Demersal Fish Committee

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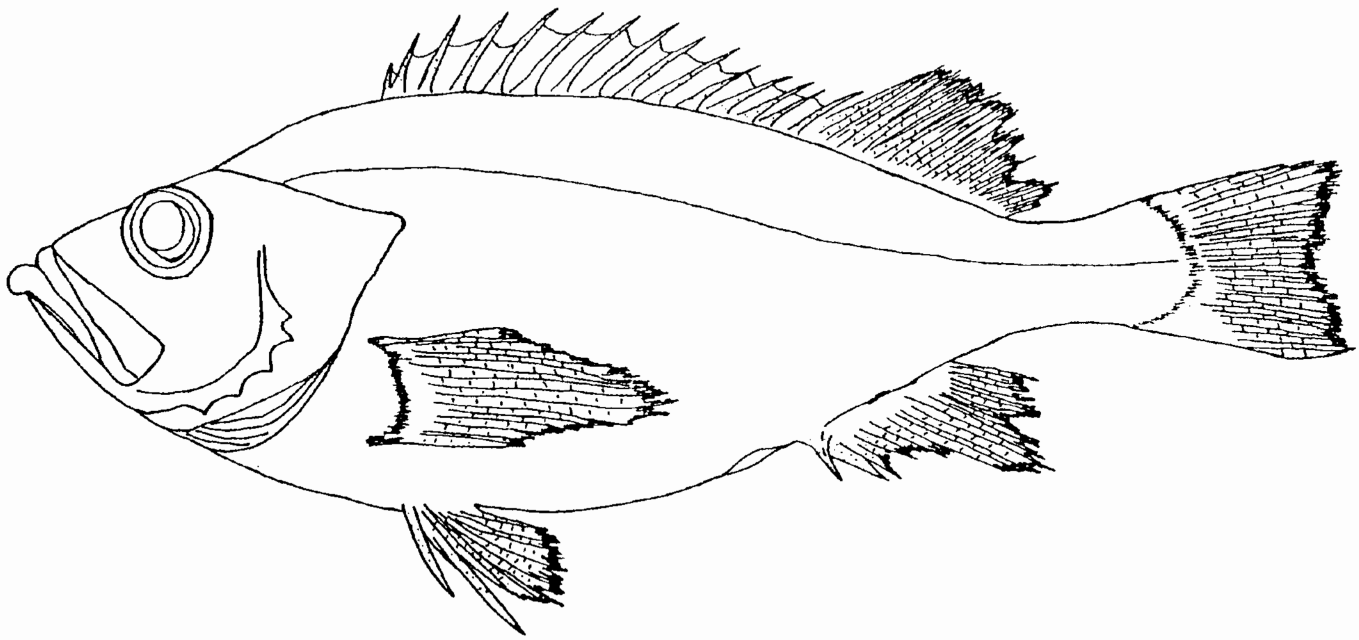
REPORT OF THE WORKSHOP ON AGE READING OF *SEBASTES* spp.

Bremerhaven, Germany
4 - 8 December, 1995



International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1. INTRODUCTION

1.1 Terms of Reference

The Workshop on Age Reading of *Sebastes* spp. met in Bremerhaven, Germany at the Branch Office of the Institute for Sea Fisheries during 4 - 8 December, 1995. The terms of reference for the Workshop as set by the Council's resolution, passed at the 82nd ICES Statuatory Meeting (C. Res. 1994/2:33) were to:

1. evaluate the various methods for determining age in redfish species and investigate the reasons for the differences in results between methods;

2. examine the validity of using age-conversion factors between different parts of the time series.

1.2 Participants

D.B. Atkinson	Canada (co-chair)
B-K. Berntsen	Norway
K. Drevetnyak	Russian Federation
K. Kosswig	Germany (co-chair)
W. Legge	Canada
S.E. MacLellan	Canada
J. Magnusson	Iceland
K.H. Nedreaas	Norway
A. Post	Germany (part time)
H-J. Rätz	Germany
F. Saborido-Rey	Spain
V.N. Shibanov	Russian Federation
Th. Vidarsson	Iceland

A complete list of participants' mail addresses, phone and FAX numbers, and e-mail addresses can be found in Appendix I.

1.3 Goals

In the past, the workshops held to address problems associated with determining the age of redfish have each resulted in a number of conclusions concerning the use of structures and their interpretation. Overall, these conclusions have been general in nature rather than specific, with the result that over time no clear resolution of the problems, nor implementation of processes to aid in timely resolution of the problems have taken place. For the 1995 Workshop to be considered successful, participants considered it necessary to achieve some very specific goals that will themselves reflect "real" progress; or that will result in the formulation of specific work activities aimed at achieving real progress within agreed time frames. Thus, goals for the Workshop were agreed upon as follows:

1. Achieve agreement on the most appropriate structure for determing the age of redfish. Assuming agreement will be reached, the Workshop results should contain a strong recommendation concerning which structure to use for the future.

2. Documenting terminology to be used when interpreting the agreed structure as is applicable specifically to North Atlantic redfish.

3. Documenting similarities/differences in interpretation of agreed structure as well as reasons for, and implications of, these differences.

4. Preparing, as much as possible, a list of preliminary protocols to be applied by readers when examining the agreed structure for North Atlantic redfish, and recommending and documenting steps required to resolve observed differences assuming they are important.

5. Age validation requirements for the agreed structure.

6. Documenting differences between interpretations of agreed structure and other structure(s) used in past as well as their implications (based on data most suitable for comparisons).

7. Compile/document information on existing databases for Northeast Atlantic redfish so as to clarify the nature and extent of the requirements for dealing with information from alternate structures.

8. Compile/document information on historical datasets for Northeast Atlantic redfish which may be useful for comparisons/conversions between structures.

9. Recommending and documenting procedures necessary for complete evaluation/conversion of historic data.

10. Recommendations for future schedule of activities.

1.4 Available Documentation

A number of working papers were available and discussed during the Workshop. These are as follows:

Atkinson, D.B. 1995. A history of redfish (*Sebastes* spp.) age determination in the North Atlantic.

Kosswig, K. and H.-J. Rätz. 1995. Evaluation of German redfish length and age data.

Saborido-Rey, F. 1995. Age and growth of redfish in Flemish Cap (Div. 3M).

Nedreaas, K.H. and V.N. Shibanov. 1995. Differences between otolith and scale readings of Oceanic *Sebastes mentella*.

Berntsen, B-K. 1995. Repeated age determination of *Sebastes mentella* caught in the Irminger Sea in June-July, 1994.

1.5 Previous Experience

A comprehensive age reading bibliography has been compiled and is in Appendix II.

The problems of age determination of redfish have been ongoing for very many years, beginning back in the 1950s. Interpretation of the different possible structures, as well as validation of interpretations, has been fairly straight forward for many fish species. However, the slow growth and long life of redfish has made the issue particularly difficult to resolve for this group of fish. Many years of focussed research and interaction between institutes was required before consensus was reached, and agreed processes accepted for Pacific Ocean redfish, but studies are ongoing. For North Atlantic redfish, the process of discussion and interaction has also occurred for many years, but not on a continuing basis. It is only within the last 10-12 years that more focussed discussion has occurred for Northeast Atlantic redfish.

The process will take time due to the life history of the fish being studied. Also, in order to properly address the problems, there must be an ongoing commitment of resources necessary to conduct work as outlined in this report. Without this commitment, there may still be some progress but it will be much slower.

Because of continuing differences in the selection of structure (scales or otoliths), as well as differences in interpretation of the same structure, ICES sponsored 3 previous workshops (Anon. 1983, 1984 and 1991) to address the issues. The reports of these workshops are available to interested readers so results will not be repeated here. Also, the above noted reports as well as some of the working papers contain material outlining the history of the important issues pertaining to the age determinations of redfish so these will not be repeated here.

It is perhaps important to note nonetheless, the first recommendation of the 1991 workshop as it formed an important starting point for the current discussions. This recommendation was as follows:

"Because of independent evidence for the correctness of using otoliths, and lack of such evidence for using other structures like scales, the Workshop recommends that broken and burnt otoliths should be the preferred structure for age determination of North Atlantic *Sebastes* species. In order to use scales in the future, these methods should be validated by independent and internationally approved methods. However, the age reading during this Workshop showed that otoliths and scales yield approximately the same age for the youngest fish (younger than approx. 12 years)."

2. GOAL 1 - MOST APPROPRIATE STRUCTURE

Workshop participants agreed that since the 1991 Workshop, no additional evidence had been produced which would alter the recommendation concerning the preference for the use of otoliths instead of scales. The only validation studies remain those for otoliths. Thus it is recommended that:

In future, all routine age reading for North Atlantic redfish should be done using otoliths. Inherent in this is the belief that proper interpretation of otoliths will yield the most accurate estimate of true age.

Participants acknowledged that an extensive database of information based on scale interpretation exists in some laboratories (Germany, Greenland, Iceland and the Russian Federation (PINRO)). These data should be used to the extent possible. Different reports in the past have suggested that otolith and scale interpretations for North Atlantic redfish are similar for ages to about 6, 12 or 15. This requires detailed clarification before the usefulness of the historical data can be determined. It is therefore recommended that:

Institutions which have collected scales in the past should continue to do so for a number of years in addition to collecting otoliths. The scales and otoliths must be taken from the same fish. Sufficient scales must be collected so both techniques (polarized light and transmitted light) can be evaluated.

Validations of scale interpretations are still lacking. If researchers wish to continue to explore the appropriateness of scale interpretation, it is essential that validation work be carried out. It is recommended that:

Until such time as validation studies of scale interpretation are carried out using internationally approved methods, age estimation based on scale interpretation should not be used (except for considerations in relation to possible conversions of historical data).

3. GOAL 2 - TERMINOLOGY

Much international work has taken place in order to standardize, as much as possible, the terminology used during otolith interpretation. Participants reviewed existing definitions (Beamish and McFarlane 1983; Chilton and Beamish 1982; Gaemers 1984; Harkonen 1986; Jensen 1965; MacLellan 1995; Secor *et al.* 1995; Wilson *et al.* 1987)), and adapted them as appropriate for interpretation of otoliths from North Atlantic redfish. It is recommended that the following definitions be adhered to when making reference to North Atlantic redfish otoliths and their interpretation:

Accuracy: The closeness of a measured or computed value (e.g. age) to its true value. Accuracy can be proven or estimated: estimates of accuracy are less valuable, but in some cases only an estimate is possible.

Age estimation, age determination: These terms are preferred when discussing the process of assigning ages to fish. The term ageing (aging) should not be used as it refers to time-related processes and the alteration of an organism's composition, structure, and function over time.

Age-group: The group of fish that has a given age (e.g., the 5-year-old age-group). The term is not synonymous with year-class.

Annulus (pl. Annuli): (Winter zone) A translucent growth zone that forms once a year representing a time of slower growth. For most of the redfish stocks the annulus is formed during the fall and winter months, but for some stocks this seems not to be the case.

Annual growth zone: A growth zone that consists of one opaque zone (summer zone) and one annulus (winter zone).

Bias: A lack of precision that is not normally distributed around the mean; it is skewed to one side or the other. For age reading it may apply to one reader's interpretations which are predominantly more or less than those of another for all ages; or it may only apply to only a portion of the age range.

Checks: Translucent zone(s) that forms within the opaque (summer) zone representing a slowing of growth. These zones are usually not as prominent as annuli and should not be included in the age estimate. More than one check per year may form, especially in juvenile growth zones where they are most prominent.

Cohort: A group of fish that were born during the same year (Jan. 1 - Dec. 31).

Edge growth: The amount and type of growth (opaque or translucent) on an otolith's margin or edge. The amount and type of growth on the edge must be related to the time of year the fish was caught and the internationally accepted and standard January 1st birthday. New opaque growth forming on the margin of the broken or sectioned otolith is often referred to as plus growth or increment growth.

Nucleus: It is the central area of the redfish otolith bounded by the first translucent zone (check). It is the central area of the otolith formed during the larval stage. The nucleus in North Atlantic redfishes is always opaque.

Opaque zone: (Summer zone) A growth zone that restricts the passage of light. In untreated otoliths under transmitted light, the opaque zone appears dark. Under reflected light it appears bright. Burning causes only a slight change in the color of the opaque zone, not changes in the transmission of light.

Precision: A process that measures the closeness of repeated independent age estimates. Precision relates to reproducibility and is not a measure of accuracy. The degree of agreement among readers is a measure of the precision of the determinations and <u>not</u> the accuracy of the technique.

Reflected light: Light that is shone onto the surface of an otolith from above, or from the side if the surface is not shadowed.

Sagitta (pl. Sagittae): The largest of three otolith pairs found in the membranous labyrinth of redfishes. It is usually compressed laterally and is elliptical in shape. The sagitta is the otolith used most frequently in otolith studies.

Sulcus acusticus (commonly shortened to sulcus): A groove that forms along the proximal surface of the sagitta. A thickened portion of the otolithic membrane lies within the sulcus. This region is frequently referred to in otolith studies because of the clarity of growth zones near the sulcus in transverse sections of sagittae.

Summer zone: Opaque growth that is normally deposited during the spring and summer seasons when fish are growing relatively quickly.

Transition zone: A region of change in an ototlith growth pattern between two similar or dissimilar regions. It is recognized as a region of significant change in the form (e.g., width or clarity) of the annual growth zones. In redfish a transition zone has been defined as the region of change from juvenile to mature growth. The juvenile annual growth zones are relatively larger than those of later adult zones. For some fishes this transition zone has been validated as coinciding with the onset of first maturity. This is also believed to be true for redfish, although not validated yet. Other areas of the broken otolith or section may also show a change in width and clarity of the annual growth zones which may be related to habitat changes (e.g., movement to deeper waters).

Translucent zone: (Hyaline zone, annulus, check) A growth zone that allows a better passage of light. The definition of the term hyaline has often been misunderstood and is not recommended for use. In untreated otoliths under transmitted light, the translucent zone appears bright. Under reflected light it appears dark.

Transmitted light: Light that is passed through the otolith from below (e.g., sections); for broken otoliths is also from the side if the surface is shadowed.

Winter zone: Translucent growth (annulus; not check) that is normally deposited during the fall and winter seasons when fish are growing relatively slowly.

Year-class: The cohort of fish that were born in a given year (Jan. 1 - Dec. 31) (e.g., the 1990-year-class).

Zone: Region of similar structure or optical density (opaque or translucent). Synonymous with ring, band, and mark. The term zone is preferred.

4. GOAL 3 - READING COMPARISONS

Otolith collections were available from a number of different sources as indicated below:

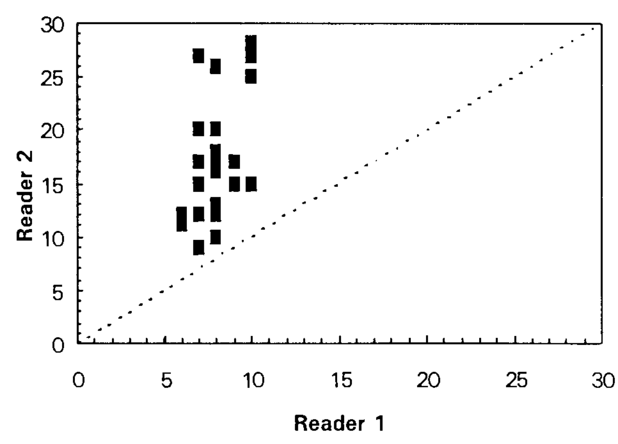
Sources of otolith and scale materials available for Workshop.

Country	Source	Species	Number	Preparation
Norway	Irminger Sea	*S. mentella*	93	broken/burnt
	Barents Sea	*S. mentella*	33	broken/burnt
	Barents Sea	*S. marinus*	18	broken/burnt
	Barents Sea	*S. viviparus*	19	broken/burnt
Iceland	Irminger Sea	*S. mentella*	50	broken/some burnt
	Irminger Sea	*S. mentella*	41	thin sections
	Iceland area	*S. mentella/ marinus*	50	whole (small fish)
Spain	Flemish Cap	*S. mentella*	25	broken/baked
	Flemish Cap	*S. fasciatus*	25	broken/baked
	Flemish Cap	*S. marinus*	25	broken/baked
Canada	NAFO 4RST	*S. mentella*	18	broken
Germany	East Greenland	*S. mentella*	234	sections
Russia	Barents Sea	*S. mentella*	30	broken/burnt
Russia	Irminger Sea	*S. mentella*	50	scales

For the most, the preparation techniques have been documented previously and need not be repeated. However, some participants were unfamiliar with the process to prepare thin sections. These were prepared following the method of Bedford (1983). The otoliths are carefully positioned in rows in specially prepared moulds which are then filled with liquid black polyester resin. The resin hardens to form solid rectangular blocks with the otoliths embedded in them. The blocks are removed from the moulds and machined with a high-speed diamond saw. Thin slices (0.6 mm thick) are cut from the blocks precisely along the lines of the centres of the rows of otoliths. The slices are mounted and fixed on standard glass microscope slides, and are then ready for reading.

Because of time constraints all of the available material could not be examined. Instead, samples of otoliths were selected from some of the material for reading comparisons (24 sectioned otoliths from East Greenland, 30 broken/baked otoliths from Flemish Cap, 18 broken otoliths from NAFO 4RST, 18 broken/burnt otoliths from Irminger Sea (Norway samples), and 30 broken/burnt Barents Sea otoliths (Russia)). Reading was done using reflected light for the broken/burnt samples, and both reflected and transmitted light for the broken/baked samples and sectioned samples.

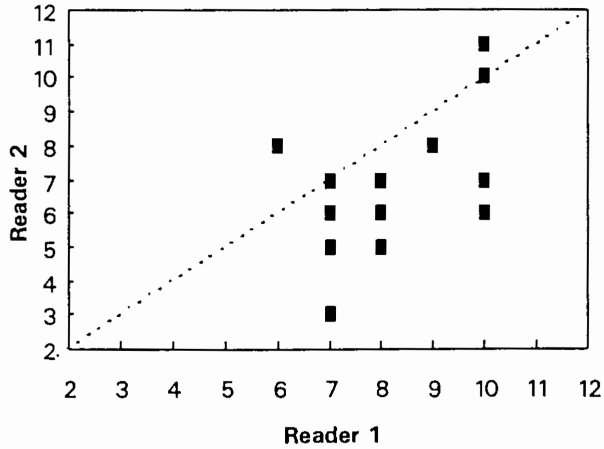
Results between readers were quite variable indicating in most instances significant biases, but also considerable variability (lack of precision). It is not considered necessary to include full details of all the comparison results. The poorest agreement was quite dramatic, and is considered a "worst case." (Note that in the figures the dashed line represents a one-to-one similarity.)



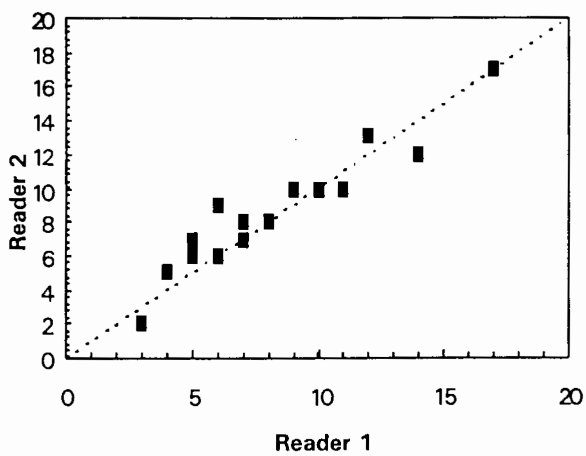
As can be seen, Reader 1 assigned ages ranging between age 5 and age 10, while Reader 2 assigned ages ranging from age 7 to age 29. These are very significant and important differences, but it should be noted that neither reader had any previous experience reading thin sections.

Nonetheless, after discussion among all readers, the differences, although still important, were reduced considerably. **This adjustment clearly indicates that**

ongoing meetings and exchanges between readers is important.

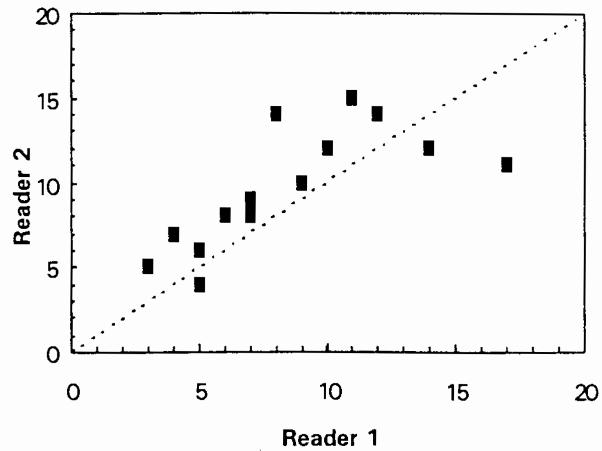


In some other instances, agreement between readers was remarkably good without any prior between reader discussions. It was considered that this was in part due to the experience of both readers with the stock/species. **This reflects the importance of experience in reading the otoliths from any particular stock/species.**

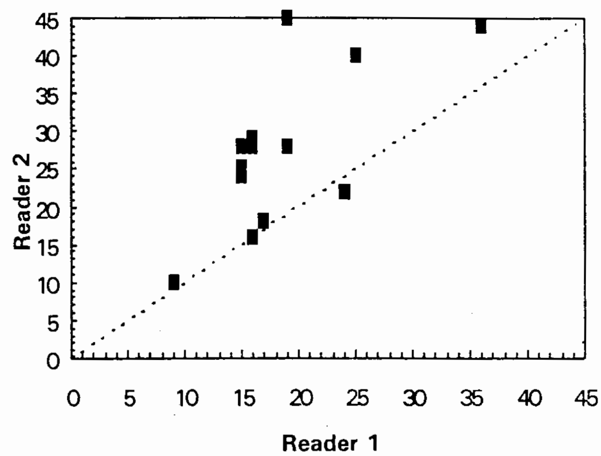


The remaining results were between these two "extremes" as indicated in the following examples. These have been selected to illustrate the types of comparison results which were achieved during first readings. (Note that "Reader 1" and "Reader 2" were not the same people in the different examples.)

Example 1:



Example 2:



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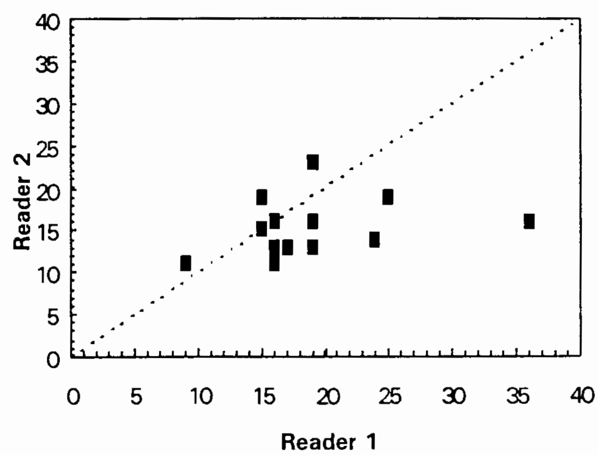

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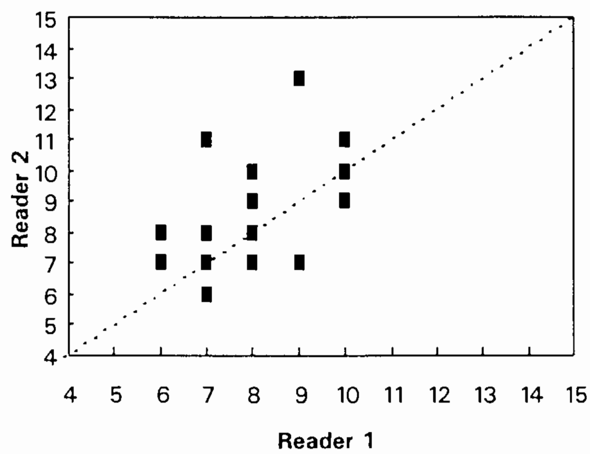
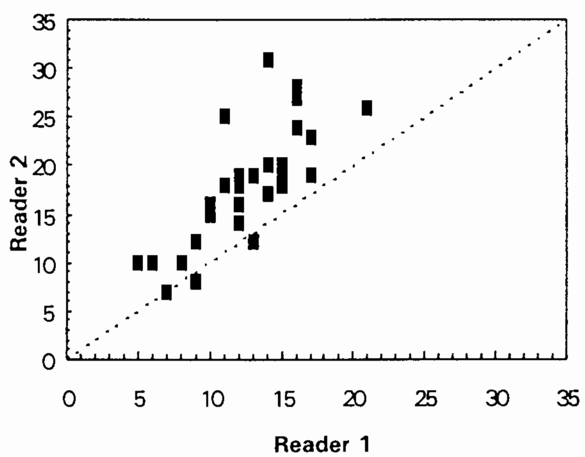
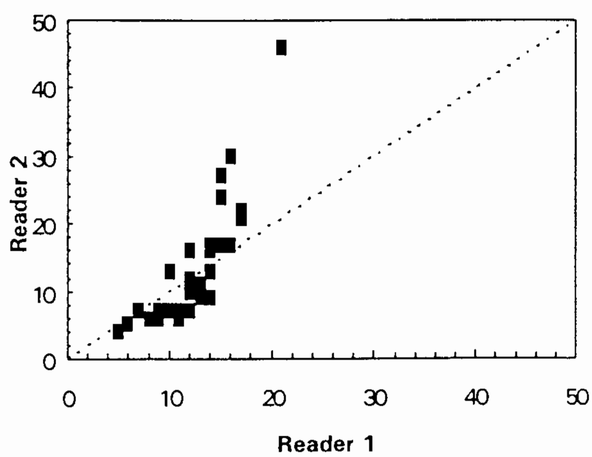


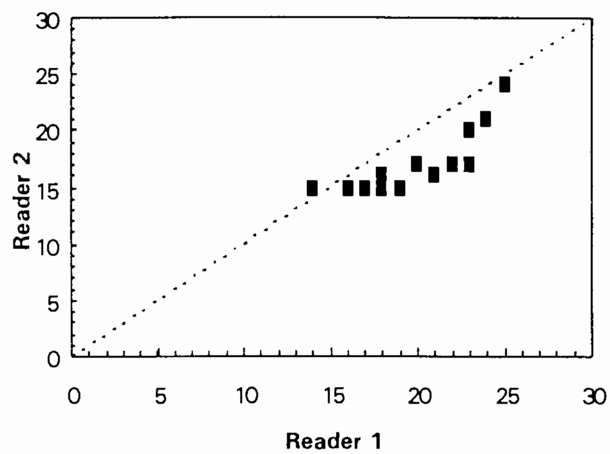
Example 5:



Example 6:



Example 7:



Although the observed differences may seem quite extreme to those unfamiliar with determining redfish ages, given the possible explanations as outlined below participants were very encouraged by the results. For example, in most instances removal of the largest "outlier" from each of the comparisons usually resulted in maximum differences of no more than about 3 years, and many of the differences were only by one year.

Reasons for the observed differences in interpretation were discussed amongst readers. Overall, it was concluded that many of the differences were attributable to the following:

1. Differences in experience levels of the different readers with regard to general otolith interpretation.

2. Differences in the preparation techniques (sections; broken/baked; broken/burnt; broken only), coupled with differences in experience examining otoliths subjected to the various treatments.

3. Differences in interpretation of otoliths from different stock areas.

4. Differences in interpretation of critical areas of the otoliths: a) the first annulus versus check(s) in the nucleus area, b) observed transition zone(s), and c) the otolith edge.

Although encouraging, the current observed differences in interpretation have significant implications with regard to studies of the life history of redfish, as well as assessment and determination of sustainable catch levels. Therefore, for any age based analyses it is important that these differences be resolved in the future.

5. GOAL 4 - AGE READING PROTOCOLS

Assuming that redfish otolith interpretation can be accurate, it is necessary to eliminate any bias between readers, then achieve as high precision as possible. In order to be successful, it is necessary that interpretation protocols be established and adhered to by all readers. Participants discussed this and concluded that the following should form the basis for future interpretations of North Atlantic redfish otoliths:

PROTOCOL FOR HANDLING AND AGE DETERMINATION OF NORTH ATLANTIC REDFISH OTOLITHS

I: SAMPLING AND STORING

Both sagittae otoliths should be collected. In the North Atlantic, they are usually stored dry in a paper envelope, whereas Pacific Ocean redfish otoliths are routinely stored in glycerin. Both procedures are acceptable. Biological data such as total length, round weight, sex, maturity stage, etc. together with a reference number should be taken.

II: EQUIPMENT

Good quality equipment is essential towards attaining the most accurate ages possible. Microscope optics and lighting are the two most important factors. In order to age otoliths, either broken, broken/burnt, broken/baked or thin-sections, the reader needs a stereo microscope (binocular) with high quality optics and resolution power. It should be capable of magnifying at least 40x (experience from age determination of Pacific rockfishes would say at least 100x). These high magnifications, especially when reading broken and burnt otoliths, require very direct and intense light, and fibre optics are recommended.

Tissues are needed to wipe the otolith surface clean and dry in preparation for burning or baking.

For **broken/burnt** otoliths, an **alcohol lamp** should be used to burn the otoliths. A pair of good quality, easily manipulated forceps with serrated tips is needed to hold the otolith during burning.

Paint brushes are used to paint oil (or glycerin/water) onto the broken otoliths (burnt, baked or unburnt). Mineral oil is recommended as it is nonreactive and nontoxic. A 50:50 mixture of alcohol and glycerine may also be used. A **probe or forceps** can be used to manipulate the position of the otolith in its plasticine or clay holder. A **small dish** can be used to hold the plasticine. Contrasting colours such as black or green **plasticine** are preferred.

III: PREPARATION TECHNIQUES

It is important to break or section the otolith as closely through the nucleus centre as possible (Figure 1). Always look at the surface of the otolith half after breaking to be sure this is the case. Breaking or sectioning the otolith too far from the centre may cause the loss of the first annulus on the cross-section. A diagonal break or section will distort the whole pattern. It is important that the reader learn to recognize if these problems have occurred and adjust interpretation of the pattern accordingly, and remember, there is always the other otolith to work with. Burning causes translucent zones to turn brown in reflected light, and still to remain bright in transmitted light. For experienced readers, it is not necessary for the reading to be conducted on the two otolith halves. The reader should assess the quality of the pattern regardless of preparation technique, and determine if further preparations are necessary. A reader should read the same section 2-3 times before deciding on the most appropriate age assignment.

a) Breaking

The otoliths can be broken by the fingers, a tong, by sawing etc. By sawing you will get a smooth surface. Sawing may be necessary for very thick and very small otoliths. Some laboratories prefer the clarity of a "rough" broken surface to a polished surface.

i) Broken Only

No additional preparation beyond breaking is required. The otoliths are mounted then read.

ii) Broken and Burnt

Before breaking an otolith, wipe or wash off all fluids, dirt or membranes using a tissue. Take special care to clean out the sulcus which often retains membranous materials. Fluids, oil or membranes that get onto the broken surface will burn black and obscure the growth zones.

Only alcohol lamps should be used. It is important to get an "even" burn; that is, a uniform darkening of the growth zones over the whole broken surface. This can be difficult to achieve. Hold the otolith in forceps with the cross-section surface facing the eye so that it can be observed as it burns. It doesn't matter whether the sulcus is closest to or away from the flame. The thinner parts and outer edges burn more quickly, and the farther away the otolith is held from the flame the slower it burns. To achieve an optimum burn the otolith must be moved vertically or horizontally over the flame of the alcohol lamp. Burning the otolith beyond dark brown will cause the otolith to turn black, grey and then ash white, all of which are considered over-burnt. Different degrees of burning can be utilized to enhance certain characteristics on some broken areas.

Generally, small or young otoliths should be burnt more lightly than bigger, older otoliths. Deliberately "over-burning" big, old otoliths can make older annual zones near the proximal edge clearer, and also eliminate checks.

Double otolith samples are advised. This is recommended both due to the fact that one burn attempt may become unsatisfactory.

iii) Broken and Baked

Before breaking an otolith, wipe or wash off all fluids, dirt or membranes using a tissue. Take special care to clean out the sulcus which often retains membranous materials. Fluids, oil or membranes that get onto the broken surface will bake black and obscure the growth zones.

Otolith halves are then baked in an oven for at least one (1) hour at 200° C. This causes a light burn that is too light for being recommended for reading in reflected light. However, baking will enhance the surface of the otolith cross-section when the otolith is read in transmitted light by keeping the surface shadowed. The darkening of the growth zones is always uniform over the whole broken surface.

iv) Oil

Addition of oil to the cross-section surface is necessary to clarify the growth pattern. An oil which is non-toxic, inexpensive and nonreactive should be used. Mineral oil is recommended since the otoliths treated with this kind of oil can be reburnt or rebaked.

b) Thin Sections

For preparing thin sections of large numbers of otoliths it is recommended to use the method described by Bedford (1983). The otoliths are carefully positioned in rows in specially prepared moulds which are then filled with liquid black polyester resin. The resin hardens to form solid rectangular blocks with the otoliths embedded in them. The blocks are removed from the moulds and machined with a high-speed diamond saw. Thin slices (0.6 mm thick) are cut from the blocks precisely along the lines of the centres of the rows of otoliths. The slices are mounted and fixed on standard glass microscope slides, and are then ready for reading.

IV: READING

As routine practise, the age reading should take place without prior information on the fish length being available to the reader. Nonetheless, some checking after interpretation, especially for old fish and juveniles may help reduce outliers. The length, however, should not be the guiding factor for age determination.

It is recommended that the reader use the dorsal side of the redfish otolith for age determination. The ventral side is generally more unreliable, and should therefore only be used for corroboration.

A control of the reading can be achieved by reading towards the proximal edge closer to the sulcus (although not as close as axis III in Figure 2) in addition to reading towards the dorsal tip. Checks are usually less prominent in the sulcus area.

a) Position angles of light and otolith; reflected versus transmitted light

It is usual to observe broken only and broken/burnt otoliths using reflected light. For broken/baked otoliths, the light burn is considered too light to be recommended for reading in reflected light. However, baking will enhance the surface of the otolith cross-section when the otolith is read in transmitted light by keeping the surface shadowed.

Thin sections may be read by either transmitted or reflected light.

b) Axes to count along

The annual growth pattern is often easier to interpret on the otoliths from older or mature fish than those from juveniles. Mature fish otoliths exhibit a pattern "frame of reference" that is not seen on juvenile structures. A reader can see the transition where juvenile annual growth slows down. Because this development is lacking on juvenile otoliths, they are often over-aged with checks being mistaken for annuli. Therefore, it is important to keep a picture of a mature adult pattern in mind when interpreting young otoliths. These changes in pattern deposition are usually easier to interpret on certain areas of the broken or sectioned otolith.

The dorsal side of the otolith section is preferred for age determination. However, one should read along the axes which show the most consistent and clearest pattern. Counts should be confirmed by reading as many clear axes as possible. If no axes are clear from nucleus to edge, count in clear areas and use a prominent growth zone to trace from one clear area to another.

Figure 2 shows possible counting axes for redfish broken or sectioned otoliths. Note that axis III near both sides of the sulcus can be confusing due to prominent checks.

When determining the age of older redfish otoliths, some readers find it easier to count from the proximal edge towards the nucleus. In any case, a reader should be able to confirm any age by reversing the direction of a count.

c) Definition of the nucleus

The nucleus is the central area of the redfish otolith bounded by the first translucent zone (check). It is the central area of the otolith formed during the larval stage. The nucleus in North Atlantic redfishes is always opaque.

d) Determination of the first annulus

It is very useful to have otoliths from known-age juveniles on hand (e.g., following a strong year-class through its length-modes) in order to determine the size range and shape of the first year's growth. This will help to establish the location of the first annulus. Size of the first annual growth zone differs between species, but can also differ between individuals, year-classes and stocks. Shape combined with relative size however, is often characteristic for each species.

i)Procedures useful in helping to locate and identify the first annulus

1. For broken otoliths, line up the broken otolith halves with the distal surface of one close to the broken surface of the other. Then compare the extent of the first year's growth.

2. Use a micrometer in one of the oculars. Measure the first year's growth as the distance across the nucleus from the dorsal tip to the ventral tip of the annulus (see double arrows marking first annulus in Figure 2). As an example, refer to Nedreaas (1990) who showed the first 5 annuli for *S. Mentella*.

3. When comparing the same measurement on different otoliths, remember to do that at the same magnification. It is important to remember that there will be between-specimen variability so a range rather than particular distance should be defined. Also, it is important to remember that there will be differences between stocks/species.

4. Use a convenient mark on a probe or forceps as a "measuring stick." By doing this it doesn't matter what magnification is used. The cautions given in #2 above also apply here.

Inexperienced readers should get used to finding the first annulus at one magnification, preferably a lower power, 10-20 x. They should only increase magnification once they are sure of its location.

It is important for each stock/species to validate correct interpretation and counting of the annual juvenile growth zones by e.g., following the length modes of a strong year-class.

e) Opaque and translucent zones

An opaque zone and a translucent annulus together form one year's growth. Opaque zones of younger (juvenile) fish are much larger than those found within the annual growth zones of older fish, and checks are often prominent in juvenile opaque growth zones. Juvenile opaque zones gradually decrease in size towards the transition and mature zones, and this should be kept in mind when reading to avoid including checks.

f) Checks

Translucent zone(s) that forms within the opaque (summer) zone representing a slowing of growth. These zones are usually not as prominent as annuli and should not be included in the age estimate. More than one check per year may form, especially in juvenile growth zones where they are most prominent.

A check is usually discontinuous, or merges with annuli. In many cases these checks are most prominent on the distal side of the cross-section. They are often not as visible on the proximal side of the boundary. Checks usually merge with annuli at the point where the annulus bends towards the sulcus and continues parallel to proximal edge. Annuli and checks both burn dark.

g) Transition zones

A region of change in an otolith growth pattern between two similar or dissimilar regions. It is recognized as a region of significant change in the form (e.g., width or clarity) of the annual growth zones. During this period of growth, the width of the annuli and opaque zones become significantly reduced.

In redfish a transition zone has been defined as the region of change from more rapid to slower growth. For some fishes this transition zone has been validated as coinciding with the onset of first maturity. This still remains to be validated for North Atlantic redfish

Within this transition zone checks become less prominent. The widths of the annuli and the opaque zones are closer to equal. From this transition zone and outwards the reader should bend off and read towards the proximal edge.

h) Mature growth zones

Annuli and opaque zones become more similar in size. The growth is so small that it is rare to see checks. Oxytetracycline (OTC) tagging of Pacific Ocean *Sebastes* spp. (e.g., Leaman and Nagtegaal 1987, Wallace and Tagart 1995) has shown that all the narrow translucent zones in this part of the broken or sectioned otolith should be included and counted as true annuli.

i) **Edge growth**

Proper identification of the amount and type of growth on an otolith's margin is necessary in order to assign the correct year-class. The amount of new (plus) growth on the edge must be related to the time of year the fish was caught and the internationally accepted and standard January 1st birthday (see Figure 3). It is therefore important to have available, during any age reading work, information on the time of capture of the samples.

Usually, juvenile fish have a proportionately larger amount of growth on their otolith's edge than older fish, at any time of the year. Also, juvenile otoliths tend to start showing new growth sooner in the year and may continue to show new edge growth later in the year than what is seen on adult otoliths.

Often new growth is hard to see on a broken surface or section's preferred counting axis because it presents a more "compacted" dimension than that of the distal-whole surface. Edge growth may be very difficult to interpret on older otoliths at any time of the year as the growth zones are so small. The reader should try to trace the last annulus seen from the sulcus area, along the proximal edge to the dorsal tip where the new year's (summer) growth will be most visible. In the sulcus area the new year's growth may only appear to be an extra thickening of the last year's annulus.

A problem with the broken/burnt technique is that often, whether or not there is an annulus on the edge, the edge burns dark. Possibly this is because the edges are more directly exposed to the flame, Be aware of this and take into account the time of year and what the unburnt distal surface edges look like as a reference. This should help to interpret broken/burnt otoliths correctly.

In some redfish stocks (e.g., Norwegian and Barents Sea stocks of *S. mentella* and *S. marinus*) the new year's growth (plus growth, summer zone) may first appear on the otolith surface in June. In the months January-May it may, however, be difficult to detect the annulus at the edge (separate from the edge itself). Therefore, in the period January 1st - May 31st the edge itself has been counted as being the last year's annulus.

It is emphasized that this protocol is general in nature at this point in time. It is well documented (and supported from results of the comparative readings made during this Workshop) that otoliths and their interpretation are often different for different stocks as well as for different (but closely related) species. Thus readers should continually evaluate and refine the protocol so it is most appropriate for the stock/species under examination.

For those interested in determining ages of North Atlantic redfish, it is necessary to continually compare readings and to resolve differences in interpretation when they occur. To achieve this, a number of routine steps should be followed, and testing systems and processes must be developed at different levels. It is important that these be stock/species specific.

a) For the same reader: a reader should not demonstrate self-bias, and must exhibit self-precision between readings. There should not be "drift" (change in interpretation) with time.

b) Between readers: readers of similar experience/ability should not demonstrate bias, and must exhibit precision. This can apply either between different readers in the same laboratory, or between readers in different laboratories.

c) Reference collections should be made and maintained to allow checking for reader "drift." Again it is emphasized that these must be stock/species specific.

d) Each laboratory should develop a <u>routine bias/precision testing system</u> that is made part of the agency's overall protocol. Some examples of procedures and analyses may be found in Anon. 1994, Campana *et al.* 1995, and Hoenig *et al.* 1995.

e) Regular inter-laboratory first hand discussion and comparison is necessary. This should apply most frequently to those involved with the same stock/species, but on a less frequent basis should involve all those involved with the same species regardless of stock.

f) There is a need for continued discussion between the age readers and the biologists familiar with the stock/species.

6. GOAL 5 - VALIDATIONS

There are five important areas of an otolith with regard to the process of interpretation. These are the nucleus, the younger (juvenile) portion, the transition portion, the older (mature) portion, and the edge. Each of these must be studied in order to make accurate interpretations. Studies of each of these have been made for various stocks and species of *Sebastes*. For example, validations of the juvenile ages have been done for Flemish Cap (Saborido-Rey 1995), Gulf of Maine (Mayo *et al.* 1981) and Barents Sea (Nedreaas 1990) redfish based on lengths and modal analysis. Data have also been collected from the redfish around Iceland which would permit similar comparisons. Although year-classes have been proposed based on modal analysis, the otolith (and scale) materials have not been examined to date.

Some work on older ages has been conducted using oxytetracycline (OTC) marking of Pacific Ocean *Sebastes* (Leaman and Nagtegaal 1987, Wallace and

Tagart 1995) but similar work is generally not possible with North Atlantic species because of the difficulties in capturing then releasing these fish live. Radiochemical work on Northwest Atlantic redfish (Campana and Zwanenburg 1990) has indicated that they can be very old.

All of these point to the overall situation for redfish species. However, because of the limited amount of work completed, it is still necessary to make the assumption that these are applicable for all stocks/species. As noted elsewhere, there is no reason not to expect differences between different stocks/species. In order to fully understand the differences which may exist, it is necessary to conduct studies on each stock/species. These studies can include modal analysis of younger (juvenile) fish as well as older fish if the data are appropriate. Also, it would be useful to conduct radiochemical work in order to ensure that the stock/species can indeed live to be as old as assumed.

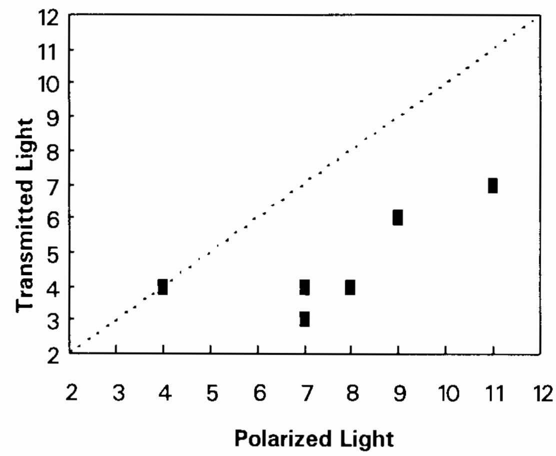
It is recognized that these studies will take time, and in some cases may even have to await the development of new tools or techniques. Nonetheless, in order to fully determine the accuracy of otolith interpretation as an indicator of true age, these are essential. All laboratories interested in age determination of redfish should be continually looking for opportunities to conduct such studies.

7. GOAL 6 - SCALE/OTOLITH COMPARISONS

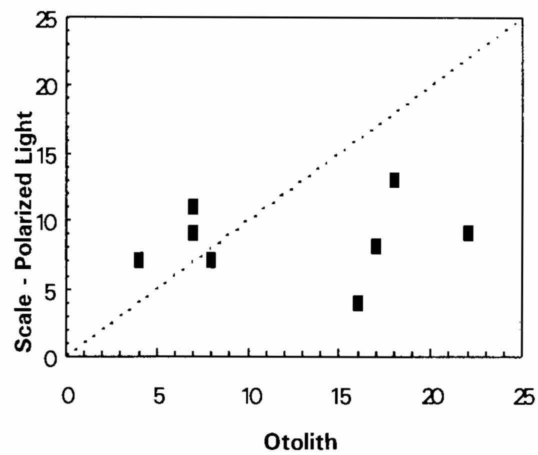
The Working Paper by Nedreaas and Shibanov contained information on comparisons between scale and otolith readings from the same fish. The results indicated that differences were greatest for ages 13 and older, but that there was a general tendency to obtain older estimates from otoliths than scales for all samples examined. The procedures of study presented represent the type of work that must be conducted in the future in order to determine if and how conversions may be possible.

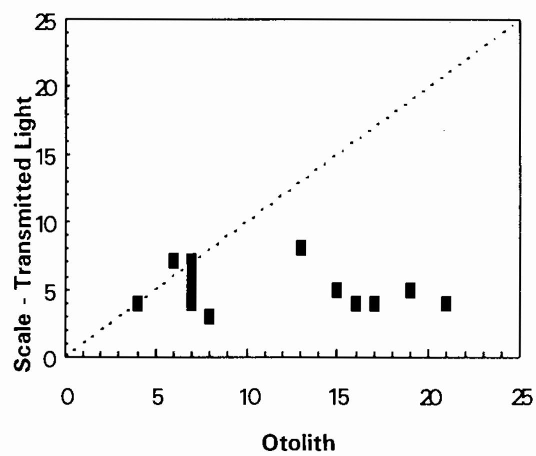
Only limited data were available for comparisons between scale and otolith interpretation during the Workshop. One sample of scales was available from the Northwest Atlantic although there were only small numbers of these. Otoliths from the same fish had been previously read by Canadian readers. The scales were read using transmitted light, as well as with polarized light. Problems with the sample made it impossible to carry out the silver nitrate staining.

Although problems with the scale samples are acknowledged, the limited results indicate considerable differences between scale interpretations based on the viewing technique (transmitted versus polarized light).



There were also significant differences between scale and otolith interpretations, particularly when transmitted light was used to view the scales.





These limited results clearly indicate that more work in this area is essential before decisions can be made concerning the possiblr conversion and use of the historical data.

8. GOAL 7 - EXISTING HISTORICAL AGE/GROWTH DATABASES

The following tables indicate the age and growth information for redfish in the ICES area held in the databases of the various institutions. The structure sampled was generally either scales or otoliths with littleo no overlap. It is the historical information based on scale interpretation which requires evaluation for possible conversions.

1. Germany

Species	Area	Lengths (Commercial)	Lengths (Research)	Age/Growth Data	Structure
S. marinus	ICES I, IIb			1974-81	scales
S. marinus	ICES IIa	1962-92		1976-90	scales
S. marinus	ICES Vb, XIVb	1962-91	1982-93	1974-91	scales
S. marinus	NAFO 1	1962-78	1982-94	1973-89	scales
S. marinus	NAFO 2-3			1975-83	scales
S. mentella	ICES I, IIb			1974-82	scales
S. mentella	ICES IIa	1976-93		1980-90	scales
S. mentella	ICES Vb, XIVb	1962-93	1982-93	1974-91	scales
S. mentella (oceanic)	ICES XII, XIVb			1974-91	scales
S. mentella	NAFO 1		1982-94	1975-88	scales
S. mentella	NAFO 2-3	1962-93		1974-85	scales

2. Iceland[1]

Species	Area	Lengths (Commercial)	Lengths (Research)	Age/Growth Data	Structure
S. marinus	ICES Va	1952-95	1955-95	1952-95	otolith/scale
S. mentella	ICES Va	1975-95	1971-95	1971-95	otolith/scale
S. marinus	ICES XIVb	1955-82	1954-95	1954-83	otolith/scale
S. mentella	ICES XIVb	?-1975	1973-82	1973-82	otolith/scale
S. mentella (oceanic)	ICES XIVb, XII, Va	1989-95	19720-95	1972-95	otolith/scale

[1] The time series are not complcte in all cases, and mainly scales were collected during the 1980s.

3. Norway

Species	Area	Lengths (Commercial)	Lengths[1] (Research)	Age/Growth Data	Structure
S. marinus	ICES I	1988-95	1986-95	1989-95	otoliths
S. marinus	ICES IIb	1988-95	1986-95	1989-95	otoliths
S. marinus	ICES IIa	1988-95	1986-95	1989-95	otoliths
S. mentella	ICES I		1986-95	1990-95	otoliths
S. mentella	ICES IIb	1988-95	1986-95	1990-95	otoliths
S. mentella	ICES IIa	1988-95	1986-95	1990-95	otoliths
S. mentella (oceanic)	ICES XII, XIVb	1991-94	1994	1991-94	otoliths
S. viviparus	ICES Iia		1986-95		

[1]Also are sporadically collected samples from 1970-85; but with some risk of species misidentifications during this period.

4. Russian Federation

Species	Area	Lengths (Commercial)	Lengths (Research)	Age/Growth Data	Structure
S. mentella (oceanic)	ICES XII, XIVb		1981-95	1981-95	scales
S. mentella	ICES Iia, IIb		1953-95	1953-91	scales
				1992-95	otoliths
S. marinus	ICES IIa, IIb		1953-95	1953-56	scales
				1964-65	
				1981-89	

5. Spain

Species	Area	Lengths (Commercial)	Lengths (Research)	Age/Growth Data	Structure
S. mentella	ICES XII, XIVb	1995	1995	1995	otoliths

9. GOAL 8 - EXISTING AGE/GROWTH DATABASES FOR SCALE/OTOLITH COMPARISONS

The following tables contain information on the databases available where both scales and otoliths have been collected from the same fish from within the ICES area. The information contained in these databases must be fully evaluated as part of the process to determine the usefulness of the historical database of scale reading information.

1. Germany

Species	Area	Year	No. fish sampled	Length Range (cm)	Aged (Y or N)
S. mentella (oceanic)	ICES XII, XIVb	1994	173	26-47	N
S. mentella (oceanic)	ICES XII, XIVb	1995	604	25-49	N

2. Iceland

Some data have been collected (otoliths and scales from the same fish) mainly from the oceanic *S. mentella*, but also from the other stocks in particular from smaller fish. These data have not been evaluated yet.

3. Norway

Species	Area	Year	No. fish sampled	Length Range (cm)	Aged (Y or N)
S. marinus	ICES IIa	1985-88	44	8-61	Y
S. marinus	ICES IIa	1987	434[1]	30-70	Y
S. mentella	ICES IIa, IIb	1985-88	47	13-47	Y
S. mentella	ICES IIa	1990	8	35-41	Y
S. mentella	ICES I, II	1991	10	25-29	Y
S. mentella (oceanic)	ICES XII, XIVb	1990	124	26-42	Y
S. vivparus	ICES IIa	1985-88	68	13-27	Y
S. marinus *S. mentella* *S. viviparus*	ICES I, II	1985-1988	hundreds	complete	N

[1] Not individual otolith/scale comparisons, but age-length keys from the same fish from both otolith and scale (polarized light) readings.

4. Russian Federation

Species	Area	Year	No. fish sampled	Length Range (cm)	Aged (Y or N)
S. mentella	ICES XII, XIV	1994	214	25-45	Y

10. GOAL 9 - REQUIREMENTS FOR SCALE/OTOLITH COMPARISONS

It has been recommended above (see Goal 1) that in order to fully evaluate the usefulness of the historical databases of age determinations based on scales, that collections of both scales and otoliths from the same fish are necessary. This can be expanded, and it is recommended that:

Separate collections of scales and otoliths from the same fish be collected from those stocks/species for which there are historical information from scales, and for which possible conversion of these data is considered useful. For these collections, stratified sampling of 5 fish per cm. per sex should be made.

The collection of this material should not go on indefinitely. Instead, it is recommended that: **Collections be made for the next two (2) years after which time the necessary analyses are carried out. The examination of material, and analysis of results should be done by small working groups of experts familiar with the stock/species in question. This must include those most familiar with the "traditional" age determination technique(s) applied to the different structures for the stock/species in question. It is possible that different degrees of usefulness may exist for the different stocks/species.**

11. GOAL 10 - FUTURE ACTIVITIES AND TIMETABLE

1. The time limit for collection of material for comparison of scale and otolith interpretation is two years. During that time period, analyses of existing material should be ongoing. Small working groups

of experts, as is appropriate for each stock/species in question should meet during the second half of 1998 to examine results and determine, to the extent possible from the data, possible conversions. These working groups should comment on the usefulness of any such conversions including limitations.

2. There is an ongoing requirement for those involved with age determinations (otoliths) of particular redfish stocks/species to meet and discuss their work. These small working groups should meet to examine interpretation consistency (within and between reader), bias and precision of their interpretations. These meetings should occur annually until such time as an acceptable level of ongoing agreement has been achieved after which time the meetings may be less frequent.

3. There is also a requirement for those involved with age determinations of redfish (otoliths) generally to meet and discuss their work. These larger working groups should meet every second or third year. The topics for discussion should be varied and reflect current research activities as well as any general problems or other discussion points. An example of this type of meeting is the Pacific Ocean experience (Canada/USA) (e.g., Saunders 1995).

4. All institutes interested in age determinations of redfish should continuously be searching for information and opportunities to conduct age validation work. Even if it is not possible to process the data in a timely fashion after collection, the material should be collected.

12. SUMMARY

The Terms of Reference of the Workshop were:

1. Evaluate the various methods for determining age in redfish species and investigate the reasons for the differences in results between methods.

The Workshop participants reviewed available information and re-itterated the recommendation of the 1991 Workshop that the **otolith** is the most appropriate structure to be used for age determination of redfish. Although more thorough analyses are desirable, at present the only validation work has been done for otoliths. Those wishing to use scales on a routine basis must first validate their interpretations using internationally acceptable methods. Until such time as this validation work has been carried out, scale interpretations should <u>not</u> be used on a routine basis.

Otoliths have been shown in many studies to reflect the true age of redfish. It is considered that for these slow growing fish, after a certain age there is little or no need for increased scale growth and therefore older fish can be underaged using scales. Also, there is often significant regeneration of scales in the older fish. This too will affect age estimations.

2. Examine the validity of using age-conversion factors between different parts of the time series.

There are only limited datasets and/or analyses currently available to allow for evaluation of possible age-conversion factors being applied to the historical data sets. These have suggested different results; similarities to age 6, similarities to about age 13, and similarities to about age 15. It is not possible at this time to evaluate any further the possibility of making conversions, and it will remain impossible to evaluate this until the collections and analyses identified above are completed. As part of this process, it is also necessary to identify the use to which the converted data may be put as this could affect the usefulness of any conversions.

The slow growth and long life of North Atlantic redfish has made the issue of accurate age determination particularly difficult to resolve. This Workshop has resulted in important progress toward the resolution of the age determination problems as they now exist in the Northeast Atlantic. Although there are still important issues to be addressed with regard to age validation, repeatability, bias elimination and precision, commitment of institutes to the procedures and timetable outlined above will result in significant progress in the future. It must be emphasized that without commitment the goal of "resolution" will not be achieved, or at best will only be achieved at a slow pace.

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Figure 1: Whole redfish otolith.

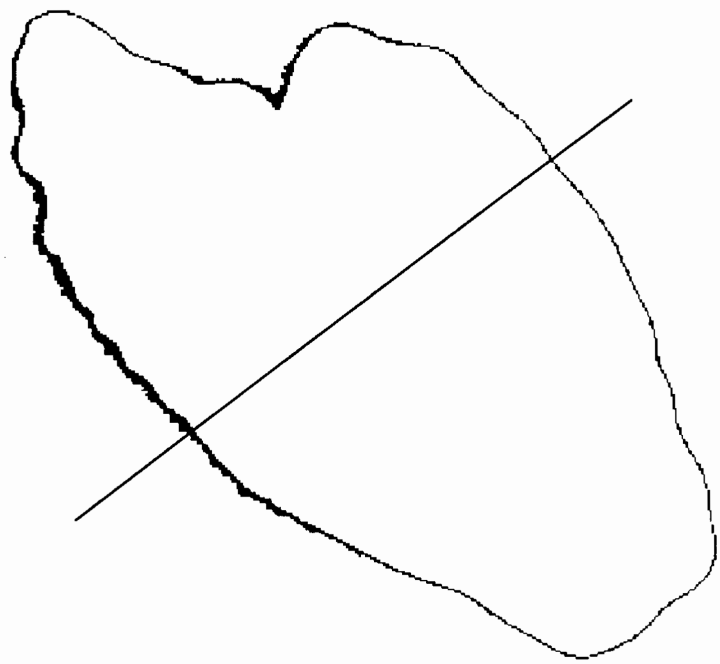
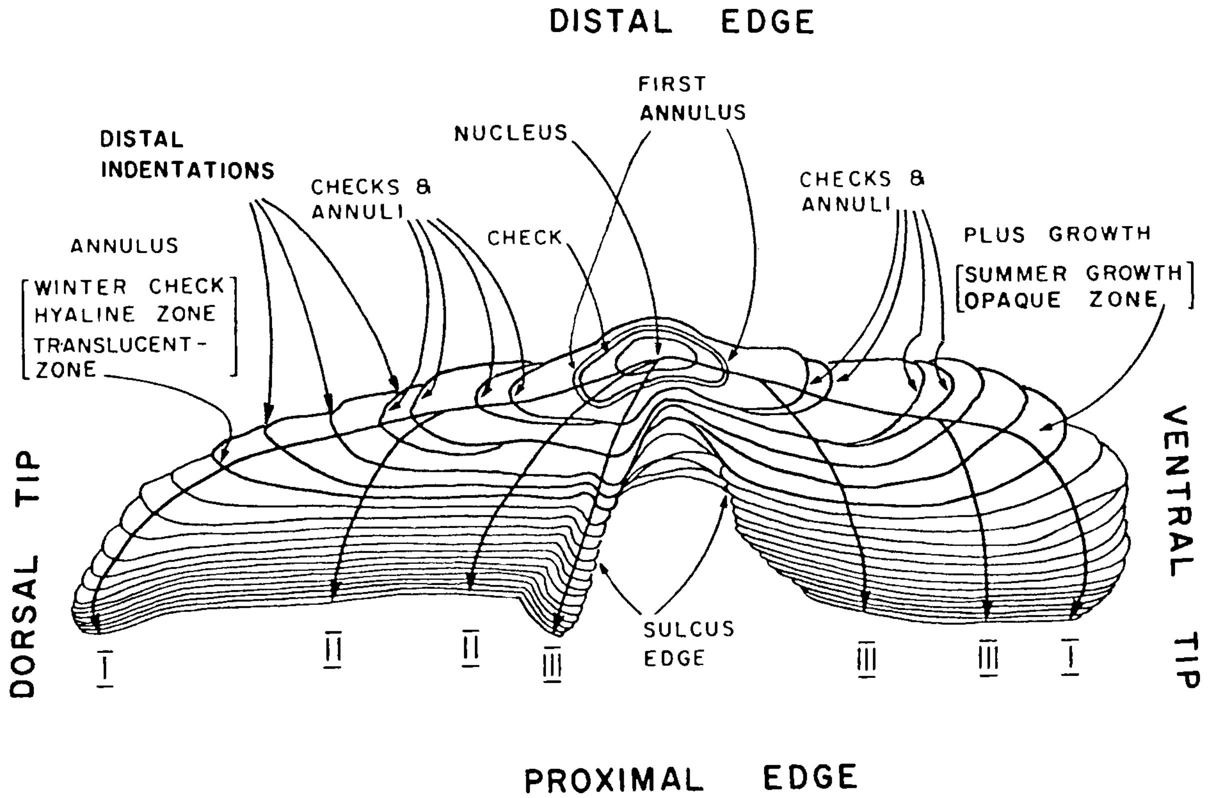
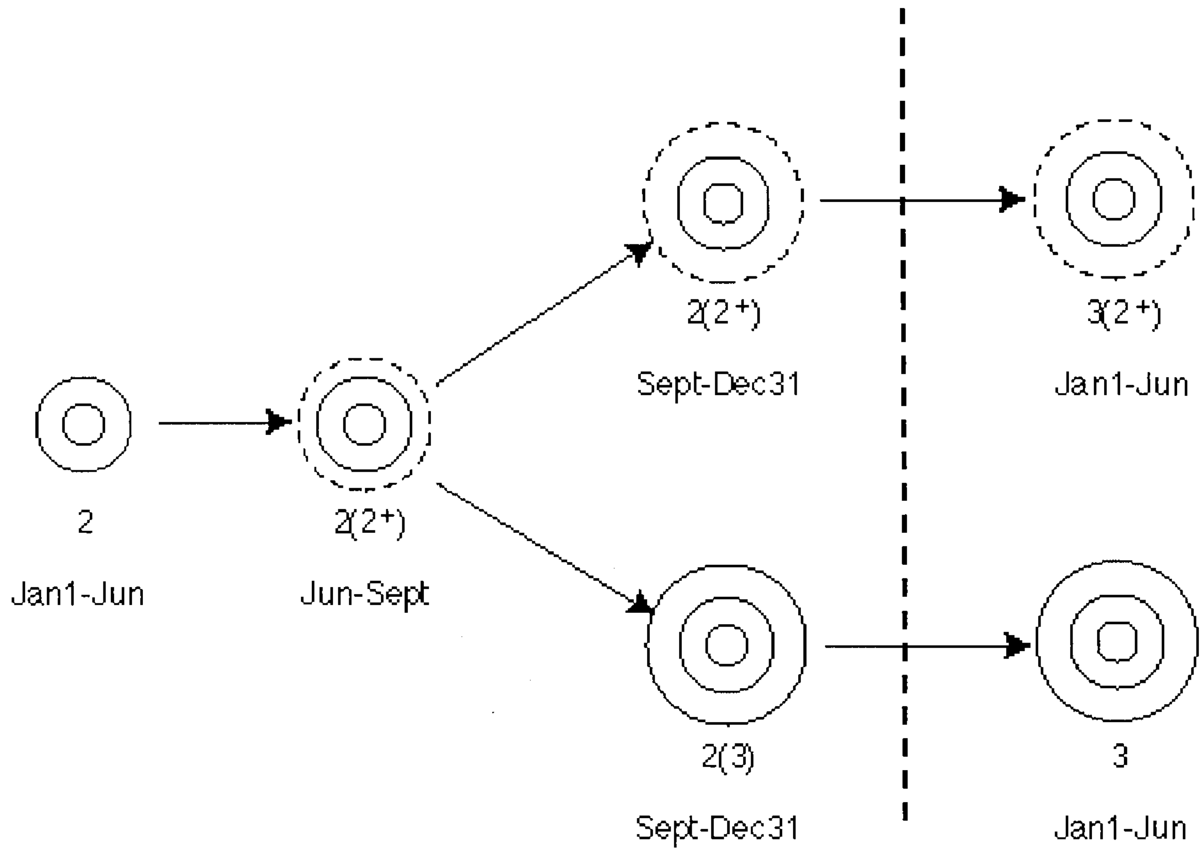


Figure 2: Surface of broken or sectioned otolith.



(modified from MacLellan 1995)

Figure 3: Growth designations.



These drawings represent a "figurative" otolith showing growth stages from one January to the next. The **black** zones are annuli, and the **white** are summer zones. The dashed lines represent incomplete summer growth. Take note as to how the age designation changes as the months progress. The number within the brackets indicates the number of annuli actually seen, as well as the summer growth represented by a "+". The number before the brackets is the age class interpreted according to the time of the year when the fish was caught with reference to the January 1 birthday (from MacLellan 1995). Note that the time periods indicated apply to redfish generally, but have not been confirmed for all North Atlantic stocks/species.

APPENDIX I

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APPENDIX II

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