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Answers to the Questionnaire on Age Reading

by R.S. Keir

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ANSWERS TO THE QUESTIONNAIRE ON AGE READING

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Introduction

A questionnaire on the techniques of otolith reading and on the terminology and symbolism used in describing the structure of the otoliths was circulated to member countries by the Secretariat on 21st December, 1959. The questionnaire was based on one drafted by Dir. G. Rollefsen and followed from the following recommendation endorsed by the Standing Committee on Research and Statistics.

> That the Secretariat should approach the proper institutes in ICNAF member countries and request data on otolith terminology and symbols, and photographs of otoliths and scales with interpretations. This material should be distributed as soon as possible to age readers concerned, and treated at the proposed meeting of otolith specialists.
>
> (1959 'Redbook', page 49)

The meeting of otolith specialists referred to in this recommendation was proposed in another recommendation:

> That age readers should be given opportunity to meet when preparatory work has been done and be given sufficient time to study the age reading techniques used by different countries, to discuss the introduction of a standard terminology and symbol system. The possibility of using spawning zones as a means for stock assessment, and stock prediction, should be given attention.
>
> (1959 'Redbook', page 49)

The questionnaire follows a Cod Otolith Exchange Programme begun by ICNAF in 1958. A second Otolith Exchange Programme, on Halibut was begun in 1959 but has not yet developed. In the Cod Otolith Exchange Programme, samples of otoliths were circulated among interested scientists. Each scientist reported his readings independently to the Secretariat. These results were published in ICNAF Documents Nos. 636 and 637. They showed that, on the whole, age estimates by different scientists were consistent, that ease of reading (and agreement between scientists) varied greatly between cod of different stocks, that variation in readings increased with size (age) of fish, and that some differences in interpretations existed between the scientists taking part in the programme.

There is a need for exchange of otoliths and for continued comparison of interpretations to prevent isolation, causing non-comparability of readings. Also, new techniques are continuously being investigated and the useful results of these investigations require to be disseminated. A particular field where more might be done is otolith photography, where special skills have been acquired by only a few scientists.

The complete replies to the questionnaire, including photographs, have been reproduced as appendices to this document to serve as background material for discussions on techniques and for proposals on standardisation of terminology and symbolism.

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Replies to the questionnaire have been received (5th April, 1960) as follows:

Country	Species	Specialists	Appendix Number (Page Nos. in parenthesis if more than one species is dealt with in the same appendix)
Canada (St. Andrews)	Cod	A.C. Kohler, A. Maillet, R. Robicheau	VII
	Haddock	A.C. Kohler, N.J. McFarlane	VII
	Plaice	P.M. Powles	VII
	Halibut	F.D. McCracken	VIII
Canada (St. John's)	Cod	A.M. Fleming	II
	Labrador Cod	A.W. May	III
	Haddock	V.M. Hodder	IV
	Redfish	E.J. Sandeman	V
	Am. Plaice	T.K. Pitt	VI
Denmark	Cod	Paul M. Hansen, Inge Meldal	XVI (1)
	Halibut	Paul M. Hansen, Aa. Horsted	XVI (2)
	Redfish	Paul M. Hansen, Aa. Horsted	XVI (3)
Germany	Cod	Arno Meyer, Margot Hilden	IX
	Haddock	Arno Meyer, Margot Hilden	IX
Norway	Cod	O. Annaniassen, R. Hovland, A. Frøland	XV
	Haddock	O. Bostrøm	XV
United Kingdom	Cod	G.C. Trout	XVII (1)
	Plaice	R. Margetts	XVII (5)
	Haddock	R. Blacker	XVII (7)
	Coalfish	B. Jones	XVII (7)
	Redfish	G.C. Trout	XVII (6)
		M.J. Holden	
United Kingdom (Scotland)	Halibut	A.D. McIntyre	XIII
	Whiting	Ray Gamble	XIV
United States	Haddock (otoliths)	A.C. Jensen	X
	Haddock (scales)	A.C. Jensen, J.B. Skerry, J.P. McDermott	XI
	Redfish	George F. Kelly, Allan M. Barker	XII

Techniques

The table on pages 4, 5, 6 summarises very briefly the techniques on otolith preparation and reading reported in the survey.

In all studies binocular microscopes are used except by McIntyre, who uses a hand lens. Staining is never employed. Otoliths are usually preserved dry, but occasionally they are preserved in 50% glycerine and water with thymol; 70% alcohol; or 90% alcohol. The descriptions of lighting and shading are frequently condensed in the table, and reference should be made to the appendices for a detailed description.

Interpretations

To make them more accessible, the following comments have been selected from the replies on the subject of interpretations.

Cod Otoliths

1. We think cod spawned in Unit areas 4TK, 4TL and 4TN can be picked out. Otoliths from these unit areas are distinguished by an unusually small first annulus. (Kohler, Appendix VII)

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Scientist	Species	Appendix No.	Cut, Broken, or Whole	Treatment and Wetting of Broken Surface	Magnification	Filters	How Fixed on Microscope Stage	Lighting
Fleming	Cod	II	Cut with sharp scalpel*	Rough break may be ground on emery stone. Surface of break covered with 70% Ethanol.	Objectives 1.6X Ocular 10X	Blue or green	One portion of otolith is completely embedded in plasticine so that only the broken surface is visible, level with the top of the block. The other is only partially embedded.	On the broken surface, which is at the level of the plasticine, light is directed at a 45° angle: reading is thus by reflected light. The other portion is illuminated by light directed against the concave surface and transmitted up through the broken surface which is shaded from direct light by a scalpel.
May	Cod	III	Cut with serrated scalpel* or broken with fingers	Surface of break covered with 50% Ethanol.	15X	Blue	Embedded in plasticine with concave side facing away from observer.	Transmitted light - the otolith is shaded and light directed onto the concave side of the otolith at an angle of 45°.
Sandeman	Redfish	V	Cut with well-worn razor blade.* Occasionally ground. (See fig. App. V.)	Surface wetted with 95% Ethyl alcohol.	Age 1-15: 1.3 objective, X10 ocular. Age 15-30: 3.0 objective, X10 ocular. Age 30-50+: 3.0 objective, X20 ocular.	Blue	The four halves are embedded in a plasticine block, labelled, and prepared a short time before being read.	Reflected light - directed onto the otolith at an angle of 45°, allowing as little light as possible to fall on the plasticine block.
Pitt	Am.Plaice	VI	Otolith cracked* after making guide scratch with scalpel.	Surface wetted with 90-95% alcohol.	15X - 20X	No	Embedded in black plasticine with only the broken surface showing.	On the broken surface at about a 45° angle.
Kohler	Cod Haddock Am.Plaice	VII	Broken with fingers (or with scalpel for very large or small fish). Am.Plaice otoliths usually read whole.	Surface covered with 50% glycerine.	9X - 27X	Blue sometimes	Otolith embedded in modelling clay, with fracture surface flush with upper surface.	The light beam is directed from above so that light is reflected from the surface of the otolith.
McCracken	Halibut	VIII	Whole	Surface covered 50% glycerine	-	-	-	Reflected light.
Meyer, Hilden	Cod Haddock	IX	Cut with copper-bladed saw and diamond dust.	Dry	Objectives 16X Ocular 10X	No	Otoliths embedded in blue plasticine.	The light comes from the side away from the observer, nearly parallel to and a little below the surface of the otolith. The beam is usually directed on the concave side of the otolith.

Scientist	Species	Appendix No.	Cut, Broken, or Whole	Treatment of Wetting of Broken Surface	Magnification	Filters	How fixed on Microscope Stage	Lighting
Jensen	Haddock	X	Broken (see Kohler)	Submerged	Objectives 1X Ocular 10X	Medium Blue	See (Kohler)	Light beam directed downward at 30° from the vertical onto the broken surface.
Kelly, Barker	Redfish	XII	Cut across long axis* with dull edge razor. Broken surface ground smooth.	Glycerine on cut surface. Whole otolith read in 70% alcohol against a dark background.	Objectives 0.7X -3.0X Ocular 10X-15X	No	Inserted in slot on cork stopper.	Light directed against side or end of otolith - surface shade with shield. Whole otolith read with direct light reflected.
McIntyre	Halibut	XIII	Whole.	Soaked 2 hours in water. Read submerged.	Hand Lens X10	None	In dish.	General illumination, otolith against black background.
Gamble	Whiting	XIV	Broken with fingers.	Moistened with turpineol - may be ground.	X25	Yellow	In putty.	Horizontal light beam: broken surface shielded by finger so that light shines only through the lower part of the otolith.
Hovland, Møller	Cod Haddock	XV	Broken with fingers or pliers.	Surface wetted with 50% glycerine - water.	15X	Yellow	Moulding wax.	The light beam is directed partly on the side of the otolith broken surface, and shaded with a pencil so that the surface comes in shadow.
Hansen, Meldal	Cod	XVI	Broken with fingers.	Submerged in 96% alcohol. Surface rarely ground.	Objectives 16X -25X Ocular 10X	No	The otoliths are fixed in a fine clamp which is held in the right hand, making it possible to turn the otolith so that the light falls onto the broken surface at various angles.	Oblique, in-falling light about 45°. Surface may be shaded.
Hansen, Horsted	Halibut	XVI	Whole or broken.	Submerged in 72% alcohol.	Objectives 16X Ocular 10X	No	Otolith held in clamp.	Transmitted light if whole; reflected light if broken.
Hansen, Horsted	Redfish	XVI	Broken with fingers.	Half-hour in 70% alcohol. Read in alcohol.	Objectives 16X Ocular 10X	No	Otolith held in clamp.	Spotlight; oblique, nearly horizontal reflected light. Convex side faces light. Shading with finger or clamp.
Holden, Trout	Cod	XVII	Broken with fingers. May be sawn.	Rough surface moistened with Xylol: may be ground.	14X	No	Plasticine mount.	Transmitted light - see photographs in Appendix XVII. A horizontal bar is used to screen the surface from direct light. The light beam is directed horizontally to strike the otolith just below broken surface.

Scientist	Species	Appendix No.	Cut, Broken, or Whole	Treatment and Wetting of Broken Surface	Magnification	Filters	How Fixed on Microscope Stage	Lighting
Margetts	European Plaice	XVII	Whole	Immersed in water	X7 ocular X2.5 objective	No	Otolith in black watchglass	Reflected light; angle of light about 45°.
Trout	Redfish	XVII	Whole	Immersed in alcohol	X7 ocular X2.5 objective	No	Otolith in black watchglass	As for European Plaice above.

*Otolith supported by plasticine while cutting.

APPENDIX XVIII

The following reply was received at the last minute.

QUESTIONNAIRE ON AGE READING

Biologische Anstalt Helgoland, Abteilung Fischereibiologie, Bremerhaven

Dr. Adolf Kotthaus: Sebastes marinus (all types) and Sebastes viviparus

Techniques

1.a. During scientific cruises a random sample of 200 specimens – if available – is measured. Otoliths are taken from the first hundred of that sample. Sex and maturity stages are taken.
b. At the fish market the same techniques are used for each market category; the maturity stages, however, are not determined and the immature fish cannot be sexed. The same holds true for "giant fish" if gutted.
2.a. Otoliths are removed through the gill cavity.
b. They are preserved in Ethyl alcohol (of 96%) at sea, whereas they are put in dry condition into paper bags at the market.
c. Otoliths are stored either in 10 ml. bottles with plastic stoppers (Ethyl alcohol) or dry in paper bags.
3. Dry otoliths are cleared in alcohol (96%) some hours before reading.
4. Binocular microscope.
5. They can be read whole.
6. –
7. The otolith lies in a Boveri-dish.
8. It is submerged in alcohol.
9. Mona lamp.
10. Blue filter.
11. A light screen (black cardboard with a hole the size of the otolith) is laid under the dish, in order that only the otolith is illuminated.
12. Normally transmitted light; whole surface of the otolith is illuminated. Small otoliths and those of S. viviparus are investigated in reflected light inside a black Boveri-dish.
13. A combination of 8X oculars and 1 or 2X objectives is used.
14. The hyaline zones are counted along both the longitudinal and the transversal axis.
15. No staining nor sectioning is done.
16. Scales and otoliths have been compared in the beginning of the studies, but age reading from scales has been abandoned.

Interpretations

1. Yes – otoliths are used for definition of stocks.
2. Yes – otolith types can be defined.
3. Size, breadth, shape, indentation and transparency.
4. Yes – special types of zones can be defined.
5. Spawning zone, the following zones become very narrow.

Terminology

No

Symbols

The following symbols are used to designate the different types of redfish:

Ma – marinus
Me – mentella
Int – intermediate
R – giants

2. At least two classes of otoliths can be set up in our area. Those which have well-defined hyaline zones throughout, no serious check zones and are quite easily read, often called Type A. The remainder (often called Type B) may, after more study, be further subdivided. This type contains otoliths with many check zones, "split" hyaline zones, etc., and are generally more difficult to read.
(Fleming, Appendix II)

3. Otoliths of cod from certain fjords can be distinguished from otoliths of cod from the banks. Otoliths of fjord cod have indistinct hyaline zones and many check rings. Frequently the hyaline zones are placed very densely and are very narrow; this is caused by slow growth.
(Hansen and Meldal, Appendix XVI)

4. Otoliths have been used to distinguish between different stocks, i.e. the coastal cod and the "skrei". We have defined the following otolith types:

a. Type I } "Skrei"
b. Type II }
c. Coastal cod

Type I: The zones are uniform in structure, and the opaque and hyaline zones are sharply delimited from each other. Regularity characterizes the otolith.

Type II: Both opaque and hyaline zones are "split" by hyaline and opaque material respectively.

Coastal cod: The bright nucleus and the shape of the first year are characteristic. (Hovland and Møller, Appendix XV)

5. Otoliths are not normally used for definition of stocks. When reading Icelandic otoliths an attempt is made to differentiate fish from Greenland by using differences in otolith structure and age/length relationships, but this differentiation cannot be done with certainty.

Mr. Trout, in a study of the Spitzbergen-Bear Island cod, used differences in otolith structure to split fish into three sub-populations (see Trout 1957, The Bear Island Cod, Migrations and Movements. M.A.F.F. Fish. Invest. Ser. II, Vol. XXI, No. 6).

All segregation of otoliths into types is done by attempting to classify patterns of otolith growth. In the example referred to above, Trout used variations in the opaque zone, calling uniform opaque zones with one, or occasionally two, hyaline check rings (see "Terminology" for definition) as "Western-Split", and opaque zones merging into each other "Eastern", the terms Western, Western-Split and Eastern referring to the areas in which the pattern was most common. (See photographs 3 and 4, Appendix XVII.) Such segregation is always difficult because there is always considerable variation from the chosen type pattern, which makes classification very difficult.

On the broader question of separating stocks, it is possible with experience to differentiate between otoliths from fish from the main fishing area, e.g. between North Sea and Bear Island Cod (compare photographs 5 and 3, Appendix XVII), but not between fish from two areas within the North Sea. This is only of academic interest because the area from which the otoliths are collected is known. The exception is that of Icelandic otoliths, where it is desirable to know the admixture of fish from Greenland. Otoliths from Greenland fish are regular, with well-defined annual opaque and hyaline zones and with few if any splits compared with Icelandic otoliths, that have

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typically ill-defined zones with many splits (compare photographs 6 and 7, Appendix XVII).

It is, however, possible from time to time to distinguish certain years by peculiarities in the structure of the otolith. 1954 was a good year for plankton production in the Arctic and some cod otoliths from Region IIb show an exceptionally large opaque zone. This is more noticeable because the preceding two years, 1952 and 1953, were years of below average plankton production and the otolith growth was less than normal. Where this structure occurs in an otolith, it is possible to use it as a check against the age determination by counting zones only and it is useful in older fish, where the outer growth may be difficult to interpret. This check can only be used positively because not all otoliths showed the same pattern of growth for these years, i.e. some fish whose otoliths showed them to be definitely spawned in 1952 had normal-sized 1952, 1953 and 1954 rings. (see Photograph 8, Appendix XVII.) (Holden, Appendix XVII)

Haddock Scales

6. We believe scale types can be defined, but this has not been verifie as yet. Three special zones can be defined:

   a. An accessory mark formed in October of the first year.
   b. The zone of narrow (so-called winter type) circuli.
   c. The zone of broad (so-called summer type) circuli.

   The accessory mark is defined by the number of circuli (from the focus) involved and the narrow, dark appearance of the circuli forming the mark. The narrow zone and broad zone are defined only by the appearance of the circuli forming the zone.
   (Jensen, Appendix XI)

Redfish

7. Can special types of zones be defined? - only zones concerned with change in growth pattern of the otolith (Regions).
   (Sandeman, Appendix V)

8. The otoliths have been used for age determination only. Their gross structure, i.e. length in relation to fish length, has been used for speciation purposes but no interpretation of the type requested in questions 1-5 has been attempted. (Holden, Appendix XVII)

Halibut

9. Otoliths can be arranged into types on the basis of general shape and length/breadth ratio and presence or absence of double rings, but these types seem often to be mixed in single samples and it has not yet been found possible to use them for stock differentiation.
   (McIntyre, Appendix XIII)

European Plaice

10. The otoliths are used solely for age determination and no attempt has been made to use them for defining stocks or to classify them into types. (Holden, Appendix XVII)

American Plaice

11. The possibility exists that otoliths may be used for definition of stocks. (Pitt, Appendix VI)

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Whiting

12. A distinction can be made between the otoliths of whiting in the Clyde area and those in the North Sea. The former are much more difficult to read, because the first two winter zones tend to be made up of many fine hyaline rings with little summer growth separating them. The corresponding winter growth in North Sea whiting are much more solid in appearance, with the second winter zone commonly having a double structure. (Gamble, Appendix XIV)

Terminology

Pertinent comments and definitions are set out below.

1. Check Ring

Cod and Haddock

1.1. Hyaline ring which, because of its incomplete or indistinct formation, is not considered a true "winter" mark. (Fleming, Appendix II)

1.2. Narrow hyaline zone or band of very fine hyaline zones which do not show up as clearly as the "normal" type of hyaline zone. Width of the preceding and/or succeeding opaque zone(s) may aid in definition of a check ring. (May, Appendix II)

1.3. A hyaline ring that is not counted as an annulus - usually because of poor definition or irregular spacing. (Kohler, Appendix VII)

1.4. Check rings are often indistinct and incomplete. (Hansen, Appendix XVI)

1.5. Any hyaline zone laid down that is considered not to be an annual hyaline zone. This definition assumes that the normal pattern of otolith growth is one opaque zone, normally laid down in the summer, and one hyaline zone, normally laid down in the winter, each year. Any hyaline zone within the opaque zone is a check ring. (See photograph 4, Appendix XVII.) (Holden, Appendix XVII) Also used for Redfish otoliths.

Haddock Scales

1.6. A zone of narrow circuli composed of only two or three circuli and obviously not as wide as the adjacent zones of narrow circuli. (Jensen, Appendix XI)

Redfish

1.7. Hyaline band which cannot be regarded as a true hyaline year band. (Sandeman, Appendix V)

1.8. The term is not used in our work. Year zone is used to denote one year of growth in the otolith represented by an opaque and a hyaline zone. I question whether 'check ring' should be proposed for universal usage, since it is an inexact term that is only loosely associated with age and growth. (Kelly and Barker, Appendix XII)

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2. Nucleus

Cod and Haddock

2.1. The opaque centre of the cross-section of a cod or haddock otolith. (Kohler, Appendix VII)

2.2. The nucleus is found only in few otoliths. It shows as a small distinctly delimited kernel, often of yellow colour. (Hansen, Appendix XVI)

2.3 The opaque material in the centre of the otolith is called nucleus. (Hovland and Møller, Appendix XV)

2.4 Nucleus. The whole of the first year's growth of the otolith, including all opaque and hyaline zones. (See photograph 4, Appendix XVII.) (Holden, Appendix XVII) Also used for Redfish nucleus.

Haddock Scales

2.5. Nucleus (focus) - central point of the scale around which the concentric circuli are arranged. (Jensen, Appendix XI)

Redfish

2.6. Not used. Centre of otolith is not, as yet, fully understood. All readings are based on the "first definite hyaline year zone which corresponds to the first zone of narrowly-spaced circuli of the scales". Centre or origin is loosely used to describe the area inside the first definite hyaline zone. (Sandeman, Appendix V)

2.7. This term applies to the smallest opaque zone at the center of the otolith. Focus is sometimes used to indicate the first center of otolith growth. (Kelly, Appendix XII)

Halibut

2.8. The part inside the first ring. (McIntyre, Appendix XIII)

European Plaice

2.9. The central opaque zone up to but excluding the first hyaline zone that corresponds in the opinion of the observers to the first winter. In the simplest cases, the nucleus is the opaque core of the otolith, but in other cases, the nucleus may contain some hyaline zone, if, again in the opinion of the observer, these hyaline zones do not correspond to winter growth. (See photographs 10, 11 and 12.) (Holden, Appendix XVII)

American Plaice

2.10. The small translucent center of the otolith. (Pitt, Appendix VI)

Whiting

2.11. Nucleus or false ring - a small hyaline ring lying inside the first winter zone, and probably laid down when the young fish adopt the bottom habitat. (Gamble, Appendix XIV)

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3. Opaque and Hyaline Edge

Cod and Haddock

3.1. Whether the edge is opaque or hyaline is determined by observation of the narrower edge of the cut surface (to the right as mounted). (May, Appendix III)

3.2. Opaque edge means the outermost material laid down at the edge of the cross-section of the cod or haddock otolith is opaque.

Hyaline edge means the outermost material laid down at the edge of the cross-section of the cod or haddock otolith is hyaline.

Narrow hyaline means the hyaline edge zone does not look to be fully formed.

Narrow opaque means the opaque edge zone does not look to be fully formed.

(Kohler, Appendix VII)

3.3. The otolith has opaque edge if the edge is of opaque material.

The otolith has hyaline edge if the edge is of hyaline material.

(Hovland and Møller, Appendix XV)

3.4. Opaque edge: an otolith edge appearing dark by transmitted light and bright by reflected light. When high light intensities are used, an opaque edge often appears light by transmitted light due to the general diffusion of light. (See photograph 5, Appendix XVII.)

Hyaline edge: an otolith edge appearing light by transmitted light and dark by reflected light. (See photograph 3, Appendix XVII.)

(Holden, Appendix XVII) Also Redfish and European Plaice.

Haddock Scales

3.5. Opaque edge (winter-type edge in scales): edge characterized by a zone of narrow circuli.

Hyaline edge (summer-type edge in scales): edge characterized by a zone of broad circuli. (Jensen, Appendix XI)

Redfish

3.6. Opaque edge: edge of otolith made up of opaque material following previous hyaline year zone or check.

Hyaline edge: edge of otolith made up of hyaline material. It is my conviction that the edge can be more clearly observed using reflected rather than transmitted light.

(Sandeman, Appendix V)

3.7. Opaque edge: indicates that opaque material comprises the major portion of the otolith periphery. Opaque material is deposited during fast or summer growth.

Hyaline edge: indicates that hyaline material, representing slower winter growth, comprises the periphery of the otolith.

(Kelly and Barker, Appendix XII)

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Halibut

3.8. Opaque edge: the white appearance of the edge when the black viewing beckground is not able to shine through due to the structure of the otolith. Usually found during the period of summer growth.

Hyaline edge: the dark appearance of the edge when the black viewing background is able to shine through due to the structure of the otolith. Usually found in winter, but some times occurs when you least expect it. (McIntyre, Appendix XIII)

3.9. Opaque edge: new growth or indication of a rapid growth area at the outer edge of the otolith.

Hyaline edge: translucent edge indicating a slow growth period at the outer edge of the otolith. (Pitt, Appendix VI)

Whiting

3.10. Opaque edge: the appearance of the edge when a new summer ring is being laid down. It is not easy to see using transmitted light, in which it appears as a dark rim to the otolith.

Hyaline edge: the appearance of the edge when a new winter ring is being laid down. Appears as a light-coloured rim in transmitted light. (Gamble, Appendix XIV)

4. Spawning Zones

Cod

4.1. Spawning zones can be recognized, but quite often difficulty is encountered in recognition of the first zone. Spawning zones have relatively wide hyaline bands generally much broader than the opaque bands in between the hyaline bands, they are evenly spaced, and are, naturally, at the outer part of the otolith. (Fleming, Appendix II)

4.2. Spawning marks: narrow, plain opaque zones with hyaline zones usually wider than these. Absence of check rings and split zones. An attempt is made to define spawning zones, but this definition is as yet uncertain. The first spawning zone only is noted as "age at first spawning" (age at previous hyaline zone) in a column opposite age. (May, Appendix III)

4.3. Spawning zones can most easily be distinguished in cod which have spawned several times. In otoliths of first spawners, where the spawning zone is placed at the edge, it may be difficult to distinguish it as a spawning zone. The spawning zones are narrow and the hyaline zones are distinctly depicted. (See photograph, Appendix XVII.) (Hansen, Appendix XVII)

4.4. The hyaline rings, which are more bright than the inner ones and often wider than the opaque rings, are called spawning marks. (Hovland and Møller, Appendix XV)

4.5. Spawning marks: these are referred to as spawning zones and are regular patterns of hyaline and opaque zones, the pattern commencing with wide hyaline zone corresponding to the winter preceding spawning, followed by a very narrow opaque zone corresponding to the summer of spawning. Further narrow

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hyalines and opaque zones then follow according to the number of times the fish has spawned. Typically, the hyaline zones are very bright, by transmitted light, and wider than the dark opaque zones. (See photograph 9, Appendix XVII.)
(Holden, Appendix XVII)

Redfish

4.6. Spawning marks: spawning zone is used in this reference in our work. It denotes a change in the year zone form, generally between the 6th and 12th zones. Both the opaque and hyaline zones become narrower than before and the subsequent year zones become more uniform in size and form. Year zones representing growth before first maturity can be distinguished from those of later years. More work must be done on this.
(Kelly, Appendix XII)

5. Certain, Uncertain and Unreadable

Cod and Haddock

5.1. An age reading is termed certain if the observer has little doubt but that the reading is correct, uncertain if the reading might be greater or lesser by one or two years. No otoliths are termed unreadable. Uncertain otoliths are those with a number of check rings, uncertain check rings, diffuse zones, etc. (May, Appendix III)

5.2. Certain otoliths: otoliths in which the annuli are so clearly defined as to give only one possible reading.

Uncertain otoliths: otoliths in which some check rings might be annuli or vice versa.

Unreadable otoliths: otoliths in which annuli and "check" rings are so poorly defined as to be indistinguishable.
(Kohler, Appendix VII)

5.3. Certain otoliths: no doubt about age determined from otolith, all observers agreeing. (See photographs 3, 5, 6, 8, and 9, Appendix XVII.)

Uncertain otoliths: readable but leaving the interpretation of some of the hyaline and opaque zones open to question so that it is only possible to state the probable age of the fish from which the otolith was taken with probable alternatives. (See photograph 7, Appendix XVII.)

Unreadable otoliths: impossible to state even a probable age of the fish from which the otolith was taken due either to an absence of hyaline or opaque zones or the presence of an irregular sequence of zones which fits with no annual pattern.
Also Redfish. (Holden, Appendix XVII)

Haddock Scales

5.4. Certain: annuli (zones of narrow circuli) distinctly different from adjacent zones of broad circuli, well separated and readily identified.

Uncertain: annuli only slightly different from broad circuli; one or more annuli norrow, having appearance of check rings.

Unreadable: scales with regenerated centers or badly eroded and damaged edges. (Jensen, Appendix XI)

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Redfish

5.5. Otoliths graded according to readability, not with regard to certainty of reading obtained. As the two aspects are obviously related, the scheme in Appendix V is probably representative. No analysis has been made of this yet, and the table in Appendix V can only be regarded as a relatively educated guess. Asterisks are also used to indicate exceptionally clear otoliths. (Sandeman, Appendix V)

5.6. Certain: both otoliths show - read in various directions - the same result. If two readers, both get the same results.

Uncertain: the two otoliths read in various directions give results which vary with plus or minus 2.

Unusable: greater variation than plus or minus 2.
(Hansen and Meldal, Appendix XVI)

5.7. These terms are little used with redfish. Most otoliths having more than 6 or 8 year zones are read with some degree of uncertainty. Unreadable otoliths are those in which a portion of the material is not differentiated into zones or is separated into zones in a confused pattern that permits either high or low counts. (Kelly, Appendix XII)

Halibut

5.8. Certain otoliths are those that can be read with confidence. Uncertain otoliths are those which for one reason or another have to be read as x+ a number of years. Unreadable otoliths can't be read even with confidence. (McIntyre, Appendix XIII)

American Plaice

5.9. Unreadable: impossible to distinguish between rings;or otolith in a crystallized condition. (Pitt, Appendix VI)

Whiting

5.10. Certain otoliths are those with clear and distinct winter zones, which can be easily counted.

Uncertain otoliths are either those with indistinct hyaline zones which are difficult to count; or otoliths with two or more hyaline rings so closely spaced that it is doubtful if they represent separate winters' growth.

Unreadable otoliths are those in which it is impossible to differentiate between successive winter zones, so that they cannot be counted. (Gamble, Appendix XIV)

6. Official Birthday

6.1. The <u>birthdate</u> of cod and haddock in Subarea 4 is taken to be February 1st. Thus our quarters of the year are: February-April, May-July, August-October, November-January. In order to be able to assign fish that form hyaline rings early in the winter to the correct age group we use the scheme explained in the table in Appendix VII. (Kohler, Appendix VII)

.../15

6.2. Official birthday. The day upon which a fish is presumed to have been spawned. It usually bears some relation to the actual spawning period. For all cod it is taken as 1st January. (See table in Appendix XVII) (Holden, Appendix XVII)

7. Split

Cod and Haddock

A narrow hyaline zone occurring within a wide opaque zone or vice versa. When the split is a narrow hyaline zone in an opaque zone the terms "split" and "check ring" are synonymous, although the term "split" is usually applied to a narrower zone than "check ring", although this differentiation is subjective. (See photograph 5, Appendix XVII)
Also Redfish and Plaice. (Holden, Appendix XVII)

8. Double Ring

Where the winter zone is made up of two hyaline rings separated by a more opaque area. Commonly found in the second winter ring of North Sea whiting. (Gamble, Appendix XIV)

Symbols

The descriptions of symbols used by scientists in recording their interpretations of otoliths are set out below.

Cod and Haddock

1. Examples below include all symbols in routine use for recording age readings at St. Andrews.

Sample Reading	Definition
6 + N.O. C_2	6 - is number of hyaline annuli + - indicates opaque growth at edge of otolith N.O. - indicates narrow band of opaque material at edge C_2 - indicates hyaline check ring between 1st and 2nd annuli
6 + W.O.	W.O. - indicates wide band of opaque material at edge
6 N.H. C_3	N.H. - indicates narrow band of hyaline material at edge C_3 - indicates hyaline check ring between 2nd and 3rd annuli
6 S.C.	6 - indicates 6 hyaline annuli, the one at the edge being fully formed S.C. - (small centre) - indicates 1st hyaline ring smaller than average, probably due to late spawning

(Kohler, Appendix VII)

2. Asterisks mark otoliths where age reading is certain. Other features described in writing. (Fleming, Appendix II)

.../16

3. (1) C — Check ring.
$2C_2$ — Two check rings in second year.

(2) *4 — Exceptionally clear otolith.
*3 — Fair to good otolith. Age reading reliable.
*2 — Poor otolith. Age may be one year more or less.
*1 — Very poor otolith. Age may be two years more or less than age assigned.

(May, Appendix III)

4. Each otolith is classified according to its readability with 1, 2, 3, and 4. 1 means very clear rings; 2 means clear rings; 3 not so good, some difficulties in age determination; 4 bad, great difficulties, results of age determination uncertain. All otoliths classified with 4 are not used for further treatment.

(Meyer and Hilden, Appendix IX)

5. Age. This is always written in Roman numerals. In the case of uncertain otoliths, the probable age is written first, followed by the probable alternative which is queried, e.g. V ? VI.

Spawning. The symbol "S" is used to denote a fish that is assumed to be about to spawn and is detected by the presence of the bright pre-spawning hyaline zone (see Terminology (7) above). The symbols S1, S2, etc. are then used to denote fish that have spawned once, twice, etc. and have completed the full year's growth of the year of spawning. Assuming a fish with the same otolith pattern of growth as in the paragraph on "Official Birthday", the symbols would be used as follows:

Date of capture of fish	Number of opaque zones*	Number of hyaline zones*	Type of edge	Symbol
1st January	0	1	Bright hyaline	S
August	1	1	Narrow opaque	S
31st December	1	2	Hyaline	S
1st January	1	2	Hyaline	S1

* Only spawning zones are referred to.

Where the official birthday is set later in the year than 1st January, e.g. 1st May, the symbol "S" is used for the period of twelve months, from the time the pre-spawning edge is first visible, after 1st May until a complete spawning zone has been completed by the 30th April twelve months later. The sequence is thus shifted five months later than in the table above.

(Holden, Appendix XVII)

6. (1) Edge type: Wh (wide hyaline); Nh (narrow hyaline); Wo (wide opaque); No (narrow opaque).

(2) Check marks: $C_{1,3}$ (checks on the first and third annuli).

(3) Age 7(8): fish is probably 7 years but there is a strong check mark which may be an 8th annulus.

(4) Age 9(?): best estimate of age from an ambiguously marked otolith.

(Jensen, Appendix X)

../17

Redfish

7. The following system is used to designate the edge structure of the otoliths. Edge structure is normally only noted to about 10 years of age.

Age

3	Hyaline year zone fairly well or well developed.
3-	Signs of appearance of opaque edge.
3+	Opaque edge clear.
3+-	Signs of development of start of hyaline zone following opaque.
3++	Definite appearance of hyaline zone.
4	Hyaline year zone fully established.

It should be noted that the end of each zone of winter growth is coincident with the "birthdate" of the fishes and thus a straight count of the number of complete "winter" zones provides the estimate of age. Because so little growth takes place during the months January to May, it is convenient to regard the "birthdate" as in the previous January and so put the estimated ages on a calendar year basis.

(Sandeman, Appendix V)

8. Age without symbol: certain. This includes possibility of error up to plus or minus 1.

Underlining of age: absolutely certain.

Question mark after age: uncertain.

Question mark without age: unusable.

(Hansen and Horsted, Appendix XVI)

American Plaice

9. In reading otoliths we indicate the approximate degree of accuracy to which we consider the otolith to have been read by numbers 1 to 5; also the condition of the edge, whether transparent or opaque, is indicated. Thus 6, T, 3 indicates an otolith 6 years old with a translucent edge and read with an acceptable degree of accuracy; 6, O, 5 indicates an otolith 6 years old with new growth at the edge extremely readable, the zones very clear; 6, T, 1 and 6, T, 2 would be rejected as being too doubtful to include in further calculations. Completely unreadable otoliths would be indicated with a dash (---)

(Pitt, Appendix VI)

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Document No. 4.
Appendix I.

TECHNIQUES FOR READING OTOLITHS

Forrest Building,
Carleton Street,
Halifax, N. S.,
21st December, 1959.

Dear Sir,

Following on the Cod Otolith Exchange Programme, the Committee on Research and Statistics has asked that the attached questionnaire be circulated to scientists and institutions interested in the study of otoliths for age determination or for stock definition (see Appendix III of the Report of the Committee on Research and Statistics, June 1959: 'Red Book', page 48). The questionnaire is not limited to cod studies, but should be completed at least for cod, haddock, redfish, and halibut.

Part of the purpose of the questionnaire is to help determine the best methods for collecting, preserving and studying the otoliths and also to provide a basis for the introduction of a standard terminology and symbol system for describing otoliths.

A standard terminology will especially simplify the comparison of results obtained by different scientists. It would also remove some confusion from the field and thus simplify the work of the novice being introduced to the study of otoliths for the first time.

The Committee on Research and Statistics also requested that photographs of otoliths and scales, to illustrate interpretations, terminology and symbols, be forwarded with your replies to the questionnaire.

Your co-operation in answering the enclosed questionnaire and in forwarding suitable photographs will be greatly appreciated.

Yours sincerely,

Ronald S. Keir,
Biologist-Statistician.

Circulated to: Dr. J. Ancellin, Dr. H. Graham, Dr. P. Hansen, Dr. J. Jónsson, Dr. C. E. Lucas, Dr. J. Lundbeck, Dr. A. Marcotte, Dr. J. Marti, Dr. W. R. Martin, Dir. G. Rollefsen, Dr. M. Ruivo, Mr. M. Sunico, Dr. W.R. Templeman, Mr. G.C. Trout.

QUESTIONNAIRE ON AGE READING

This questionnaire concerns the use of otoliths for age reading or stock definition. The same form may be used to respond on scale reading techniques if scales are used.

1. Name of institutes using otoliths for age determination.
2. Name of persons responsible for age estimates, and the species studied.

Techniques

1. How are the fish, from which the otoliths are taken, selected? What data are collected (length, sex, etc.)?
2. In what way are they removed from the fish? How are they preserved? How are otoliths stored (envelopes, boxes or special devices when collected by one person)?
3. How are they treated before reading?
4. What kind of microscopes or other instruments are used to study the otoliths?
5. If the otoliths are not read whole, how are they cut or broken?
6. Is the broken surface treated before reading and, if so, how?
7. How is the otolith fixed on the microscope table?
8. Is the otolith read submerged or in air?
9. What light source is used?
10. Are filters used and, if so, which colour is used?
11. Are other special devices used to facilitate the reading?
12. How is the light beam directed, and on what part of the otolith?
13. What magnification is used (objectives, oculars)?
14. When counting the zones, is the hyaline or the opaque zone used, and along which axix or direction?
15. Is staining or sectioning used?
16. Are scales and otoliths from the same individuals compared?

Interpretations

1. Are otoliths used for definition of stocks?
2. Can otoliths types be defined?
3. What features are used for segregation of otolith types?
4. Can special types of zones be defined?
5. What features are used to define special zones?

.../2

Terminology

Are special expressions used to point out characteristics of certain parts of the otolith or certain features of patterns of the zones?

Provide definitions of (1) Check ring

(2) Nucleus

(3) Intermediate zone

(4) Secondary zone

(5) Opaque edge

(6) Hyaline edge

(7) Spawning marks

(8) Certain, uncertain, unreadable otoliths

(9) Other terms used

Symbols

When otoliths are read and data tabulated, are certain symbols used to characterize certain features? If so, please specify and describe or define as necessary.

--ooOoo--

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Appendix No. II

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

A. M. Fleming

Cod

Techniques

1. Fish, from which otoliths are to be taken, are selected by means of random numbers from samples of commercial or research vessel catches being measured for length distribution, sex, stage of maturity and sometimes weights.

2. Otoliths are extracted through the ventral side of the skull after removal of the gills and cutting through the occipital capsule containing them.

3. No treatment before reading.

4. Microscope - Zeiss Stereo (complete with demonstration tube).

5. Otolith cut transversely through the nucleus using a sharp scalpel, otolith first being placed on a block of plasticine for support to prevent splintering.

6. Broken surface not treated, except in cases of very uneven breakage when surface is ground flat on a rotating emery stone.

7. One broken portion of otolith completely embedded in plasticine block so that only broken surface is visible, level with the top of the block of plasticine. The other broken portion is only partially embedded and stands vertically in the block.

8. Otolith read with broken surface covered by a drop of ethanol (about 70%).

9. American Optical 6-volt focusing illuminator.

10. Blue filter or green filter.

11. No.

12. On the broken surface which is at the surface of the block light source is directed at about a 45° angle, reading is thus by reflected light. On the broken surface standing above the plasticine surface (other portion of otolith) light is directed against the concave side, and transmitted up through the broken surface which is shaded from direct light by means of a scalpel blade.

13. Magnification. Objectives 1.6X, ocular 10X.

14. Hyaline zones used in counting, usually from nucleus outward toward longest axis, but often have to shift in counting from one direction to another.

15. No.

.../2

16. Scales and otoliths have been compared where, in reading otoliths, doubt concerning the first year exists. For baby cod scales and otoliths are being compared.

Interpretations

1. Not yet.

2. At least two classes of otoliths can be set up in our area. Those which have well-defined hyaline zones throughout, no serious check zones and are quite easily read, often called Type A. The remainder (often called Type B) may, after more study, be further subdivided. This type contains otoliths with many check zones, "split" hyaline zones, etc. and are generally more difficult to read

3. See 2.

4. Spawning zones can be recognized, but quite often difficulty is encountered in recognition of the first zone.

5. Spawning zones have relatively wide hyaline bands generally much broader than the opaque bands in between the hyaline bands, they are evenly spaced, and are, naturally, at the outer part of the otolith.

Terminology

1. Check ring: Hyaline ring which, because of its incomplete or indistinct formation, is not considered a true "winter" mark.

2. to 6. No special definition.

7. Spawning mark described in Interpretation, No. 5.

8. and 9. No special definition.

Symbols

Asterisks mark otoliths where age reading is certain. Other features described in writing.

--ooOoo--

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Document No. 4
Appendix III.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

A. W. May

Labrador Cod

Techniques

1. Inshore - Samples of 120 fish selected at random from trap catches or a sample of 120 is jigged.
   Offshore - Entire catch sampled if it is small, otherwise random selection in baskets for length measurements, and random selection of 1 in 10 of these for otoliths.
   Data collected include length, sex and maturity, and sometimes weights.

2. The otoliths are removed by cutting into the otolith capsule from the ventral side near the midline of the body. The knife is then twisted anteriorly and laterally to expose the otolith, which is then lifted out with forceps. The otoliths are stored dry in envelopes.

3. No treatment before reading.

4. Spencer-cycloptic binocular stereoscopic microscope fitted with magni-changer.

5. The otolith is cut across the long axis at the center by means of a scalpel, the blade of which has been serrated by striking the cutting edge 10 to 15 times with the edge of a heavier blade. Small otoliths are easily cut. Larger ones may be easily broken between the thumb and forefinger of each hand once a notch has been cut with the scalpel.

6. A few drops of about 50% ethanol are applied to the cut surface before reading.

7. Each piece of the otolith is embedded vertically in a small piece of plasticine with the concave side facing away from the observer.

8. The otolith is read in air (transmitted light).

9. American Optical 6-volt focusing illuminator with 30-watt transformer.

10. One blue glass filter is used.

11. The edge of a scalpel blade is applied to the further edge (edge nearest the light source) of the cut otolith. Thus the otolith surface is shaded and is read with transmitted light.

12. The light beam is directed at about a 45° angle onto the surface and concave side of the otolith.

13. Magnification used is 15X.

14. The hyaline zones are counted to both edges (to left and right as mounted). If the readings differ the reading to the narrower edge is taken (to the right as mounted).

.../2

15. Neither staining nor sectioning is employed.

16. Scales and otoliths are sometimes compared if the otolith age is uncertain, but only for fish smaller than 50 cm.

Interpretations

1. No.

2. No.

3. ---

4. An attempt is made to define spawning zones but this definition is as yet uncertain.

5. The first spawning zone only is noted as "age at first spawning" (age at previous hyaline zone) in a column opposite age.

Terminology

1. Check ring: Narrow hyaline zone or band of very fine hyaline zon which do not show up as clearly as the "normal" type of hyaline zone. Width of the preceding and/of succeeding opaque zone(s) may aid in definition of a check ring.

2. ---

3. ---

4. ---

5 and 6. Whether the edge is opaque or hyaline is determined by observation of the narrower edge of the cut surface (to the right as mounted)

7. Spawning marks: Narrow, plain opaque zones with hyaline zones usually wider than these. Absence of check rings and split zones.

8. An age reading is termed certain if the observer has little doubt but that the reading is correct, uncertain if the reading might be greater or lesser by one or two years. No otoliths are termed unreadable. Uncertain otoliths are those with a number of check rings, uncertain check rings, diffuse zones, etc.

9. ---

Symbols

1. C Check ring
   2 C_2 Two check rings in second year.

2. *4 Exceptionally clear otolith.
   *3 Fair to good otolith. Age reading reliable.
   *2 Poor otolith. Age may be one year more or less.
   *1 Very poor otolith. Age may be two years more or less than age assigned.

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Appendix IV

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

V. M. Hodder

Haddock

Techniques

1. Two types of samples are taken on research vessel surveys: (a) At sea during the measuring (length only) 1 in every 10 measured is examined for length, sex, maturity and both otolith and scales are taken (the selection is made by using a system of random numbers); (b) In order to supplement the sea collections and to fill in for weaknesses in the data due to variations in year-class strength a non-random sample is collected (a number of fish at each centimenter size covering the general range of lengths present in the catches).

   The sampling of the commercial catches and landings is the same as for type (a) above.

   Remainder 2-16 same as shown for Labrador cod.

   Interpretation, terminology and symbols the same as for Labrador cod.

--ooOoo--

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Appendix V.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

E. J. Sandeman

Redfish (Sebastes, mentella and marinus types from ICNAF area)

Techniques

1. Various types of samples depending on purpose for which samples were obtained.

   (a) Spot Random Sample. Random sample of 120-200 fish taken from a particular locality and depth usually a single set (research vessel).

   (b) Random samples obtained by sampling 1/10, 1/20, etc. while obtaining length measurements. Random numbers or cards used for randomization (research vessel and commercial landings).

   (c) Category samples (non-random). Selection of a specific number of fish of each sex at specified length groups.

   (d) Special samples of small fish (total catch of a set at random).

   Data collected: Only in a few instances is sampling of redfish conducted for age estimation as the primary requirement. Thus the complete data collected will vary from sample to sample bu in most cases the following basic data pertaining to studies involving the ages of fish are taken.

Length:	-	(Fork length - Tip of lower jaw with mouth closed to the end of the median caudal rays - measured to the nearest cm.)
Whole Weight:	-	Spot random samples and category samples in particular. (Weight to nearest oz. for fish greater than 8 oz. in wt., to nearest gram when 8 oz. or less.)
Sex:	-	
Maturity:	-	
Type:	-	(mentella, marinus, etc.
Otoliths:	-	
Scales:	-	Most fish less than 20 cm. in length.

2. Removed from ventral side after removal of the gills.

   Preservation:

   (a) Small fish. Special collections stored in glass vials with plastic stoppers preserved under 95% Ethyl alcohol. Scales included in vial till mounting.

   (b) Large fish. Otoliths removed from fish, washed briefly in fresh water, dried in a cloth and placed in a coin envelope. Envelopes are stored in cardboard boxes or i drawers.

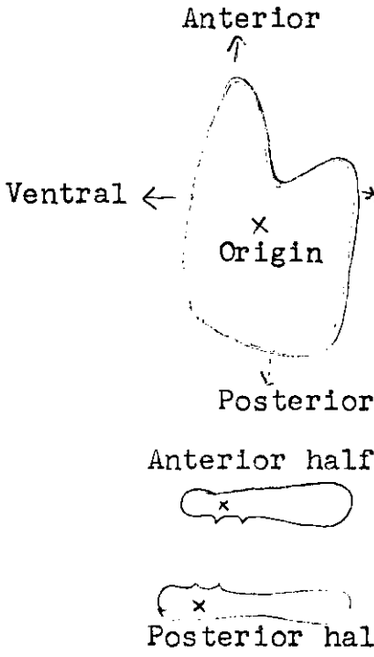
3. Routine age reading - no treatment.

4. Stereoscopic Binocular (Bausch and Lomb extendable arm) for routine reading.

5. Otoliths never read whole. Cracked transversely (dorso-ventral axis) through the origin.

Method: Otoliths held for cutting in a film of plasticine on a board (plasticine about 1/32 - 1/10 inch thick). A drop of alcohol (95% Ethyl) is first placed on the plasticine surface to prevent the otolith sticking to the plasticine and to provide some lubrication for the blade while cutting. Otolith is cut and broken by means of a well-worn safety razor blade (the type having a single cutting edge). The razor blade should be used for sharpening pencils and the edge should have a fairly sharp but saw-toothed quality. When used like a saw such a blade neatly cuts and breaks an otolith. (See following diagram.) Otolith is cut with the concave or distal surface uppermost.

Redfish Otoliths - Approximate Axes



Left hand otolith ⟶ concave surface upward

(ie.) Concave surface is distal
Convex surface having the sulcus is proximal and growth of otolith in older fish takes place mostly on this surface.

6. Routine age reading. Only with the occasional otolith is the cut surface ground flat on an abrasive stone before reading - normally no treatment.

7. The four otolith halves are embedded in a block of plasticine in such a manner that only the cut and cracked surfaces are visible and these are in the same plane as the surface of the plasticine block. A separate block is used for each fish and each block is labeled by means of a cardboard tag. The otoliths are removed from the envelopes, cut and mounted by a technician a short time before being read. The blocks are stored in wooden trays during this period.

8. For routine age reading the surface of the otolith is moistened with 95% Ethyl alcohol.

9 and 12.

Otoliths normally read using reflected light, the source usually being one or two "Spencer universal lamps" directed at an angle of about 45° to the surface of the otoliths. The brightness of the light source used is varied to suit the extraneous light conditions present at the time and the mood of the reader. The light beam is focussed to illuminate the cut and cracked otolith

.../3

surface allowing as little light as possible to fall on the plasticine block.

10. Blue filters are used.

11. An eyepiece micrometer is always used.

12. See (9) above.

13. For reading the otoliths of small fish a magnification of 13X is used (1.3 objective with a X10 wide field ocular).
When reading the otoliths of large, old fish various combinations of ocular and objective are used. No hard and fast rules are followed but the following type of increase in magnification would I think be typical.

Age (years)		
	1-15	1.3 objective and X10 wide field ocular
	15-30	3.0 objective and X10 wide field ocular
	30-50 and above	3.0 objective and X20 wide field ocular

In examining the otoliths of such large, old fish the magnification used is changed often in both counting and examining specific features of the otoliths.

14. Hyaline zones used (dark bands). Usually along the longer arm (Dorsal) of the dorso-ventral axis. This can usually only be followed clearly for about the first 12-15 years. A change in growth pattern of the otolith usually allows the later years to be more easily followed toward the proximal surface of the otolith and it is in this portion of the otolith that best counts of the later years are obtained. (See sketch).

15. Not for routine reading.

16. Scales and otoliths are not compared for fish older than about 5 years. Otoliths from fish likely to be less than 5 years old from other areas than Hermitage Bay, where many such scale otolith comparisons have been made, are normally compared against scales.

Interpretations

1. No.

2. Not as yet.

3. ---

4. Only zones concerned with change in growth pattern of the otolith (Regions).

5. ---

Terminology

Definitions.

1. Check ring. Hyaline band which cannot be regarded as a true hyaline year band.

2. Nucleus. Not used. Centre of otolith is not, as yet, fully understood. All readings are based on the "first definite hyaline year zone which corresponds to the first zone of narrowly-spaced circuli of the scales". Centre or origin is loosely used to describe the area inside the first definite

.../4

hyaline zone.

3. Intermediate zone. Not used with rigorous definition.

4. Secondary zone. Used to describe the general growth pattern of the otolith. - Regions.

5. Opaque edge. Edge of otolith made up of opaque material following previous hyaline year zone or check.

6. Hyaline edge. Edge of otolith made up of hyaline material. it is my conviction that the edge can be more clearly observed using reflected rather than transmitted light.

7. Spawning marks. Not used.

8. Otoliths graded according to readability not with regard to certainty of reading obtained. As the two aspects are obviously related the following scheme is probably representative. No analysis has been made of this yet and the following table can only be regarded as a relatively educated guess.

Index of readability and estimated reliability

Grade	Description	Maximum estimated error of age reading at the age groups below			
		1-10	10-15	15-30	30-50 /above
A	Good clear otolith, no difficulties encountered in reading.	0	±2	±4	±6
B	Clear otolith, but difficulty encountered at one or more places. Difficulties resolved with fair confidence.	±1	±3	±6	±10
C	Relatively clear. Difficulties in reading resolved, but issue still felt open to some doubt.	±2	±4	±8	±14
D	Otolith difficult to read. Tentative reading only. Not normally used in age studies.	±3	NBG	NBG	NBG=No good
E	Not readable		NOT USED		

It should be noted in connection with the table above that all otoliths of fish with ages greater than 10-15 years are read some 3 to 8 times during the one examination, all readings are recorded as well as a best estimate of age. The best estimate of age provides the age to be used while the range of readings provides information on the reliability.

Asterisks are also used to indicate exceptionally clear otoliths and otoliths which show any special features. The special features are designated by notes.

Symbols

The following system is used to designate the edge structure of the otoliths. Edge structure is normally only noted to about 10 years of age.

..../5

Age

3	-	Hyaline year zone fairly well or well developed.
3-	-	Signs of appearance of opaque edge.
3+	-	Opaque edge clear.
3+-	-	Signs of development of start of hyaline zone following opaque.
3++	-	Definite appearance of hyaline zone.
4	-	Hyaline year zone fully established.

It should be noted that the end of each zone of winter growth is coincident with the "birthdate" of the fishes and thus a straight count of the number of complete "winter" zones provides the estimate of age. Because so little growth takes place during the months January to May, it is convenient to regard the "birthdate" as in the previous January and so put the estimated ages on a calendar year basis.

Note on Scales.

Scales are only used for young fish (less than 5 years of age). They are not used per se, but only to complement age estimations from otoliths.

Scales from the small redfish are preserved in the same vials as the otoliths under 95% Ethyl alcohol. For age reading the scales are cleaned in a solution of Sodium Peroxide and mounted in a gum-arabic solution on glass slides under coverslips.

The scales are normally removed from the region on the lateral body surface situated at the end of the pectoral fin (when flattened against the body) and slightly below the lateral line.

Symbols, etc. used in reading are similar to those used with otoliths.

--ooOoo--

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Document No. 4
Appendix VI.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada
Biological Station, St. John's, Nfld.

T. K. Pitt

Plaice

1. (1) (a) Random samples from research vessels
(b) Selected categories
(c) Random samples 1 in 10, 1 in 20 from commercial landings selected by random numbers.

   (2) Length, gutted weight, sex and maturity.

2. (1) By first removing the gills and then making a cut on each side of the occipital bone.

   (2) Envelopes.

3. No treatment.

4. Low-powered binocular (Spencer cycloptic stereoscopic).

5. A mark is made across the otolith with a sharp painted scalpel and the otolith then cracked. During the cracking the otolith is placed on a thin layer of plastinine on a microscope slide.

6. No treatment.

7. The otoliths are embedded with only the broken surface showing in a small block of black plasticine.

8. A drop of 90-95% ethyl alcohol is placed on the broken surface of the otolith.

9. Reflected light from a microscope light (American Optical 6-volt focusing illuminator).

10. No.

11. No.

12. On the broken surface at about a 45° angle.

13. 15X to 20X total (Spencer cycloptic microscope).

14. The hyaline zones are counted along the long axis.

15. No.

16. No.

Interpretations

1. No, but the possibility exists.

2. No attempt has as yet been made to do so but it probably can be done.

3. ---

4. No.

.../2

5. ---

Terminology

(1) Not used.

(2) The small translucent center of the otolith.

(3) Not used.

(4) Not used.

(5) New growth or indication of a rapid growth area at the outer edge of the otolith.

(6) Translucent edge indicating a slow growth period at the outer edge of the otolith.

(7)

(8) Unreadable - impossible to distinguish between rings or in a crystallized condition.

Symbols

In reading otoliths we indicate the approximate degree of accuracy to which we consider the otolith to have been read by numbers 1 to 5, also the condition of the edge, whether transparent or opaque, is indicated. Thus 6, T, 3 indicates an otolith 6 years old with a translucent edge and read with an acceptable degree of accuracy, 6, 0, 5 indicates an otolith 6 years old with new growth at the edge extremely readable, the zones very clear, 6, T, 1 and 6, T, 2 would be rejected as being too doubtful to include in further calculations. Completely unreadable otoliths would be indicated with a dash (---).

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Appendix VII.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada,
Biological Station, St. Andrews, N.B.

Cod	Haddock	Plaice	Halibut
A. C. Kohler A. Maillet R. Robicheau	A. C. Kohler N. J. McFarlane	P.M. Powles	F. D. McCracken

Techniques

1. Random sampling of commercial catches.
Stratified sampling of survey catches.
Only length and otolith collected for commercial samples.
For survey catches we may take any combination of length, sex, maturity, stomach contents, parasites, meristic and morphometric data.

2. Otoliths are bared with the help of a splitting knife, then removed with the aid of forceps.
Otoliths from cod under 30 cm in length are stored in separate small vials in a 50% solution of glycerin with a little thymol added, or in ethyl alcohol. Those from cod larger than 30 cm are stored in brown Kraft paper coin envelopes.
This procedure is also followed for haddock.
Flatfish otoliths are kept in the above-mentioned solutions in separate vials, regardless of size of fish.

3. No treatment now. Our London, Ontario Station has just investigated the possibilities of heating. The results are evidently good. A note on the technique will be published in the Journal of the Fisheries Research Board in the near future.

4. Two types of microscopes are now in use:

   (a) American Optical "Spencer" stereoscopic with 9X eyepieces, and 1X, 2X, and 3X objectives;
   (b) Zeiss stereo-microscope with 10X eyepieces - various objectives to give 6.3, 10, 16, 25X magnification.

   We have a #6E146 Stereoscopic Binocular on order from W. Watson and Sons, England, with modified 6X eyepieces, one containing a screw micrometer and 2.5X and 5X objectives. This is to be used for measuring distances between rings on otoliths.

5. Cod and haddock otoliths are broken across the interruption in the longitudinal groove (sulcus acusticus) on the convex side. They are usually broken by exerting pressure with the thumbs and forefingers, although a sharp scalpel is sometimes used on exceptionally large and small fish.

   Flatfish otoliths are usually read without breaking.

6. No treatment of broken surface before reading.

7. The otolith is fixed in a cube of modelling clay with the fracture surface set flush with the horizontal upper surface of the clay.

.../2

8. The fracture surface of the otolith is covered with 50% glycerin solution for reading.

9. Light sources are either:

   (a) American Optical Co. Universal microscope lamp with 4.5-7.5 volt transformer;
   (b) Zeiss low voltage microscope lamp.

10. Blue filters are sometimes used on the lamps.

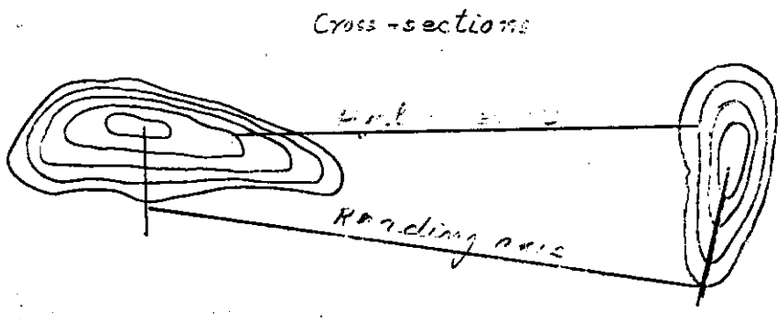
11. No other special devices are used.

12. The light beam is directed from above so that light is reflected from the surface of the otolith to be examined.

13. (a) On American Optical stereoscopic - 9 X eyepiece, 1X, 2X, and 3X objectives.
    (b) On Zeiss Stereo-microscope - 10X eyepiece, various objectives to give 6.3, 10, 16X magnification.

14. Cod and haddock are age by counting hyaline zones along the axes indicated in the diagrams.

Cod Haddock



15. No staining or sectioning.

16. Comparison of haddock scales and otoliths has been made with help of U. S. Fish and Wildlife personnel (see Kohler and Clark, J. Fish. Res. Bd. Canada, Vol. 15, No. 6). It was found that more reliable age estimates for older haddock were possible when otoliths were used.

INTERPRETATIONS

1. No definition of stocks using otoliths.

2. We think we can pick out cod spawned in unit areas 4TK, L, N.

3. Otoliths from the above-mentioned unit areas are distinguished by an unusually small first annulus.

4. We do not attempt to define special types of zones such as spawning zones at the present time.

5. ---

TERMINOLOGY

Definitions:

(1) Check ring - a hyaline ring that is not counted as an annulus - usually because of poor definition or

.../3

irregular spacing.

(2) Nucleus - the opaque centre of the cross-section of a cod or haddock otolith.

(3) Intermediate zone - not used.

(4) Secondary zone - not used.

(5) Opaque edge - the outermost material laid down at the edge of the cross-section of the cod or haddock otolith is opaque.

(6) Hyaline edge - the outermost material laid down at the edge of the cross-section of the cod or haddock otolith is hyaline.

(7) Spawning marks - not used.

(8) (a) "certain" otoliths - otoliths in which the annuli are so clearly defined as to give only one possible reading;

(b) "uncertain" otoliths - otoliths in which some check rings might be annuli or vice versa.

(c) "unreadable" otoliths - otoliths in which annuli and "check" rings are so poorly defined as to be indistinguishable.

(9) (a) Narrow hyaline - hyaline edge zone does not look to be fully formed.

(b) Narrow opaque - opaque edge zone does not look to be fully formed.

SYMBOLS

Examples below include all symbols in routine use for recording age readings at St. Andrews.

Sample reading	Definition
6+N.O. C_2	6 - is number of hyaline annuli + - indicates opaque growth at edge of otolith N.O. - indicates narrow band of opaque material at edge C_2 - indicates hyaline check ring between 1st and 2nd annuli
6+W.O.	W.O. - indicates wide band of opaque material at edge
6 N.H. C_3	N.H. - indicates narrow band of hyaline material at edge C_3 - indicates hyaline check ring between 2nd and 3rd annuli
6 S.C.	6 - indicates 6 hyaline annuli, the one at the edge being fully formed S.C. (small centre) - indicates 1st hyaline ring smaller than average - probably due to late spawning.

The birthdate of cod and haddock in Subarea 4 is taken to be February 1st. Thus our quarters of the year are: Feb.-Apr., May-July, Aug.-Oct., Nov.-Jan. In order to be able to assign fish that form hyaline

..../4

rings early in the winter to the correct age group we use the scheme explained in the table on the following page.

Time of capture: Quarter of year	Months	Number of hyaline zones	Type and width of edge zone		Age group assignation
I	Feb.	5	narrow	hyaline	5
	Mar.	5	wide	hyaline	5
	Apr.	5	narrow	opaque	5
		5	wide	opaque	5
II	May	5	narrow	hyaline	5
	June	5	wide	hyaline	5
	July	5	narrow	opaque	5
		5	wide	opaque	5
III	Aug.	5	narrow	hyaline	4[a]
	Sep.	5	wide	hyaline	5 (4[a] in Oct.)
	Oct.	5	narrow	opaque	5
		5	wide	opaque	5
IV	Nov.	5	narrow	hyaline	4[a]
	Dec.	5	wide	hyaline	4[a]
	Jan.	5	narrow	opaque	5
		5	wide	opaque	5

[a]In these cases it is assumed that a new hyaline zone has formed at the edge of the otolith before the birthday of the fish in the following February. Therefore, it is not counted as a year-zone for age-groups assignation.

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Appendix VIII

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B.

Halibut otoliths

Halibut otoliths have not been examined to any extent since a special study of halibut in ICNAF Subdivisions 4T and 4X was carried out in 1946 and 1947. No otoliths have been collected from the commercial landings (we have plans for such a program in 1960). Otoliths have been collected routinely in 1958-59 from all halibut taken during research vessel survey hauls. These have been stored in a 50% solution of glycerin with thymol added. Otoliths taken for the 1946-47 study were stored in a similar manner. We have no comparative evidence to show whether this method of preservation is better or worse than preserving dry. Otoliths collected in 1946-47 were read by reflected light, unbroken, and with the surface covered with 50% glycerin solution.

Currently we are planning more intensive work on halibut. We expect to review and examine our halibut age-reading techniques in relation to recent developments and improved equipment. Until such a program is carried out the remainder of the questions are mainly non-applicable. Presumably we will follow the listed definition of terminology wherever possible.

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Appendix IX

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Bundesforschungsanstalt für Fischerei,
Institut für Seefischerei, Hamburg,
Germany.

1. Institut für Seefischerei

2. Dr. Arno Meyer, Margot Hilden; cod and haddock.

Techniques

1. Most otoliths are taken from market landings. The rest on board of "Anton Dohrn" or trawlers. We always try to get otoliths of all sizes, especially of those sizes which are dominating in the landings or catches. For a good sample we measure 300-500 fish and from 200 we take the otoliths.

2. We only take one otolith per fish, the otolith from the right side. The otoliths are removed through the gills. On the market and when collected by one person the otolith is put into the corresponding cm-hole of the otolith-box (with 100 holes in 10 rows). When all otoliths are taken they are put into small paper bags, the otoliths of each cm-group in one paper bag. Thus each otolith in the paper bag represents one fish of that cm-group which is written on the paper bag. On board or when the otoliths are collected by more than one person each otolith is put into <u>one</u> paper bag and in this case length, sex, maturity and stomach content can be written on the paper bag, if the fish are not gutted.

3. Before reading the otoliths are cut by a special otolith-saw (copper blade with diamond dust). If there are more than one otolith in the paper bag, each otolith is numbered twice before cutting, in order that later on the corresponding halves of each otolith can be identified.

4. We use a binocular.

5. See 3

6. Not treated before reading, for the cut after sawing is better than polished.

7. We fix the halves of the otolith (mostly 6 halves in one row) in blue plastiline. The plastiline is put into a small glass-vessel (4 cm diameter, 2 cm high). Thus the otoliths can easily be moved and turned on the binocular table with the thumb and the 3rd finger of the right hand, whilst the 2nd finger can shade the light beam in a suitable way.

8. In air

9. Monla lamp or plug-in lamp on binocular

10. No filters

11. None

12. The light comes from the opposite side, nearly parallel to the surface of the cut of the otolith. The beam is directed a little bit beneath the cut of the otolith and mostly the beam is directed

..../2

to the convex side of the otolith.

13. 16^{x} objectives, 10^{x} oculars.

14. Hyalin zone (winter-ring), always all directions!

15. No

16. No

Interpretations

1. No

2. May be, but very difficult.

3-5. ---

Terminology

No

Symbols

Each otolith is classified according to its readability with 1, 2,3 and 4. 1 means very clear rings. 2 means clear rings. 3 not so good, some difficulties in age determination. 4. bad, great difficulties, results of age determination uncertain.

All otoliths classified with 4 are not used for further treatment.

When spawning zones can be defined with certainty they are tabulated.

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Appendix X.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Institute, U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Biological Laboratory, Woods Hole, Massachusetts, USA.

A. C. Jensen

Species: Haddock, Melanogrammus aeglefinus (L.).

Techniques

Our techniques are identical with those used at the Fisheries Research Board of Canada Atlantic Biological Station, St. Andrews, N. B. Mr. Jensen was trained in otolith reading at St. Andrews.

1. Twenty large haddock and 15 small (scrod), representing all sizes, are selected from each trawler's trip. The length and sex of each fish are noted.

2. The otoliths are removed through cuts made in the roof of the fish's mouth and placed dry in small envelopes. The length and sex of the fish are written on each envelope which is returned to the laboratory and stored dry.

3. The otoliths are not treated before reading.

4. A Spencer-American Optical binocular microscope is used to study the otoliths.

5. The otoliths are broken by hand as described by Kohler (1958).

6. The broken surface is not treated.

7. See Kohler (1958).

8. Submerged.

9. and 10. A 30-watt American Optical microscope lamp is used with a medium blue filter.

11. No other devices are used.

12. The lamp is attached to the microscope, between the oculars, so that the light beam is directed downward at about a 30° angle from the vertical.

13. A combination of 1X objectives and 10X oculars is used.

14. The hyaline zones are counted from the center out, along the longitudinal axis of the cross-section.

15. No staining or sectioning is done.

16. Scales and otoliths from the same fish are compared only for special studies, not for routine age determinations.

.../2

Interpretations

1. No.

2. Not in the present stage of our studies.

3. (Not applicable.)

4. Not at present.

5. (Not applicable.)

Terminology

Our otolith studies are extremely limited in scope and we have not found it necessary to use special terms. Hence, we do not have definitions for the terms listed.

Symbols

1. Edge type: Wh (wide hyaline); Nh (narrow hyaline); Wo (wide opaque); No (narrow opaque).

2. Check marks: $C_{1,3}$ (checks on the first and third annuli).

3. Age 7(8): Fish is probably 7 years but there is a strong check mark which may be an 8th annulus.

4. Age9(?): Best estimate of age from an ambiguously marked otolith.

Reference

Kohler, A. C. 1958. The validity of otolith age determinations.....J. Fish. Res. Bd. Canada, 15(6), pp. 1229-1238.

Prepared by A. C. Jensen, December 23, 1959.

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Appendix XI

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Institute, U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Biological Laboratory, Woods Hole, Massachusetts, USA.

A. C. Jensen, J. B. Skerry,

J. P. McDermott.

Species: Haddock, Melanogrammus aeglefinus (L.).

Techniques

1. Twenty large and 15 small (scrod) haddock, representing all sizes, are selected from each trawler's trip. The length and sex of each fish are noted.

2. The scales are scraped off with a knife and placed between slips of absorbent paper in small envelopes. The length and sex of the fish are written on each envelope which is returned to the laboratory and stored dry.

3. Scales from small (scrod) haddock are not treated before reading. Scales from large haddock are impressed in plastic slides (see Arnold, 1951).

4. The plastic scale impressions are projected in a Rayoscope microprojector. The scrod haddock scales are examined under a Spencer-American Optical binocular microscope.

5. (Not applicable.)

6. (Not applicable)

7. The plastic impressions and the scrod scales are placed on a mechanical stage.

8. (Not applicable.)

9. and 10. Scrod scales are examined with a 30-watt American Optical microscope lamp (with medium blue filter) as sub-stage illumination. The Rayoscope projector transmits light down through the impression.

11. No other devices are used.

12. See 9. and 10. above.

13. Microscope for scrod scales: 1. 3X objectives; 15X oculars. Rayoscope: 25X magnification (occasionally 40X).

14. The zones of narrow circuli are counted from the focus out, along the longitudinal axis of the scale.

15. No staining is done.

16. Scales and otoliths from the same fish are compared only for special studies, not for routine age determinations.

.../2

Interpretations

1. Scales are not used for stock definition.

2. We believe scale types can be defined but this has not been verified as yet.

3. (Not applicable.)

4. Three special zones can be defined:
   a. an accessory mark formed in October of the first year.
   b. the zone of narrow (so-called winter type) circuli.
   c. The zone of broad (so-called summer type) circuli.

5. The accessory mark is defined by the number of circuli (from the focus) involved and the narrow, dark appearance of the circuli forming the mark. The narrow zone and broad zone are defined only by the appearance of the circuli forming the zone.

Terminology

A few special terms are used to describe special features of the pattern of scale markings. These terms are defined below:

1. Check ring--a zone of narrow circuli composed of only two or three circuli and obviously not as wide as the adjacent zones of narrow circuli.

2. Nucleus (focus)--central point of the scale around which the concentric circuli are arranged.

3. Intermediate zone--(no definition).

4. Secondary zone--(no definition).

5. Opaque edge (winter-type edge in scales)--edge characterized by a zone of narrow circuli.

6. Hyaline edge (summer-type edge in scales)-- edge characterized by a zone of broad circuli.

7. Spawning marks--(no definition).

8. Certain, uncertain, unreadable scales--
   a. certain--annuli (zones of narrow circuli) distinctly different from adjacent zones of broad circuli, well separated and readily identified.
   b. uncertain--annuli only slightly different from broad circuli; one or more annuli narrow, having appearance of check rings.
   c. unreadable--scales with regenerated centers or badly eroded and damaged edges.

9. No other terms or symbols are used.

Reference

Arnold, E. L. 1951. An impression method for preparing fish scales for age and growth analysis. Prog. Fish-Cult. 13(1): pp. 11-16.

Prepared by J. P. McDermott, December 23, 1959.

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Appendix XII.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Institute, U. S. Department of the Interior, Fish and Wildlife Service, Bureau of Commercial Fisheries, Biological Laboratory, Woods Hole, Massachusetts, USA.

Redfish

George F. Kelly and Allan M. Barker

Techniques

1. A random sample of 100 fish is taken from interviewed fishing trips known to have fished in a single statistical area. Data recorded includes fork length to nearest millimeter, sex, gonad condition and incidence of parasite Sphyrion lumpi.

2. Otoliths are removed by cutting through the skull from the mid-dorsal point laterally across the preopercle. The cut exposes the otoliths so they can be picked out with forceps. They are dry-stored in coin envelopes. Some special collections are stored in 90 percent ethyl alcohol.

3. Dry-stored otoliths are cut for reading whereas the wet-stored otoliths are read whole.

4. Bausch and Lomb or Spencer binocular microscopes with stage light.

5. Otoliths are cut across the long axis with a dull single edge razor blade using a plastic strip as a cutting block. The blade is sawed across the otolith until it fractures along the line of cutting.

6. The broken surface is ground smooth on the flat side of a one-inch high-speed, carborundum cutting disk, and a small drop of glycerin is placed on the cut surface.

7. The sectioned otolith is inserted into a slot in a small cork stopper.

8. The whole otoliths are read submerged in 70 percent alcohol under direct light against a black background. The cut otoliths are read in air.

9. A variable intensity microscope stage light is used employing a 30 watt transformer.

10. No light filters are used.

11. No special devices are used.

12. The light is directed against the side or end of the otolith and the surface is shaded with a shield. The otolith may be rotated to attain better illumination of the year zones.

13. Between 12 X and 15 X magnification is generally used, occasionally higher to 30 X. (oculars 10 X to 15 X, objectives 0.7 to 3.0).

14. Pairs of opaque and hyaline zones are counted, generally along the long axis of the otolith. The zones are traced around the sides of the long axis whenever possible.

.../2

15. No staining technique is employed. Many stains have been tested but none was successful in staining the otolith zones differentially. Sectioning is difficult and time consuming. It would not improve redfish otoliths sufficiently for age reading to warrant the added effort. Sectioning would be worthwhile for preparing otoliths for photography where greater flexibility of lighting is possible with thin sections than with otolith halves.

16. Not routinely - only on special studies.

Interpretations

1. Otoliths are not used for definition of stocks at present.

2. No systematic work has been done on definition of otolith types but this would appear to hold some promise.

3. Nothing to report.

4. Year zones representing growth before first maturity can be distinguished from those of later years. More work must be done on this

5. Nothing to report.

Terminology

1. Check ring - this term is not used in our work. Year zone is used to denote one year of growth in the otolith represented by an opaque and a hyaline zone. I question whether check ring should be proposed for universal usage since it is an inexact term that is only loosely associated with age and growth.

2. Nucleus - this term applies to the smallest opaque zone at the center of the otolith. Focus is sometimes used to indicate the first center of otolith growth.

3. and 4. Intermediate zone and secondary zone are not used in our work.

5. Opaque edge - indicates that opaque material comprises the major portion of the otolith periphery. Opaque material is deposited during fast or summer growth.

6. Hyaline edge - indicates that hyaline material, representing slower winter growth, comprises the periphery of the otolith.

7. Spawning marks - spawning zone is used in this reference in our work. It denotes a change in the year zone form, generally between the 6th and 12th zones. Both the opaque and hyaline zones become narrower than before and the subsequent year zones become more uniform in size and form.

8. These terms are little used with redfish. Most otoliths having more than 6 or 8 year zones are read with some degree of uncertainty. Unreadable otoliths are those in which a portion of the material is not differentiated into zones or is separated into zones in a confused pattern that permits either high or low counts.

Symbols

No special symbols are used to designate features of the otolith zones.

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Appendix XIII

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Scottish Home Department, Marine Laboratory, Aberdeen.

A. D. McIntyre.

Halibut

Techniques

1. From market samples. All the fish in an 8 st. box (usually 50-150 fish) are otolithed, measured, weighed and sexed. From research vessels - all fish not required for tagging are otolithed, measured and sexed.

2. Otoliths removed from fish by cutting diagonally through the head from just behind the eyes. Extracted with forceps. Kept dry in envelopes in partitioned cardboard boxes.

3. If not read immediately on extraction they are first soaked in water for 2 hours.

4. A small hand lens x10.

5-13. Otoliths read whole, in water, against a black background. Lighted by general illumination from a Prior microscope lamp without filter.

14. The hyaline zones are counted and an attempt is made to follow these round the whole otolith.

15-16. No.

Interpretations

1-3. Otoliths can be arranged into types on the basis of general shape and length/breadth ratio and presence or absence of double rings, but these types seem often to be mixed in single samples and it has not yet been found possible to use them for stock differentiation.

4-5. No.

Terminology

Numbers 1, 3, 4, 7 not used.

2. Nucleus - the part inside the first ring.

5. Opaque edge - the white appearance of the edge when the black viewing background is not able to shine through due to the structure of the otolith. Usually found during the period of summer growth.

6. Hyaline edge - the dark appearance of the edge when the black viewing background is able to shine through due to the structure of the otolith. Usually found in winter, but sometimes occurs when you least expect it.

8. Certain otoliths are those that can be read with confidence. Uncertain otoliths are those which for one reason or another have to be read as x+ a number of years. Unreadable otoliths can't be read even with confidence.

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Appendix XIV.

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Scottish Home Department, Marine Laboratory,

Aberdeen.

Ray Gamble

Whiting. (G. merlangus)

Techniques

1. From market samples - 50 fish are chosen at random, otolithed, measured and weighed. Of the remaining fish in the box, which holds 8 st., ten at each centimetre length are otolithed and measured. From research vessel hauls - up to 5 fish at each centimetre length are otolithed, measured and sexed.

2. Otoliths are removed from the fish by cutting diagonally down through the head from a point on the dorsal surface just behind the eyes, through the hind part of the brain. The otoliths are then extracted with forceps.

   They are kept, dry, in envelopes, and stored in partitioned drawers.

3. Untreated.

4. Beck binocular microscope.

5. Otoliths are broken by snapping them across transversely with the fingers..

6. The broken surface is brushed with turpineol before reading. Occasionally it is ground flat before applying the turpineol, if theotolith is difficult.

7. The otolith is stuck upright in putty.

8. In air.

9. Prior microscope lamp.

10. Yes; yellow.

11. The broken surface is shielded from the light by a finger, so that the light shines only through the lower part of the otolith.

12. Horizontally; and see 11.

13. Magnification X25. Objectives - 49 mm; oculars - wide field X10.

14. The hyaline zones are counted. For direction, see line marked on figure.

15. No.

16. No.

.../2

Interpretations.

1. No.

2. Yes.

3. A distinction can be made between the otoliths of whiting in the Clyde area and those in the North Sea. The former are much more difficult to read, because the first two winter zones tend to be made up of many fine hyaline rings with little summer growth separating them. The corresponding winter growth in North Sea whiting are much more solid in appearance, with the second winter zone commonly having a double structure.

4. No.

5. -

Terminology

Some special terms are used.

1. Not used.

2. Nucleus or false ring - a small hyaline ring lying inside the first winter zone, and probably laid down when the young fish adopt the bottom habitat.

3. } Not used.
4. }

5. Opaque edge - the appearance of the edge when a new summer ring is being laid down. It is not easy to see using transmitted light, in which it appears as a dark rim to the otolith.

6. Hyaline edge - the appearance of the edge when a new winter ring is being laid down. Appears as a light coloured rim in transmitted light.

7. Not used.

8. Certain otoliths are those with clear and distinct winter zones, which can be easily counted.

   Uncertain otoliths are either those with indistinct hyaline zones which are difficult to count; or otoliths with two or more hyaline rings so closely spaced that it is doubtful if they represent separate winters' growth.

   Unreadable otoliths are those in which it is impossible to differentiate between successive winter zones, so that they cannot be counted.

9. Double ring - where the winter zone is made up of two hyaline rings separated by a more opaque area. Commonly found in the second winter ring of North Sea whiting.

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APPENDIX --- XV

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Techniques, interpretations and terminology used for age reading and stock definition of cod and haddock otoliths.

O. Annaniassen	cod
O. Bostrøm	haddock
R. Hovland	cod
A. Frøland	cod

Techniques

1. Onboard the research vessels every fish of the catch is measured before the otoliths are collected. Then the otoliths of ten fish from each 5-cm group are taken. The otoliths are put into small envelopes where length, sex and stage of maturity of the fish are written. In Lofoten one part of the catch, that makes about hundred fish, is measured and the otoliths are put into envelopes where data are written as above.

2. Usually the otoliths are removed by cutting through the otic bones from the inside. On smaller fish a cut from the bridge of the nose through the eyes into the otoliths is used.
The otoliths are preserved in small envelopes which are placed in a box, one sample in each box.

3. The otoliths are kept in the boxes untill the reading.

4. For reading following stereo-microscopes are used: Zeiss opton, Leitz and Reichert.

5. The otolith is broken either with fingers or by means of a pair of pliers, through the nucleus.

6. The broken surface is often moistered with a mixture of half and half glycerine and water.

7. The otolith is fixed on the microscope table on a bit of mouldingwax.

8. The otolith is read in air.

9. As light source are used microscope-lamps (Zeiss, Reichert).

10. A yellow filter is used for the reading.

11. No other special devices are used to facilitate the reading.

12. The light beam is directed partly on the side of the otolith broken surface, and shaded with a pencil or one finger so that the surface comes in shadow.

13. The magnification used is about 15-16x.

14. When reading the age, the opaque zones are counted, usually from nucleus to the concave edge and from nucleus to both tips.

15. Staining or sectioning is not used.

16. Scales and otoliths from the same individuals are not compared.

.../2

Interpretations

1. Otoliths have been used to distinguish between different stocks, i.e. the coastal cod and the "skrei".

2. We have defined following otolith types:

   a. Type I } "Skrei"
   b. Type II }
   c. Coastal cod

3. Type I: The zones are uniform in structure, and the opaque and hyaline zones are sharply delimited from each other. Regularity characterizes the otolith.

   Type II: Both opaque and hyaline zones are "split" by hyaline and opaque material respectively.

   Coastal cod: The bright nucleus and the shape of the first year are characteristic.

4. Special types of zones are not defined.

5. None.

Terminology

(2) The opaque material in the centre of the otolith is called nucleus.

(5) The otolith has opaque edge if the edge is of opaque material.

(6) The otolith has hyaline edge if the edge is of hyaline material.

(7) The hyaline rings, which are more bringth than the inner ones and often wider than the opaque rings, are called spawning marks.

R. Hovland D. Møller

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APPENDIX --- XVI

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading.

Fisheries Researches of Greenland

Paul M. Hansen and Inge Meldal

COD

Techniques

1. Representative samples of commercial catches. Catches from research vessels.

   From commercial catches otoliths are collected, together with total lengths and sex.

   From all catches of research vessels are collected otoliths, total length, sex, and sometimes weight and stomach contents.

2. A transverse cut is made with a knife through the skull just behind the eyes and obliquely backwards. The otoliths are extracted with forceps and stored in small bags.

3- 4. The otoliths are broken across with the fingers. If the break is wrong pincers are used.

5. Zeiss stereo-microscope (Zeiss Opton). Ocular 10x. Objective 16x.

6. No treatment. In very rare cases the broken surface is ground with a fine rotating stone.

7 + 8. The otoliths are fixed in a fine clamp, which is held in the right hand, making it possible to turn the otolith so that the light falls on to the broken surface at various angles. During the reading the otolith is submerged in 96% alcohol.

9. Zeiss low-voltage microscope lamp.

10. No filter.

11. Obliquely in-falling light (about 45°).

12. See 5.

13. Hyaline zones. Reading along an axis across the cut from the centre towards the concave surface and along the longitudinal axes of the broken surface from the centre in both directions.

14. See 7 and 8. Sometimes shading with a finger is necessary to ensure that the light is falling through the otolith.

15. No.

16. No.

.../2

Interpretation

1. No.

2. Otoliths of cod from certain fjords can be distinguished from otoliths of cod from the banks.

3. Otoliths of fjord cod have indistinct hyaline zones and many check rings. Frequently the hyaline zones are placed very densely and are very narrow; this is caused by slow growth.

4 +5. Spawning zones can most easily be distinguished in cod which have spawned several times. In otoliths of first spawners, where the spawning zone is placed at the edge, it may be difficult to distinguish it as a spawning zone. The spawning zones are narrow and the hyaline zones are distinctly depicted. (see attached photograph).

Terminology

1. Check rings are often indistinct and incomplete.

2. The nucleus is found only in few otoliths. It shows as a small distinctly delimited kernel, often of yellow colour.

3 -6. No special definition.

7. See "Interpretation" 4 and 5.

Symbols

No special symbols are used.

Halibut

(Dr. Paul M. Hansen and Sv. Aa. Horsted)

Techniques

1. Catches from research vessels (long-lines and a few hand-lines). From the whole catch otoliths are collected together with information on total length, sex, eventually stomach contents and weight. Sometimes the colour of the blind side (clear white or grey is also noted).

2. Extraction - as for the cod. Kept dry in small bags.

3. Nothing special.

4. Read whole, for supplement sometimes one otolith is broken across.

5. Zeiss stereo-microscope (Zeiss Opton).

6. No treatment.

7. The otolith is held by a small clamp so that its position can be varied.

8. Submerged in 70% alcohol.

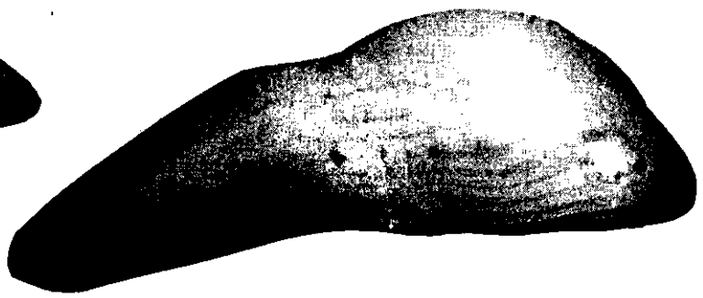
9. Zeiss low-voltage microscope lamp. Concentrated light beam.

.../3

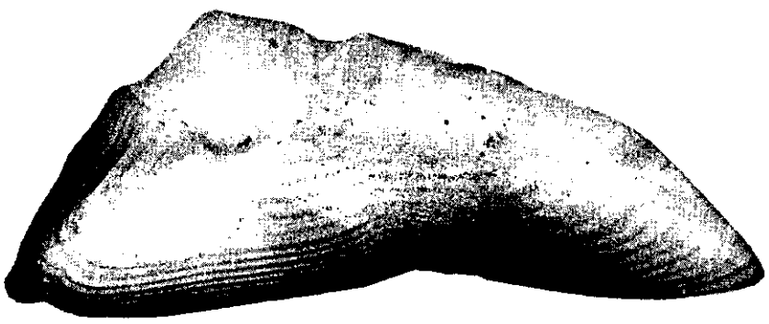
Otolith Number 8, 27 July 1957
37 cm, male, 3 years.
(check rings)

Otolith Number 12, 28 July 1957.
67 cm, male, 7 years.

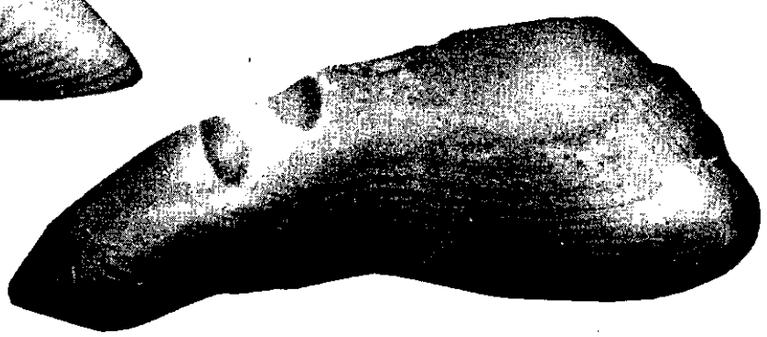
Otolith Number 14, 28 July 1957.
74 cm, male, 8 years.



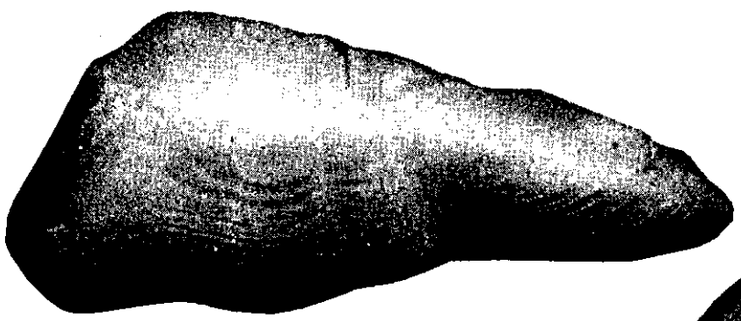
Otolith Number 15, 27 July 1957.
79 cm, female, 9 years, Spawning 8



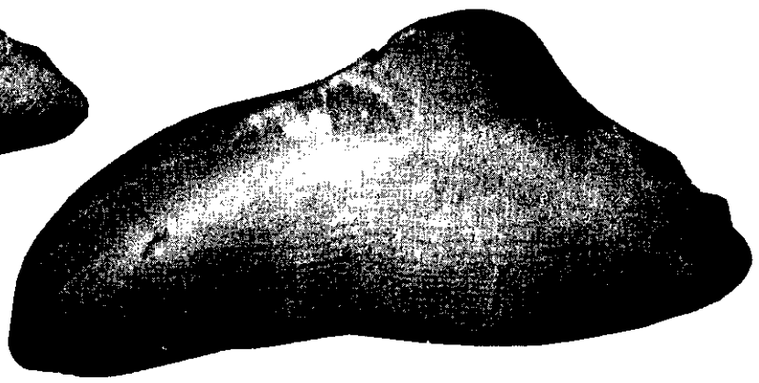
Otolith Number 22, 28 August 1957.
88 cm, female, 15 years, Spawning 7



Otolith Number 23, 27 August 1957.
94 cm, female, 16 years, Spawning 7



Otolith Number 35, 9 April 1957.
80 cm, female, 17 years, Spawning 6 or 9.



Otolith Number 35a, 14 September 1957.
131 cm, female, 21 years, Spawning 8.
(2 innermost rings disturbed by shadow).

10. No.

11. Transparent light. If broken surface is used, eventually reflected light. When this transparent light is used, the surface is read but only one surface can be used.

12. Ocular 10x. Objective 16x.

13. Both hyaline (mainly in reflected light), and opaque (mainly by transparent light). No special direction.

14. No.

15. No.

16. Only otoliths used.

Interpretation

1. No.

2-5. Do not know, experience and material too limited.

Terminology

No remarks as experience and material are too limited.

Symbols

No remarks.

Redfish

(Dr. Paul M. Hansen and Sv. Aa. Horsted)

Techniques

1. Catches from research vessels. Most catches are taken with shrimp trawl, a few by hand or long line.
The whole catch is measured. Now and then otoliths are collected and the whole catch, or a representative part of it, if it is more than 200 specimens, is sexed. For some samples a bone postcleithrum from the shoulder skeleton is used as well as the otoliths.
In a few samples additional weight determinations and analyses of stomach contents are made.

2. The otoliths are extracted as for the cod. Normally they are kept dry in small bags, but it seems to be better to keep them in sea water for about two months and then to transfer them to 70% alcohol. If they are kept in sea water for more than two months, the decomposition is too strong and the enclosed label may be destroyed. When transfering into alcohol, the label must be replaced. The advantage of keeping the otoliths in sea water for some time is that then they are more easily broken in the correct way, and the reading of them is possibly easier - at any rate not more difficult.

3nd4. Otoliths which have been kept dry are deposited in 70% alcohol for about half an hour, after they have been broken across with the

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fingers. Small otoliths are broken with the fingernails, the larger eventually with pincers.

5. Zeiss stereo-microscope (Zeiss Opton). Ocular 10x. Objective 16x (eventually 25x).

6. No treatment.

7. The otoliths are held with a small clamp so that the position can be varied until the light is at its best.

8. Submerged in 70% alcohol.

9. Zeiss low-voltage microscope lamp. Concentrated light - almost spotlight.

10. No.

11. Oblique, nearly horizontal reflected light. The flat surface of the otolith is farthest away from the light. With the finger or with the small clamp a more or less shading of the light can be achieved. Eventually transmitted light is used until the lighting is the very best possible. In most cases a strong reflected light is the best.

12. Ocular 10x. Objective 16x, sometimes 25x.

13. Normally from the centre towards the flat side of the otolith along the line lying between the cross axis and the shortest length's axis. Possible corrections in other directions. Most often hyaline zones with some kind of illumination if opaque.

14. See above (2, 7, 8, 11 and 13).

15. No.

16. Scales cannot be used. In a few samples comparison of the otolith with the postcleithrum has been made. The postcleithrum is kept dry. During drying rings show up which possibly are annual rings. Good agreement between otolith and postcleithrum up to six years. For older redfish, the innermost rings on the postcleithrum are difficult to distinguish - most often it is impossible to do so. Kept dry in small bags, or wet in small glasses (70% alcohol).

Interpretation

1. No.

2-5. Do not know. Material and experience too limited.

Terminology

1-7. No special definitions. Too limited experience.

8. Certain: both otoliths show - read in various directions - the same result. If two readers, both get the same results.

Uncertain: The two otoliths read in various directions give results which vary with plus or minus 2.

Unusable: Greater variation than plus or minus 2.

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Symbols

Age without symbol: certain. This includes possibility of error up to plus or minus 1.)

Underlining of age: absolutely certain.

Question mark after age: uncertain.

Question mark without age: unusable.

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APPENDIX ---
XVII

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Fisheries Laboratory, Lowestoft, Suffolk, England.

G. C. Trout	redfish, cod
R. Blacker	haddock
B. Jones	coalfish
R. Margetts	plaice
M.J. Holden	cod and genereal co-ordination of methods and techniques within the laboratory.
J.A. Gulland	(Statistician responsible for target allocations).

Techniques

I Cod

1 A. Sampling of commercial catches.

a. Initial sampling is by areas. White Sea (Region I) Norway Coast (IIa) Bear Island and Spitzbergen (IIb), North Sea (IV) Iceland (Va) and Greenland (XV).

b. Stratified sampling of the fish on the market is used. For regions I, IIa, IIb and Va monthly target figures are 30 otoliths from 40 cm fish, 40 otoliths from 50 cm, 60 cm and 70 cm fish, 30 otoliths from 80 cm fish and as many as possible from 90 cm and 100 cm fish. From region IV the target is 10 otoliths from each 10 cm length group. There is no set target for region XV.

A few otoliths are taken each day so that sampling is spread over the ships landing within a month. This also ensures that all the grounds within a statistical region are sampled. There are no rules governing this procedure it being left to discrimination of the collectors except that not more than five otoliths in any one 10 cm length group are taken from the fish landed by any one ship.

c. Data collected. Port of landing, date of collection, name of ship, region, statistical square in which fishing occurred, total length of fish.

B. Sampling from research vessel catches.

Stratified sampling is employed with the same targets as stated above but no more than five fish in each ten centimetre length group are sampled at any one station unless fishing occurs at one station for several days.

The data collected are:- Date of collection, name of ship, cruise number, station number, serial number of otolith, total length of fish, sex and maturity as immature, maturing or mature.

2.A. Removal

This varies according to the whim of the collector except with collection from commercial samples when the brain case is cut away from around the otolith, access being obtained through the opercula opening. This is to prevent disfigurement of the fish because the fish are not bought by the Ministry.

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Other methods employed are:-

a. Cutting vertically between the eyes with a knife or guillotine.

b. Cutting transversely behind the eyes - this method is liable to damage the otoliths if the cut is misjudged.

c. Cutting the top off the head by inserting a knife through the eye sockets and slicing backwards.

B. Preservation

Placed into small envelopes two inches square, the envelopes being kept in boxes 12" x 10" x 2" divided internally into four compartments 12" x 2 1/2" x 2".

3. Treatment. None

4. Low power binocular microscopes.

5. The otolith is broken transversely across the middle using the fingers. If the otolith is difficult to break it is sawn in two using an electrically driven, water cooled jeweller's saw.

6. If the break is satisfactory no treatment is given. If it is rough the surface is painted with xylol to improve the contrast. If the break is very rough or does not pass through or almost through the middle of the otolith it is ground down using a fine carborundum stone driven by an electric motor.

7. Vertically using a "Plasticene" mount so that the broken surface is observed. The otolith is read by transmitted light. (See photograph 1 and 2).

8. In air.

9. High powered filament lamp, 6V 8 amp run from mains through a variable transformer.

10. No.

11. A horizontal bar is used to screen the broken surface of the otolith from direct light. This bar is moveable in the vertical plane. A shield is used to prevent light falling upon the otolith from other sources except the lamp.

12. The light beam is directed horizontally to strike the otolith approximately at right angles to the vertical axis just below the shielded, broken face. (See photograph 1).

13. x5 wide angle eyepiece and 54 mm objectives. Total magnification is approximately x14.

14. This depends upon the whim of the observer, some counting the hyaline zone others the opaque zone. No particular axis is used that giving the clearest reading being used and checked off against other axes.

15. No.

16. No.

Interpretations

1. Not normally. When reading Icelandic otoliths an attempt is made to differentiate fish from Greenland by using differences in oto-

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lith structure and age/length relationships but this differentiation cannot be done with certainty.

Mr. Trout in a study of the Spitzbergen-Bear Island cod used differences in otolith structure to split fish into three sub-populations (see Trout 1957). The Bear Island Cod. Migrations and Movements. M.A.F.F. Fish Invest. Ser. II, Vol. XXI No. 6).

2. Yes.

3. All segregation of otoliths into types is done by attempting to classify patterns of otolith growth. In the example referred to in "1" above Trout used variations in the opaque zone, calling uniform opaque zones with one, or occasionally two, hyaline check rings (see "Terminology' for definition) as "Western-Split" and opaque zones merging into each other "Eastern", the terms Western, Western-Split and Eastern referring to the areas in which the pattern was most common. See photographs 3 and 4. Such segregation is always difficult because there is always considerably variation from the chosen type pattern which makes classification very difficult.

On the broader question of separating stocks it is possible with experience to differentiate between otoliths from fish from the main fishing area e.g. between North Sea and Bear Island Cod, (Compare photographs 5 and 3) but not between fish from two areas within the North Sea. This is only of academic interest because the area from which the otoliths are collected is known. The exception is that of Icelandic otoliths where it is desirable to know the admixture of fish from Greenland. Otoliths from Greenland fish are regular with well defined annual opaque and hyaline zones with few if any splits compared with Icelandic otoliths that have typically ill defined zones with many splits. (Compare photographs 6 and 7).

4 and 5. The term zone in this laboratory is used entirely in the connection opaque, hyaline or spawning zones. (See "Terminology").

It is however possible from time to time to distinguish certain years by peculiarities in the structure of the otolith. 1954 was a good year for plankton production in the Arctic and some cod otoliths from Region IIb show an exceptionally large opaque zone. This is more noticeable because the preceding two years 1952 and 1953 were years of below average plankton production and the otolith growth was less than normal. Where this structure occurs in an otolith it is possible to use it as a check against the age determination by counting zones only and it is useful in older fish where the outer growth may be difficult to interpret. This check can only be used positively because not all otoliths showed the same pattern of growth for these years, i.e. some fish whose otoliths showed them to be definitely spawned in 1952 had normal sized 1952, 1953 and 1954 rings. (Photograph 8).

Terminology

1. Checking. Any hyaline zone laid down that is considered not to be an annual hyaline zone. This definition assumes that the normal pattern of otolith growth is one opaque zone, normally laid down in the summer and one hyaline zone, normally laid down in the winter, each year. Any hyaline zone within the opaque zone is a check ring. (See photograph 4).

2. Nucleus. The whole of the first year's growth of the otolith, including all opaque and hyaline zones. (See photograph 4).

..../4

3 and 4. Terms not used in this laboratory.

5. Opaque edge. An otolith edge appearing dark by transmitted light and bright by reflected light. When high light intensities are used an opaque edge often appears light by transmitted light due to the general diffusion of light. (See photograph 5).

6. Hyaline edge. An otolith edge appearing light by transmitted light and dark by reflected light. (See photograph 3).

7. Spawning marks. These are referred to as spawning zones and are regular patterns of hyaline and opaque zones, the pattern commencing with wide hyaline zone corresponding to the winter preceding spawning followed by a very narrow opaque zone corresponding to the summer of spawning. Further narrow hyalines and opaque zones then follow according to the number of times the fish has spawned. Typically the hyaline zones are very bright, by transmitted light, and wider than the dark opaque zones. (Photograph 9).

8. Certain otoliths. No doubt about age determined from otolith, all observers agreeing. (Photographs 3, 5, 6, 8 and 9).

Uncertain otoliths. Readable but leaving the interpretation of some of the hyaline and opaque zones open to question so that it i only possible to state the probable age of the fish from which the otolith was taken with probable alternatives. (See photograph 7).

Unreadable otoliths. Impossible to state even a probable age of the fish from which the otolith was taken due either to an absence of hyaline or opaque zones or the presence of an irregular sequence of zones which fits with no annual pattern.

9. Other terms a. "Split". A narrow hyaline zone occurring within a wide opaque zone or vice versa. When the split is a narrow hyaline zone in an opaque zone the terms "split" and "check ring" are synonymous although the term "split" is usually applied to a narrower zone than "check ring" although this differentiation is subjective. (Photograph 5).

b. Official birthday. The day upon which a fish is presumed to have been spawned. It usually bears some relation to the actual spawning period. For all cod it is taken as 1st January. Thus assuming an otolith pattern of growth of one opaque summer zone laid down between 1st April and 30th September and one hyaline winter zone laid down between 1st October and 31st March an otolith showing the following pattern upon examination would be aged as follows:

Date of capture of fish	Number of opaque zones	Number of hyaline zones	Type of edge	Age
mid February	2	2	Hyaline	II
mid June	3	2	Opaque	II
mid November	3	3	Hyaline	II
31 December	3	3	Hyaline	II
1 January	3	3	Hyaline	III

Symbols

1. Age. This is always written in Roman numerals. In the case of uncertain otoliths the probable age is written first followed by the probable alternative which is queried e.g. V ? VI.

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2. Spawning. The symbol "S" is used to denote a fish that is assumed to be about to spawn and is detected by the presence of the bright pre-spawning hyaline zone (See Terminology (7) above). The symbols S1, S2 etc. are then used to denote fish that have spawned once, twice etc. and have completed the full year's growth of the year of spawning. Assuming a fish with the same otolith pattern of growth as in the paragraph on "Official Birthday" the symbols would be used as follows:

Date of capture of fish	Number of* opaque zones	Number of* hyaline zones	Type of edge	Symbol
1st January	0	1	Bright hyaline	S
August	1	1	Narrow opaque	S
31st December	1	2	Hyaline	S
1st January	1	2	Hyaline	S1

*Only spawning zones are referred to

Where the official birthday is set later in the year than 1st January e.g. 1st May the symbol "S" is used for the period of twelve months, from the time the pre-spawning edge is first visible, after 1st May until a complete spawning zone has been completed by the 30th April twelve months later. The sequence is thus shifted five months later than in the above table.

Techniques

II. Plaice

1. Only sampling of commercial catches from one area, the North Sea, is carried out.

The following stratified sampling is carried out on fish bought from the market.

Port cm length grouping	Lowestoft No. of fish each month	Grimsby No. of fish each month
25-29	30	20
30-34	30	30
35-39	30	30
40-44	20	30
45-49	20	20
50-	20	20

No more than three otoliths in any one 5cm length group are taken from any one ship. Otherwise the fish are selected at random throughout the month.

The data collected are total length, sex, weight and gonad weight.

2. The otoliths are removed by cutting vertically down between the

...../6

eyes with the fish in its normal position i.e. white side down. Cutting is facilitated by placing the fish on a notched board. The otoliths are preserved as with cod.

3. Treatment. None.

4. Low power binocular microscopes.

5. Read whole.

6. Inapplicable.

7. The otolith is placed in a black solid watchglass and read by reflected light.

8. It is immersed in water.

9. High powered filament lamp, 6V 8 amp run from mains through a variable transformer.

10, 11. No.

12. The beam is directed down at an angle of approximately 45° from the horizontal on to the whole otolith.

13. Magnification is x7 oculars with x2.5 objectives.

14. This depends upon the whim of the observer, some counting the hyaline zone others the opaque zone. No particular axis is used that giving the clearest reading being used and checked off against other axes.

15, 16. No.

Interpretations

The otoliths are used solely for age determination and no attempt has been made to used them for defining stocks or to classify them into types.

Terminology

(2) Nucleus. The central opaque zone up to but excluding the first hyaline zone that corresponds in the opinion of the observers to the first winter. In the simplest cases the nucleus is the opaque core of the otolith but in other cases the nucleus may contain some hyaline zone, if, again in the opinion of the observer, these hyaline zones do not correspond to winter growth. (See photographs 10, 11 and 12).

5 and 6. As for cod.

The other terms are not used.

The term "split" is used as defined for cod.

III Redfish.

Techniques

1. Fish are sampled on research vessels only and otoliths are taken from fish selected as required by the research workers' programme. The data collected are as for cod on research vessels.

.../7

2. The fish are deep frozen on the research vessel and their otoliths removed direct into tubes of 70% alcohol. The tubes are numbered, the data being kept on sheets.

3. Treatment. None.

4. Low power binocular microscopes.

5. Read whole.

6. Not applicable.

7, 8. As for plaice accept that the otolith is read in alcohol.

9. High powered filament lamp, 6V 8 amp run from mains through a variable transformer.

10, 11. No.

12. The beam is directed down at an angle of approximately 45° from the horizontal on to the whole otolith.

13. Magnification is x7 oculars with x2.5 objectives.

14. Opaque zones.

15, 16. No.

Interpretations

The otoliths have been used for age determination only. Their gross structure i.e. length in relation to fish length has been used for speciation purposes but no interpretation of the type requested in questions 1-5 has been attempted.

Terminology

As for cod except that spawning marks have not been noticed.

IV. Haddock

Work on haddock otoliths has only recently started and the techniques employed to date are those for cod. No special terminology or interpretations have been adopted.

Stratified sampling is employed, ten fish from each 10 cm length group a month being taken from two areas Regions I and II combined and Region Va.

V Coalfish

The same remarks apply as to haddock but no routine samples are taken, otoliths being taken on research vessels as and when available.

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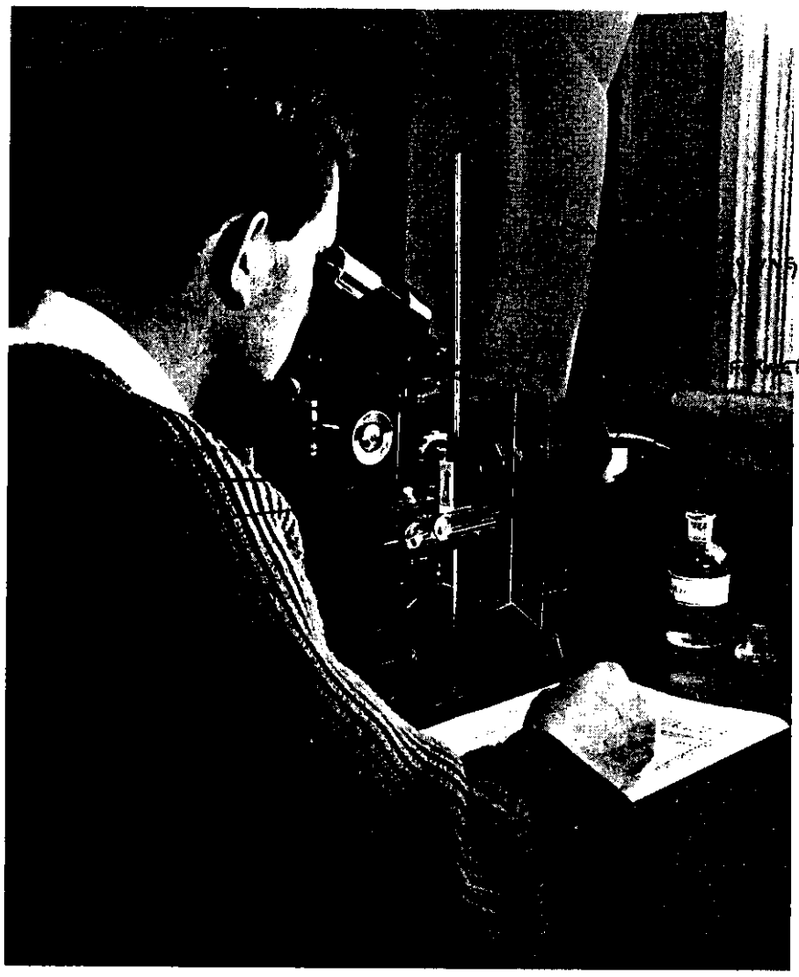

Fig. 1. Microscope set up for reading cod, haddock and coalfish (saithe) otoliths.

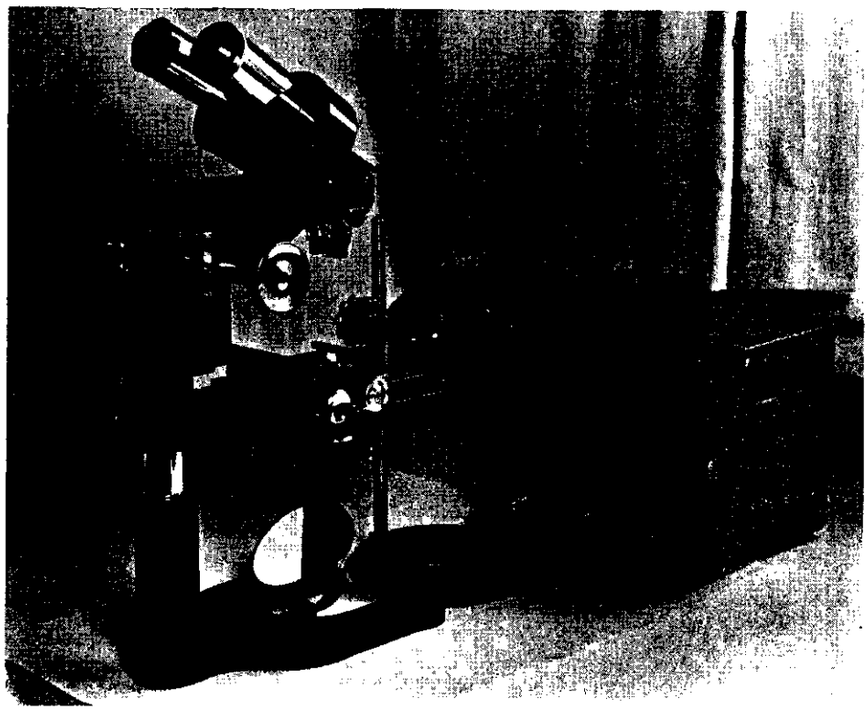

Fig. 2. Close-up of microscope set up for reading cod, haddock and coalfish otoliths.

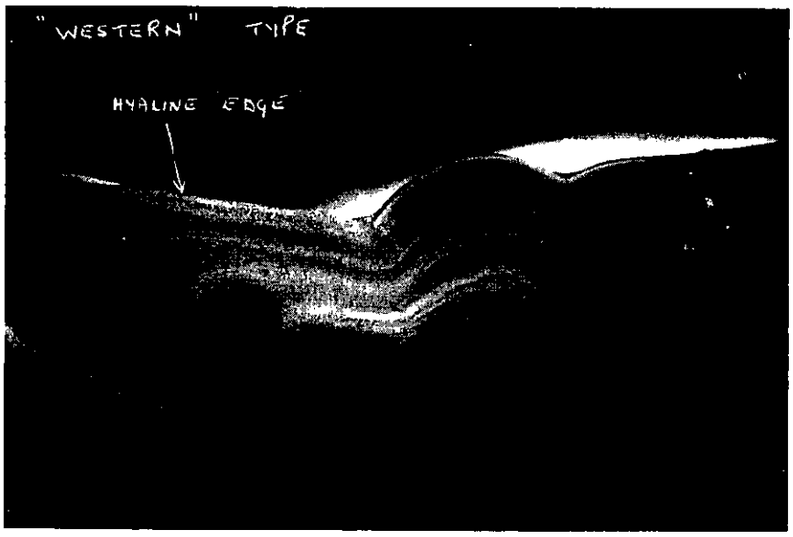

Fig. 3. Cod, 40 cm. (approx.); Region IIB, Bear Island, Spitzbergen; **age 4 years;** April (approx.).

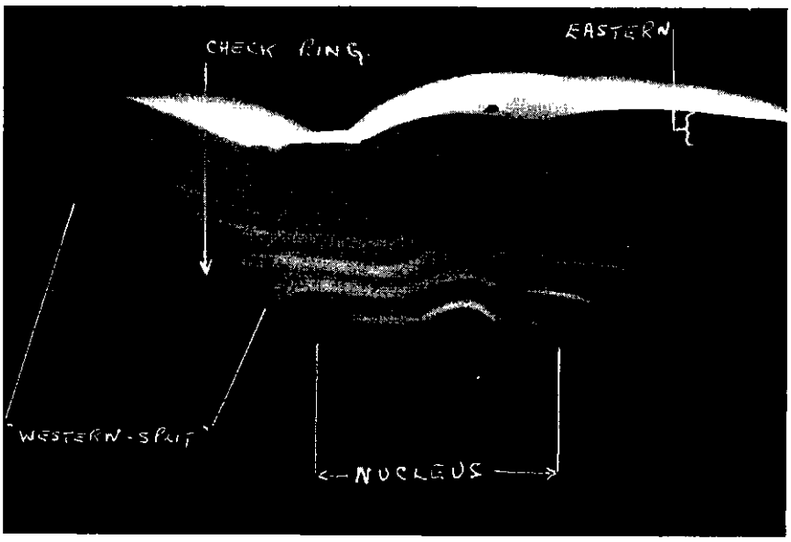

Fig. 4. Cod, 85 cm. (approx.); Region IIB, Bear Island, Spitzbergen; **age doubtful;** March (approx.).

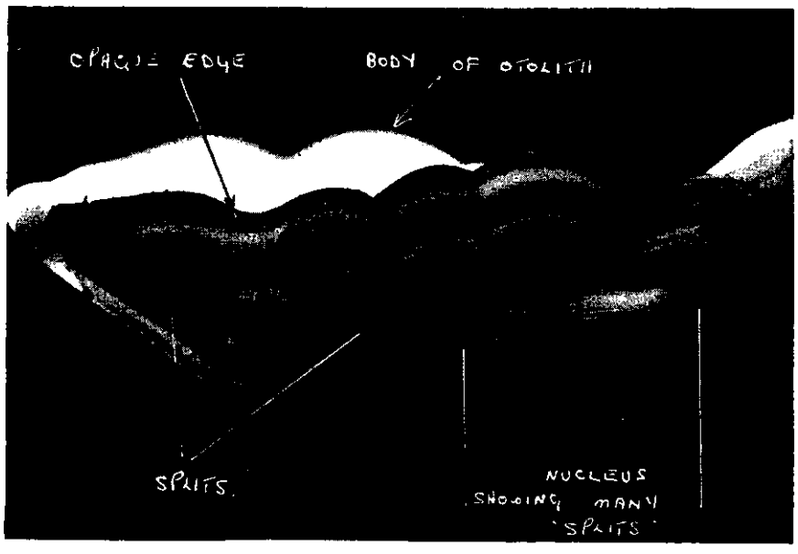

Fig. 5. Cod, 69 cm.; Region IV, North Sea; age 3 years; March, 1950.

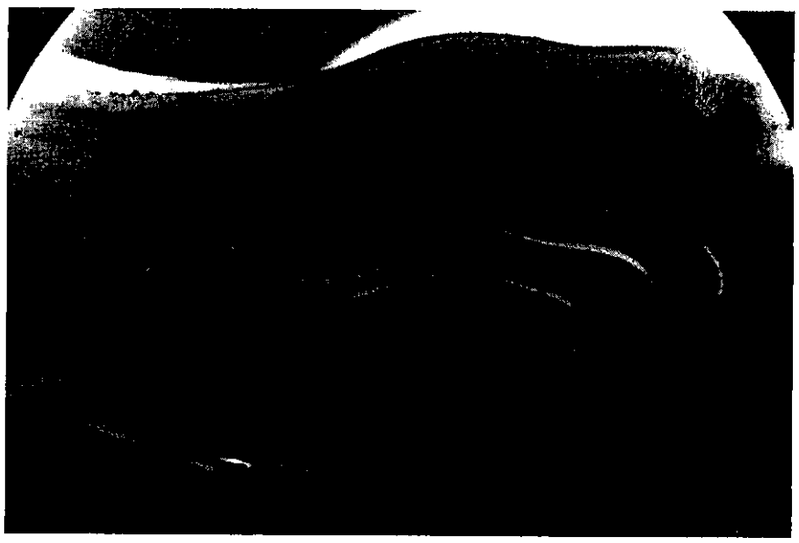

Fig. 6. Cod, 54 cm.; Region XV, Greenland; age 7 years; October, 1957.

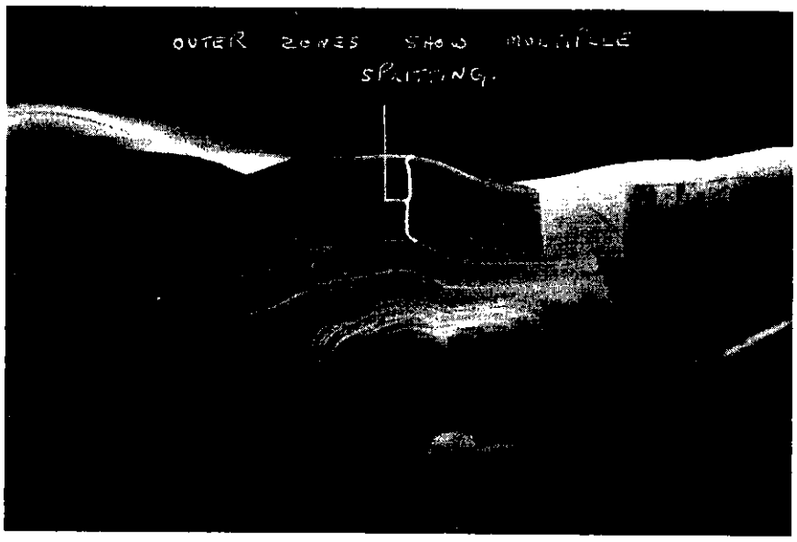

Fig. 7. Cod, 85 cm.; Region Va, Iceland; age 6 or 7 years; February, 1959.

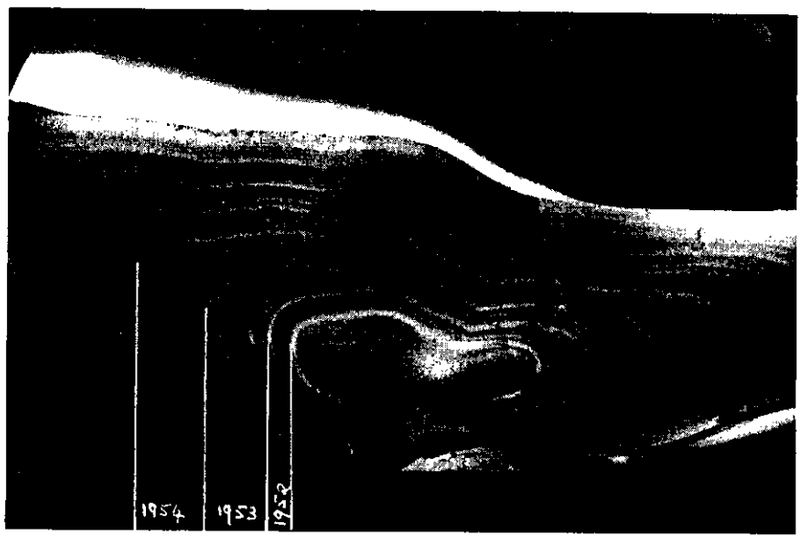

Fig. 8. Cod, 77 cm.; Region IIB, Bear Island, Spitzbergen; age 8 years; November, 1959.

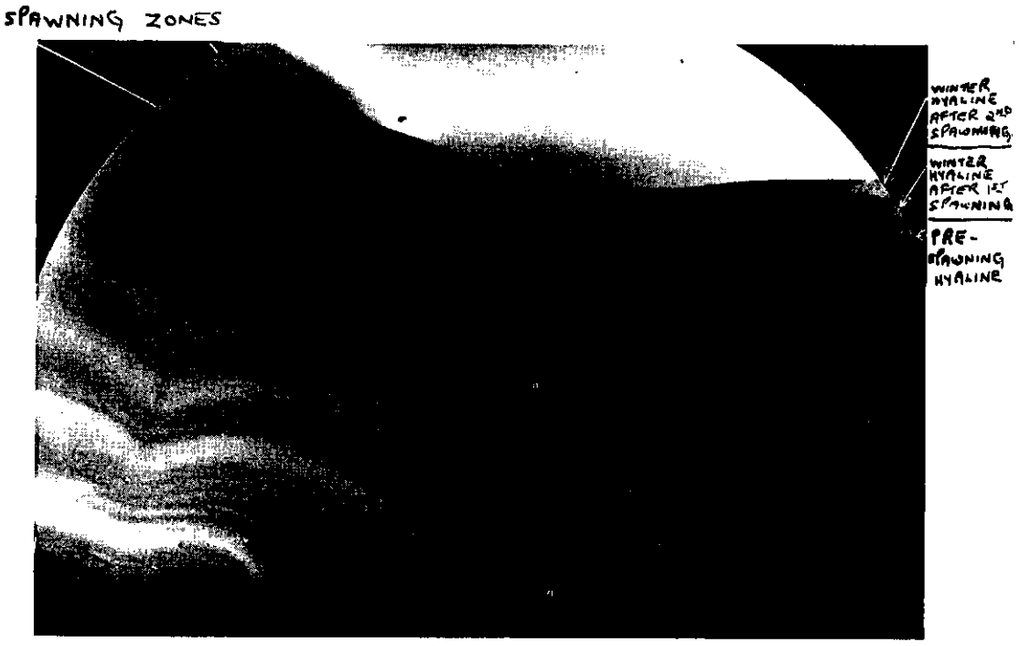

Fig. 9. Cod, 96 cm.; Region Va, Iceland; age 10 years; February, 1959. This is a Greenland fish that has spawned twice (see text).

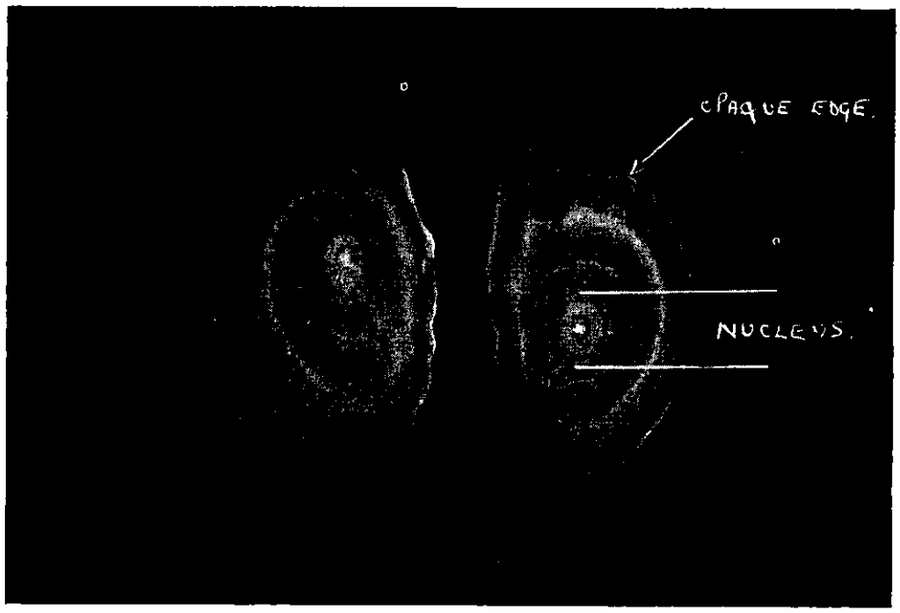

Fig. 10. Plaice, 29 cm.; Region IV, North Sea; age 3 years?; May (approx.).

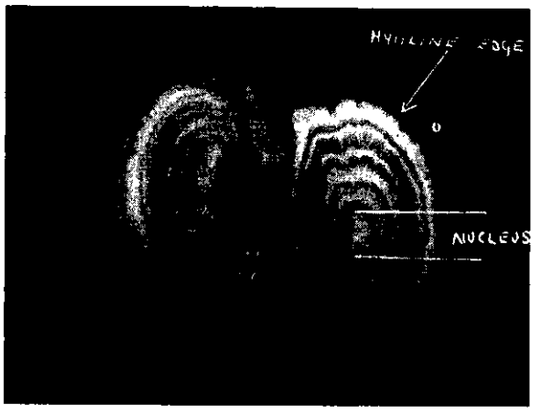

Fig. 11. Plaice; Region IV; male 35 cm.; one otolith V, one VI; March, 1954.

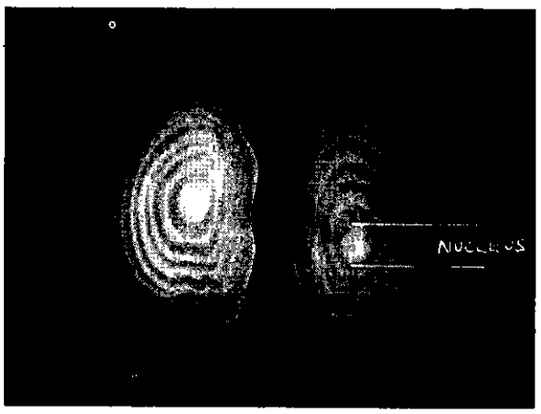

Fig. 12. Plaice; Region IV, North Sea; age 11 years; May (approx.).

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Direccion General de Pesca Maritima, Madrid

Mr. A. Figueras is responsible for age estimation. The species studied is principally cod.

Techniques

1. The fish, from which the otoliths are taken, are selected randomly and the data collected are: fish species, ship, date, geographic situation, size, sex, and stomach contents.
2. The otoliths are removed from the fish. They are preserved dry and stored in envelopes.
3. They are not treated at all before reading.
4. To study the otoliths we use a binocular microscope.
5. The cod otoliths are not read whole, but are broken by Rollefsen's method: pressing the otolith with the two thumbs against the two forefingers.
6. Sometimes, if we wish to measure the annual rings, we polish the two surfaces with a glazer.
7. To fix the otolith on the microscope table we use a slide with a mound of "plastilyne" (a sort of mastic) black coloured, fixed in the center of the slide. The "plastilyne" does not require any substance to fix to the glass.
8. We study the cod otolith in air.
9. We use as light source an ordinary table lamp of 100 W.
10. We use neither filter or other special devices to facilitate the reading.
12. The light beam is directed to the side of the otolith, with the cut surface observed to stay in darkness; the annual rings then appear luminous.
13. Usually for the cod otoliths we use a 9.7 ocular and a 3.5X or 1.8X objective for the larger.
14. We count the hyaline zones along both the axis from the center to the ventral and dorsal edge of the otolith.
15. We use neither staining nor sectioning.
16. We do not usually compare scales and otoliths from the same individuals.

Interpretations

1. We use otoliths for definition of stocks.
2. For the time being we have not defined otolith types in the cod.

Terminology

There are special expressions used to point characteristics of certain parts of the otolith or certain features of patterns of the zones.

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We should say that:

Check ring: a narrow hyaline zone in the section of the otolith that point at a check in the fish growth.

Nucleus: the central zone limited by the first check ring.

Intermediate zone: the zone between two consecutive rings.

Secondary zone: the zone limited by two secondary rings.

Opaque edge: edge without hyaline zone.

Hyaline zone: often is difficult to distinguish if there is or is not the hyaline zone at the edge.

Interpretation

1. Not usually. An attempt is made to identify some cod stocks in Subarea 4 (St. Lawrence).

2. Yes. But only in certain geographical areas.

3. For segregation of otolith types we use: differences in the structure of opaque zones and hyaline zones (rings); patterns of the otolith growth.

   In the St. Lawrence we distinguish two types: (1) Photograph 1 - opaque zones large and uniform; hyaline rings clearly defined, without differing structure or with gradual decrease in the amount of hyaline material; read easily; (2) Photograph 2 - opaque zones weakly defined, heterogeneous structure due to the distribution of hyaline material, which forms broad rings with pronounced discontinuities; reading difficult.

4. Spawning zones.

5. We consider as spawning zones the outer zones showing an accentuated change in the growth rhythm; the opaque zones become narrow and the hyaline zones (rings) are homogeneous, well depicted under transmitte light.

Terminology

1. Check ring: hyaline zones showing incomplete or indistinct formation or irregular spacing; its position is abnormal in relation to the general pattern of the other rings (winter rings).

2. Nucleus: small central opaque zone, normally pale yellow-coloured under transmitted light and delimited by a narrow hyaline ring usually.

3. Not used.

4. Not used.

5. When the marginal zone (the last) is of opaque material, corresponding to a growth period.

6. When the marginal zone (the last) is of hyaline material, correspond. to a winter ring.

7. See Interpretation, 5.

8. -

9. Not used.

Symbols

Age: Roman numerals (I, II, III, etc.).

Spawning: S. S_6, ...S_7... means the age of first maturity.

S_0 means immature

$S_?$ means uncertain or unreadable spawning.

Nucleus: N

Check ring: PA (vis. PA-III/IV; this means a check ring between the third and fourth annual rings.

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INTERNATIONAL COMMISSION FOR THE NORTHWEST ATLANTIC FISHERIES

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Appendix XIX

TECHNIQUES FOR READING OTOLITHS

Questionnaire on Age Reading

Research Group of the National Commission of Fisheries for the Northwest Atlantic, Lisbon, Portugal

Mario Ruivo and Glicinia Quartin

Techniques

1. The fishes from which otoliths are collected are obtained by random sampling from commercial catches before discarding. Data collected include: length, sex and stage of maturity, and sometimes total weight, weight of livers and gonads.

2. In order to avoid spoiling the fish, a transverse cut (Fig. 2) is made with a knife through the skull just behind the eyes and obliquely backwards. The cut is widened by bending the fish (Fig. 1), to the extent that the otoliths become exposed (Fig. 3). When shaking the fish with its head downwards over a table, the otoliths are freed. If need be, a pair of pincers are used for extracting the otoliths.

   The otoliths are cleaned and kept dry in a small envelope, carrying the data of the fish.

3. Untreated before reading.

4. Stereomicroscope Hensoldt-Wetzlar; ocular x5, objective x4.

5. The otoliths are broken across with the fingers. If the break is wrong, a pair of pliers are used.

6. No treatment. In some cases the broken surface is ground with a fine stone.

7. The otolith is fixed on the microscope table in plasticine, or it is held in the right hand, with pliers, and turned so that the beam of light falls on its surface at various angles. This technique is only used for easily read otoliths.

8. The broken surface is moistened with 70% alcohol.

9. A microscope lamp.

10. Blue filter.

11.12. The light beam is directed on the surface to be read at an oblique angle (45°); reading is thus by reflected light. The source of light is placed in front of the observer.

   A scalpel blade is used, sometimes, to screen the broken surface in order to obtain a reading by transmitted light.

13. See 4.

14. The opaque zones are counted, usually from the center to the periphery. No particular axis is used; the one giving the clearest reading is chosen.

15. No. In the case of difficulties of interpretation, a thin slide is cut, using a plier (corta arame).

16. No.

To distinguish the characteristics of the edges, we use the symbols below:

A numeral representing the age, without any other symbol: hyaline edge.

A numeral with the symbol +: opaque edge.

A numeral with the symbol ': hyaline edge in formation.

Example: 8
8^{+}
8'