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Report of the Workshop on Age Reading of North Sea Cod (WKARNSC)

5-7 August 2008

Hirsthals, Denmark

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CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The overall result of the workshop exercises is that there are significant variations in North Sea cod age estimates between readers. Both low precision, and relative biases between readers were found and overestimation of fish ages seems to be the problem.

The image analysis exercise clarified that the lack of agreement can be referred to the perception of the first age structure (O1) and the very widespread appearance of some age structures resulting in several translucent bands within one year (split rings).

Exploring the application of image analysis, the group agreed that applying such tools in the routine age estimation of North Sea cod may prove very valuable. It gives the opportunity to use metrics to rule out doubt when defining the age structures to count and also gives a very useful exchange tool for the individual readers both within and between laboratories.

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, some of the major problems in ageing otoliths of North Sea cod. The group reached agreement on a definition of an ageing protocol/guidelines mentioned in the present report and the aim is to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures.

A collection of Agreed Age cod otoliths were started at the workshop. The reference collection will have to be expanded considerably through exchange of otoliths and images. Additionally, the collection of agreed age otoliths should not stand alone, but be a part of a larger compilation of data on 'typical' otoliths for the species and area, in which measurements of O1, typical distances between age-structures, edge development over season, and general growth curves for the sub-stocks of cod in the North Sea.

1 Terms of reference

2007/2/ACFM A Workshop on Age Reading of North Sea Cod (WKARNSC) (Chairs Lotte Worsøe Clausen, Denmark and Hans Høie, Norway) will be established and take place in Denmark, 5-7 August 2008 to:

a) Compile information on laboratory procedures. Prior to the workshop participants are asked to supply the Chairs with their national ageing manual. These will form the base for the creation of a common agreed ageing manual to be applied by all laboratories ageing North Sea cod;

b) Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation of North Sea cod;

c) Evaluate the use of traditional age determination combined with recent development in image analysis in order to include back-calculation of length at age and growth based on age structures defined by age-readers;

d) Collate agreed age reference collection. The otoliths applied under ToR b) and c) will be the first otoliths in an agreed age reference collection. Digitized images will be saved in a database format along with annotated agreed ages;

e) Make recommendations for further cooperation between North Sea Cod age readers, otolith sample exchange, bilateral cooperation, and workshop.

WKARNSC will report to ACFM, WGNSSK and PGCCDBS by early September 2008.

Supporting information

Scientific justification and relation to action plan:	The recent exchange of North Sea Cod otoliths under PGCCDBS during 2005/2006 showed an agreement percentage well below the acceptable limit of 90% (Woods 2006). The age estimations were positively biased giving an unacceptable overestimation of ages compared to the modal age and even experienced readers did not show a high agreement within and between laboratories. Clarifying the diverging perception of age structures among age readers and explore the usage of metric measurements of otolith structures as solutions to minimizing the divergence in age estimation would potentially improve the agreement between readers and form the basis of an agreed method to interpret growth structures in otoliths of North Sea cod.
Resource requirements:	DCR data collection system.
Participants:	North Sea Cod age determination experts from 8 countries (Denmark, Norway, Belgium, UK, France, Germany, the Netherlands, and Sweden). Experts from national institutes of all these countries participated in the exchange program.
Secretariat facilities:	None
Financial:	Attendance to the Workshop eligible under the 2008 DCR
Linkages to advisory committees or groups	There is a direct link to ACFM through WG on the Assessment of Demersal Stocks in the North Sea and Skagerrak (WGNSSK) and PGCCDBS
Linkages to other organisations:	There is a direct link with the EU DCR

2 Agenda and participation

The agenda is presented in Annex 1, and list of participants in Annex 2.

3 National cod age reading manuals (Tor a)

Cod age reading manuals from different countries is given in Annex 4.

The group reached agreement on a definition of an ageing protocol/guidelines mentioned in the below points and the aim is to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures. The group strongly recommends that all ageing laboratories processing blue whiting should include the guidelines developed during the workshop in their ageing manuals

A list of recommendation for methodological aspects when preparing and reading sectioned North Sea otoliths was compiled during the workshop.

3.1 Sampling otoliths

- Sample the two sagittae otoliths. It is always useful to have two otoliths from the same fish even if only one is prepared for reading.
- Otoliths should be cleaned for tissue and blood by gently wiping between the fingers or using paper and water.

3.2 Storing otoliths

- In paper envelopes or plastic trays
- Store dry for short time storage
- Labeling each otolith pair with unique name
- Store information of accompanying fish data etc in database

3.3 Embedding

- First make a resin base and let it dry until sticky. Thereafter place the otoliths in the tray with convex side down. Align the otolith correctly using a live camera according to a line on the monitor, or using a laser line, so the nuclei on the otoliths are in line. Check the numbers of otolith are correct. It is useful to insert a "dummy" object in case of missing otoliths so the numbers in database and resin block match (no missing number).
- Transparent or black resin can be used for reading sections under a microscope. Transparent resin preferred when taking pictures of the sections in order to obtain good image quality and avoid light scattering. Place the resing in a fume cabinet for health reasons.
- Label the mould with a unique number. After cutting, also name the slides and each otolith in the slide with unique numbers.

3.4 Sectioning

- Use diamond saw blade, high speed cutting (~1000 rpm) with water. 400-500µm blade thickness is recommended.
- Cut the slide to approximately 400-500µm thickness. Thin slides will reveal more detailed otolith structures, but with the risk of damaging the sections. Aim for two slides per otolith, and choose the best slide which is closest to nucleus for interpretation.

- Store slides in bags, or mount on glass-slides with transparent resin. Covering the slides with glass on both sides is also used.

3.5 Reading otoliths from slides

- Relevant data of fish size etc should be available and used in case of doubt when assigning fish age.
- Use microscopes with good quality optics. Stick to one microscope that the reader is familiar with. Use generally low magnification, but zoom when necessary.
- Preference of source of light, transmitted or reflected, varies between institutions. Some use both transmitted and reflected light, others only transmitted or only reflected. Features in the otoliths, especially at the edge, might look different using different light settings.
- Otolith surface is covered with a thin layer of oil when reading otoliths not covered with glass.
- Consider information from other otoliths caught at the same time when interpreting the growth structures.
- Otolith axis to interpret: Often useful to look at the distal side (Figure 3.5.1). For older fish it is also useful to interpret the latest years along the dorsal radius. Sometimes the otoliths look different when turning up-side-down, it can therefore be useful to turn around otoliths that are difficult to interpret.

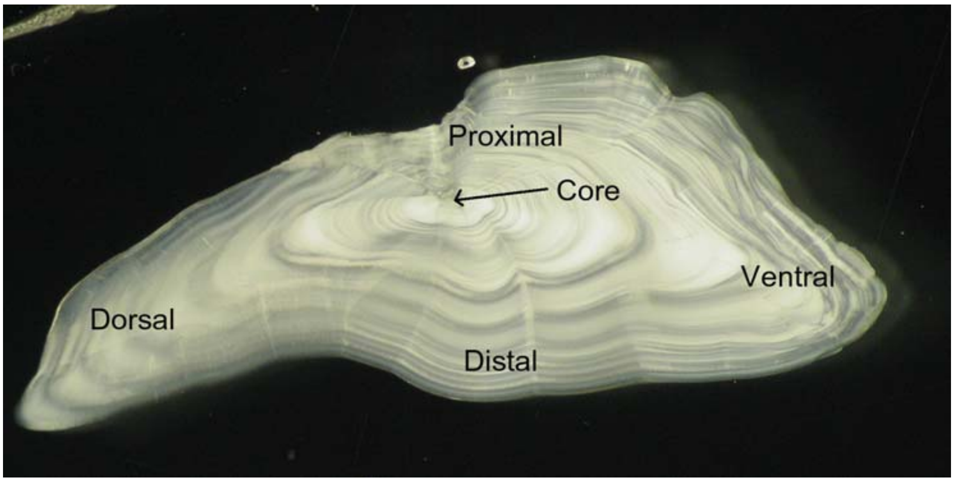

Figure 3.5.1. Directions on a sectioned cod otolith.

3.6 Quality control

- New readers should go through an extensive teaching program lead by an experienced reader.
- Fish ages estimated by inexperienced readers should often be controlled by experience readers the first years. The experienced readers should also be controlled by other experienced readers as an extra control. Randomly chosen otoliths should also be read twice of the same reader to control for inter-reader precision.

3.7 Photographing

- Photographing and annotating pictures are important for documenting how readers interpret the otolith structures. However, photo quality is very important and proper preparation of otoliths is necessary for obtaining good photographs. Avoid over-exposed pictures. Remember to calibrate image, information of resolution in the file name is recommended. Pictures should be saved in Tiff-format. Use only one microscope for each stock, there might be microscope-specific calibration variance. Recalibrate the setup regularly.
- Minimum camera specification:
- At least 6 MP
- Good light sensitivity
- High speed connection between camera and computer is recommended.
- Processing pictures can be done with specialized software as TNPC, or more general software as ImagePro, ImageJ, or others. A high resolution screen is important.

3.8 Interpretation

- Be aware of false translucent ring near the centre which can be confused with the first annuli (Figure 3.8.1). The false ring is generally thinner and well defined, approximately 1 mm diameter. On the other hand the first annulus is approximately 2 mm in diameter and less well defined.

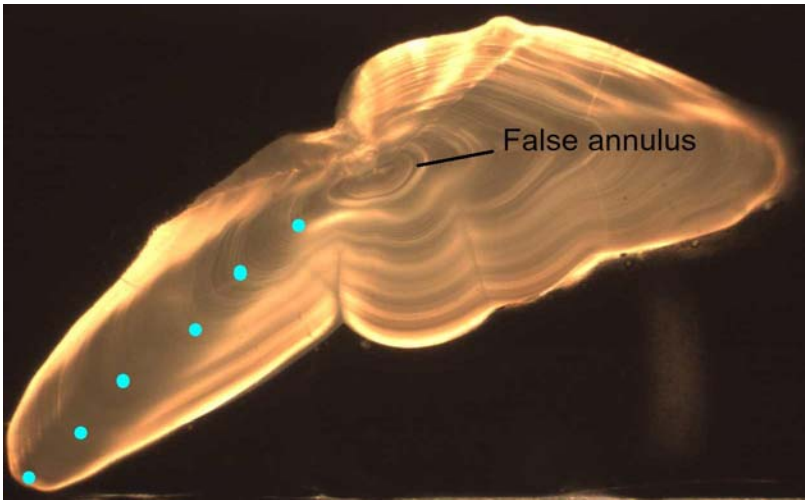

Figure 3.8.1 Photo of a six year-old cod otolith. The inner translucent band is deposited during summer, and must not be interpret and an annulus.

- Edge. The first translucent annuli is deposited in late autumn and often completed before New Year (Figure 3.8.2). Thus, there can be quite wide opaque marginal increment in quarter 1, especially for young fish due to high growth rates.

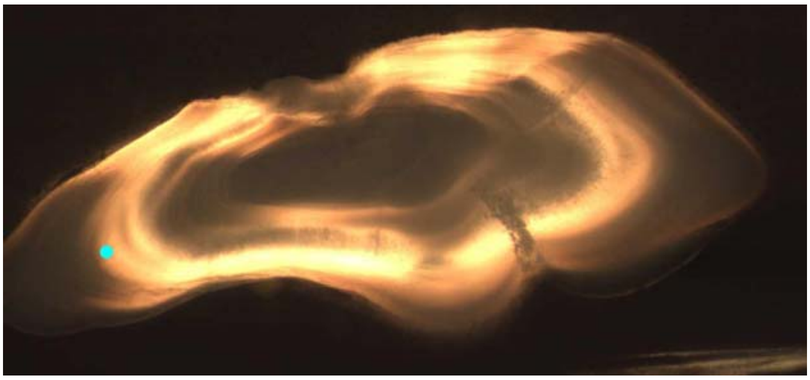

Figure 3.8.2. Photo of a one-year old cod otolith, captured in quarter 1. The wide opaque growth after the translucent band was formed in the period from end of translucent band in December and until capture in quarter 1.

- Split rings. Multiple translucent banding within an annual structure can occur within the same otolith (Figure 3.8.3). This can also re-occur for several years. Counting the multiple translucent bands as annuli will result in overestimated fish age. Split rings are often confused with true annulus when interpreting the growth structures at the dorsal and ventral part of the otoliths.

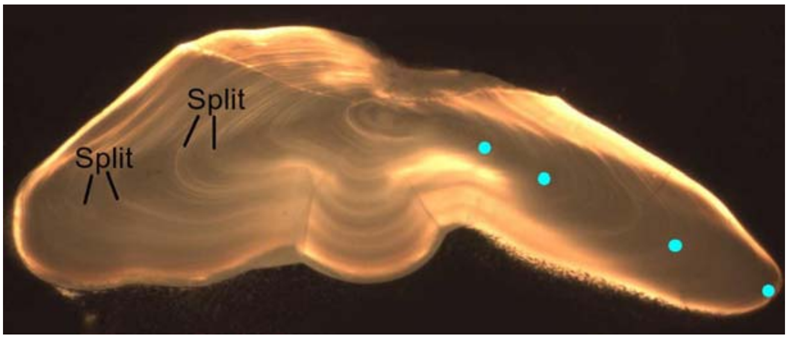

Figure 3.8.3. Photo of a four(??)-years old cod otolith. Split rings in the second and third annulus are visible.

- The appearance of the otolith edge varies seasonally and between age classes (Figure 3.8.4). The translucent annuli is deposited in autumn and often completed before New Year for young fish. Older fish might deposit the translucent band over a longer period. Note that there are also regional differences in timing of otolith zone formation, and annual temperature variations will also affect timing of translucent growth (e.g. Pilling et al., 1997).

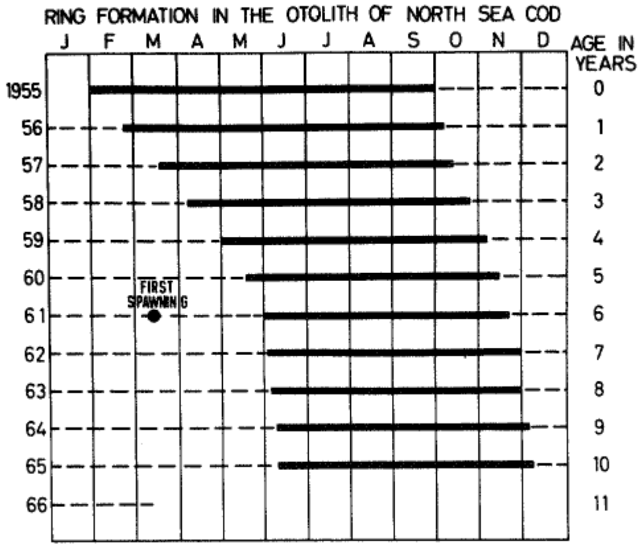

Figure 3.8.4. Timing of the growth of opaque and translucent zones in the otoliths of cod from the North Sea. Solid lines are opaque growth, dotted lines are translucent growth. From Williams and Bedford, 1974.

4 Resolve interpretation differences between readers and laboratories (Tor b)

Pictures of cod otoliths with the respective nations annotations were discussed in plenum as part of the image calibration exercise described in section 5.2. The key issues relevant for consistent age interpretation were identified and are illustrated in section 3.8.

4.1 General disagreements

A key issue was identified as interpretation of the first annuli which can be confused with a first translucent band most likely deposited in the time the juvenile cod identified settles to the bottom. The first annulus is wider than the first translucent band, approximately two and one millimetre in diameter respectively.

Another source of confusion was interpretation of age 1 cod captured during quarter 1 which have quite wide opaque edge growth. Some aged these fish to be two years old since they assumed the translucent band was deposited after New Year, and the opaque edge represented a summer growth period. The agreed interpretation is that the translucent band is deposited in the period autumn – New Year, and the opaque edge growth is deposited during the winter months in quarter 1.

A third source of misinterpretation is the occurrence of split rings. Some of the translucent annulus can consist of several thinner translucent bands that can be misinterpreted as true annulus which leads to overestimation of fish age. These bands can be identified as being thinner that true annulus and with less distance between them.

4.2 Recommended actions for resolving interpretation differences between readers

As part of the discussion of the otoliths in the group, the following guidelines or recommendations for age estimation of North Sea cod were produced.

4.2.1 Transmitted vs reflected light

Two types of light settings can be used when viewing otolith in microscope, transmitted or reflected light. Detailed structures in the otolith upper surface are seen when using reflected light and less detailed structures in the whole section are seen when using transmitted light. For interpreting the structures and assigning a fish age the reader should be able to use both sources of light. Using only one source of light, transmitted or reflected light, means that valuable information will not be available.

4.2.2 Switching otolith side.

The growth structures in otolith sections may vary considerable due to differences in growth such as lobes. The two surfaces of an otolith section might therefore look quite differently. Otoliths that are difficult to age should therefore be also be examined on the other side since new information can then be visible.

4.2.3 Seasonality of otolith zone formation.

An important requirement for accurate age estimation is knowledge of timing of translucent annuli growth. Regional differences in seasonality of otolith growth within species, together with age- and temperature-specific otolith growth are all factors important to consider when assigning fish age (Wilson and Beckman, 1974, Pilling et al., 2007). More information of seasonal timing of translucent zone formation is therefore needed. Appendix 2 shows an example of what should be produced for all major regions from where cod are landed.

4.2.4 Reference collections of validated otoliths

To our knowledge, no validated otoliths of North Sea cod otoliths exist. Accuracy of age estimates can therefore not be calculated. Validated cod otoliths should be produced for each cod stock i.e. from catch-recapture studies where otoliths are marked with i.e. oxytetracycline.

4.2.5 First translucent ring

It seems like much of the discrepancies in estimating age is caused by different perception of the first translucent band. Some interpret that as the first annuli, while other interprets that as the assumed settlement check. The group recommends exploring the natural variability in the size of the first translucent band in order to separate it from the settlement check and second annual ring. The first ring should be validated by using otolith microstructure (daily increments) and size measurements.

4.2.6 Terminology

It is common to use the terms winter ring and summer ring for translucent and opaque zone respectively. However, that terminology implies that the translucent band is formed at winter which is not always the case. It is therefore recommended to only use the term translucent and opaque bands which do not imply formation at a specific season.

4.2.7 Photographing otoliths routinely.

There are several advantages with photographing all cod otoliths on a routinely basis like documentation, image databases, estimating fish growth rates etc. However, this would mean that most laboratories must completely re-organize their way of working, including developing new infrastructure. The different laboratories could consider using image analyses on a more routinely basis.

Otolith growth curves and measurements of distances between rings can be a good help for evaluating translucent bands that are difficult to interpret as the first annual band or false checks. The interpretation of otolith structures will then move towards a more objective manner when the readers have measurements to support interpretation of the otolith structures.

5 Traditional age determination combined with recent development in image analysis (Tor c)

5.1 Summary of the exchange prior to WKARNSC

A thorough report of the results of the North Sea cod otolith exchange 2005/2006 has been produced (Analysis of Data from the North Sea Cod (*Gadus morhua*) Otolith International Exchange Scheme 2005/2006, by Fiona Woods, Ireland). At the WKARNSC Workshop first day a summary of the results from the otolith exchange was presented, both based on the previous report and new analyses. The main results are given below.

5.1.1 Objectives

Objectives of the North Sea cod otolith exchange were to:

1) Investigate the levels of agreement on age readings

2) Analyse the relative differences between reader ages

3) Examine the effect of preparation method on reader performance since some laboratory reads sectioned otoliths, and some reads broken otoliths.

5.1.2 Material and methods

Nine institutions and 22 age readers participated in the otolith exchange, four of the institutions reads broken otoliths, and five reads sectioned otoliths (Table 5.1.1).

Table 5.1.1. Overview of institutions and age readers participating in the North Sea cod otolith exchange 2005/2006.

Name	Institute	Country	
Ilse Maertens	ILVO-Sea Fisheries Department	Belgium	Sectioned otoliths
Bart Maertens	ILVO-Sea Fisheries Department	Belgium	
Martine Moermann	ILVO-Sea Fisheries Department	Belgium	
Mick Easey	CEFAS Lowestoft Laboratory	England	
Dave Brown	CEFAS Lowestoft Laboratory	England	
Gary Burt	CEFAS Lowestoft Laboratory	England	
Brian Harley	CEFAS Lowestoft Laboratory	England	
Mary Brown	CEFAS Lowestoft Laboratory	England	
Robert Bellail	IFREMER (Lorient)	France	
Jean-Louise Dufour	IFREMER (Boulogne)	France	
Friederike Beussel	BFA fur Fisherei	Germany	
Dorit Schroeder	BFA fur Fisherei	Germany	
Gerrit Rink	RIVO	Netherlands	
Yolanda Jongejans	RIVO	Netherlands	
Harald Laresen	Institute of Marine Research	Norway	Broken otoliths
Lisbet Solbakken	Institute of Marine Research	Norway	
Gordon Henderson	FRS Marine Laboratory	Scotland	
Maria Mathewson	FRS Marine Laboratory	Scotland	
Anne-Marie Palmén-Bratt	National Board of Fisheries	Sweden	
Raffie Sjöberg	National Board of Fisheries	Sweden	
Tommy Henriksen	Danish Institute of Fisheries Research	Denmark	
Knut Jensen	Danish Institute of Fisheries Research	Denmark	

The exchanged otoliths were from ICES areas IVb and IVc. All readers should assign age to 62 sectioned otoliths samples in quarter 1, and 46 broken or sectioned otoliths, depending on the respective laboratories ageing procedure, sampled in quarter 3.

Data was analysed using the EFAN-sheet developed by Guus Eltink (AGE COMPARISON.XLS excel workbook by Guus Eltink, RIVO, Ijmuiden, The Netherlands).

5.1.3 Results

Modal age groups 0-7 were presented in the otolith exchange, with relatively most of the modal age groups 1, 2 and 3 (Table 5.1.2).

Table 5.1.2. Number of fish per modal age group.

Modal age	Number of fish	Percent fish
0	10	9.3
1	21	19.4
2	21	19.4
3	18	16.7
4	13	12.0
5	12	11.1
6	12	11.1
7	1	0.9

There was varying number of otoliths read by each person, ranging from 44-108. The readers were positioned in the EFAN-sheet according to experience level, the most experienced readers to the left. 22 age readers were participating, but one common German age was given. Thus 21 readers were compared.

5.1.4 Precision

Mean precision of age estimate for individual fish were Coefficient of Variation (CV) of 39.8% and percent agreement to modal age of 74%. There were large variations in precision of age estimate between individual fish, with CV ranging from 0 – 246% and percent agreement range from 30 to 100%.

Precision of individual age readers, given as precision of age estimate within each modal age group, also varied significantly (Figure 5.1.1 and 5.1.2). Two readers had CV below 10% and seven readers had CV between 10 and 15% respectively, while five readers had CV higher than 45%.

Percent agreement showed similar variation. Five age readers had values above 90%, while four readers had values below 60%. These precision measures demonstrate that there are large variations in age reader’s performance.

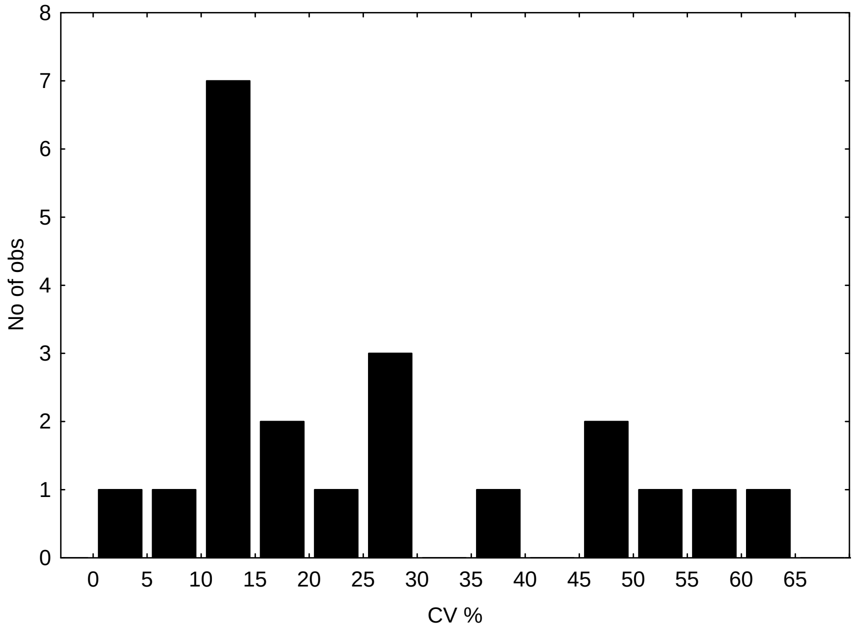

Figure 5.1.1. Histogram of age readers Coefficient of Variation (CV%) with all modal age groups combined.

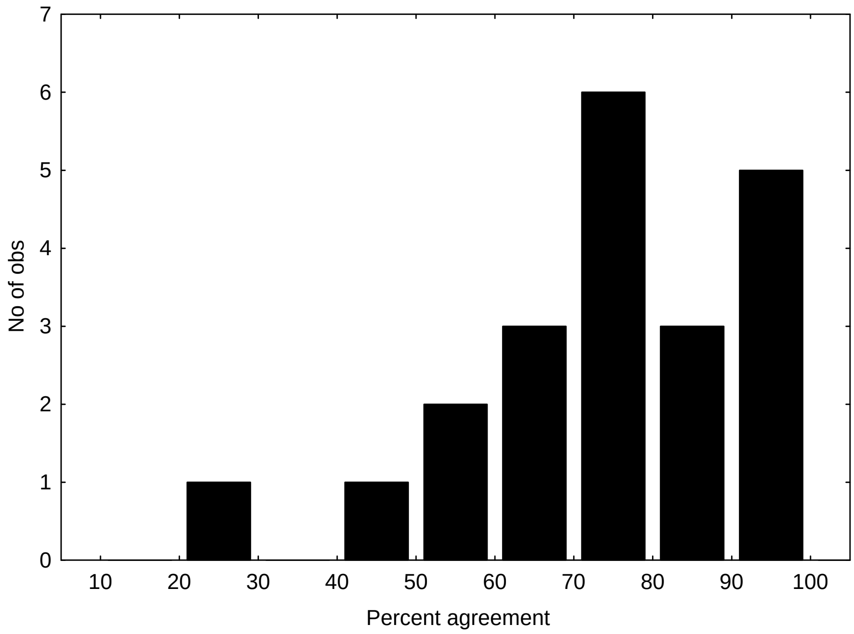

Figure 5.1.2. Histogram of age readers percent agreement with all modal age groups combined.

5.1.5 Relative bias (accuracy)

Two measures of relative bias are given. Mean estimated age for each modal age group is given for each reader. The difference between mean and modal age is then a measure of relative bias. When combining all modal age groups the majority of the age readers have values above 0 (Figure 5.1.3), meaning that fish age is overestimated compared to modal age. Most age readers overestimate age less that 0.1 year, but three overestimate ages by more than 0.6 years.

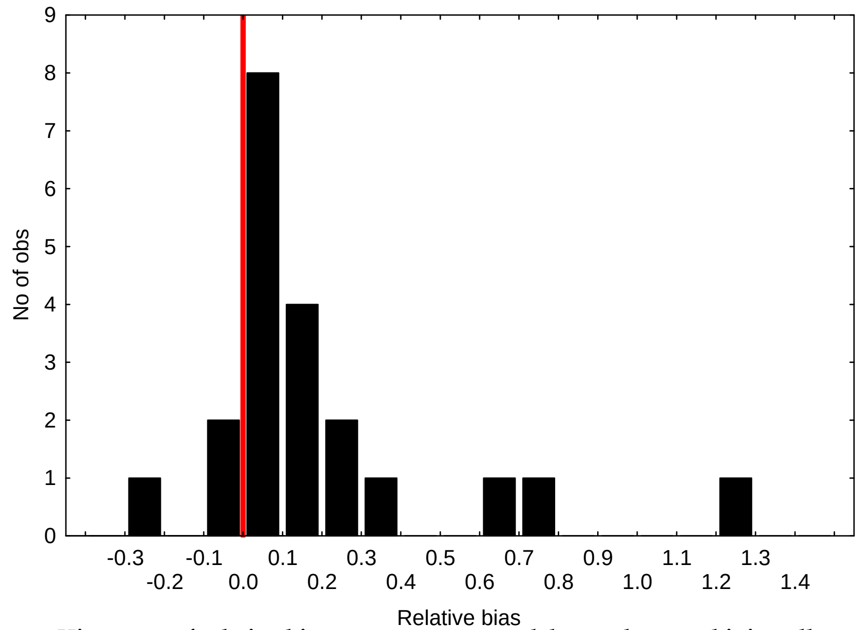

Figure 5.1.3. Histogram of relative bias, as mean age – modal age when combining all age classes. Age readers to right of 0 (red line) overestimate age, while readers to left underestimate age.

Relative bias was also compared between individual age readers by Wilcoxon`s test. With a total of 21 age readers, there are 210 combinations of intra-readers comparisons (Table 5.1.3). 40% of the comparisons showed certainty of bias between readers, 13% showed possibility of bias, and 47% showed no sign of bias. When comparing each reader to modal age the percent showing certainty of bias, possible bias and no bias were 24, 9 and 67% respectively.

Relative bias was also examined within laboratories. Four of the eight laboratories showed no sign of bias between readers (England, Scotland, France and Belgium). Three laboratories showed certainty bias between readers (Norway, Sweden and The Netherlands), but two of the three were then comparing an experience and an inexperienced reader. Denmark showed possible sign of bias between the readers, but only 44 otolith otoliths were read by both readers. Thus, there seems to be consistent age estimates between experienced individual age readers within laboratories.

Table 5.1.4. Inter-reader bias test and reader against modal age test. Readers 1-10 were used for calculating modal age.

	Mick Easey Reader 1	Jean Louis Dufour Reader 2	Gerrit Rink Reader 3	Anne Marie Palmén Bratt Reader 4	Rajlie Sjoberg Reader 5	Schroeder/ Beussel Reader 6	Maria Mathewson Reader 7	Kurt Jensen Reader 8	Ilse Maertens Reader 9	Robert Bellail Reader 10	Harald Larsen Reader 11	Gordon Henderson Reader 12	Dave Brown Reader 13	Brian Harley Reader 14	Mary Brown Reader 15	Martine Moerman Reader 16	Tommy Henriksen Reader 17	Gary Burt Reader 18	Yolanda Jongejans Reader 19	Bart Maertens Reader 20	Lisbet Solbakken Reader 21
Reader 1																					
Reader 2	–																				
Reader 3	**	*																			
Reader 4	–	–	*																		
Reader 5	**	**	**	**																	
Reader 6	–	–	*	–	**																
Reader 7	**	–	*	–	–	–															
Reader 8	–	–	–	–	–	–	–														
Reader 9	**	*	–	–	**	–	**	–													
Reader 10	–	–	*	–	**	–	–	–	*												
Reader 11	**	**	–	**	**	**	**	**	**	**											
Reader 12	–	–	*	–	–	–	–	–	*	–	**										
Reader 13	–	–	**	**	–	–	–	–	–	–	**	–									
Reader 14	–	*	**	*	–	–	–	–	**	–	**	–	–								
Reader 15	–	–	**	–	*	–	–	–	*	–	**	–	–	–							
Reader 16	**	*	–	–	**	–	*	–	–	*	**	*	–	**	*						
Reader 17	**	**	–	–	–	*	–	*	**	**	**	–	–	*	**	**					
Reader 18	–	–	*	–	**	–	–	–	*	–	**	–	–	–	–	*	**				
Reader 19	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**			
Reader 20	*	–	–	–	**	–	*	–	–	–	**	*	–	**	*	–	**	–	**		
Reader 21	**	**	*	**	**	**	**	**	**	**	–	**	**	**	**	**	**	**	**	**	
MODAL (R1-R10)	–	–	*	–	**	–	–	–	**	–	**	–	–	–	–	*	**	–	–	–	**

– = no sign of bias (p>0.05)
* = possibility of bias (0.01<p<0.05)
* * = certainty of bias (p<0.01)

5.1.6 Comparison of broken versus sectioned otolith reading method

A robust comparison between the two reading methods was not possible with this otolith material since only 36 otoliths were prepared for both methods. Also the age range of those otoliths was very narrow: 87% were in the modal age interval 0-3 years old. Thirdly, there were different numbers of age readers using the two preparation methods, eight using the broken otolith method and 12 using the sectioned method. However, keeping these limitations in mind some vague analysis and conclusions might be drawn.

CV of age estimate for individual fish was 39.3 and 22.5 % for the broken and sectioned reading method respectively. Percent agreement (to modal age) was 56.8 and 80% respectively.

Relative bias between readers of the broken otolith methods showed 43% certainty of bias, and 25% were different from modal age. The same numbers for readers using the sectioned otolith method were 26 and 17% respectively. However, it should be mentioned that the relative biases were dominated by readers from Norway and Netherlands being different from the other countries.

5.1.7 Conclusion

- Significant variations in North Sea cod age estimates between readers were found. Both low precision, and relative biases between readers were found. Overestimation of fish ages seems to be the problem.
- There were generally consistent ageing within laboratories. This suggests that there is laboratory-specific different interpretation of the otolith structures. Hence, there is a need to agree on how to interpret the cod otolith growth structures.
- There are possibly better precision and less relative bias among readers using the sectioned versus broken otolith method, but firm conclusions cannot be draw due to limitations of the otolith material.

5.2 Image analysis exercise

5.2.1 Materials and methods

The image analysis system tool makes use of XY-coordinates corresponding to the points, the reader marks as age structures on the digitised image of the otoliths.

Prior to the exercise the readers agreed on one axis from the centre and towards the edge along the rostrum along which all points should be placed. All reading on the digitised images were done by marking the first age structure as the first point and then marking all identified age structures along the agreed axis. All points logged on each individual otolith were then transferred into an Excel spreadsheet with the correct ID (otolith number, picture number and reader ID). The readers agreed to mark the outer edge of each translucent ring identified as an annual structure.

From the XY coordinates recorded by the age readers in the image analysis programme the first ring was calculated as the mean X and mean Y for each otolith and each reader. This starting point was then used to compare individual reader interpretations of translucent rings. The data coordinates were further subjected to statistical analyses for the variance in different interpretations of the age structures and the span of different positions of the actual structures.

5.2.2 Results and discussion

The spreadsheet program, which combined image analysis and plots, made it possible to demonstrate where the individual age readers interpret the rings directly on the digitised images of the otoliths. Some otoliths showed to be very difficult to reach a common interpretation of the age and the points counted as age structures were scattered along the otolith, however, most otoliths were agreed upon by the readers.

Section 3.8 gives a thorough discussion of the issues raised during the evaluation of the exercise results; however, the following gives the more general patterns of disagreement as expressed by the X-Y coordinates by reader.

Definition of the first ring: The width of O1 was subject for discussion. Figure 5.2.1 illustrates the most frequent disagreement in interpretation of O1. Some readers defined the first translucent zone as O1, whereas other readers discarded this structure as a 'settling' ring. The group agreed that otolith microstructure analysis of these structures would be useful as such analysis would rectify the interpretation of O1.

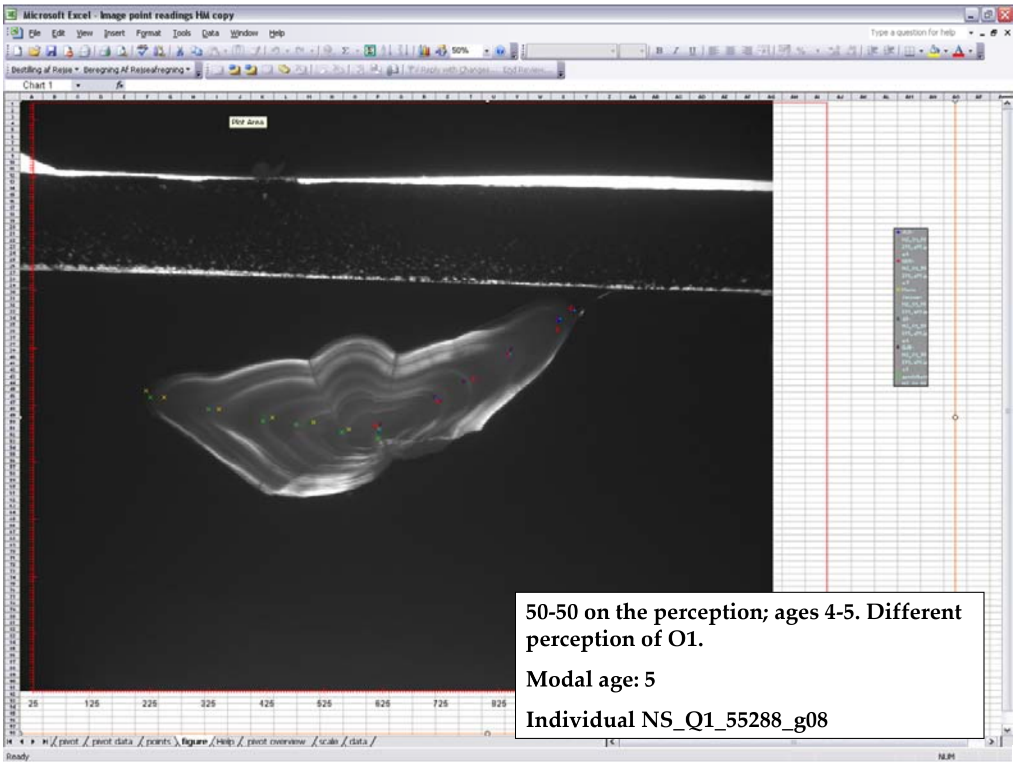

Figure 5.2.1. Example of disagreement of the definition of the first age structure (O1). Coloured marks are the X-Y coordinates set by the readers.

Definition of 'false' rings: Some annual rings appeared to be 'too narrow' compared to the remaining rings were not counted by some readers. Some distinction between 'true' and 'false' rings were performed, but the pattern is not easily recognized based on the present material. The group agreed that a combination of validation of age structures through otolith microstructure analysis and a set of average distances between consecutive age structures obtained by measurements of agreed age otoliths would help resolve these discrepancies in perception of which structures to count as annual structures. Figure 5.2.2 illustrates examples of differentiated perception of annual structures.

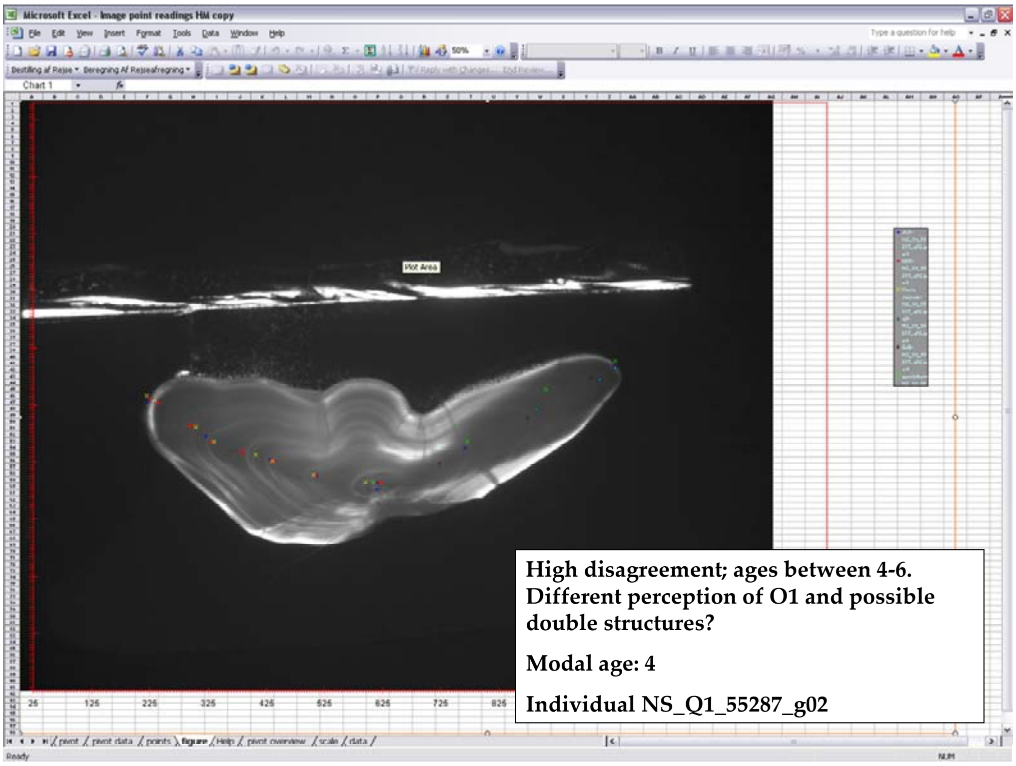

Figure 5.2.2. Example of different perception of age structures between readers. Coloured marks are the X-Y coordinates set by the readers.

Definition of the annual structure: The 'boundaries' for each annual structure were not so well defined in some of the otoliths, which again potentially gave rise to the discussion of 'true' and 'false' structures. Figure 5.2.3 show an otolith with agreed age 5, but with some variation in definition of O1. This was seen in a dominant part of the otoliths in the exercise.

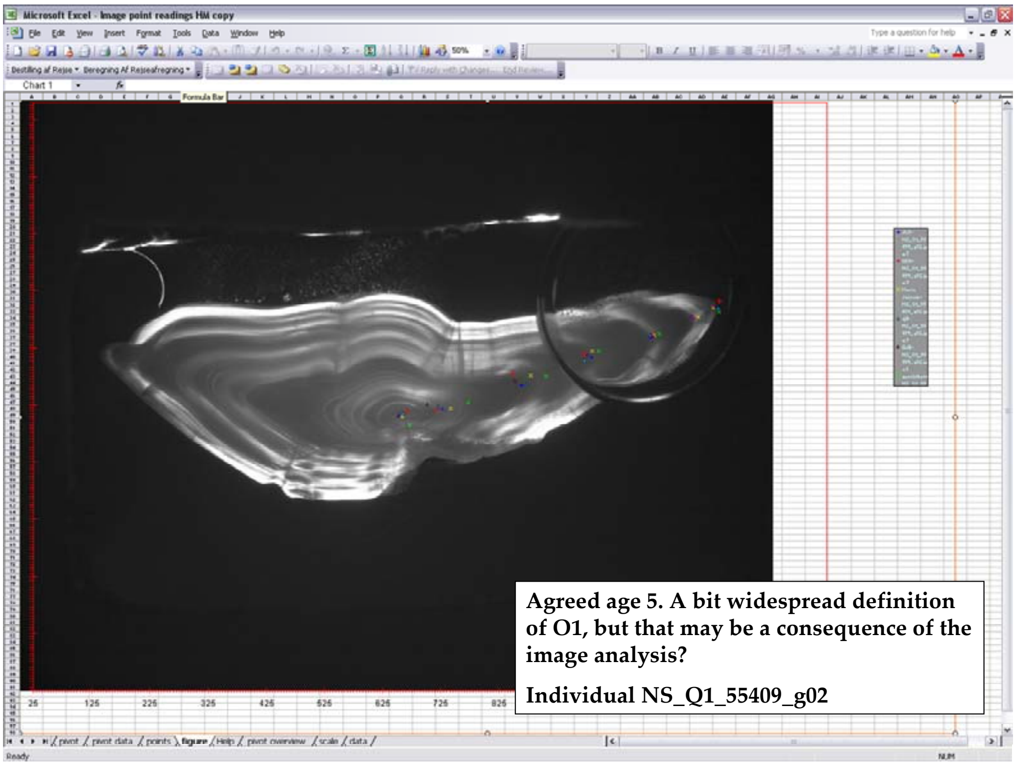

Figure 5.2.3. Example of a widespread definition of O1. Coloured marks are the X-Y coordinates set by the readers.

In cases where a reasonably common interpretation of individual rings existed, disagreement arose where some readers choose to leave out specific rings identified by other readers as true annual rings. Identification of ring position is in general varying between readers, even in readings, which estimate age equal to the modal age, do not all have the same interpretation of ring position.

The quality of the images used in the exercise were in some readers mind not as good as they potentially could be and thus a second round of readings were performed on new images of the same otoliths under improved settings in terms of light, image information, etc. This did, however, not give a clear signal in percentage agreement and it was not possible to directly conclude that improved image quality gave a different interpretation of the individual otoliths (Table 5.2.1). However, the improved image quality did facilitate the image analysis for the readers and it must be underlined that the quality of any images used for the purpose of age estimation, image calibration exercises and the like is of outstanding importance and the guidelines outlined in section 3.7 should be followed.

Table 5.2.1. Comparison of the modal age and percentage agreement reached in the two image analysis calibration exercises during the workshop.

ID	Modal age 1st WS reading	1st WS reading agreement	Modal age 2nd WS reading	2nd WS reading agreement
Cod 26 IVb.tif	4	75%	4	88%
Cod 29 IVb.tif	3	63%	4	75%
Cod 4 IVc.tif	1	75%	1	63%
Cod 40 IVb.tif	4	38%	6	38%
Cod 50 IVb.tif	6	63%	6	75%
Cod 54 IVb.tif	6	100%	6	75%
Cod 58 IVb.tif	6	38%	6	50%
Cod 59 IVb.tif	5	88%	5	100%
Cod 6 IVc.tif	3	100%	1	71%
Cod 7 IVc.tif	1	63%	1	63%
Overall agreement		**70%**		**70%**

As part of the second image analysis exercise, at new software program TNPC was applied to the readings performed in ImageJ. The X-Y coordinates were transferred to TNPC and the program was applied to calculate general growth for each otolith by reader and then compare these growth curves to examine for differences in interpretation of growth structures. If the curves line up, the readers seem to have the same interpretation of the age structures (Figure 5.2.4a), whereas if the curves differ, the interpretation of the age structures are different and thus the average growth (Figure 5.2.4b). In cases, where the interpretation is not unison, a general growth curve, produced as the sum of a number of growth curves from individuals from the exact same stock and area, can be applied to 'force' the annotation of the age structures upon the digitized otolith image and from this get an age estimation. A very important prerequisite for this method is a firm knowledge of the general otolith growth from cod in that particular area and at best known-age material matching the sample of wild cod.

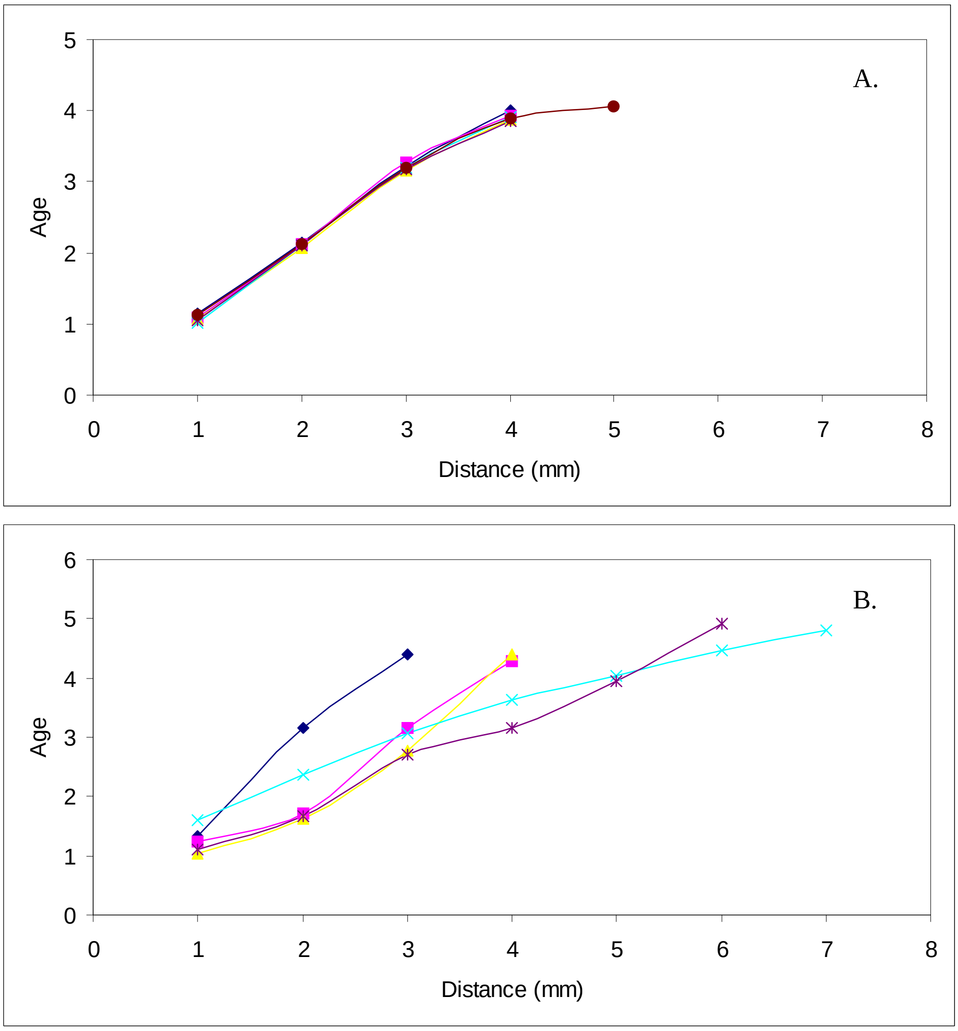

Figure 5.2.4. Comparison of the distance between rings for the Cod26IVb (A.) and Cod40IVb (B.). Each line coloured is a fitted growth curve from the age estimation made by the individual readers.

5.2.3 Conclusions

The exercise pointed out some of the major problems in ageing otoliths of North Sea cod, clarifying the predominant sources of disagreement as outlined in sections 4 and 5.2.2.

The application of image analysis in a calibration exercise showed to be a very valuable tool to resolve interpretation differences between readers. The importance of image quality and clear definition of the tasks of the readers was underlined. Using image analysis not only adds the possibility to use measurements and other tools in the reading process, it also 'forces' the reader to look more objectively at the otolith, reducing the subjectivity in the ageing process. If the image analysis programme used gives the opportunity to calculate general growth curves based on the markings of the age structures, this aids considerably in the interpretation of otoliths with deviating structures.

6 Collate agreed age reference collection (Tor d)

A set of agreed North Sea cod otoliths were produced during the workshop, though it turned out to be difficult to achieve 100% agreement on the age of most otoliths. A selection of the agreed otoliths is shown below, and all agreed otoliths were distributed to the workshop partners on a DVD.

This is to be regarded as the merely beginning of a set of agreed age otoliths for North Sea cod. The reference collection should be expanded considerably through exchange of otoliths and images. Additionally the group agreed that the collection of agreed age otoliths should not stand alone, but be a part of a larger compilation of data on ‘typical’ otoliths for the species and area, in which measurements of O1, typical distances between age-structures, edge development over season, and general growth curves for the sub-stocks of cod in the North Sea.

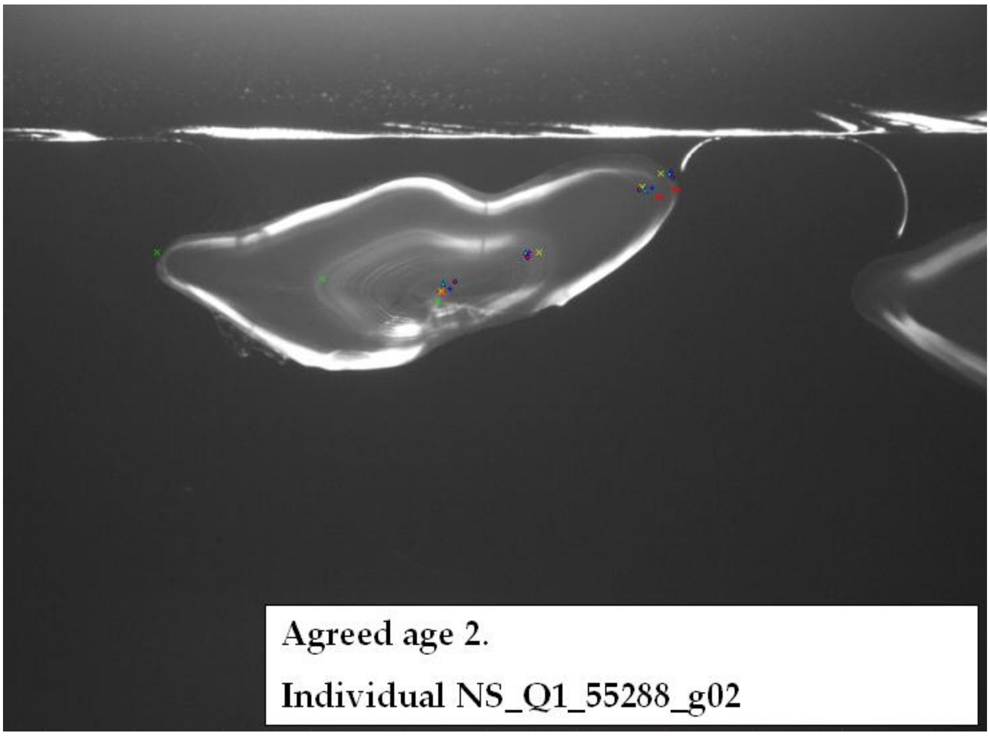

Figure 6.1. Agreed age 2. Coloured marks are the X-Y coordinates set by the readers.

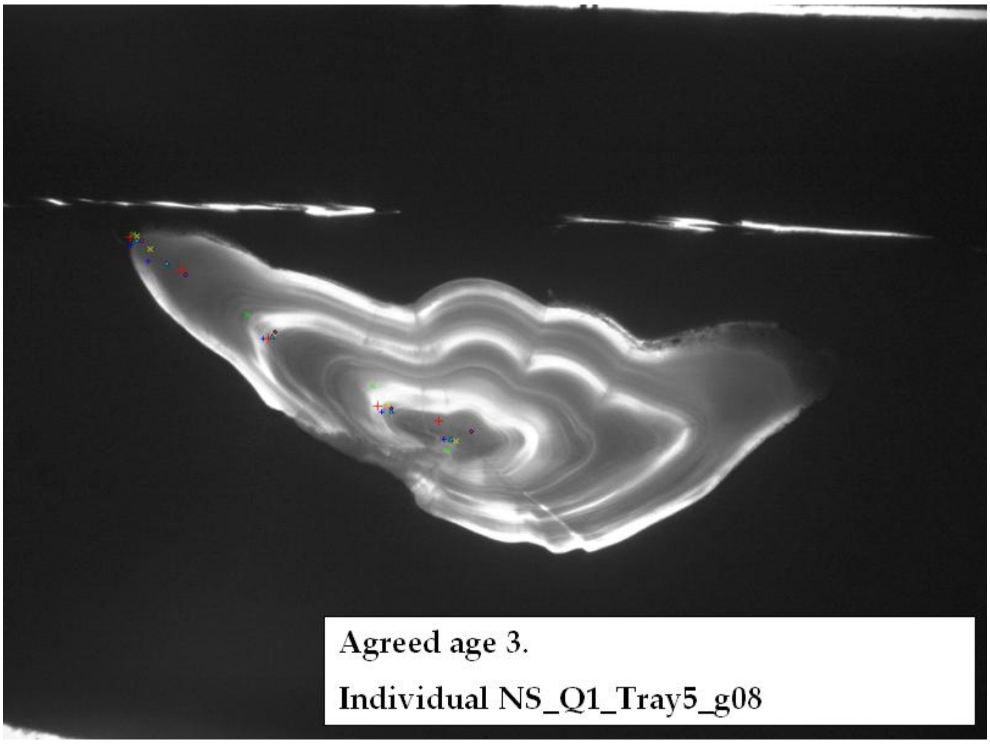

Figure 6.2. Agreed age 3. Coloured marks are the X-Y coordinates set by the readers.

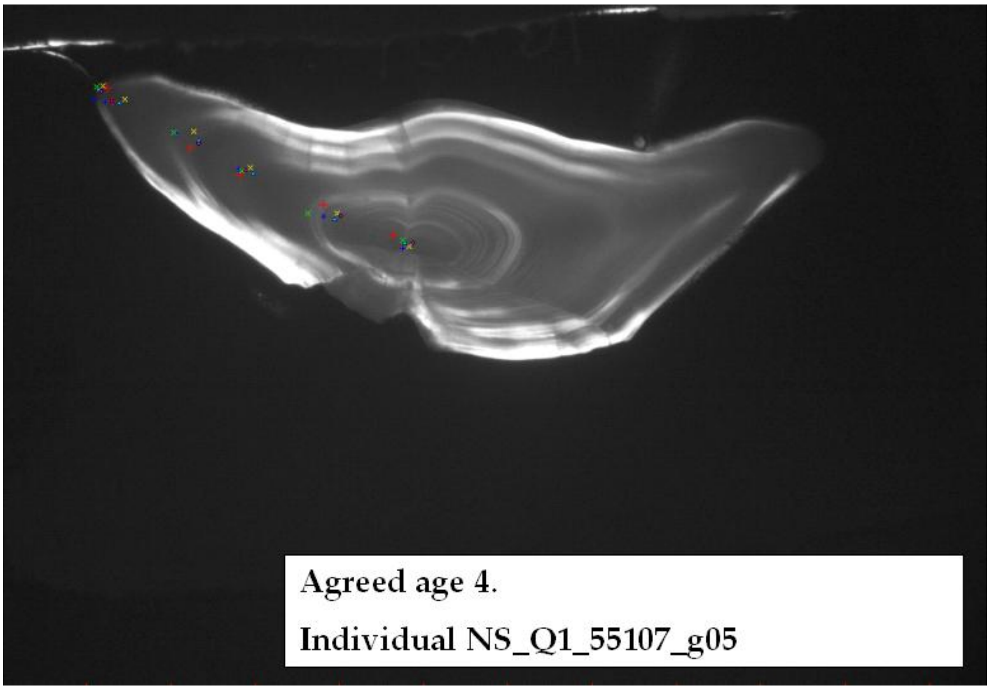

Figure 6.3. Agreed age 4. Coloured marks are the X-Y coordinates set by the readers.

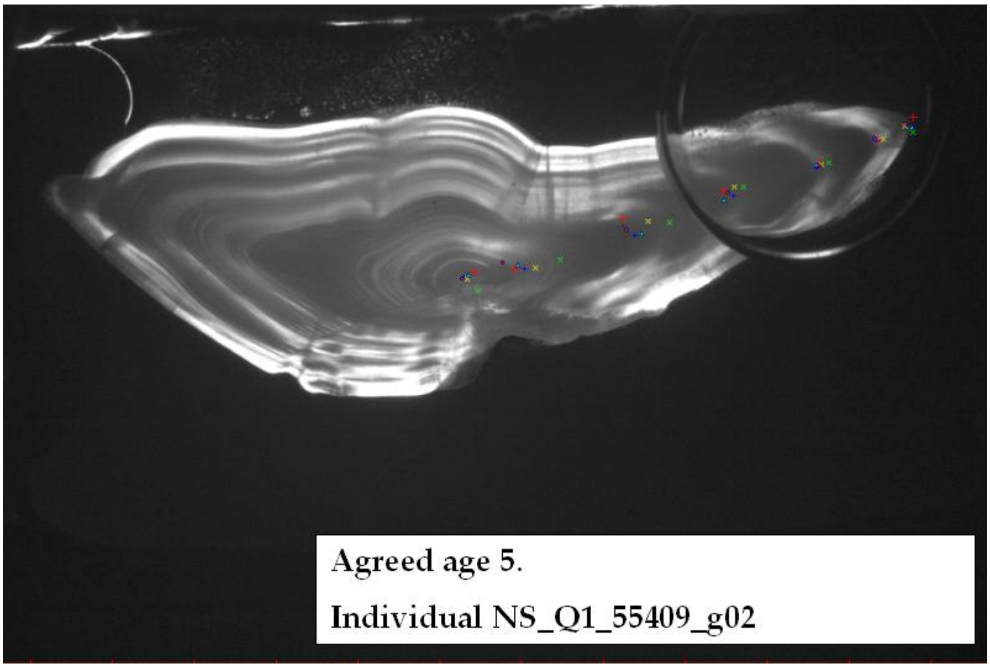

Figure 6.4. Agreed age 5. Coloured marks are the X-Y coordinates set by the readers.

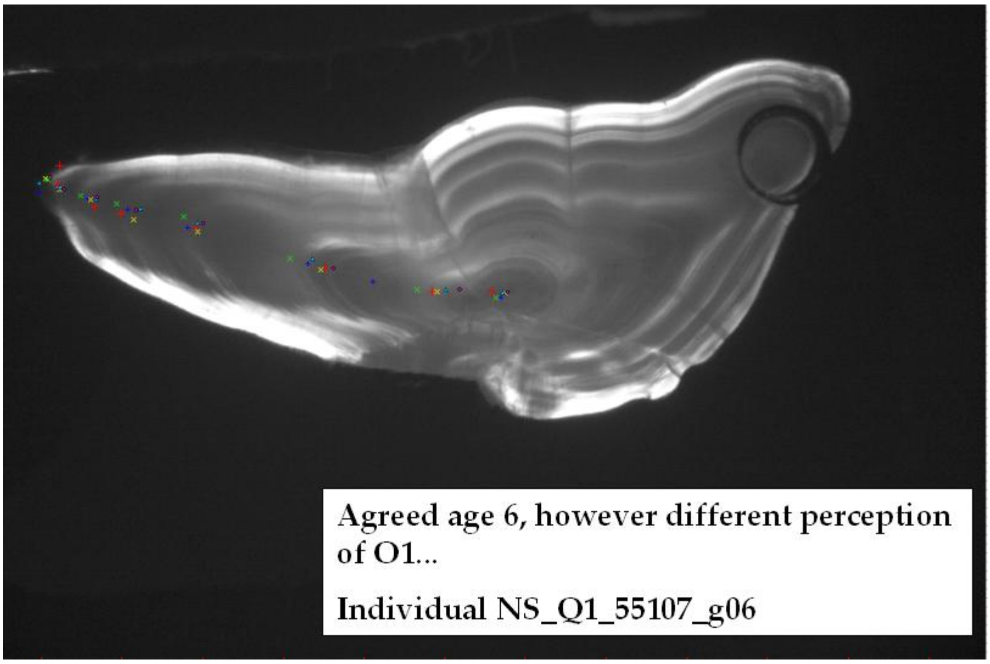

Figure 6.5. Agreed age 6. Coloured marks are the X-Y coordinates set by the readers.

7 Recommendations for further cooperation, exchanges, workshops and other actions in relation to the age estimation of North Sea cod (Tor e)

7.1 General recommendations

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, some of the major problems in ageing otoliths of North Sea cod. The group reached agreement on a definition of an ageing protocol/guidelines mentioned in the present report and the aim is to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures. The group strongly recommends that all ageing laboratories processing North Sea cod should include the guidelines developed during the workshop in their ageing manuals. If possible the ICES system should facilitate the distribution of these guidelines to all relevant laboratories.

All participants in the workshop agreed to follow the defined guidelines in the present report.

All labs are recommended to use image analysis in the process of ageing North Sea cod to the extent possible for the individual laboratories. It is the intention to compile a dataset consisting of measurements on distances between age-structures from all stocks and areas from which the groups get samples of North Sea cod. This will be the basis of an international reference collection and is intended to be used in future workshops.

Through the discussions at the workshop it became apparent that the various life history traits for the North Sea cod may differ within the North Sea and that knowledge of this is highly important for the age readers. In addition, all age readers would benefit from more information on the formation of otolith structures in North Sea cod, especially the formation of split rings. Thus, the group recommends the inclusion of studies on otolith formation in general and the North Sea cod physiology/growth/behaviour in relation to this as a part of the training and updating of all North Sea cod age readers.

Below are some general recommendations by the group for further action.

7.1.1 Manual

The age reading manual produced at this workshop should be maintained and further developed in the future. The report should be published e.g. through CORDIS.

7.1.2 Standardized reading within laboratories.

New readers should be trained by experienced readers for a long time order to ensure agreement within laboratories. Regular documentation for new readers should be performed, and a minimum level of agreement must be achieved before readers can estimate fish age independently. New readers should focus on one species at time in order to avoid confusion between species.

7.1.3 Quality control between labs.

Otolith exchanges should also be performed regularly every second or third year, but with rather few otoliths (e.g. 25 otolith pictures) involved each time. In case of disagreement in the age readings, larger exchanges should be performed. The PGCCDBS should appoint a coordinator to lead this work.

7.1.4 Regular workshops

In order to follow up work it is important to have workshops at regular intervals at 3-5 years intervals. This would lead to increased focus on each fish species and a continuation of the work

It should be obligatory to attend at workshops and otolith exchanges for all laboratories contributing age estimates for assessment of a fish stock.

8 References

Pilling, G.M., Millner, R.S., Easey, M.W., Maxwell, D.L., Tidd, A.N., 2007. Phenology and North Sea cod *Gadus morhua* L.: has climate change affected otolith annulus formation and growth? J. Fish Biol. 70, 584-599.

Williams, T., Bedford, B.C., 1974. The use of otoliths for age determination. In: Bagenal, T.B. (Ed.), Ageing of Fish, Unwin Brothers, England, pp. 114-123.

Annex 1: Agenda

Workshop on Age Reading of North Sea Cod (WKARNSC) (Chairs Lotte Worsøe Clausen, Denmark and Hans Høie, Norway). North Sea Centre, Hirtshals, Denmark. August 5th to August 7th, 2008.

Agenda:

Tuesday, 5th of August 2008

10:00 – 13:00: Welcome and introduction to the workshop

ToR's

Introduction to a new approach to age estimation of cod

Exchange results

13:00 – 14:00: Lunch

14:00 – 17:00: Age reading exercise applying image analysis

Wednesday, 6th of August 2008

09:00 – 11:00: Evaluation of the age reading exercise

Resolve interpretation differences between readers and laboratories by performing an in depth analysis of difference in age reader interpretation of otolith spatial patterns and explore the usage of metric measurements of otolith structures as a solution to minimize the divergence in age estimation of North Sea cod

11:00 – 12:00: Selection of an Agreed age collection from the calibration material

12:00 – 13:00: Lunch

13:00 – 17:00: Evaluate the use of traditional age determination combined with recent development in image analysis in order to include back-calculation of length at age and growth based on age structures defined by age-readers;

Thursday 7th of August 2008

09:00 – 12:00: Compilation of a manual for age estimation of North Sea cod

12:00 – 13:00: Lunch

13:00 – 16:00: Workshop round off;

Make recommendations for further cooperation between North Sea Cod age readers, otolith sample exchange, bilateral cooperation, and workshops

Annex 2: List of participants

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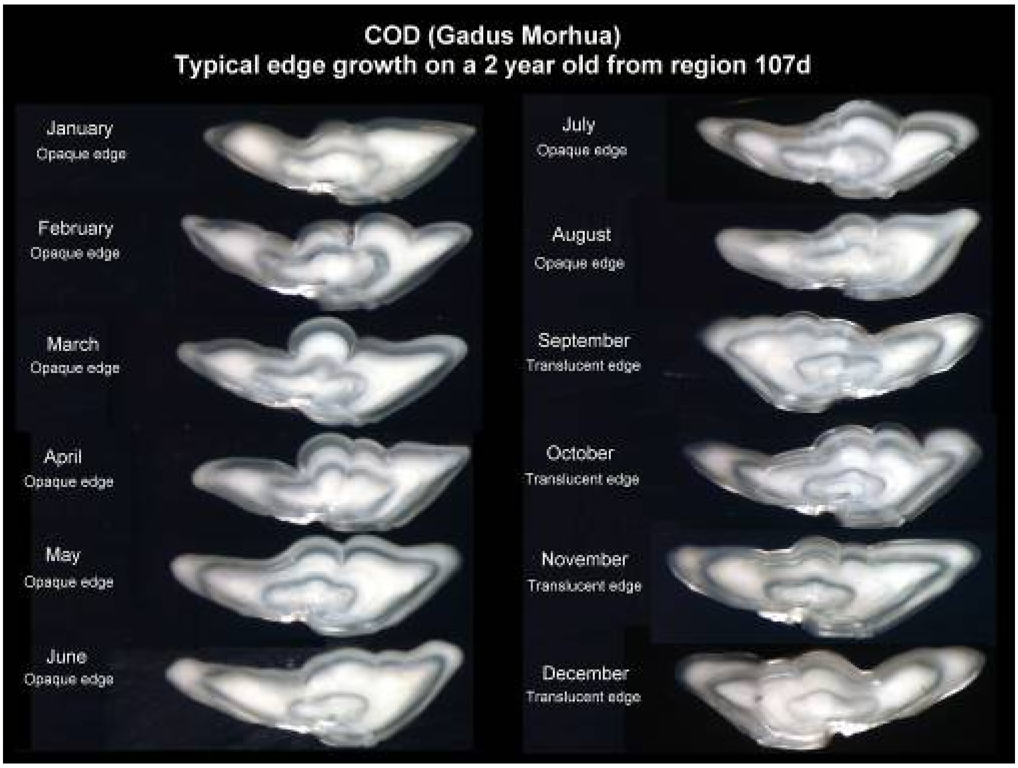
Tor Ivar Halland	tor.ivar.halland@imr.no

Annex 3: COD (Gadus Morhua) – Typical edge growth on a 2-year-old from region 107d



Annex 4:

Age reading protocols from different laboratories

Germany

Description of age reading procedures: Gadoid age determination at the Johann Heinrich von Thünen Institute, Institute of Sea Fisheries, Hamburg, Germany

Species:	Cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*), whiting (*Merlangius merlangus*), Norway pout (*Trisopterus esmarkii*)
Stock(s):	North Sea (ICES areas IIIa, IV, VIId), Northeast Arctic (ICES Sub-areas I, II), Greenland (ICES Div. XIVb, NAFO Sub-area 1)
Last update of description:	29 July 2008
Average no. of otoliths read:	North Sea cod: approx. 2000/year
Description by:	Dr. Christoph Stransky Johann Heinrich von Thünen Institute [vTI] Federal Research Institute for Rural Areas, Forestry and Fisheries Institute of Sea Fisheries Palmaille 9, 22767 Hamburg, Germany Tel. +49 40 38905-228, Fax: +49 40 38905-263, e-mail: christoph.stransky@vti.bund.de
Contact details of reader(s):	Dorit Schröder / Friederike Beußel Johann Heinrich von Thünen Institute [vTI] Federal Research Institute for Rural Areas, Forestry and Fisheries Institute of Sea Fisheries Palmaille 9, 22767 Hamburg, Germany Tel. +49 40 38905-101, Fax: +49 40 38905-263, e-mail: friederike.beussel@vti.bund.de / dorit.schroeder@vti.bund.de

1. Introduction

Since the founding of the Institute of Sea Fisheries in 1938 and the re-establishment of the Federal Research Centre of Fisheries after 1948, the age of fish has been determined on the basis of hard structures. For over 20 years, only the otoliths (sagittae) of the species to be investigated have been used for this purpose. Presently, two technicians determine the age of gadoid fish (plus two on blue whiting only, on an irregular basis) and take part in international otolith exchanges (*e.g.* Woods 2007, Mahé 2008) and age reading workshops (*e.g.* Easey *et al.* 2006). Both technicians are experienced readers with 20 and 7 years ageing experience, respectively. For each fish species group, at least two persons in charge are available to verify any unclear or doubtful readings. Samples are obtained from commercial fishing trips and research surveys at sea.

North Sea cod otoliths have been thin-sectioned since the late 1970s. Otoliths had been embedded into black resin, and the sections were read using normal transmitted light. Since 2004, however, transparent resin is being used for embedding, and the sections are read using polarised transmitted light.

2. Methods

2.1. Otolith removal

In the case of small gadoid fish (and Norway pout in general), the cut can be made through the cranium. The knife is applied to the head behind the eyes and brought down through the head until it can be folded forwards. The brain is visible then. The otoliths are to the right and left next to the brain in a pocket. Only the largest otolith, the sagitta, is being removed (which as a rule is also the only one which can be seen), and in each case only one otolith is removed, either the right or the left one.
In the case of large fish, the cranium is often too hard and the otoliths have to be removed by cutting the gills.
For North Sea cod, otoliths of two fish are collected per station and length category, but with a maximum of 10 per Roundfish Area.
The otoliths are stored individually in a small paper bag, on which the following information is given:

- trip number
- station number
- area
- fish species
- length, weight, sex and maturity stage

2.2. Embedding

The centre (the nucleus) of the otolith is marked with a lateral pencil mark on the convex (sulcus) side of the otolith. The otoliths marked in this way are embedded into rectangular blocks (300 x 40 x 10-15 mm) with a mixture of synthetic resin and hardener. A silicon casting mould is used with three depression lines, with 13 linear grooves (3 mm wide and 2 mm deep at 2 cm intervals) for marking the nucleus positions. Some spare area of the mould depressions is being used for labelling the block.
The following work is carried out under a fume hood for health and safety reasons.
Application of the resin-hardener mixture: A block contains 120-130 ml GTS polyester casting resin (35-40% styrene) and 6 ml MEKP hardener (Voss Chemie GmbH, Uetersen, Germany). The two products are mixed together carefully (to avoid air bubbles) in a plastic receptacle (N.B.: Beware of the heat build-up during the mixing process). Wait a few minutes until any air bubbles have risen to the surface. The resin mixture is then poured out into the bottom of the mould depression. The mould is placed so that the label side is to the left. It is necessary to wait for about 20 minutes until the mixture has started to set: hard enough for the otoliths not to sink in when placed on top, soft enough to fix them.
The otoliths are placed along the 13 grooves, starting in the right-hand bottom corner and working row by row from bottom to top up to the edge of the label area to the left. The otolith record sequence is kept to and transferred row by row numerically into a mould recording sheet so that the position of each otolith is traceable from both records. Since in the further processing the block is turned round, the mould record is made from top left to bottom right. The otoliths are arranged in 13 vertical rows in such a way that the lateral nucleus marking on their underneath is congruent with the groove on the bottom. In this way, 13 rows are created, each with 4-13 otoliths (depending on size) arranged. Their marked underside faces the person carrying out the embedding. This prevents air bubbles forming under the otoliths during the embedding process. After an approx. 10 minute drying period, the above-mentioned mixture is poured onto the aligned otoliths, until they are covered. The thickness of the blocks therefore varies between 1 and 1.5 cm, depending on the thickness of the otoliths.
After a further 20 minutes, a label is placed on the left three cm of the resin block, on which the fish species, trip, date and block numbering are noted. After about 24 hours, the solid blocks are removed from the container and left for a further week in the fume hood for complete hardening and evaporation.

2.3. Sectioning

In the next process, thin-sections are produced from the nucleus region of the otoliths. For this purpose, the block is turned so that the left-hand side with the label faces bottom right, the bottom of the grooves therefore now becomes visible on the top.
The block is clamped into the carriage of a half-automated (projection motor) mineralogy sawing machine (Conrad, Clausthal-Zellerfeld, Germany) at the first of the 13 ridges of the block. Thin-sections of the block are produced between two diamond-tipped saw blades of 0.3 mm thickness and 100 mm diameter, rotating at 6000 rpm and using tab water for cooling. The distance between the blades (and thus the thickness of the sections) can be adapted with plastic or steel spacers of 0.5, 0.6 or 0.75 mm thickness. The usual section thickness for gadoid otoliths is 0.5-0.6 mm. Then the block is shifted one ridge further and the next section is produced.

2.4. Mounting of the thin-sections

For reading (and archiving purposes), the thin-sections are affixed in sequence between two glass plates. For this purpose, a glass plate (89 x 119 x 1 mm) is edged with cut glued (general purpose glue) cardboard strips or spaghetti. On the left-hand size, two narrow vertical paper strips are affixed for the inscription. Then a correspondingly small quantity of the resin-hardener mixture described above is applied. This is spread using a small piece of cardboard as a thin film on the base of the glass plate with the omission of the inscription strips. Now the thin-sections are applied. No. 1 top left, No. 2 to the right of it, No. 3 under No. 1, No. 4 under No. 2, etc. The left-hand inscription strip is inscribed again with fish species, vessel, date, quarter and current number of otoliths of the entire plate, the right-hand strip counts the rows (1, 3, 5, ...). The rest of the mixture is then poured onto the plates, and another unprocessed plate is placed on top, free of air bubbles, carefully from one side to avoid the inclusion of air bubbles. Superfluous glue escaping in the process can be removed from the edge as tacky matter after 30 minutes. The plates should dry and evaporate for at least one more day in the fume hood, and then be cleaned (ceramic scraper, acetone).

2.5. Reading

The reading is carried out with a binocular microscope at approx. 7-10x magnification with transmitted light and built-in polarising filter. The hyaline areas (winter rings) are read from the nucleus (centre) to the edge along a so-called A-axis (Fig. 2.5.1). In the case of difficult identification of growth rings on the A-axis, the B-axis (Fig. 2.5.2) can be used for control purposes. Only yearly growth increments are to be recorded. False or intermediate rings can be observed during eventual growth periods in winter, and ‘bottom rings’ (Fig. 2.5.1) during the settlement of juvenile cod onto the bottom (*e.g.* Jantschik 2007) can confound readings. To determine the age, the last winter ring is included in the first and second quarter, but not in the third and fourth quarter of the year (unless a summer zone follows the winter ring, then the marginal winter ring is recorded). Information on the fishing area and date of sampling, as well as biological data (length, sex, maturity, individual weight), is given when the reading is carried out. To ensure good quality of the readings, it is preferred that two readers work together at the discussion binocular microscope. Results are recorded in an otolith recording sheet.

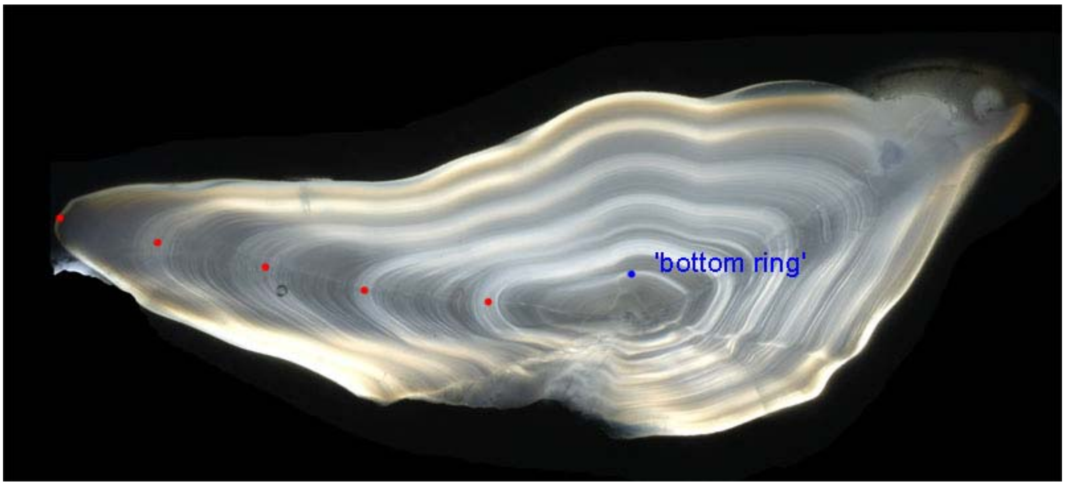


Fig. 2.5.1. Otolith thin-section of a five-year old cod with ‘bottom ring’ (blue dot), reading along the A-axis (red dots).

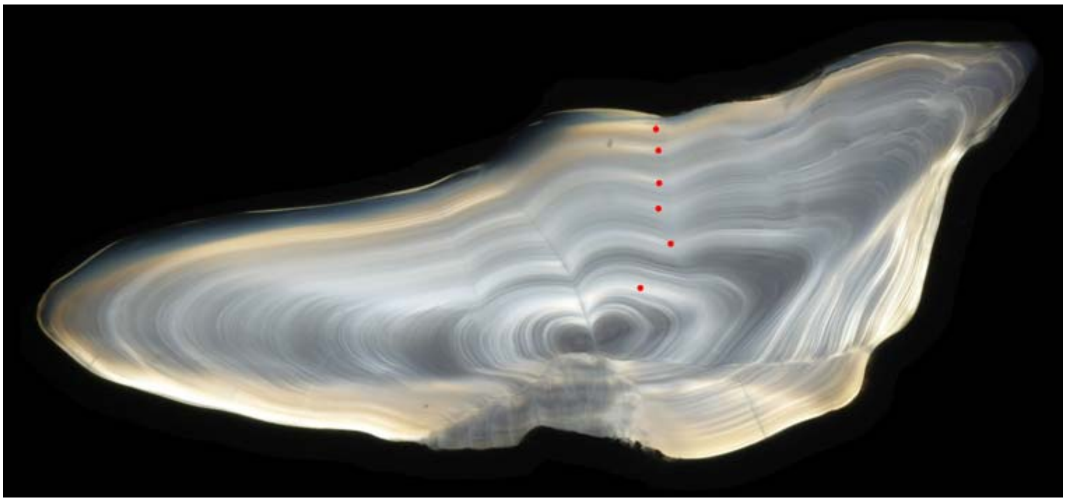


Fig. 2.5.2 Otolith thin-section of a six-year old cod, reading along the B-axis (red dots).

3. Validation

Are there validated otoliths of this species available at the institute?
NO

Have there been exchanges and/or workshops for this species within the past 15 years? Please give references as detailed as possible. If possible, please state who took part (if event is not too ancient).

- North Sea Cod Otolith Exchange Scheme 1997-1998 (Co-ordinator: Scotland; see Newton 1999)
- North Sea Cod (*Gadus morhua*) Otolith International Exchange Scheme 2005/2006 (Co-ordinator: Ireland; Participants: Belgium, Denmark, France, Germany, Netherlands, Norway, Sweden, United Kingdom-England, United Kingdom-Scotland; see Woods 2007)

Are sets of otoliths available for training? If yes, for which age group?

- Otoliths easy to interpret: YES (all age groups)
- Otoliths difficult to interpret: YES (all age groups)

4. Literature:

Easey, M., Henderson, G. and Shanks, A.M. (2006): Report of the Whiting (*Merlangius merlangus*, L.) Otolith Exchange Scheme 2004 and Workshop 2005, CEFAS, Lowestoft, England, 17-20 October 2005, 80 pp.

Jantschik, P. (2007): Ist das Wachstum des Kabeljaus (*Gadus morhua*) an klimatische Oszillation gekoppelt? Diploma Thesis, University of Rostock, Germany, 62 pp.

Mahé, K. (2008): Report of the Saithe (*Polliachius virens*, L.) Otolith Exchange Scheme 2007-2008. IFREMER, Boulogne-sur-Mer, France, 36 pp.

Newton, A.W. (1999): North Sea Cod Otolith Exchange Scheme 1997-1998, EFAN Report 1-99.

Woods, F. (2007): Analysis of Data from the North Sea Cod (*Gadus morhua*) Otolith International Exchange Scheme 2005/2006. Marine Institute, Galway, Ireland, 48 pp.

France

Age estimation of cod from the North Sea, subdiv. IVb, IVc and the Eastern Channel VIId

Introduction

Age determination of cod is performed at IFREMER for the estimation of the demographic structures of landings (North Sea, Eastern Channel, Celtic Sea, 3 PS, etc…) and surveys (IBTS, CGFS, EVHOE) since a very long time.

Age determination is based on the number of annual structures (one opaque and one translucent) as observed in thin slides of sectioned otoliths embedded in translucent polyester resin.

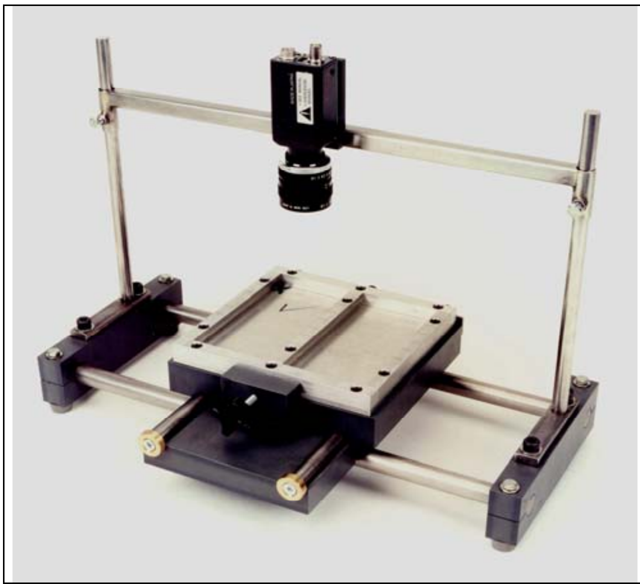
This document describes the methodology which is used to embedded, sectioned and observed the otoliths.

Method

Both sagitta are taken from each individual and stored in small bags of paper. Otoliths needs to be dry before embedding in resin.
Of course, all the biological data of the fish are stored in a database (length, date of capture, weight, area, etc …)

Preparation.

- The otoliths are embedded in polyester resin using a positioning system (stand with a camera and a monitor). The objective is to perfectly align all the nuclei

	
Positioning system	Blocs of otoliths

- The blocs of resin with the otoliths are sectioned in thin slices (0.4 mm of thickness) using a semi automatic sawing machine.

semi automatic sawing machine

- The thins slices are observed on the monitor of a PC connected to a numerical camera fitted on a binocular.

- Each otolith is individualized with a name, an image and the treatment are made with a specific software developed by IFREMER (TNPC which mean numerical treatment of calcified pieces). This software has many features such as :
    - A database of images
    - Drawing the axes of reading
    - Plotting the zones
    - Measurements of distance (from the nucleus to the rings, inter rings, …), of surface, …
    - It can drive a motorized microscope stage
    - And so on …

Scotland

Age estimation of North Sea Cod; Current practice at Fisheries Research Services, Aberdeen, Scotland.

Gordon Henderson, July 2008. DRAFT VERSION.

North Sea Cod otoliths are collected at various fish markets around Scotland and the outer islands.

ICES divisions IVa an IVb are further divided into demersal sampling areas unique to Scotland. Otoliths from cod are collected from each of these sampling areas at a rate of 3 per cm length up to 90cm; beyond 90cm all fish measured are otolithed. The sampling target for each month is 3 otoliths per cm, per sampling area, per gear type.

Only one of the pair of sagittal otoliths is collected from each fish, and placed in a paper envelope for storage. The fish length is recorded on the front of the envelope. A label bearing information on port of landing, species, vessel, date, gear type and fishing area and sample identification number, is placed on the front of the bundle of otoliths from each sample.

Following verification against the length recording sheet used at the fish market, the otoliths are ready for processing. The steps required to prepare the otoliths for reading are set out below:

1) PREPARE THE OTOLITH MOUNT.

The first step in the process is to flatten and smooth the plasticene in the otolith mounts. This is necessary in order to provide a continuous flat surface of sufficient width around the edge of the otolith mount, in to which the prepared otoliths will be placed. The plasticene may be smoothed using the ball of the thumb, or with the handle of a scalpel.

Photo 1: Image of prepared mount ready to receive the processed otoliths.

2) PREPARING THE SAMPLE FOR MOUNTING.

If only one size category is present in the sample, mount the otoliths sequentially, from smallest length to largest in groups of five.

If multiple size categories are present, mount the otoliths in sequential order, by category, in groups of five. By mounting in groups of five, any discrepancies are easier to resolve than if the otoliths were mounted sequentially.

In both cases, retain all the packets as the otoliths are removed from them, and keep them in the original order. The processed otoliths will be returned to the packets when they have been read, and then placed in storage.

Where multiple categories are present within a sample, each category will possess a label preceding the packets containing the otoliths. These “label” packets will bear either a single letter or an alpha-numerical code denoting which category they represent e.g. W, G1, G2 etc. Alpha character denotes fish presentation “whole” or “gutted”; numeric character is assigned sequentially to denote different size categories. These do not necessarily relate to fishing industry size classifications, which may include two size categories as used by FRS. When mounting the otoliths, record the number of otoliths in each category on the top left hand side of each label packet, corresponding to the category. If no category label is present, write the number of otoliths on the front label, on the upper left hand side.

3) MOUNTING PROCEDURE.

Remove the otoliths carefully from their packet, by inserting a finger or a blunt object such as a scalpel handle, into the packet and dislodging the otolith. This process may prove difficult in some instances, as the otoliths can adhere to the packet. If a packet is found to be empty or the otolith is smashed, retain the packet in its correct place within the bundle and write “NO OTO” on the bottom right hand side. This will alert the reader that an age is required for that length, thus avoiding problems with later data processing.

Place the otoliths on a cutting mat in sequential order with their distal surface uppermost. If the otolith has paper residue adhering to the surface, this should be removed by scraping it off with a scalpel blade.

Identify the position of the nucleus as indicated in photograph 4, by the small, raised dome (arrowed) on the otolith surface. Having identified this structure, hold the otolith down on the cutting mat and score a line across the position of the nucleus at right angles to the anterior – posterior axis, with a scalpel. Two or three scores in succession may be required to separate the otolith into two parts. Do not attempt to cut through the otolith using pressure on the blade. This may cause the otolith to shatter, rendering it unusable, or cause the blade to break resulting in personal injury. The importance of placing the break in the correct position is demonstrated by the diagrams on page 6.

If the procedure has been performed correctly, the otolith piece containing the posterior end is normally retained for reading purposes, the other is discarded. In the event that there is only a partial otolith in the packet, or that the otolith breaks in the wrong place, it is possible to carefully trim the otolith back to the position of the nucleus. Occasionally it may be necessary to use the anterior end of the otolith for reading purposes.

When all otoliths in a sample have been processed, mounting can proceed. Taking a plasticene filled mount, mark it with an “L” shaped mark. This is the start point indicator, and all otoliths will be mounted in a clockwise direction from this mark. The cut otolith piece as detailed above is mounted vertically in the plasticene, with the proximal surface facing outwards. Otoliths should be held between the index finger and thumb, placed in position and secured in place with gentle thumb pressure. Care should be taken to ensure that otoliths are inserted in to the mount at a uniform depth. Otoliths are normally mounted in groups of five, with a small gap between each group, continuing around the circumference of the mount. Mounts should be numbered sequentially in Roman numerals, by marking the plasticene with a sharp object such as a scalpel blade or pencil point.

When mounting is complete assemble the packets in the correct order, secure with a rubber band and place in a storage box along with completed mounts.

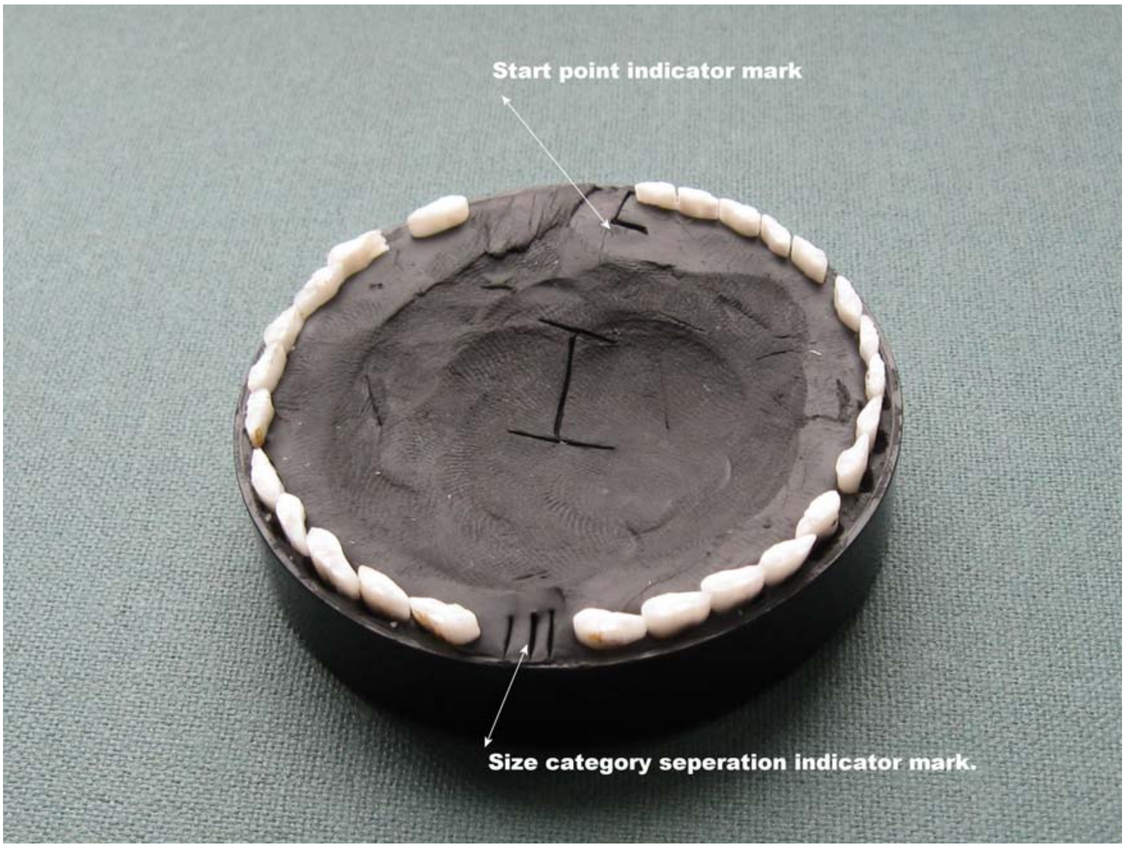


Photo 2: Illustration of completed mount.

An illustration of the location of the structures and surfaces of the otolith.

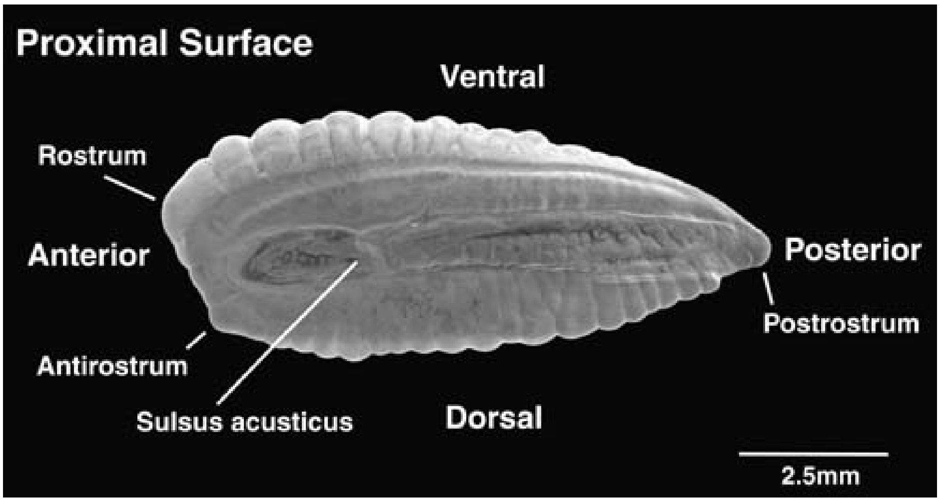


Photo 3: Image courtesy of Dr. Steven Campana, Canada.

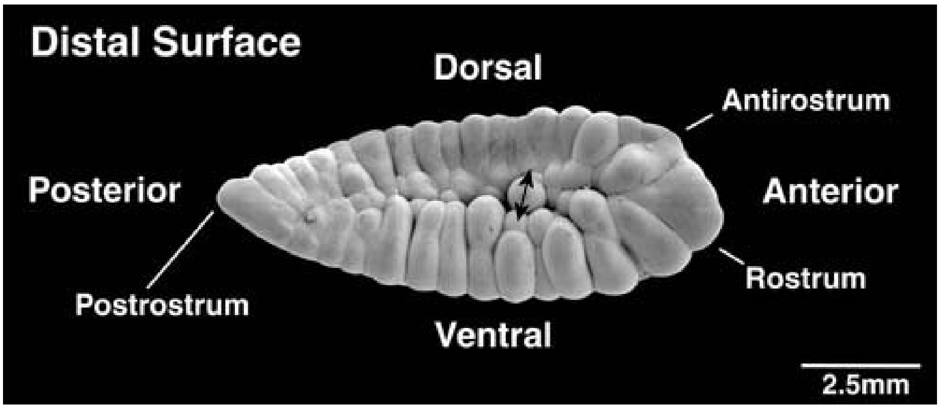
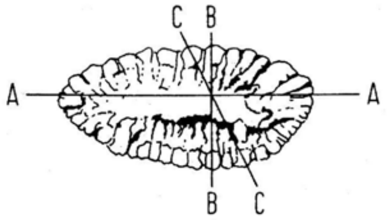
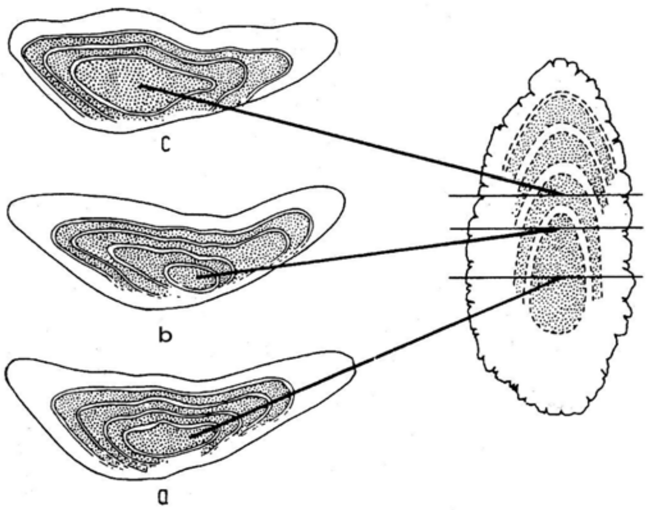


Photo 4: Original image courtesy of Dr. Steven Campana, Canada.

The importance of cutting the otolith in the correct position is illustrated below.



Three possible sections through a cod otolith:
A-A longitudinal; B-B lateral; C-C diagonal.



The varying appearance of the ring structure of a cod otolith when broken.

(a) through the centre of the nucleus
(b) through one end of the nucleus
(c) missing the nucleus completely

Image modified from original in; Williams, R. & Bedford, B. C. (1974). The use of otoliths for age determination.

Age estimation procedure:

The broken surface of each otolith is painted with a thin film of mineral oil to improve clarity of the surface and remove residual debris. The processed otoliths are placed on the stage of the microscope and illuminated by the beam of a free standing bench lamp. By interrupting the "side transmitted" light beam with a finger to alter contrast and brightness, the individual growth zones can be made to stand out. One reader prefers to have a blue filter in the light path as it removes the yellow cast of the lamp.

Otoliths are examined at a total magnification of 10x to 16x, depending on individual reader preference. Age estimation is based on the premise that one translucent and one opaque zone together form a years growth, and that the birthday of the fish is January 1st. Age estimations are produced by counting the opaque zones, preferably along the axis from nucleus to dorsal edge, as illustrated below;

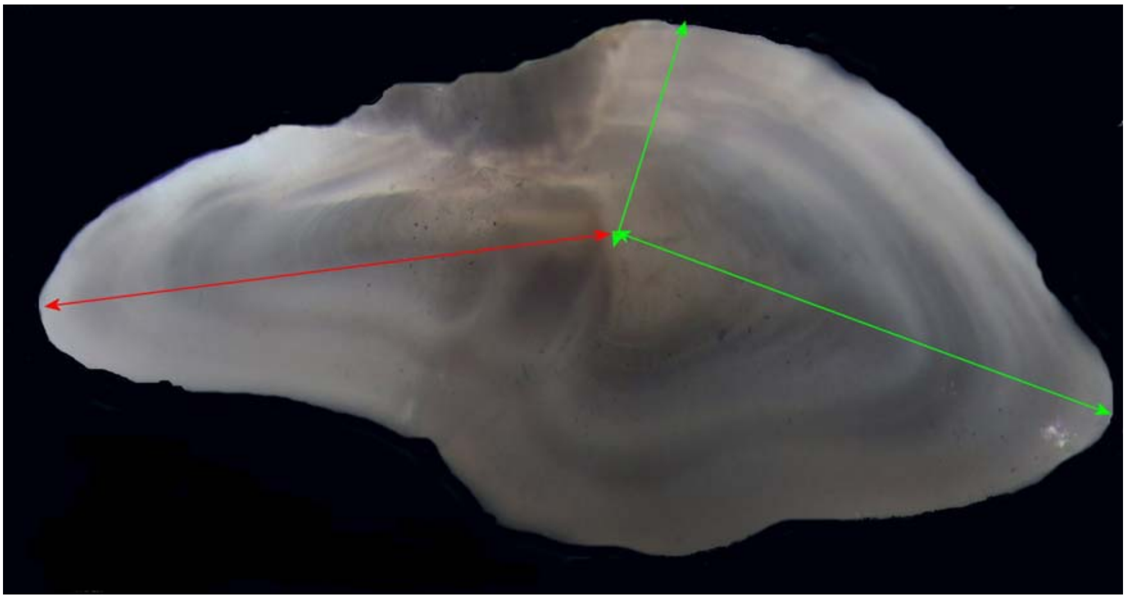


Photo 5: Preferred reading axis shown in red, alternative axes which can be used to corroborate age estimate shown in green. Picture courtesy of A. Edridge.

References:

Williams, R. & Bedford, B. C. (1974). The use of otoliths for age determination. In *The Ageing of Fish* (Bagenal, T. B., ed.), pp. 114–123. London: Unwin Brothers.

The Netherlands

Cod ageing as done by Wageningen-IMARES (The Netherlands)

Cod otoliths from the southern part of the North Sea are relatively easy to read. The growth pattern is very clear because cod is a fast and regularly growing fish.

Since 1998, the otoliths are prepared by means of transverse sections (described in further detail below). For the age determination a binocular with translucent light is used, therefore the translucent rings appear bright and the opaque rings appear dark. The sectioned otoliths are covered with a thin layer of oil to enhance the distinction between opaque and translucent. The translucent rings are counted, usually from the edge of the otolith towards the centre.

If the age determination is expressed as year-class (i.e. "birthday" on Jan. 1^{st}), the period in which the fish is caught has to be taken into account. For cod from the southern North Sea, in January to April, one year has to be added to the count of translucent rings. Approximately around May (when the fish starts growing again) there is a critical moment in deciding if an extra ring/year has to be added or not.

Transverse sections as done by Wageningen-IMARES (The Netherlands)

All otoliths have to be clean and dry. The nucleus is located by means of translucent light, is marked with a waterproof pen. The otoliths are glued (with the concave side up, to prevent the formation of air bubbles) on a resin bottom. The otoliths are positioned with the nucleus on the cutting line, to ensure that transverse section goes through the nucleus. This is achieved with a camera and a monitor. Once the otoliths are positioned, a layer of resin can be placed on top. After the resin is hardened, the otoliths are cut with a diamond blade cutting machine. The sections are glued on a slides with epoxy glue. The sections are not covered with a glass slip.

Sweden

3.12 Cod

3.12.1 Sampling – otoliths

General: When sampling for ageing purposes register total fish length, weight and sex (except for landed fish that is gutted). The sampling bag should be marked with individual number, haul number and vessel code or other sampling identification as well as year and month.
Otoliths: For small fishes: place an incision with approximately 45 degree inclination in the skull. For larger fishes: Preferably insert the knife and work to both sides to open up the skull. A saw may be used when handling very large fish. Break up the skull to expose the otoliths, remove both sagitta otoliths with a pair of tweezers.
Remove remains of membrane and blood by gently rubbing the otolith between your fingers or clean in water or ethanol. If required remove excessive moisture from the otoliths on absorbing paper before they are placed in the sampling bag. The sampling bags are placed standing in a sampling rack of styrene plastic for drying, after which they are gently bundled with string.

3.12.2 Preparation and reading

Otoliths, broken: One of the two sagitta otoliths is broken/parted with a pair of tongs or by hand. If required make a small cut at the nucleus position with a file before breaking. The halves are placed in a lump of clay or are held with a pair of tweezers, with the breaking surface up, during the reading procedure. Moist the breaking surface of the ototliths with water. The otoliths are read in stereomicroscope with reflected or transmitted light in magnification 6.4-16x. To receive better contrast the otolith may be shadowed from the light with a pen or a pair of tweezers. The annuli in the otolith should be studied in several directions and the magnification varies with regards to the size of the otolith. The ototlith is read from the nucleus towards the edge or edges that is most legible.

3.12.3 Assigning age – interpretation of zones (general for all labs.)

See Chapter 2 for description of age and annuli.
Juvenile zone
Close to the nucelus of the otolith is often a small, clear and even ring. This is not counted when assigning age to the fish.
Hyaline zone
The hyaline zones are regular and concentric.
False hyaline zone
False hyaline zones are possibly formed during stress like starvation or sudden temperature changes. Compared to other hyaline zones they are often not complete or diffuse and may be dispositioned compared to the more regular growth pattern of the hyaline zones.
First hyaline zone
Distinct, regular and koncentric not to be confused with the smaller juvenile zone.
Growth zone
Opaque, non-translucent zone, appears white in reflected light. The growth zones of the first years are broader than the hyaline zones.
Problem otoliths
When one or more hyaline zones occurs, with small growth zones in between that considerably deviates from other growth zones, shall the hyaline zones be counted as one..

Baltic Cod:
Juvenile zone
Close to the nucelus of the otolith is often a small, clear and even ring. This is not counted when assigning age.
Hyaline zone
A hyaline zone can be more or less translucent, it has generally the same shape as the otolith and it appears bright in transmitted light. It should in general be visible around the whole otolith but exceptions exists. It can sometimes contain two or more close rings that together are interpreted as one hyaline zone.
False hyaline zone
In general a thin often diffuse ring that is not visible around the whole otolith

<u>Growth zone</u>
Growth zones are opaque, non-translucent zones that appears dark in transmitted light.
<u>Problemotoliter</u>
Formation of false hyaline zones is common in Baltic Cod otoliths. Double and mulitple hyaline rings can lead to misinterpretation. The first hyaline zone is often a double ring especially for fish up to two years. Delayed formation of the last hyaline zone on the edge of the otolith sometimes leads to that it is included in assigning age before it is visible on the ototlih.

Denmark

TACADAR meeting, Enniskillen July 2005.

Age estimation of cod from the North Sea, subdiv. IIIa, and the Baltic.

Lotte Worsøe Clausen, DIFRES

Introduction

Since the 1950's, the estimation of annual demographic structures of landings of cod in Denmark from the surrounding sea has been performed at DIFRES. The amount of analysed individuals vary with the size of landings.

Age estimation is based on the number of annual growth structures defined as one opaque and one translucent zone as observed in broken otoliths immersed in alcohol under a binocular with reflected light.

This document describes the methodology and some results from an exploratory exercise using otolith weight as a proxy for age. Besides, results from a recent age calibration exercise are given along with a new method for calibration exercises.

Method

Both sagitta are taken from each individual, and collected otoliths are kept in trays with one otolith pair per hole from a couple of days to several months until the age determination. Following age estimation the sagitta are weighed on a fine scale weight and stored in individual marked paper-bags.

The otolith macrostructure is very different between the areas from which samples are obtained and as the accuracy and precision of the age determination relies on both a synchronously formation of one opaque and one translucent zone in all individuals and that this pattern is recognisable for all individuals in the population, the time and location of catch is considered vital information for the age estimation of cod. This information is always included in the age estimation process.

The otoliths of cod caught in the Eastern Baltic are very difficult to interprete as a broad range of structures in the otolith can be taken for age-structures. Cod migrating between the very different salinity layers in the area may have additional structures in the otoliths very similar to age-structures caused by this migration. This makes the differentiation between winter- and summer-rings difficult. In addition cod in this area tend to form double-rings in the otoliths further complicating the age estimation.

The age-structures in the otoliths from cod caught in the Western Baltic, Sub area IIIa and the North Sea exhibit much clearer and regular age-structures creating a more easily interpreted pattern. Figures 1 to 3 show otoliths from the Eastern Baltic, Western Baltic and the Kattegat (sub.div IIIaS). The metal rod is of the same size in all the pictures illustrating the differences between the otoliths.

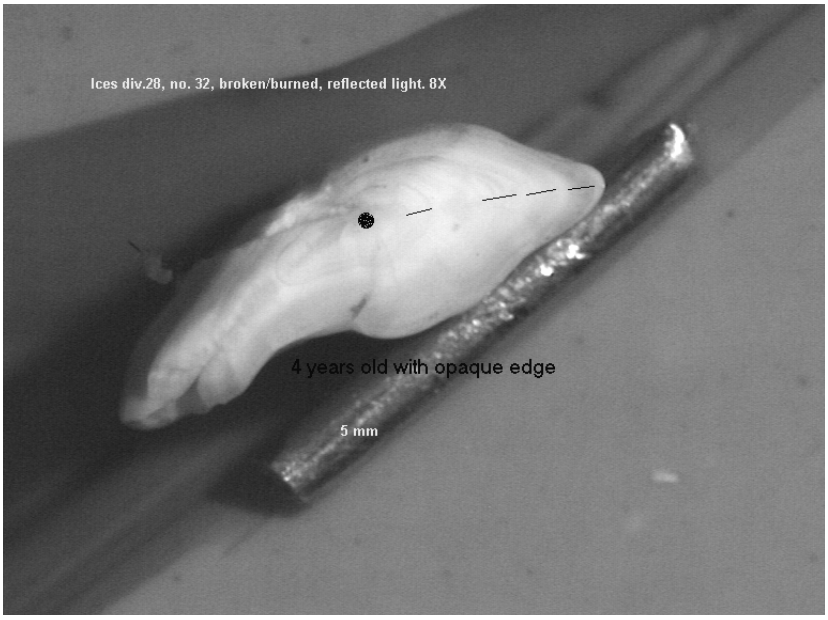


Figure 1. Cod sagitta from the Eastern Baltic. Age: 4
Metal rod size: 5mm

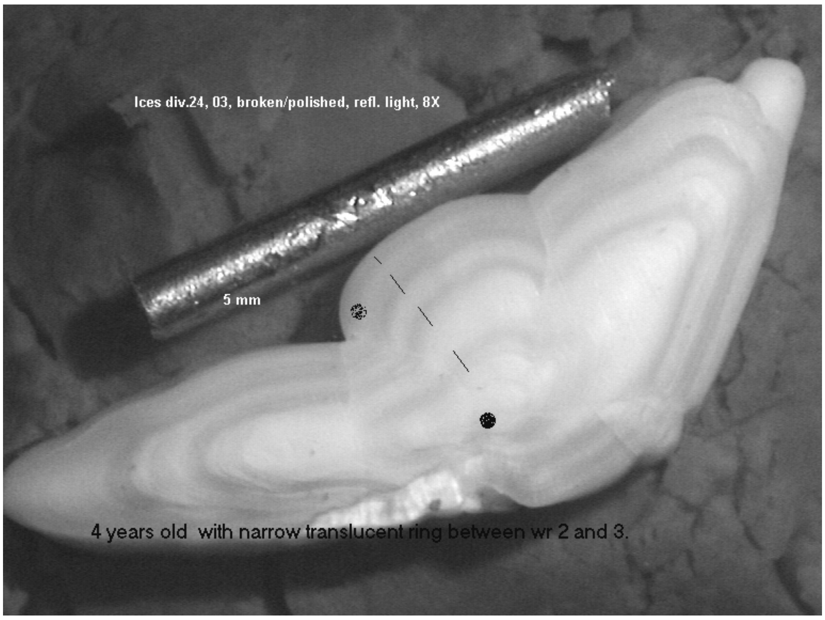


Figure 2. Cod sagitta from the Western Baltic. Age: 4
Metal rod size: 5mm

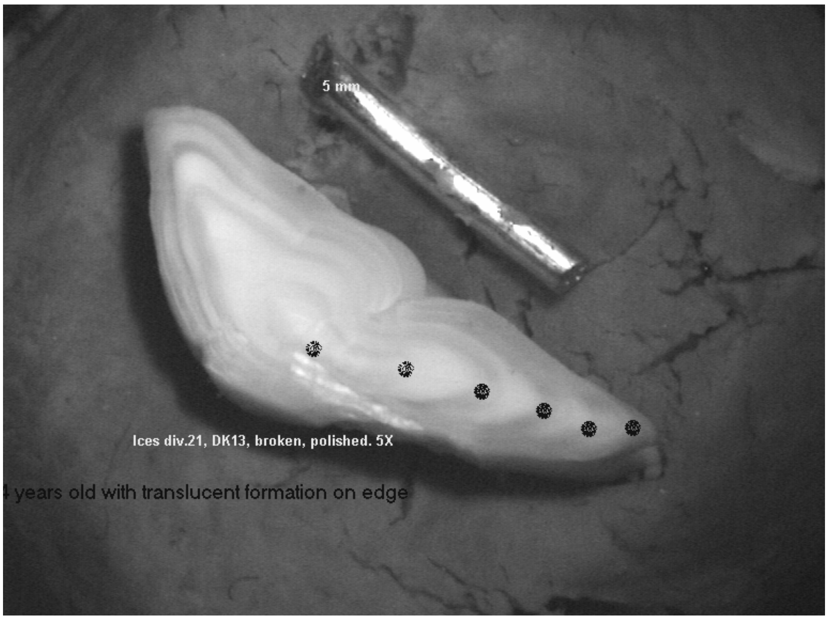


Figure 3. Cod sagitta from Kattegat (sub.div. IIIaS). Age: 5.
Metal rod size: 5mm

Prior to the age estimation the otolith is broken right in the centre and polished shortly to create a smooth surface. It is important to ensure the breakage to be right on the centre to facilitate the age reading. The otolith fixed with forceps and placed in a small glass container (20 mm high with a diameter of 44mm) filled with 96% alcohol to the point where the surface of the broken otolith is submerged in the alcohol. The reading is done using a binocular (Leica MZ6) and reflected focused light (‘cold light’) pointed to the otolith from both the left and the right side in an angle of 45 degrees and with a distance to the glass container of 22 mm (left light source) and 40 mm (right light source). The magnification varies, however the most frequently used is 0.63X. Larger magnification is occasionally used when estimating older individuals to see the edge of the otolith. The reading direction of the translucent age structures varies depending on the quality of the preparation and difficulty of the otolith. Usually the longest axis provides the best view of the translucent age structures. The occasional double-structures formed in otoliths from cod in the Eastern Baltic can be identified by shadowing out the right light source. This reduces the translucent appearance of the extra ring in the structure.

Occasionally a dissection microscope with reflected light (Leica MZ12) connected with a camera (Leica DC 300F) to an image analysis system (Leica IM50 ™) is used to facilitate the discussion of individual otoliths on a computer screen and ultimately for measurements of various features, e.g. the distance from the centre to the first winter ring.

Age estimation is done without the knowledge of fish lengths though they are recorded in the database which contains all information on sampled fish. However, when the age-length distribution has been computed prior to the delivery of data to the Assessment Working Group in ICES, the ALK for the different areas and quarters (or months) are examined in order to pick out possible errors in either age estimation or length measurements.

Otolith weight as proxy for age.

Otolith weight has been used for age determination in a number of fish stocks, and its potential for use for Eastern Baltic cod has previously been highlighted by Reeves (2003). The approach has the advantage that otolith weight can be determined objectively, whereas the interpretation of the structure of the otolith can be a highly subjective process, particularly if the environmental conditions experienced by the fish are such that they do not lay down clear, annual rings in the otoliths as appears to be the case for Eastern Baltic cod. A potential disadvantage is that otolith weight does not give a direct measure of the age of the fish in the way that the numbers of rings in a scale or otolith can (Reeves 2004).
Figure 4 shows the fit of estimated age from otolith weight to the known age of cod from the Faroese Islands.
In the Study Group on Ageing issues of Baltic Cod it was agreed to explore on the use of otolith weight as a proxy for otolith age. It was agreed that national data on otolith weight from research surveys should be obtained from the years 2001-2003 in Sub-divisions 25, 26 and 28. All weights should be measured with a resolution of 1 mg. The data will be used for a statistical evaluation of otolith weight distributions as a measure of age distributions within years (ICES CM 2004/ACFM:21 Ref. G, H). The results of this exercise will be available during 2005.

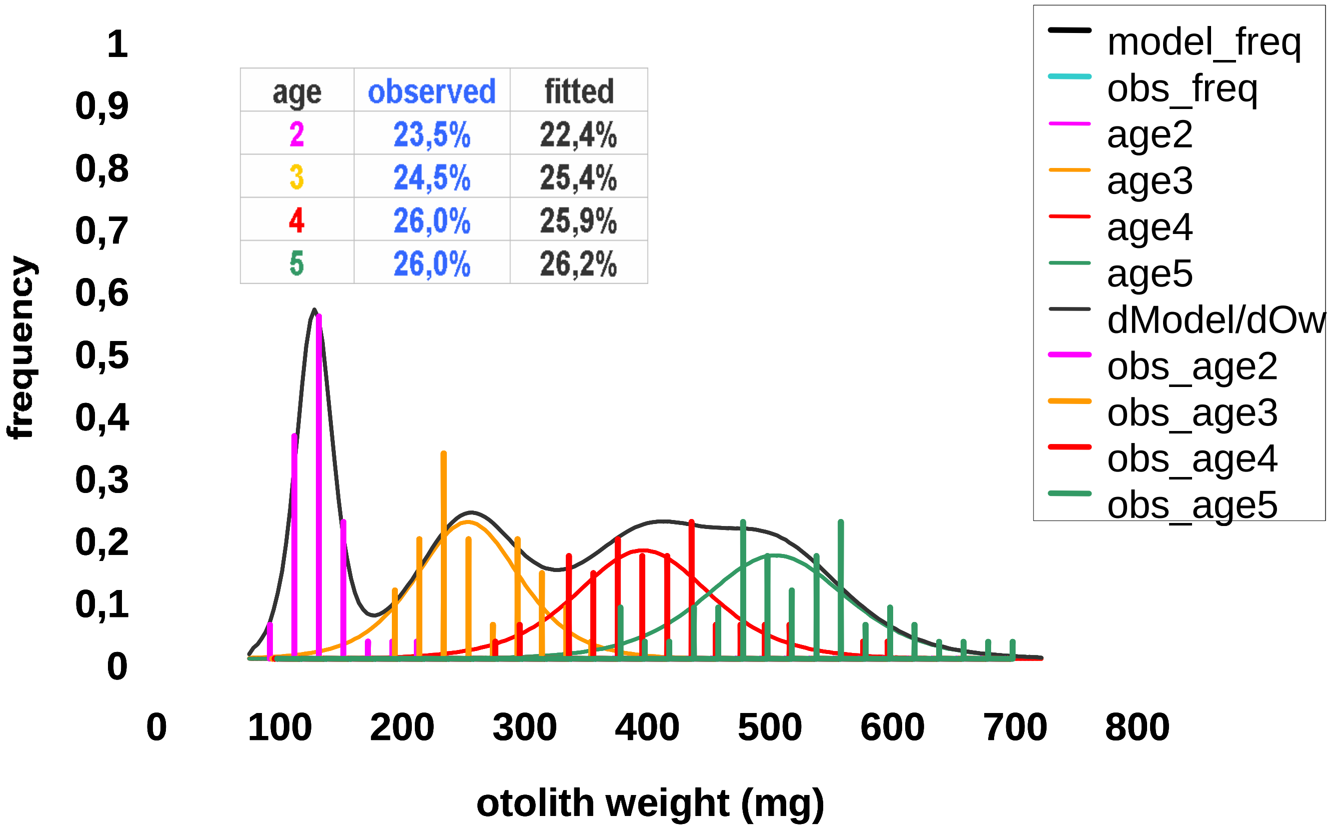


Figure 4. Otolith weight as a proxy for age. Predicted age distribution fits the known age distribution for all age classes; best fit is within the first age groups.

Age estimation calibrations

During the SGABC meeting in Riga in May 2004 a comparative exercise on reader observation on otoliths using image analysis suggested that the method an be used to disentangle and explain reader inconsistencies both between and within readers. It was agreed that such an exercise should be performed intersessionally during an exchange program comprised of both traditional age calibration and image analysis. The image analysis system tool makes use of XY-coordinates corresponding to the points, the age reader marks as age structures on the digitised image of the otoliths.

Two exchange rounds was set up, the first beginning in November 2004 and the second beginning in March 2005. Both sets consisted of two collections; an otolith pair with one broken otolith and one whole otolith and digitised images of the broken otolith on CD. Included on the CD of the otolith images were the relevant data sheets in Excel format. One for the traditional age reading and one for the X-Y coordinate from the image analysis. Also the image analysis programme ImageJ was included on the CD.

The two parts of the exercise was performed simultaneously as the age reader had the otolith exposed under the stereo microscope while pointing at the age structures on the picture using the image analysis system tool and could consult the ‘live’ otolith if the pictures did not show all the desired otolith structures clearly.
During the SGABC meeting in Riga in May 2004 the readers agreed on one axis, the longest axis, along which all points should be placed. All readings on the digitised images were done by marking the centre of the otolith as the first point and then marking all identified age structures along the

agreed axis and ending the reading by marking the edge of the otolith. All points logged on each individual otolith were then transferred into an Excel spreadsheet with the correct ID (otolith number and picture number). The readers were asked to mark the outer edge of each translucent ring identified as an annual structure.

The first exchange set consisted of 50 otoliths collected in Sub division 25 during the Danish IBTS cruises in January and March 2004. The second exchange set consisted of 25 otoliths collected in Sub division 25 during the Danish BITS cruise in November 2004.

The analysis of the traditional age determination calibration was performed using an Excel ad-hoc Workbook "AGE COMPARATIONS.XLS" from A.T.G.W. Eltink from RIVO following the recommendations of EFAN (Eltink et al., 2000). This analysis is based on a reference age when there are no validated ages available, which is the case for Baltic cod.
From the XY coordinates recorded by the age readers in the image analysis programme the otolith centre was calculated as the mean X and mean Y for each otolith and each reader. This starting point was then used to compare individual reader interpretations of translucent rings. Distances between the mean centre and each ring was calculated and compared among otoliths and readers. The data coordinates were further subjected to statistical analyses for the variance in different interpretations of the age structures and the span of different positions of the actual structures.

Results

The exchange commenced in November 2004 was finalized by the end of April 2005. A total of 7 readers completed the first set and thus all laboratories present in SGABC participated in the exchange.
The results from the traditional age calibration exercise clearly displayed the differences in perception of otolith structures between the participating age readers. The overall agreement was no more than 67.7 % with a precision of 16.7% CV and in 22% of the otoliths the agreement was larger then 80%. Figure 6.2.1.1 shows the overall pattern of the readings, showing that the divergences of the interpretations of the otolith structures were not on specific ages, but the disagreement were on all ages. Figure 6.2.1.2 shows the relative bias by modal age indicating any trends in over-or under estimation of ages by all readers combined. The younger ages seem to be overestimated whereas the ages >4 more often are underestimated compared to the modal age. Although the length of the individual is not taken into account when estimating the age from the otolith, it is worth noting that with the widespread perception of age for each individual, the length at modal age is ranging over 30 cm for ages 2 and 3 (figure 6.2.1.3).

The exchange commenced in March 2005 was not finalized prior to the scheduled SGABC meeting in May 2005. However 5 readers did manage to complete the set and the preliminary results should be mentioned.
The overall agreement was less than in the first exchange only being 56% however with a higher precision of 28.4%. The divergences of interpretation of age structures were not on specific ages (figure 6.2.1.4), however the pattern from the first exchange concerning the age-related bias pattern was repeated, overestimating the younger ages and underestimating the older ages compared to the modal age (figure 6.2.1.5).
As the otoliths in the two exchanges were collected during 3 months (January, March and November) the sampling month could potentially explain the disagreements in perception of age structures in the otoliths. This however was not the case (F=0.007, P=0.99).

The spreadsheet program, which combined image analysis and plots, made it possible to demonstrate where the individual age readers interpret the rings directly on the digitised images of the otoliths. Some otoliths showed to be very difficult to reach a common interpretation of the age and the points counted as age structures were scattered along the otolith (Figure 6.2.1.6).

The most variation in interpretation of defining rings was observed for the first ring. An example of this is illustrated by figure 6.2.1.7. The variation between otoliths in the median distance to successive rings is shown in figure 6.2.1.8 as cumulated frequency distributions of the position of each ring. This variation declined with ring number.
The distance from the centre to the first ring had no influence on the distance between the 1^{st} and 2^{nd} ring, thus the readers did not compensate for a high L1 by marking the second ring very close to the first ring.

From inspection of the position of points on otolith images it was seen that frequently some readers did not mark out rings that other readers interpreted as true annual structures. This occurred both in otoliths where the readers disagreed on the age of the individual but more interestingly also in otoliths where there were 100% agreement on the age among the readers. Figure 6.2.1.9 is an example of such a case.

Conclusions

The overall result of the age readings is that there is a general low agreement between readers. The image analysis exercise clarified that the lack of agreement can be referred to two reasons, the first being the position of the first ring. In 80% of the non-agreed otoliths the readers did among other things not agree upon which structure to point to as the first ring. In cases where a reasonably common interpretation of individual rings existed, disagreement arose where some readers choose to leave out specific rings identified by other readers as true annual rings. Identification of ring position is in general varying between readers, even in readings, which estimate age equal to the modal age, do not all have the same interpretation of ring position.

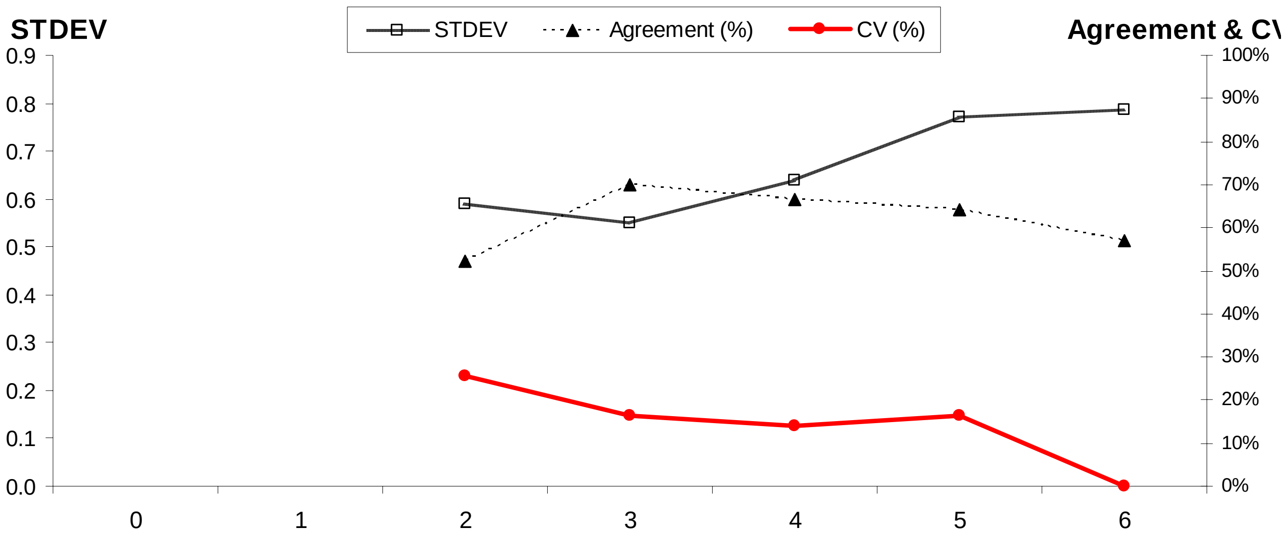


Figure 6.2.1.1. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against modal age for the ‘November exchange’. CV is much less age dependent than the STDEV and percent agreement and is thus a better index for the precision in age reading. The observed high CV’s at age indicates problems in age reading.

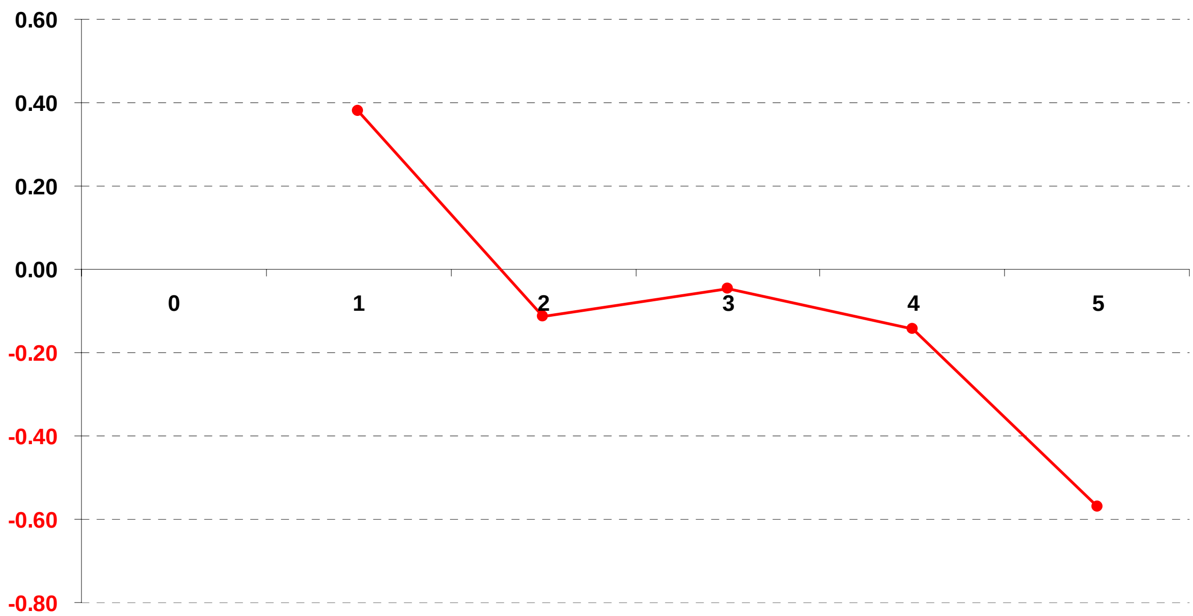


Figure 6.2.1.2. The RELATIVE bias by MODAL age as estimated by all age readers combined. Data from the ‘November exchange’.

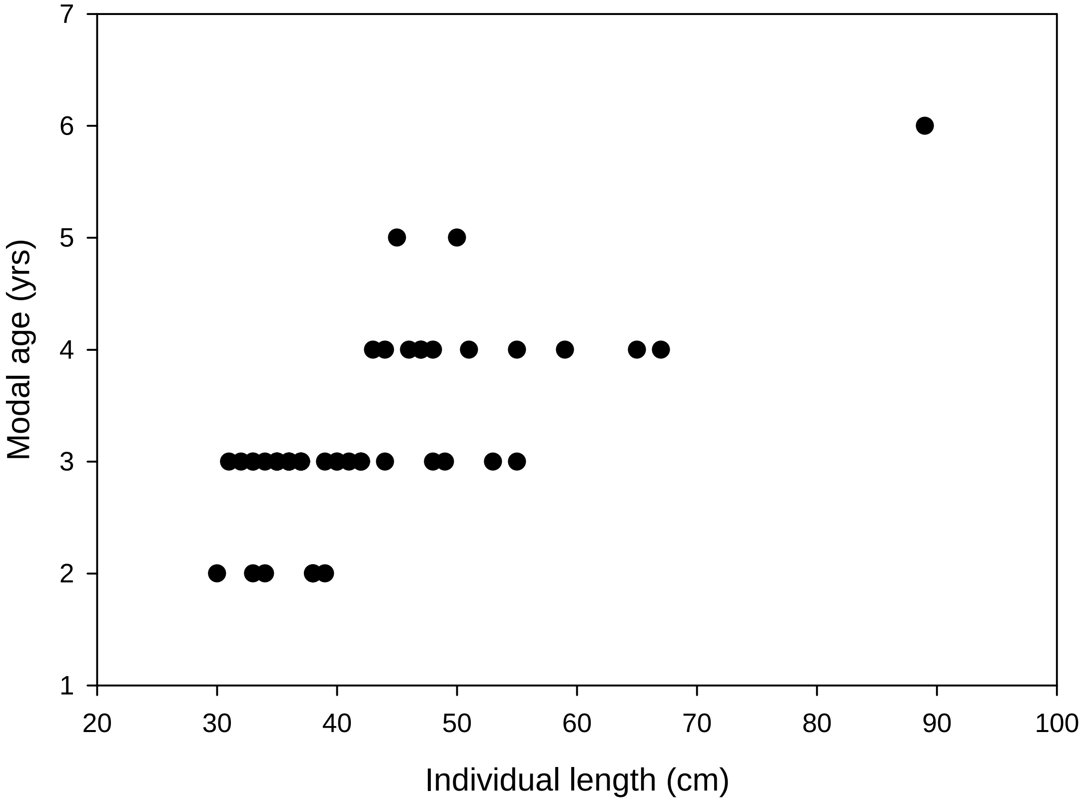


Figure 6.2.1.3. Modal age plotted against individual fish length. Data from the 'November exchange'.

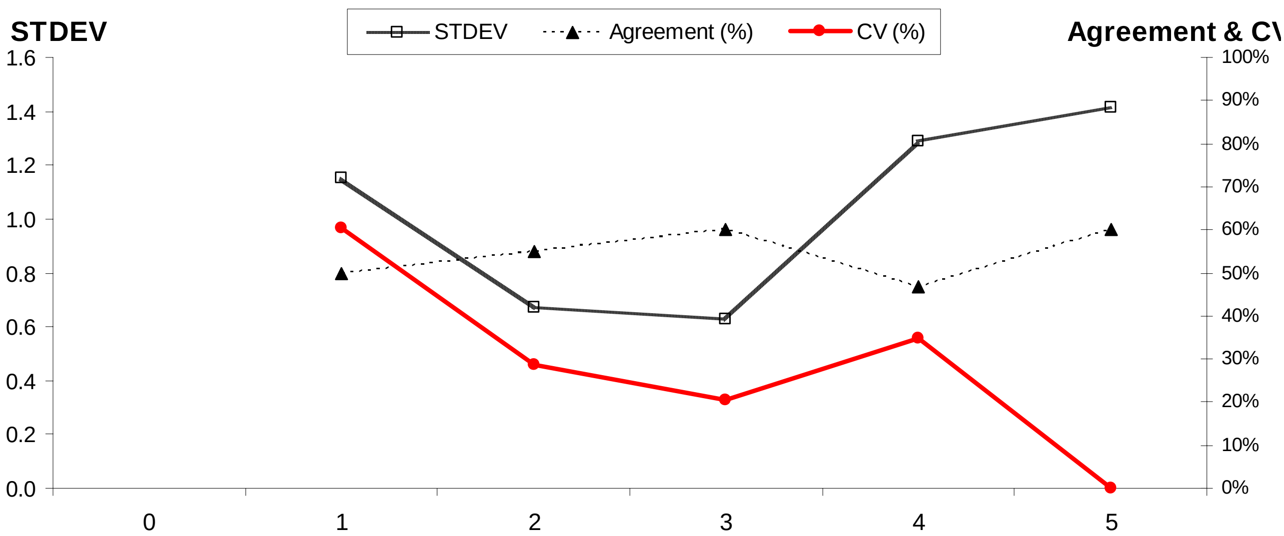


Figure 6.2.1.4. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against modal age for the 'March exchange'. CV is much less age dependent than the STDEV and percent agreement and is thus a better index for the precision in age reading. The observed high CV's at age indicates problems in age reading.

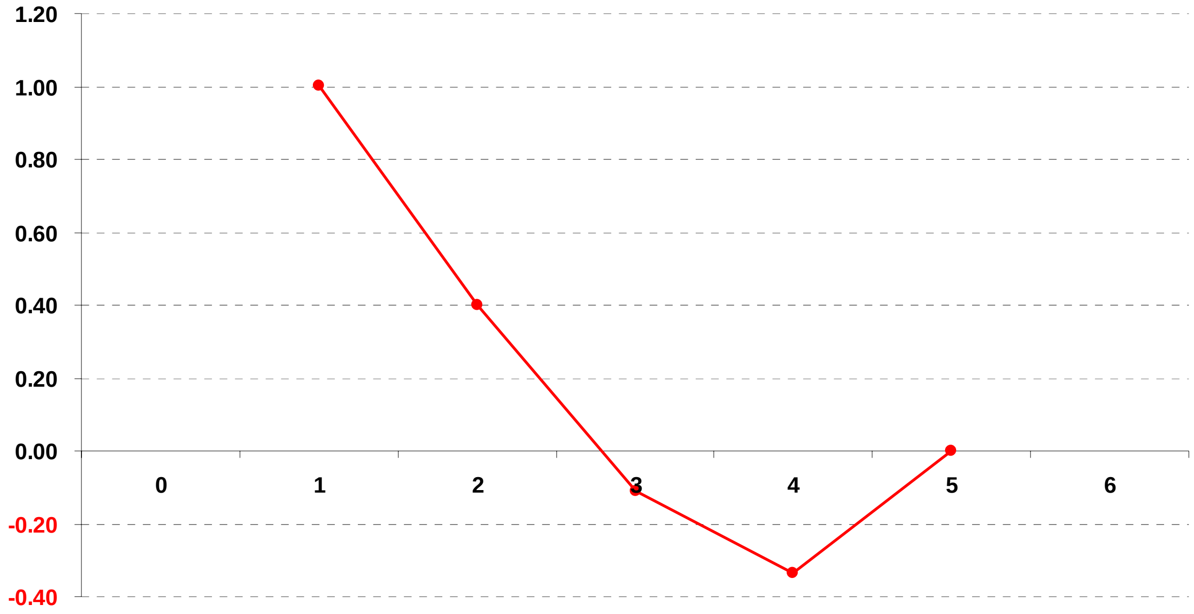


Figure 6.2.1.5. The RELATIVE bias by MODAL age as estimated by all age readers combined. Data from the 'March exchange'.

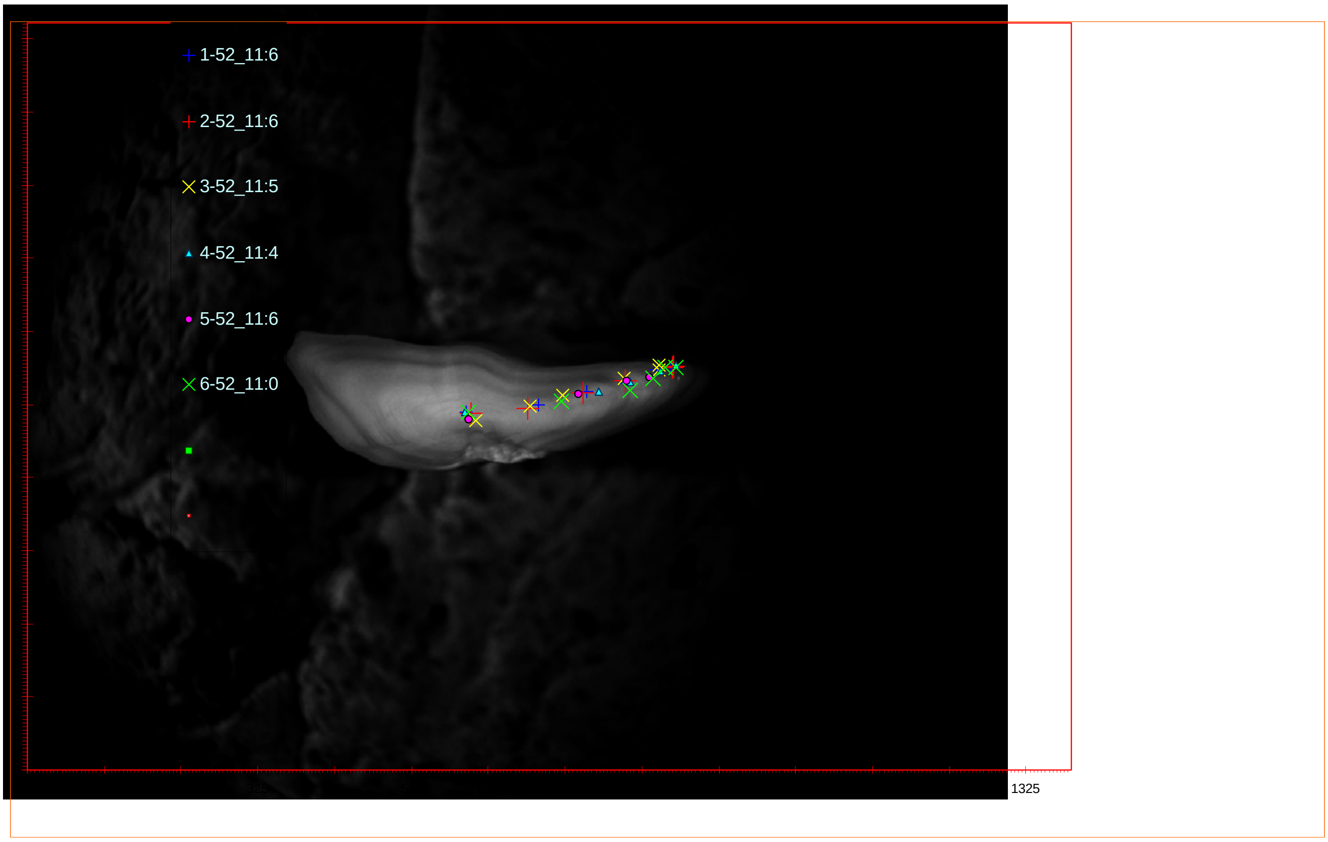


Figure 6.2.1.6. Example from the image analysis of an otolith where readers diverged to a high degree in interpretation of annual structures. Otolith from cod caught in sub.div. 25, March 2004.

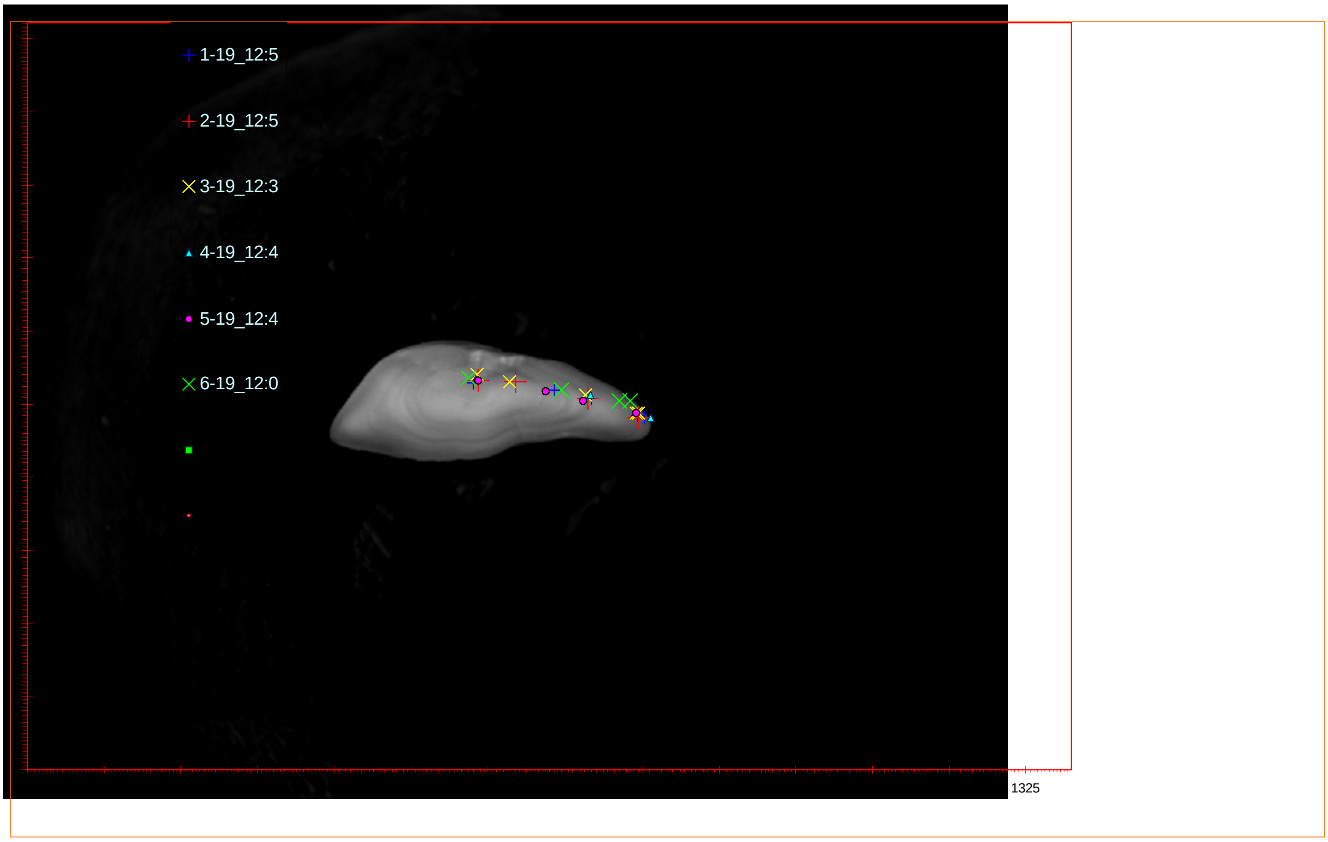


Figure 6.2.1.7. In 80% of the disagreed otoliths, the most variable structure between readers was the definition of the first ring. Otolith from cod caught in sub.div. 25, January 2004.

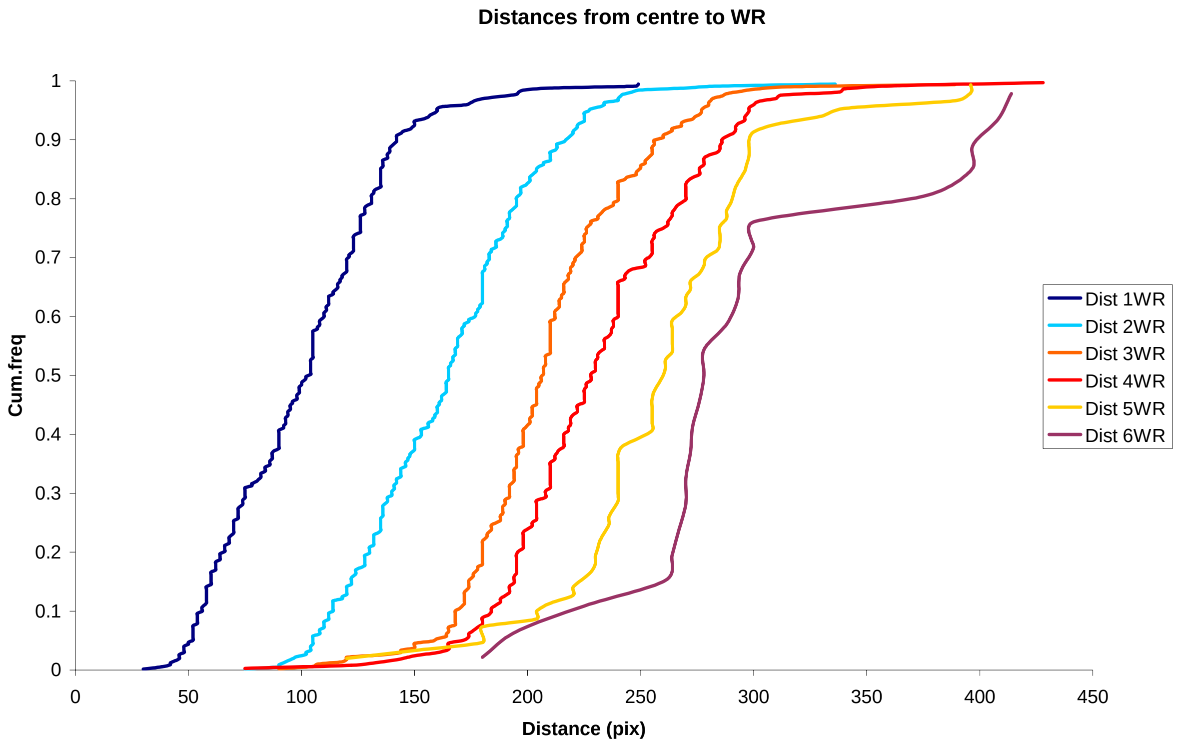


Figure 6.2.1.8. Variation in the median distance to rings from the standardised centre of the otolith. The variation is shown as a cumulative distribution.

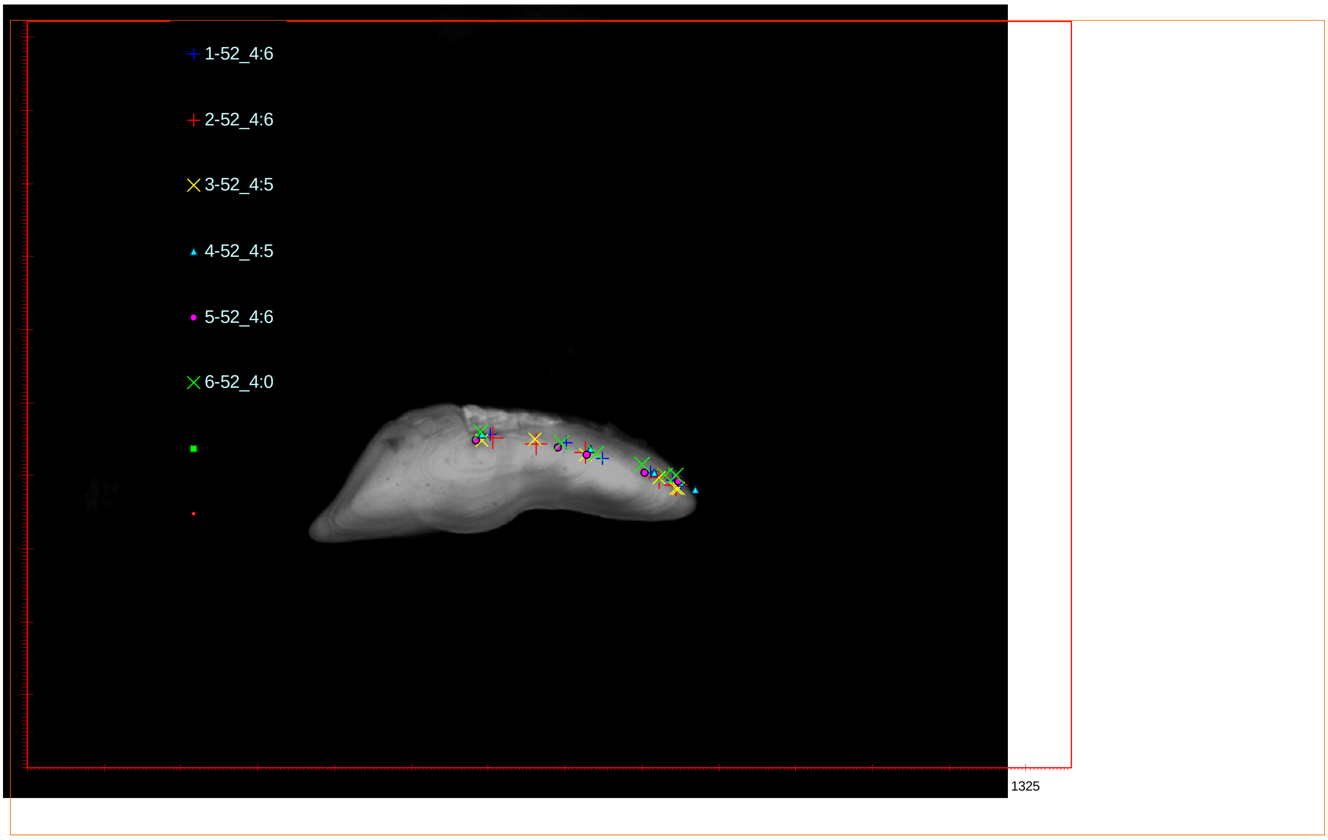


Figure 6.2.1.9. Example of an otolith, where the readers in the traditional age calibration exercise had a 100% agreement on the age of the individual, but where the structures identified as rings differ between the readers. Cod caught in Sub.div. 25, March 2004.

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Norway

PROCEDURE FOR AGE ESTIMATION OF COD, HADDOCK AND SAITHE

Version 1.0

By

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Institute of Marine Research

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1 PROCEDURE FOR AGE ESTIMATION OF COD, HADDOCK AND SAITHE

1.1 Purpose

This procedure shall assure that age estimation of cod, haddock and saithe by interpreting otoliths at the Institute of Marine Research is as accurate and precise as possible.

1.2 Extent/Scope

This procedure applies for age estimation of cod, haddock and saithe in the North Sea, the Barents Sea (both Norwegian and Russian zone), Svalbard and Norwegian fjord and coastal areas.

1.3 Definitions

Age determinatio, age estimation. To estimate the age of a fish. Both terms leave the impression of being more accurate than they really are.

Age. How old the fish is (all fish are per definition "born" on January 1).

Check. A hyaline discontinuity in the summer growth, not representing a winter growth.

Transmitted light. Light that is passed through the otolith from below. The same effect can be achieved with light from the side if the surface is shadowed.

Hyaline. That allows passage of light. A hyaline zone looks white when light passes through from below, and black in light from above, or against a dark background (e.g. a black otolith tray) where the light is absorbed by the background.

Core, nucleus. The centre of the otolith. The core is usually opaque in fish hatched in spring, and hyaline in fish hatched in the autumn.

Opaque. That does not let light pass through. In untreated otoliths under transmitted light, the opaque zone appears dark.

Otolith. Calcified structure in the inner ear of bony fishes. There are three pairs of otoliths. The largest pair is sagitta, the others are lapillus and astericus. Normally, and in this procedure the name otolith is used synonymously with sagitta.

Otolith reader. Person responsible for estimating fish age by interpreting the otolith's growth and zonation.

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

Zone. Region of similar structure or optical density (opaque or hyaline).

Summer zone. Opaque zone normally deposited during spring-summer-autumn when the fish grows relatively fast.

Winter zone. Hyaline zone normally deposited during winter when the fish grows realtively slowly.

Settlement zone. A hyaline zone that is often deposited when the fish settles in autumn. Can easily be mistaken for the first winter zone.

Annulus. One of a series of concentric zones on a structure used for age estimation. Synonymous with winter ring or winter zone.

1.4 Background

Correct age estimates of the sampled fish, whether it is for distributing an abundance estimate on age groups, to study growth or to distribute catches on age groups, are vital for fish stock estimation and management. For cod, haddock and saithe the otoliths are used for age determination.

1.5 Critical factors

To be able to carry out the tasks listed in this procedure in the best possible way, it is vital that the persons involved are acquainted with the protocol described below. Also, it is vital that they maintain their knowledge and skill by processing a reasonable amount of otoliths each year.

Acknowledgements

The authors are grateful to Bente Lundin for translation of this manual into English, comments on terms and skilful treatment of the context in this manual.

1.6 Description of standard method (Protocol)

1.6.1 Preparing

Responsibility	Step	Action/activity
Otolith reader	1	*Prepare equipment*: Microscope with incident light and 16 times magnification. A small piece of putty to fasten the otolith in. A pencil for shading the cut surface of the otolith (thus achieving transmitted light). Small envelopes for registering the age, where length, weight, sex and maturity have already been filled in,

1.6.2 Age estimation

Responsibility	**Step**	**Action/activity**
Otolith reader	1	*Localise the correct otolith*: Break the otolith in half as close to the core as possible. If the otolith is not clear, try the other one. Stick the otolith in the putty with the broken surface up.
Otolith reader	2	*Determine type*: The type is determined from the core and zone pattern. See Fig. 1-3 (p. 8). (Does not concern haddock and saithe) Type 1 = coastal cod. Even,oval shaped first winter zone, often looking like a glowing halo. Large 2nd year. The zone is more marked than in NE Atlantic cod. Rarely a ottom settling zone. See Fig. 1 (p. 8). Type 2 = Uncertain coastal cod Type 3 = Svalbard cod: NE Arctic cod which grows up in the Svalbard area. Have clear winter zones and thus easy to estimate. See Fig. 2 (p. 8). Type 4 = Uncertain NE Arctic cod Type 5 = NE Arctic cod. Long-shaped first winter zone with a small bulge on one side. Even growth with streamlined annuli. Often false zone and bottom settlement zone. See Fig. 3 (p. 8).
Otolith reader	3	*Estimate age*: Use 16 x magnification Use a pencil to shadow the whole broken surface so that light can pass through and the zones appear clearly. Age is estimated by counting the summer

		zones (opaque), including the nucleus which is darker with transmitted light. The last summer zone which is formed in May/June should not be counted before next January 1st.
Otolith reader	4	*Spawning zones*: The summer zones after spawning. These are thinner than the other zones, and the surrounding winter growth zones are more tranlucent.
Otolith reader	5	*Spawning age*: The age of the fish at first spawning.
Otolith reader	6	*Readability*: The quality of the otolith. Readability 1 = The age can be determined accurately Readability 2 = Uncertain estimate Readability 3 = Otolith not readable or missing Readability 4 = Age may be estimated but spawning zones/age not readable Readability 5 = Uncertain, but the reader has chosen the lowest of two consecutive ages Readability 6 = Uncertain, but the reader has chosen the highest age of two consecutive ages
Otolith reader	7	*Brim*: The composition (opaque or hyaline) and size of the otolith edge growth. The brim is defined by the way the edge looks. See Fig. 6 (p. 10).

1.7 Comments to the age estimation

All species have per definition birthday on 1 January.
It is generally easier to estimate the age in saithe, which have clear zones. But observe that for large saithe with several spawning zones, good light is required in order to see the narrow zones at the tip of the otolith.

Age in haddock may be a little more difficult to estimate. The core is usually large compared to the whole otolith surface, and the second year, which is also large, often has a false hyaline zone. In old haddock, like in large saithe, the zones are difficult to interpret.

Age estimates in cod are often difficult because of several false zones. We also distinguish between NE Arctic cod, coastal cod and Svalbard cod. See Fig. 1-3 (p. 8).

Especially for cod, but also for haddock, lots of training, co-reading and comparative blind readings are required.

To check for false zones, magnification may be reduced to 10x to achieve a better overall impression of the otolith.

1.8 Result

When the tasks described in this procedure have been undertaken, data from the otolith should be written down on the otolith envelop for registration and entered into the Institute of Marine Research's database.

1.9 Quality assurance

There is a "procedure for quality assurance of age estimation" dealing with comparisons of age estimates among two or more age readers. This procedure prescribes that a certain amount of saithe, haddock and cod otoliths shall be read by more than one reader, and the results compared. In addition to these compulsory comparisons, it is recommended that readers discuss difficult otoliths.

1.10 Security/environment

It is important that the work area is organised in such a way that the otolith reader can look easily into the microscope without too much tension, and that the microscope is focused correctly. Interpretation of otoliths is a demanding work and should not be carried out for too long periods without pause or change of work.

1.11 Major changes

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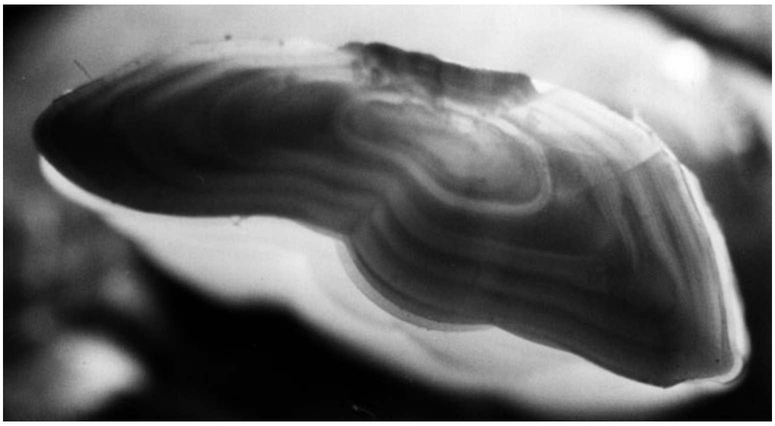
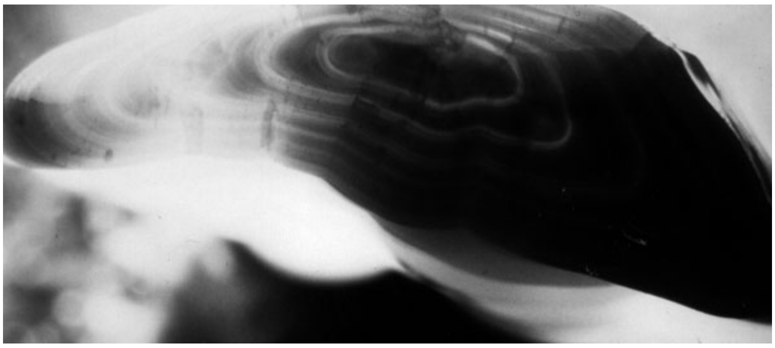
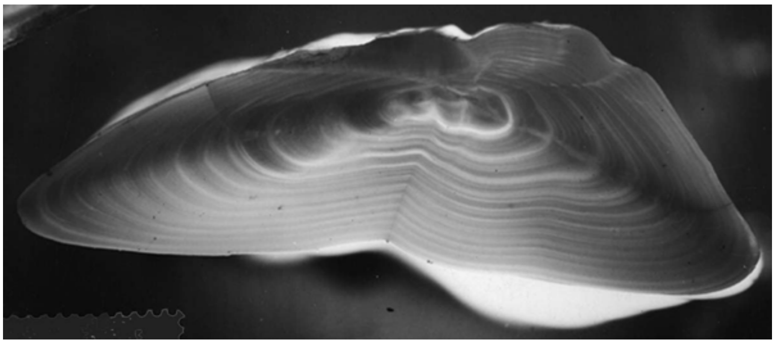
1.12 Figures of otoliths



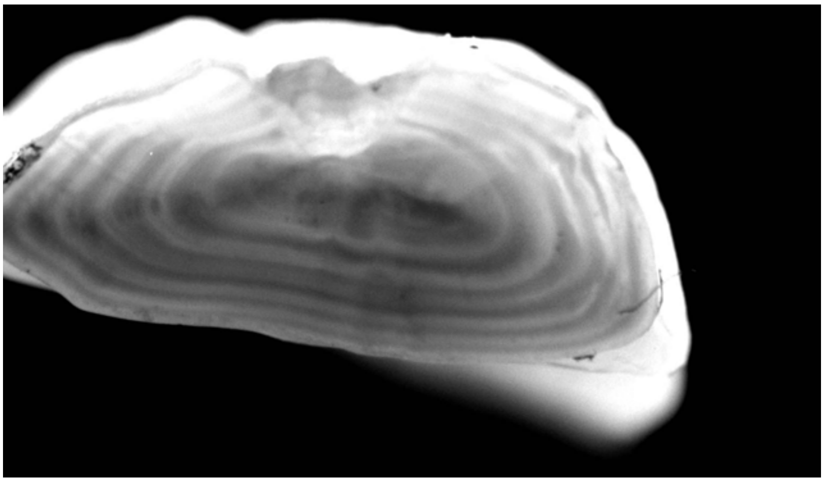
1.12.1 Fig. 1. Coastal cod



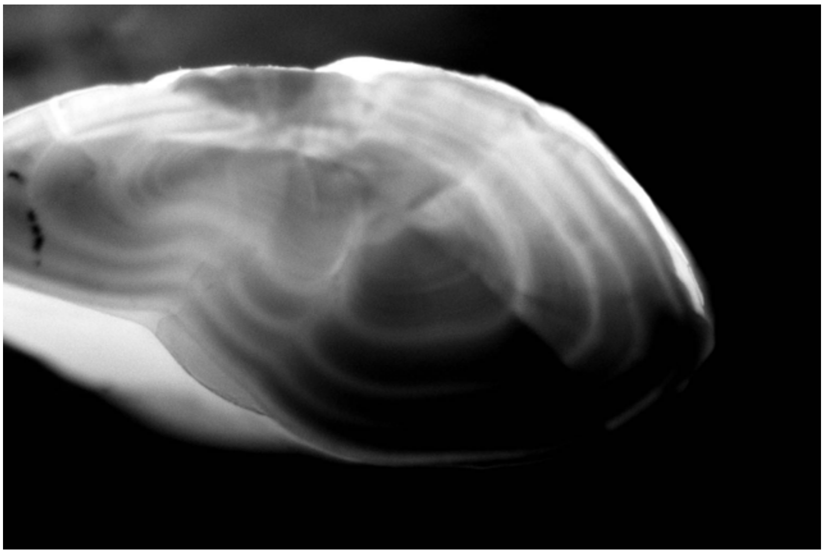
1.12.2 Fig. 2. Svalbard cod



1.12.3 Fig. 3. NE Arctic cod

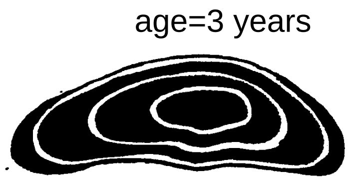


1.12.4 Fig. 4. Saithe



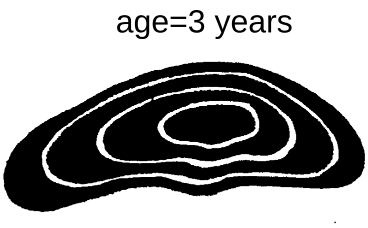
1.12.5 Fig. 5. Haddock

1.12.6 Fig. 6. Brim, the edge of the otolith (as seen in transmitted light)



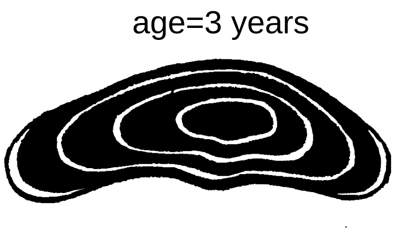
Brim-1

White edges, but black (opaque) at the brim, i.e., beginning summer zone.
The summer zone is not counted until after 1 January the following year.
Brim-1 occurs usually during May – July.



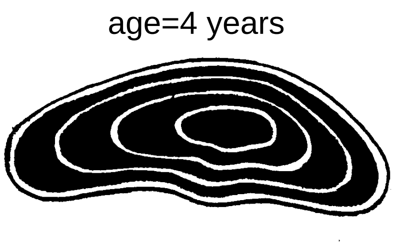
Brim-2

Black (opaque) edge around the whole otolith, i.e., full-grown summer zone.
The summer zone is not counted until after 1 January the following year.
Brim-2 occurs usually during August – October.



Brim-3

Black (opaque) at the edge, but white (hyaline) at the tips, i.e., beginning winter zone.
The summer zone is not counted until after 1 January the following year.
Brim-3 occurs usually during October – December.



Brim-4

White (hyaline) at the edge around the whole otolith, i.e., full-grown winter zone.
All summer zones shall be counted.
Brim-4 occurs usually during January - April/May.