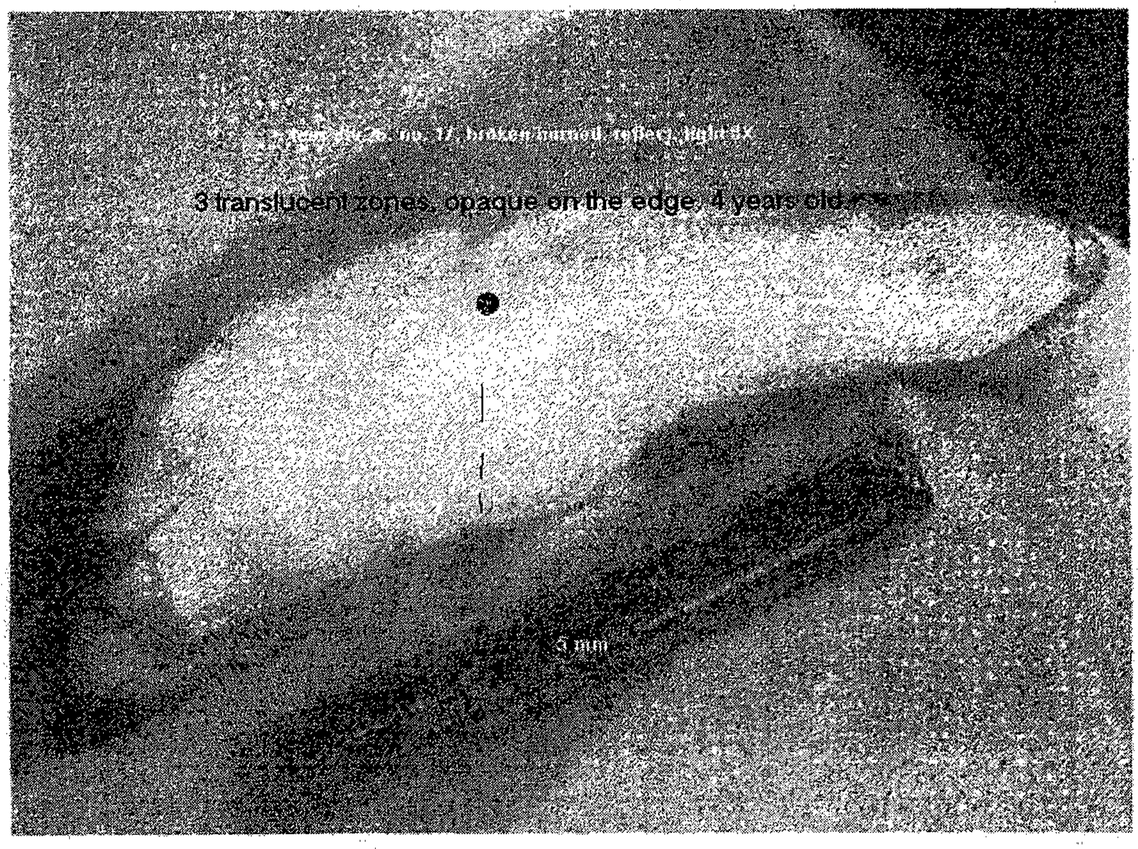

5 years old

Fig. 6.2


3 translucent zones, opaque on the edge, 4 years old

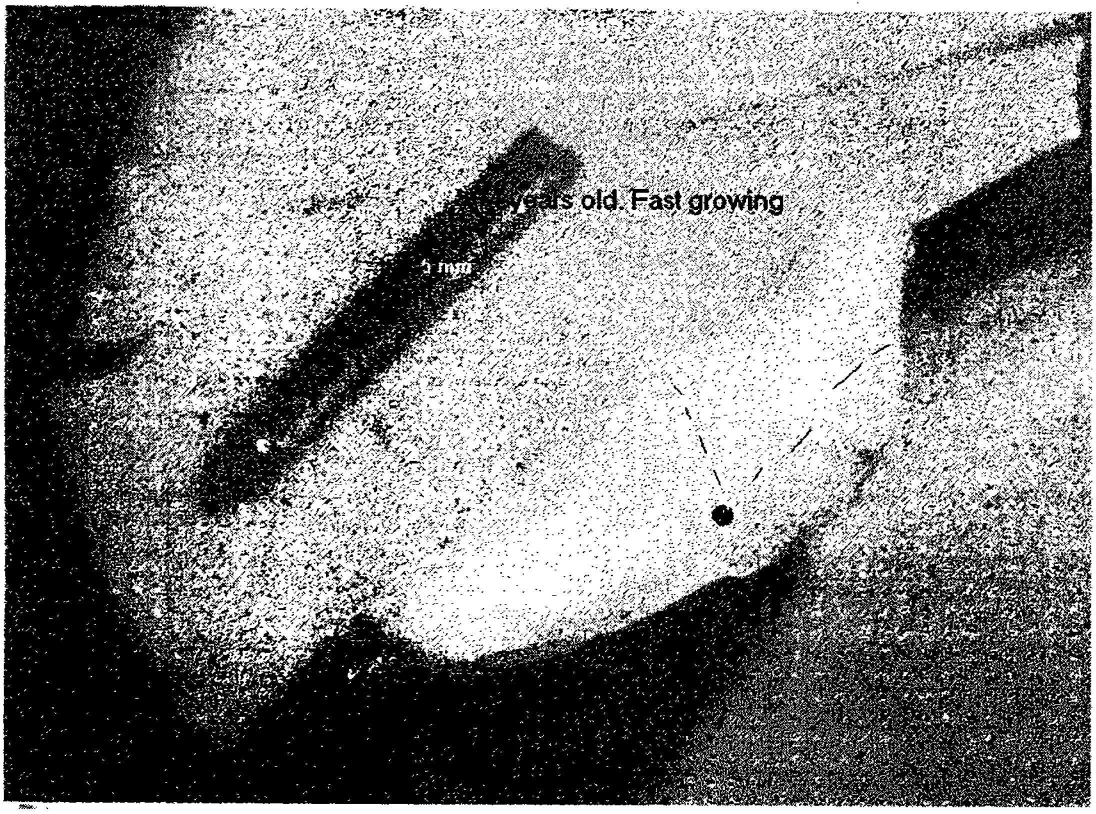


Fig. 6a

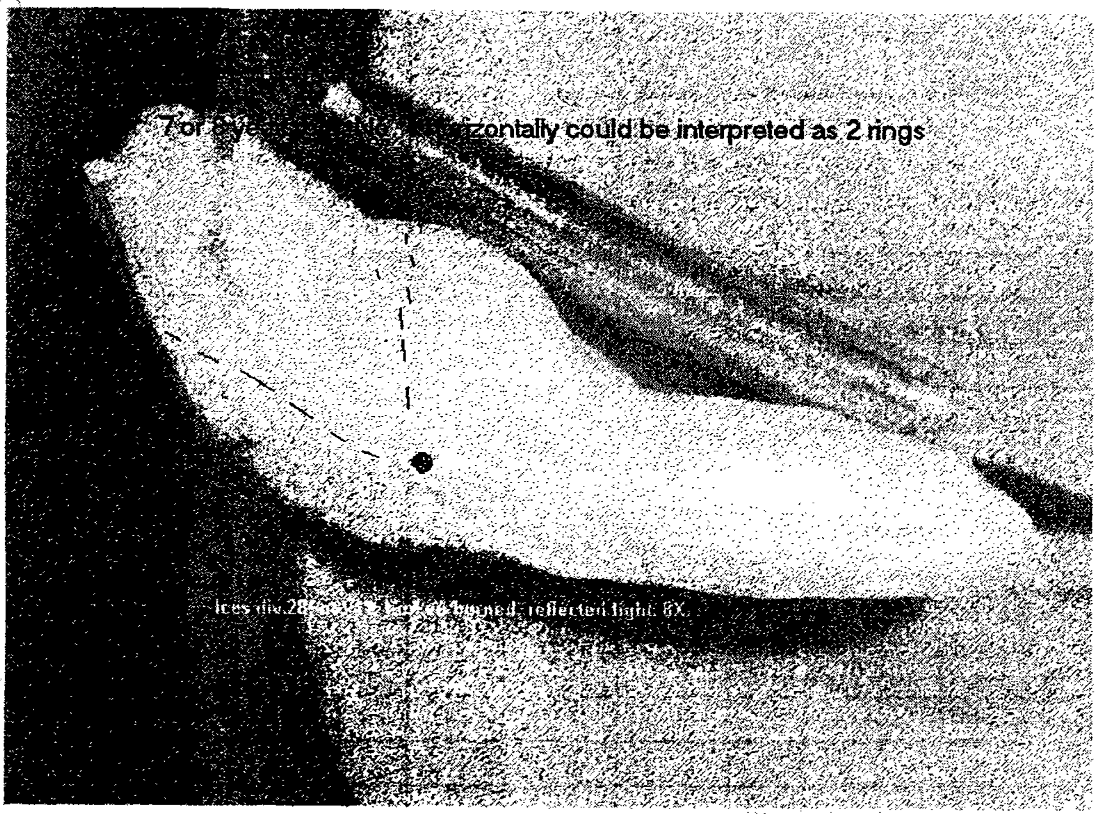


Fig. 6b

APPENDIX 1

FORM FOR AGE READING ANALYSES

Page of	

SPECIES:

ICES SUB-DIVISION:

READER Name: Nation: Experience:

METHOD: Illumination (transmitted/reflected):
Moistening mean (alcohol/water/other):
Magnification:
Preparation of otoliths:

Maturity scale used:

Otol.	Catch date		Rec-	T.length	Sex	Maturity	Characteristic of the otolith			Reada-	Age	Year-
No	Year	Month	tangle	of fish		stage	edge	nucleus	zones	bility		class

APPENDIX 2

CODES for the Age Reading Form

1. Reader experience

   Novice: < 10 000 otoliths, < 6 years
   Experienced reader: 10 000–25 000 otoliths, 7–10 years
   Senior: > 25 000 otoliths, > 10 years

2. Sex

   i) 1 male
   ii) 2 female

3. Maturity stage: According to the 8-point scale by MAIER.

4. Edge of the otolith

   i) small hyaline zone
   ii) full hyaline zone
   iii) small opaque zone
   iv) wide opaque zone

5. Nucleus

   i) nucleus present
   ii) nucleus not present
   iii) juvenile zone
   iv) no juvenile zone

6. Zones

   i) not variable
   ii) variable

1. No of the ring
H hyaline
O opaque
D double ring
S slim zone
B broad zone
N normal zone

Example: 1HDB = 1. ring, hyaline, double zone, broad zone
3OS = 3. ring, opaque, slim zone

7. Readability

   i) sure
   ii) not sure
   iii) not readable