Baltic Committee **ICES CM 2000/H:01**

REPORT OF THE

STUDY GROUP ON BALTIC COD AGE READING

Karlskrona, Sweden
27–31 March 2000

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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TABLE OF CONTENTS

Section **Page**

1 INTRODUCTION

1.1 TERMS OF REFERENCE

During the ICES 1999 Annual Science Conference in Stockholm, Sweden, it was decided (C. Res. 1999/2H01), that the Study Group on Baltic Cod Age Reading (SGBCAR) should meet in March 2000. The meeting was held at the Baltic Sea Research Station, Karlskrona in the period 27 – 31 March 2000.

The Terms of reference for the Study Group was to:

a) *compile and evaluate the magnitudes of differences in age information used for the assessment of the Eastern Baltic Cod;*

b) *examine the effect of age reading differences on the estimates of stock size and fishing mortality;*

c) *evaluate the present age interpretations, taking the recent ongoing research into account;*

d) *evaluate the procedure to correct the historical catch-at-age, mean-weight-at-age and maturity-at-age for both commercial and research survey catches to common standards. If possible, revise the databases and carry out exploratory assessments using these data;*

e) *revise and adopt the draft age-reading manual, taking the new information into account. If the manual can be finalised, recommend on the dissemination of the knowledge for the appropriate user groups, archival of the information, and possible publication in the ICES Co-operative Research Report series;*

f) *review the report of the Workshop on Otolith Ageing of North Sea Whiting.*

1.2 PARTICIPATION

The meeting was attended by:

Tatjana Baranova	Latvia
Karin Hüssy	Denmark
Jan Netzel	Poland
Henrik Mosegaard	Denmark
Maris Plikshs	Latvia
Rajlie Sjöberg	Sweden
Yvonne Walther (Chair)	Sweden

2 GENERAL

2.1 REVIEW OF PREVIOUS MEETINGS ON BALTIC COD AGE READING

The age composition of Baltic cod readings has been presented at the ICES meetings from the late 40ies. By the start of the stock assessments at the end of the 60ies it was found that the assessments were hampered by the lack of proper age composition data. Therefore a Cod Working Group met in February 1972 in Gdynia. At the meeting it was agreed that the disagreements in determination of age could be caused by different interpretation of the central part and the edge of the otoliths a problem which probably is caused by the extended spawning period of the cod and the complicated hydrographical conditions in the Baltic. The Group agreed that to solve the problem there should be an additional meeting. After many succeeding meetings there are still systematic differences between age readers in the Baltic Sea.

The next comparative cod age reading was organised in accordance to C. Res. 1981 by H. Lassen. Each laboratory was asked to sample 50 otoliths. These otoliths were circulated between the readers. The ageing material was elaborated by H.Lassen (ICES CM 1985/J:5). The analyses show consistency of the reading between most of the readers but also some cases of a rather large variation within reader. Only one reader read significantly different from the general agreed ages.

Results of the Work Shop on Baltic cod age reading in Gdynia in June 1994 showed from a cluster analysis of Alk’s that there were two main groups of Baltic cod age readers. The first cluster consisted of readers from two eastern and one western country and the second cluster mainly of readers from one western country. The pattern found occurred in readings of otolith samples from two subsequent years.

A co-ordination meeting of an EU project on improving Baltic cod assessment data was held in Copenhagen in November 1995. A Sub-group was formed and convened by J. Netzel and P. Ernst. During this meeting four area co-ordinators were assigned - P. Ernst, T. Baranova, H. Mosegaard and Y. Walther. A sampling strategy was agreed on, 50 otoliths per country and area, was to be selected. The preparation of otoliths, reading procedure and next workshop meeting was also decided.

The first meeting of Study Group on Baltic Cod Age Reading was in Rostock in 1996 (ICES CM 1997/J:1) three new items were discussed – primary increment formation in juvenile cod, daily increments in the otoliths of juvenile and adult cod and a statistical method to show discrepancies in age reading. As at the Gdynia meeting in 1994, two different groups of readers were found - that is two different schools of cod age interpretation that differed statistically. The area co-ordinators (mentioned above) organised the sampling of otoliths from different countries and the exchange between the institutes. The total sample was the basic material for the reference collection and consisted about 1 000 pairs of otoliths. From these the area co-ordinators selected a reference collection for the different areas, The reference collection consisted of 423 pairs of otoliths. To analyse the differences in age readings a couple of areas were chosen, SD 22+24 (25 otoliths) SD 26 (38 otoliths) and SD 28 (42 otoliths). During this meeting there were also a presentation and discussion about the structure of the otoliths and the influence of various environmental conditions on the formation of hyaline zones.

The next meeting of Study Group on Baltic Cod Age Reading took take place in Charlottenlund, Denmark in 1998. (ICES CM 1999/H:4) The Group summarised the results of the otolith exchange programme, established a digitised image collection based on the reference collection of otoliths and prepared a first draft of a manual for age readers based on the results of the work carried out during all meetings. Before this meeting digitised images were produced by the Danish Institute of Fisheries Research. The images were of 290 otoliths taken from the reference collection. From these images the group selected 89 images for comments. On the selected images remarks on age, ring formation, and interpretation were inserted. It was agreed that the collection of otoliths and digitised images should be stored at the Danish Institute for Fisheries Research. The Group was of the opinion that the reference collection should provide possibilities for unifying the interpretation of the age-related structures and for training of new age readers. At the meeting it was agreed that every institute participating in the age reading experiments should be provided with a CD-ROM with digitised images of otoliths of agreed age interpretation. At the ICES Annual Scientific Meeting in 1999 the first draft of the Baltic Cod Age Reading Manual was presented.

The Danish Institute for Fisheries Research have produced and delivered the CD-ROM with digitised images to the Baltic cod age reading Institutes.

2.2 STATUS OF THE REFERENCE COLLECTION

The area co-ordinators from all of the countries surrounding the Baltic Sea have collected a basic material that consists of 1000 pairs of otoliths. From this material a reference collection of 423 pairs of otoliths has been selected, which has been delivered to the Danish Institute for Fisheries and Marine Research, responsible person H. Mosegaard.

The rest of the collected otoliths are stored by the area co-ordinators:

- The otoliths from Sub-division 21 are stored at the Danish Institute for Fisheries and Marine Research, contact person H. Mosegaard.
- Otoliths from Sub-divisions 22+24 are stored in the German Institute for Baltic Fisheries, contact person P. Ernst.
- The otoliths from Sub-division 25 are missing after exchange programmes. There are however the remaining otoliths from this area that were collected by the different participating nations. Otoliths from Sub-division 26 are stored at the Polish Sea Fisheries Institute, contact person J. Netzel and otoliths from Sub-division 28 are stored at the Latvian Fisheries Research Institute, contact person T. Baranova.
- Otoliths from Sub-divisions 23, 27, 29–32 are stored at the Swedish Institute of Marine Research, contact person, Y. Walther.

The individual otolith samples in the reference collection are in a varying shape. Some of the otoliths gradually degrading by circulation in exchange programmes and different preparation methods like breaking and burning. In the circulation some otoliths have even been lost. The fact that the otoliths were collected in 1995 also makes the collection of lesser value to the present age readings since some of the year classes included in the reference collection are of low importance to the annual age readings. With this in mind the future efforts will not be concentrated on the present collection but on how we can renew and improve the reference collection to be of best use for future purposes.

3 MANUAL

The Manual for Baltic Cod Age Reading has been revised during the meeting. There are no general validated theories about the growth of the otoliths and the interpretation of Baltic Cod age structures. A number of potential mechanisms have been identified that will help understanding the growth and the formation of age related structures in the otoliths. But at this point there is nothing general enough to be included in the manual. Furthermore the group did not have time to make all the changes discussed during the meeting, which are suggested below.

The revisions made at the meeting were:

- updating of terminology and clarification of terminology by adding a picture of a schematic otolith
- description of the reference collection
- description of present use and suggestions for future use of the reference collection
- recommendations for storage of the otolith collection
- recommendation of establishing national control collections
- updating of the Form for Age Reading Analysis

Future changes that was discussed were:

- including a chapter about the biology of the Baltic cod with focus on differences by areas
- outline for updating the present reference collection
- update the description of techniques to more general terms and include more guidelines for age estimations
- develop the method for statistical analysis of age estimations
- including theories of growth of otoliths and how they may be used as an aid to interpret otolith structures

4 ONGOING RESEARCH ON MACROSTRUCTURE IN COD OTOLITHS

Otoliths are growing by extra-cellular biomineralisation, where secretion of Ca and protein plus regulation of the local ionic environment control the ongoing growth and composition of aragonite crystals. The age of Baltic cod is usually estimated by counting the supposedly seasonal pattern of translucent and opaque zones from the transverse plane of cut or broken Sagitta otoliths. Since the formation of opaque and translucent zones is controlled by the environmentally mediated physiological state of the fish, knowledge of ambient conditions is important for otolith age interpretation. Ongoing research was presented that focus on three areas important for cod otolith interpretation:

1) Experimental studies on otolith growth and structural composition in relation to food and temperature. Otolith growth rate has been suggested to be coupled to fish energy metabolism (Mosegaard *et al.* 1988) and translucent zone formation may be influenced by high temperature and/or poor feeding conditions (Mosegaard and Titus 1987; Eckman and Rey 1987). Preliminary experimental results on cod show that sagitta growth rate is approximately proportional to temperature over an intermediate range from 7 to 15 centigrade. Research on lower temperatures' effects awaits its evaluation. Also translucency increases with increasing temperatures at different somatic growth rates and feeding levels. Otolith growth continues after a cessation of feeding, the response of relative translucency being related to temperatures is retained, however, with progressing starvation the otolith formation has an increasing general level of translucency.

2) Seasonal variation in overall and local otolith growth as a tool for validation of age interpretation. It has been found that field samples of a number of temperate species support the hypothesis that translucent bands are formed during higher temperatures (Beckman and Wilson 1995). These findings have been confirmed for cod and hake by analyses of O18/O16 - otolith carbonate to estimate ambient temperatures during otolith precipitation (Javier Tomás, 2000: Old and new developments in the estimation of individual fish age by means of macrostructures in otoliths; Port Erin Marine Laboratory, University of Liverpool, Port Erin, Isle of Man IM9 6JA. *See* Annex 2 of the cell 4 Crete report *at:* http://www.efan.no/). The conservative growth of otoliths relative to variations in somatic growth (Templeman and Squires 1956) make them suitable for Baltic cod age determination through analysis of size (Cardinale *et al.* 2000).

3) Construction of an integrated model for otolith growth and composition as a function of seasonal fluctuations in ambient environmental conditions. Metabolic control of otolith formation will yield a macrostructure reflecting the combined effects of changing environmental conditions and individual energetic scope. Each combination of food and temperature conditions will give unique otolith zone formations reflecting growth, condition and spawning periods. The state at catch (otolith size and structure at the attained fish size) is the target for modelling. Simulated growth and life history trajectories limited by the physiological response to the range of likely environmental conditions are chosen to optimise the model otolith formation output to best fit the observed structures. A preliminary version of the model was presented and the output based on general knowledge about conditions in the Baltic compared to the Kattegat was discussed. The study group agreed that the main features of cod sagitta otoliths from the two areas could be reproduced.

The demonstration of ongoing research to improve the quality of age determination of Baltic cod showed that several steps are necessary for a successful result. A basic understanding of the otolith formation process is needed to rightly interpret structures like juvenile rings, the amount of otolith formation in relation to seasonal changes in translucency, and the expected structures at the edge of the otolith transverse section. Further when criteria for interpretation of annual structures are formulated this process has to be validated by one or several methods: Frequency analysis of seasonal data on marginal zone completion, analysis of progressing modes in otolith size frequencies, and/or mark - recapture experiments with vital stained otoliths. Finally a number of procedures to facilitate standardised interpretation of structures should be available ranging from updated and agreed reference collections to user adapted graphic models of otolith growth structures.

5 AGE READING DIFFERENCES

5.1 SUMMARY OF THE REFERENCE COLLECTION AGE READING DATA

5.1.1 Introduction

Joachim Gröger performed statistical analyses of the data from age readings of the reference collection 1995 in 1996. He analysed the individual readers' precision by comparison of the readings with the modal age. An investigation of the existence of different age reading schools was performed on the mean age of the data material.

5.1.2 Objectives

During the Study Group meeting in Karlskrona (March 2000), the same data material was analysed again in order to a) resolve the balance of the data distribution over the whole range of existing age classes and b) investigate possible differences in age readings related to fish age.

5.1.3 Method

The modal age was estimated from all readings of individual otoliths, in case no mode is available the rounded mean of the observations was used.

To have an indication of differences between earlier recognised institute clusters of similar interpretation (schools) GLM analyses of the following model were performed (using SAS and SAS notation):

$AGE = AGE_{modal}\ Nation\ AGE_{modal} \times Nation\ \ AGE_{modal} \times Nation \times Sub\text{-}division$

Both untransformed and log-transformed age data were tried (linear and power function), with Sub-divisions (22/24 combined and 26/28 combined) and nations as class variables.

5.1.4 Results and Conclusion

Balance of data set:

The majority of age readings were concentrated in the range 1–5 years, which reflects the age distribution of otoliths in the basic collection.

The model used in the present analysis was therefore heavily influenced by this skewed distribution of the data set, so that the first 5 year-classes determine the shape of the curve.

The second quarter was badly represented in the data set, due to a limited number of cruises.

Table 5.1.1 Age estimations from reference collection compiled as modal age per quarter.

Count of Total	agemod													
Q	0	1	2	3	4	5	6	7	8	9	10	11	#N/A	Grand Total
1		6	16	9	11	7	3	1	1	2				56
2		6	5	2	6	1	1							21
3	1	6	10	14	7	3	2	1	1		1	1	4	51
4		7	12	6	8		2	2		2			1	40
Grand Total	1	25	43	31	32	11	8	4	2	4	1	1	5	168

Difference in readings in relation to age:

The readings by most readers corresponded with the modal age. However, the age estimates of those readers, which disagreed with the modal age, were usually lower.

Table 5.1.2 Age estimations from reference collection compared to modal age.

Count of Age	Agemod												
Age	0	1	2	3	4	5	6	7	8	9	10	11	Grand Total
11											1	2	3
10										2	2	1	5
9										4			4
8									5	1			6
7							3	9	1	1			14
6					3	4	17	1		1			26
5				2	16	29	6	1					54
4				16	137	8	3	1					165
3			23	134	28	2							187
2		15	222	14									251
1	1	150	19	5	2								177
0	7	5											12
Grand Total	8	170	264	171	186	43	29	12	6	9	3	3	904

Although the method of regressing individual age readings on modal age does not seem fully appropriate the analysis indicated a discrepancy between countries. The only direct comparison of a possible western and eastern school (SWE versus LAT and POL) for Sub-divisions 26/28 indicates higher age interpretations for the eastern group.

Table 5.1.3 SAS GLM output of analysis of age estimations of the reference collection (Sub divisions 22/24 and 26/28)

The Dependent	SAS 2000 Procedure Variable:	System 184 LNA	12:20	"Wednesday," General	March Linear	"29," Models
Source		DF	Sum of Squares		Mean Square	F Value
	Pr>F					
Model		11	252.9384263	22.99440239	495.89	0.0001
Error		874	40.52722392	0.04636982		
Corrected Total		885	293.4656502			
R-Square		C.V.		Root MSE		LNA
	Mean					
0.861901		23.69198		0.21533653	0.90890043	
Source		DF	TypeIII SS	Mean Square	F Value	Pr>F
LNM		1	176.8907771	176.8907771	3814.78	0.0001
NATION*SD	5	1.17380234	0.23476047	5.06	0.0001	
LNM*NATION *SD	5	0.40428867	0.08085773	1.74	0.1221	
						T for H0
Pr>|T|	Std Error of Parameter					Estimate
	Parameter=0		Estimate			
INTERCEPT			0.101534147	B	1.78	0.0749
	0.05693969					
LNM				0.839824625	B	20.45
	0.0001	0.04105973				
NATION*SD						
		D	2224	0.069979397	B	1.07
	0.2833	0.06518686				
		DK	2224	-0.116527063	B	-1.8
	0.0726	0.0648168				
		LV	2628	-0.030956741	B	-0.38
	0.7007	0.08052488				
		POL	2628	0.067561013	B	0.84
	0.4017	0.08052488				
		SWE	2224	-0.07863805	B	-1.29
	0.1977	0.06100552				
		SWE	2628	0		B
.	.		.			
LNM*NATION *SD						
		D	2224	0.020670775	B	0.39
	0.695	0.05271027				
		DK	2224	0.101447		B
	1.94	0.053	0.0523578			
		LV	2628	0.120541204	B	2.08
	0.0382	0.05806723				
		POL	2628	0.056447035	B	0.97
	0.3313	0.05806723				
		SWE	2224	0.093426896	B	1.99
	0.0474	0.04704913				
		SWE	2628	0		B
.	.		.			

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations. Estimates followed by the letter 'B' are biased," and are Not unique estimators of the parameters.

The comparison based on log transformed age data gave a slightly better model fit than the linear data and is therefore taken as representative for the apparent differences.

With the model used, differences in age readings between nations were small for the age classes 1–4 years, while age estimates of readers from the eastern Baltic seemed to be above those from western Baltic readers in the older age classes (Figure: 5.1.1).

Only one reader from Sweden had read otoliths from both Sub-divisions 22/24 and 26/28, while readers from the western part of the Baltic had only read otoliths from Sub-divisions 22/24 and readers from the eastern Baltic had read otoliths from Sub-divisions 26/28. The otoliths of Sub-divisions 26/28 were read only by three readers (SWE, LAT, POL).

The indicated probability levels should only be used as indications of true differences in interpretation. This is due to unbalanced comparisons of countries over Sub-divisions, unbalanced distribution of individual otoliths over ages, unknown selection of otoliths for comparative age readings and the possible bias using the mode as the independent variable.

Recommendations for the structure and reading of future reference collections:

- Age distribution of collection should cover all ages.
- Readings from all nations should overlap with respect to sub division.
- Random sampling of otoliths for the collection, regardless whether or not the otolith is typical for the area etc.

5.2 DATA INDICATING AGE READING DIFFERENCES

5.2.1 Scrutinising available data

Evaluation of national basic assessment data of Baltic cod in Sub-divisions 25–32 reveal significant differences in mean weight at age and in age composition (catch in numbers). The study group noticed that discrepancies might have been caused by several reasons:

1) Age reading inconsistency.
2) Basic assessment data compilation procedure i.e., the differences in working up the catch in number at age.
3) Replacement of national data sets due to missing information i.e., from one Sub-division/quarter to others etc.
4) Difference in the selectivity features of gears used and area coverage of commercial fleets by a given country.

To investigate the possible magnitude of age reading on inconsistency of the national basic assessment data, it would be preferable to carry out analyses and comparisons of national size distribution and age-length data sets. But, since these data were not available to the Study group, the analyses were concentrated on differences between mean weight at age by countries, Sub-divisions and gears. The main questions to be answered were:

1) How significant are the differences between mean weight at age by countries.
2) Are there similar patterns of difference for all age groups that are specific for age reading “schools”.

Investigation of influence of other plausible causes (points 2, 3 and 4) was not possible due to lack of available data sets and expertise.

5.2.2 Comparisons of mean weight at age in landings by different countries

From the tables in the assessment working group report (ICES 1999/Advisory Committee on Fishery Management: 15) and the multispecies working group report (ICES 1999) data of mean weight at age (MW) were compiled by year (1996, 1997, and 1998), Sub-division (Sub-divisions 25, 26, 28), gear (trawl and gillnet) and quarter (1–4).

When sampling had not been performed MW had been assigned from other Sub-divisions, gear and quarters. To avoid double use of data in the comparisons the obvious cases where exact equal MW appeared several times only the case with the highest number of landed individuals was used. To have a sufficiently robust material only MW for age classes 2–8 was analysed.

The Swedish reported mean weights at age (MW_{SWE}) were used as a reference to which all other MW's were compared. From the available 2190 observations only 745 comparisons were found valid. The difference between each MW and the corresponding MW_{SWE} was analysed to indicate possible differences in age interpretation.

Linear and log-transformed mean weights were used in GLM's to compare country, gear, and SD effects.

Probabilities given should only be taken for their indicative value:

Table 5.2.2.1. Number of comparisons made by country, year and Sub-division:

Country/Year	Sub-division	Grand Total

	25	26	28	
DK1996	22			22
DK1997	27	26	26	79
DK1998	73	54	17	144
FRG1996	16			16
FRG1997	13			13
FRG1998	10			10
LV1996		24	17	41
LV1997		25	17	42
LV1998		59	46	105
PL1996	22	23		45
PL1997	27	20		47
PL1998	22	28		50
RU1996		24		24
RU1997		27		27
RU1998		80		80
Grand Total	**232**	**390**	**123**	**745**

The analysis of the influence of gear either as pure class information or as interaction terms with country and/or sub-division showed no significant effects. Therefore the model containing significant information was considered to be the following (by SAS and in SAS notation):

$MW = Country\ MW_{SWE}\ Country \times MW_{SWE}$

The output is shown in table 5.2.2.2

Table 5.2.2.2. SAS GLM output of analysis of differences in mean weight at age between Sweden and other countries (disagregated by country, age, quarter, gear, year and Sub-division):

```
The SAS System        12:29 Friday, March 31, 2000  21

                              General Linear Models Procedure
                                  Class Level Information
                            Class    Levels    Values
                            COUNTRY       5    DK FRG LV PL RU
                            GEAR          3    GN TG TR
                            QTR           4    1 2 3 4
                            SD            3    25 26 28
                            YEAR          3    1996 1997 1998

                         Number of observations in data set = 1113

NOTE: Due to missing values, only 797 observations can be used in this analysis.

                                    The SAS System        12:29 Friday, March 31, 2000  22
                            General Linear Models Procedure
Dependent Variable: D
Source                  DF          Sum of Squares          Mean Square  F Value    Pr > F
Model                   10      756505457.62447300     75650545.76244730   120.80    0.0001
Error                  787      492865590.73392700       626258.69216509
Uncorrected Total      797     1249371048.35840000
                  R-Square                    C.V.             Root MSE            D Mean
                  0.475625               -127.0001         791.36508147      -623.12185696

Source                  DF               Type I SS          Mean Square  F Value    Pr > F
REFSW                    1      579404621.74040600    579404621.74040600   925.18    0.0001
COUNTRY                  5      159751630.29215900     31950326.05843190    51.02    0.0001
REFSW*COUNTRY            4       17349205.59190720      4337301.39797680     6.93    0.0001
Source                  DF             Type III SS          Mean Square  F Value    Pr > F
REFSW                    1      161677280.60166100    161677280.60166100   258.16    0.0001
COUNTRY                  5       45252593.84597800      9050518.76919561    14.45    0.0001
REFSW*COUNTRY            4       17349205.59190720      4337301.39797682     6.93    0.0001
 T for H0:        Pr > |T|       Std Error of
Parameter                     Estimate       Parameter=0                        Estimate
REFSW                      -0.4339899 B           -11.41         0.0001          0.0380279
COUNTRY       DK          728.3947469               8.25         0.0001         88.2924884
              FRG         289.3456187               1.50         0.1351        193.4467223
              LV           -0.7053070              -0.01         0.9951        114.0677484
              PL           54.1857653               0.47         0.6419        116.4772386
              RU          164.0913932               1.32         0.1868        124.1884703
REFSW*COUNTRY DK            0.1115965 B             2.37         0.0180          0.0470908
              FRG           0.3425447 B             4.61         0.0001          0.0743257
              LV            0.0471247 B             0.93         0.3533          0.0507381
              PL            0.1693808 B             3.24         0.0012          0.0522441
              RU            0.0000000 B              .             .               .
NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to
      solve the normal equations.   Estimates followed by the letter 'B' are biased, and are
      not unique estimators of the parameters.
```

In the exploratory GLM runs also a significant year * MW interaction was found, indicating that in 1997 differences increased more with increasing mean weight at age than in 1996 and 1998. Both age interpretation differences as well as biological characteristics could cause this effect (e.g., differences in growth pattern within different areas as well as the inconsistency in raising methods applied in the BFAS working group). It was however decided to concentrate on the main combined effects of MW and country.

From this analysis it can be seen that apparently there could be age interpretation differences between all countries but three groups appear to exist (Figure 5.2.2.1)

1) Sweden + Germany, 2) Denmark, and 3) Poland + Latvia + Russia

However, the present analysis has some draw backs in relation to coverage of areas within Sub-divisions, different sampling and raising methods, different combinations of gear, and therefore it may only be indicative of any existing age interpretation differences.

6 EVALUATION OF THE EFFECTS OF AGE ESTIMATION ERRORS

The working group was asked to assess the importance of age reading errors on the assessments. Mr H. Hovgård (Denmark), who was not able to attain the meeting, agreed to carry out an evaluation and to submit the results and models to the group.

The evaluation was carried out on simulated catch at age data. The data set was created to simulate the eastern cod stock with respect to exploitation pattern and recruitment variability. Four scenarios of age miss readings were considered.

1) 50% of all catch at age data were shifted 1 year up.

2) 50% of all catch at age data were shifted 1 year down.

3) 50% of all catch at age data were shifted up 1.2 times.

4) 50% of all catch at age data were shifted down 0.8 times.

The cases 1) and 2) may reflect the situation where the ambiguity is confined to the way the first year or the edge of the otolith is defined. Cases 3) and 4) may reflect situations where age reading differences are gradually increasing over the life span of the fish. Shifting ages with constant factors (i.e., 0.8 and 1.2 times) require rules of how to split ages on age groups. In the simulations catches were proportionally distributed between the two adjacent ages.

Simulations were carried out using a separable VPA formulated in a spreadsheet. The effects of the biases in catch at age data are compared to a basic run with no age reading bias. The results are presented in Figures 6.1 to 6.4.

For both types of biases it is seen that

a) An over estimation of ages leads to an increase in stock size as shown by the sizes of the year-classes at recruitment. This coincides with a concurrent decrease in fishing mortality.

b) An under estimation of ages leads to the opposite result, i.e., a decrease in stock size coupled with an increase in fishing mortality.

c) All biases influence the estimates of the recruitment sizes in particular years. Potentially one may assume that age readings biases may smooth out year-class variability. This effect was not pronounced on the simulated data.

Overall, the existence age reading biases will fundamentally influence our perception of the stock and the fishery. In the case of an overestimation of the true ages we will perceive the stock as being large and subjected to a low fishing mortality. In the case of under estimations of ages we will reach the opposite conclusion, i.e., that the stock size is low but heavily fished. All age reading biases will moreover compromise our historical time series of recruitment.

7 CORRECTION OF AGE-READING DIFFERENCES

7.1 AGE VALIDATION METHODS

A prerequisite for any revision or future age estimation activities is the existence of validated methods for assignment of age to representative individuals. A number of different methods for age validation may be suggested.

1) Analysis of marginal completion of annual growth structures: The method is dependent on repeated sampling over the year and analysis of opaque/translucent structures or seasonally varying shapes at the otolith edge. When the growth and maturation processes are likely to effect the individual ambient environmental conditions then the analysis should be conducted by year- and age class. The method relies on a reasonable synchronous otolith formation in the age groups studied.

2) Identification and tracking of strong year classes: This method will provide individuals with a high probability of a known age individuals especially when the cohort grows older. It relies on a similar annual structure pattern in other year-classes (i.e., the high abundance must not influence the otolith growth).

3) Identification and tracking of special otolith features formed in early life of a year class: The method is principally comparable to 2.

4) Analysis of size frequency distributions in cod and/or their otoliths. The method is dependent on a homogeneous growth pattern of each cohort. With a time series of size frequencies the method may be expanded to follow the progression of significant differences between the frequencies of successive years. The method will mostly be applicable to follow the youngest age groups.

5) Mark recapture experiments to attain known age individuals. The method may be combined with in vivo marking of otolith growth zones using e.g., temperature coding, or incorporation of Alizarin complexion or Tetracycline.

6) Integrated otolith and fish growth analysis. The method applies a model of otolith growth controlled by variations in fish metabolism and protein turnover. The model is dependent on experimental calibration of otolith growth and structure appearance in relation to fish physiological conditions. Further data on seasonal variation in ambient environmental conditions are needed.

7) Combinations of methods. Method 4 may be used to transform otolith weight changes into widths of annual growth increments to establish the expected range of annual zone widths in relation to otolith size, thereby supporting the analysis of marginal increments (1) and assisting future routine ageing.

When a series of validated age structures are available guidelines for a standardised interpretation during routine readings are required.

7.2 REVISION OF HISTORICAL AGE READINGS

There is reason to believe that age reading differences of Baltic cod have prevailed for the last eight years or more (see Sections 2.1, 5.1 and 5.2). A revision of the age structure would therefore require methods to update the historical catch and mean weight at age. The presented analyses indicate a complex pattern of differences that is likely to remain biased by any application of additive or multiplicative factors to correct numbers at age. If e.g., the significant year effects in mean weight at age between countries is due to different age interpretation patterns among countries in different years, a revision of the numbers at age would be needed at a low level of aggregation.

There are several possible lines that suggest themselves for this process:

Rereading of historical otolith material with a standardised method for interpretation. With a stratified subsampling based on fish size this method could optimise the allocation of age reading resources for reconstruction of the age - length relationship.

Combination of otolith weights with fish length and weight data to make frequency analysis and reconstruction of the age - length structure. The method should be supported by validated age readings on a smaller stratified subsample to calibrate the size frequency analysis.

7.3 FUTURE AGE READING EFFORTS

The quality of the assessment of Baltic cod is highly dependent on accurate or at least precise age determinations. The establishment of a scientifically based model of otolith growth structures and an operational manual to guide the interpretations will facilitate this aim. The inclusion and comparison of otolith and fish size information together with age readings may give a basis for correction of national differences in sampling and working up data.

An alternative approach to standardise and unify the basic assessment data preparation by national laboratories, is creation of common age-length keys and mean weight at age for given area/Sub-division and gear/fleet. In this case national landing data are presented only as a size distribution for given strata. The age reading data (fish length, weight and age) are internationally combined from all laboratories by defined strata (area, gear etc). An advantage of such method is possibility to avoid the differences in mean weight at age and in age composition of landings caused by age reading. It allows also a standardisation of data replacements due to missing information in certain strata. This approach needs closer co-ordination, international data compilation and exchange between the national laboratories in the period prior to the Fisheries Assessment working group. However, in spite of significant advantages of this method, the consensus of age reading and growth zone interpretation between the countries is a necessary precondition. It should also be mentioned that possible shortcoming would appear if the selectivity patterns of gears used by countries differ significantly.

8 REVIEW OF THE REPORT OF THE WORKSHOP ON OTOLITH AGEING OF NORTH SEA WHITING

The Study Group on Baltic Cod Age Reading reviewed the Report of the Workshop on otolith ageing of North Sea Whiting (ICES CM 1998/G:14) with a following discussion. It was found that some of the ideas presented could be useful in the Manual for Baltic Cod Age Reading. They are as follows:

1) It would be practical to begin the Manual for Baltic Cod Age Reading with a review of Baltic cod biology in a way similar to the Report on Whiting Otolith Ageing. This would give the new readers a background of the environmental conditions of the Baltic cod. The Baltic environmental conditions are variable and thereby they are influencing the structure of the otolith. This type of information in some cases could clarify some problems with age interpretation.
2) Introduction of comparative tables between different methods can help comparisons between different readers.
3) The idea of using frequency analysis of e.g., otolith structures such as length, weight or width could be useful in age interpretation of cod.
4) It was found that the description of techniques and guidelines are very clear and understandable and it seems that this type of description could be useful in the Manual of Cod Age Reading
5) The expressed idea of exchange of age readers seems to be practical and useful
6) It was found that the interpretation of whiting age structures is not easier than that of eastern Baltic cod.

9 SUMMARY AND CONCLUSIONS

The results from Study Group on Baltic cod Age reading reveals that in general the Baltic cod age interpretation still needs to be significantly improved. Nevertheless, during the Study group meeting some progress has been achieved:

1) Revision and updating of Baltic Cod Age Reading Manual has been made. In the new version of the manual the terminology of otolith structure has been updated and a description of the present status of the reference collection has been presented. In the otolith age reading form additional otolith parameters has been included. Furthermore,

the necessity of establishing control- and agreed otolith collections on both national and international levels was stressed.

2) The images of the reference collection of otoliths have been digitised, compiled on CD-ROM and distributed to the national laboratories.
3) An evaluation of former age readings of the reference collection confirmed earlier discrepancies between the countries and “schools”. As a rule the “Eastern school” has higher age interpretation.
4) From ongoing research projects it was indicated that for age interpretation additional parameters of otolith might be used. Therefore, the Group recommends including otolith length, width and weight measurement when selecting otoliths for the reference collections.
5) A model of environmental factor and food consumption influence on growth zone formation in cod otoliths was demonstrated during the meeting.
6) The Study Group was not in a position to evaluate the magnitude of age reading inconsistency influence on basic assessment data for Baltic Fisheries Assessment Working Group. However, performed analyses revealed that three groups of countries with different age interpretation could exist. Analyses also suggest, that application of scaling factors to age reading data conversion may not be appropriate due to differences in annual patterns as well as by ages. The comparison needs to be continued including size distribution and age-length data series.
7) The Study Group made a theoretical evaluation of possible effects of age reading bias on estimated fishing mortalities and stock size. Due to deficient data information and lack of knowledge on appropriate ways of correcting basic assessment data the Study Group was not in a position to perform revision of assessment basic input data.
8) The Study group presently is not in a position to update assessment data sets backwards due to insufficient statistical evaluation of age reading differences between countries or “schools”. Due to the possibility that both annual and area based age reading differences between countries exist, it is necessary to make individual revisions of former assessment input data in this procedure. During this meeting assessment data in the required format were only available from 1996–1998.
9) Review of the applicability of the methods and results from the Report of the Workshop on Otolith ageing of North Sea Whiting has been made.

The Study Group was hampered by the lack of national representatives. Only scientists from four countries were able to attend the meeting.

10 DRAFT RESOLUTION

The **Study Group of Baltic Cod Age Reading** [SGBCAR] (Chair Y. Walther, Sweden) will work by correspondence in 2001 to:

a) continue the revision and adoption of the draft age-reading manual on Baltic Cod Age Reading, taking new information into account;

b) if the manual can be finalised, recommend on the dissemination of the knowledge for the appropriate user groups, archival of the information, and possible publication in the ICES Cooperative Research Report series;

c) prepare the case for a future Study Group to delineate the differences between the "age reading schools".

Supporting information

Priority:	The work of the Group is essential to ensure the quality of Baltic Cod Age Reading used in fish stock assessment.
Scientific Justification:	The Group plans to complete current work and evolve plans for a new group. In previous Study Groups on Baltic Cod Age Reading there has been observed divergence between age readers in different countries, often referred to as “age-reading schools”. One explanation for this has been different theories about the development of annuli in the Baltic Cod otolith depending on environmental factors. The Study Group will therefore review ongoing research on formation of annuli in Baltic Cod otoliths to update the established draft manual on Baltic Cod Age Reading with new information. Although several meetings has been held, the assumed different “age-reading schools” has not yet been clearly delineated. The Group will therefore make projections for how future work on this issue may be structured.

Relation to Strategic Plan:	This work is part of the building blocks to providing sound, credible, timely, peer reviewed advice on fishery management and as such is intimately related to a number of components of the ICES Strategic Plan
Resource Requirements:	The resource requirements are appreciated to be insignificant, since the work will be per correspondence. If terms of reference b) is fulfilled there will however be additional cost for publication of the Baltic Cod Age Reading Manual in the CRR series.
Participants:	Scientists from ICES member countries working with Baltic Cod Age Reading.
Secretariat Facilities:	None apart from normal support.
Financial:	Publication costs if required, see Resource Requirements.
Linkages To Advisory Committees:	Closely linked to ACFM activities.
Linkages To other Committees or Groups:	Directly linked to WGBFAS.
Linkages to other Organisations	None.

10.1 RECOMMENDATIONS

The Study Group recommends that:

- The national procedures for working up the data used in the Working Group of Baltic Fisheries Assessment should be described to investigate differences in assessment that are caused by other sources than age reading.
- Basic assessment data should be accompanied by the corresponding size distribution by country, Sub-division, gear and quarter.
- More work on microstructure analysis of otoliths should be encouraged
- Exchange between readers should be encouraged e.g., in exchanges programmes, workshops and visits to other institutes.
- The institutes should be encouraged to start the work by producing a guideline and start updating the reference collection as soon as possible.

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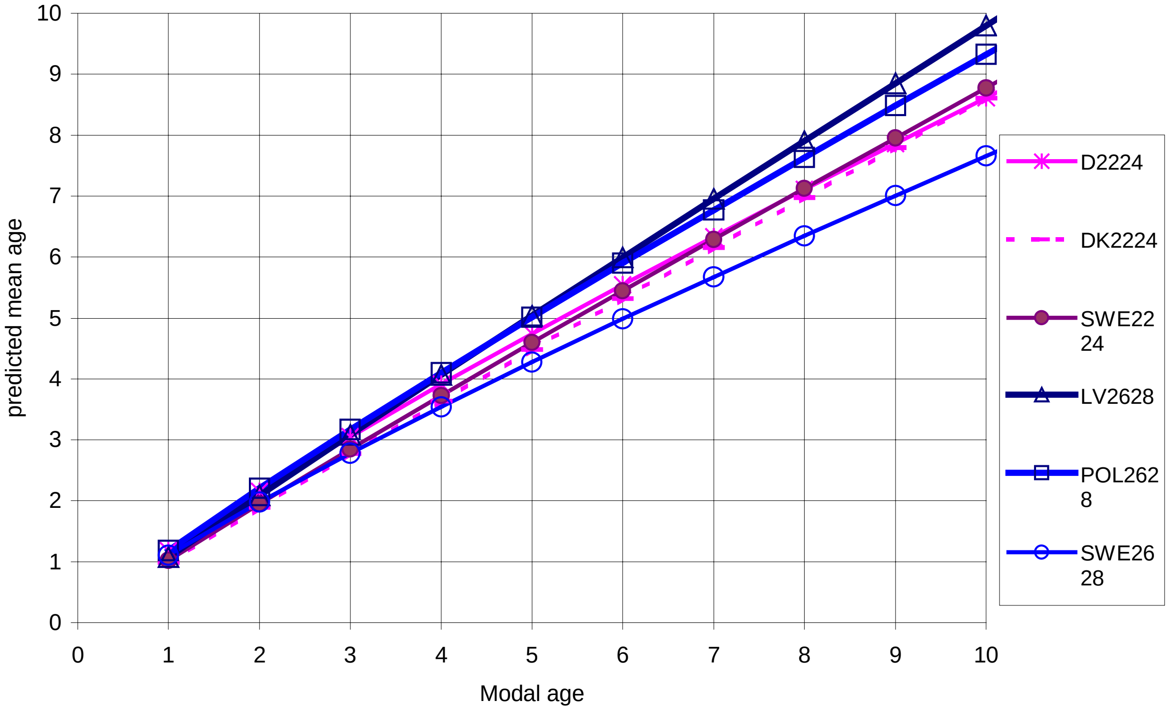


Figure 5.1.1. ANCOVA model predictions of Cod age readings by country and SD-group

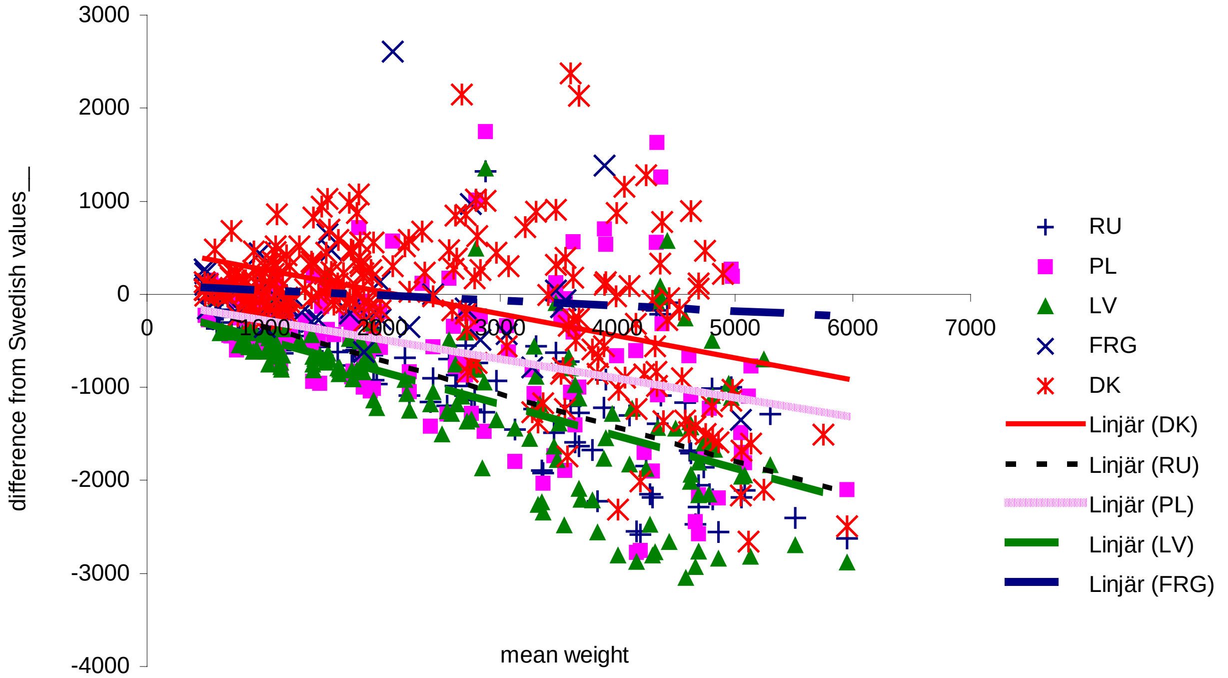
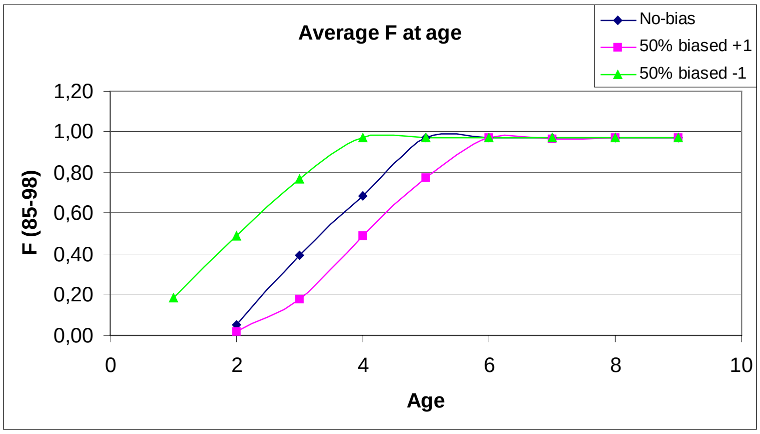
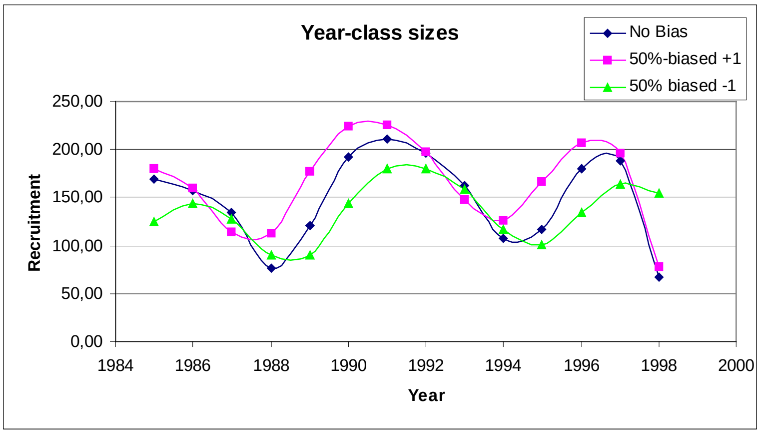
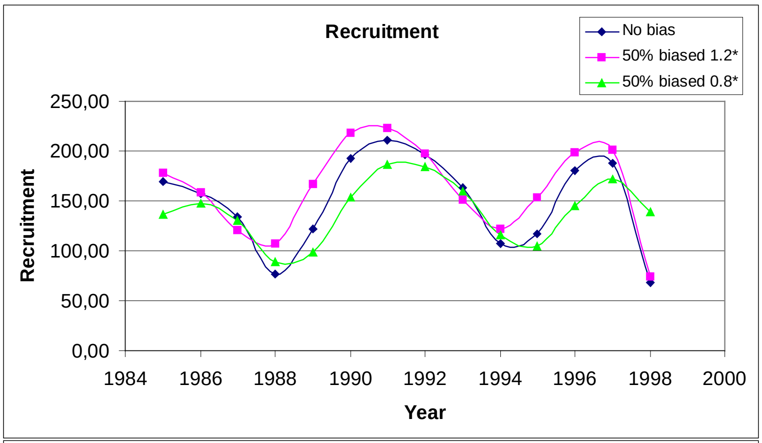
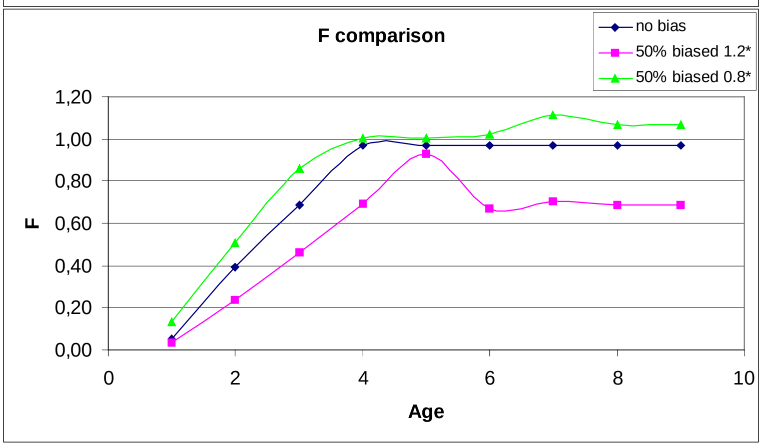


Figure 5.2.2.1 Difference in mean weight at age by SD, Q, Gear, year (from landings)









Figures 6.1 – 6.4 Results of simulated age reading errors and the effects on Average Fishing Mortality at age, Year class sizes and Recruitment estimates.

International Council for the
Exploration of the Sea

Addendum to ICES CM 2000/H:01

DRAFT II

MANUAL
FOR BALTIC COD AGE READING

REVISED during the meeting of
the Study Group on Baltic Cod Age Reading (SGBCAR),
Karlskrona, Sweden 27–31 March 2000

KARLSKRONA – March 2000

1 STANDARD METHOD OF AGE READING OF BALTIC COD

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

1.1 METHODS USED IN DIFFERENT COUNTRIES

1.1.1 Denmark

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

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

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

1.1.2 Sweden

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

1.1.3 Estonia and Latvia

The Estonian and Latvian readers generally use broken otoliths. The otoliths are broken by hand. If the otolith is difficult to read it is burned on an electric heating plate until receiving a light brown colour. But if it is still not readably the second otolith is cut to a slice (0.2–0.5 mm) by a sawing machine and embedded in pertinox. The broken otoliths are read by aid of a binocular microscope in reflected light and focused on the surface with water. The age from slices is determined in transmitted or reflected light with a dry surface. In all cases the magnification is 16.

1.1.4 Poland and Germany

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

1.2 GENERAL COMMENTS

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

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

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

1.3 EQUIPMENT AND TECHNIQUES USED

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

1.3.1 Broken otoliths

There are three general methods to prepare broken ototlihs, untreated, burned and polished.

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

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

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

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

1.3.2 Otolith slices

The technique to produce otolith slices used in the Danish Institute of Fisheries Research was demonstrated:

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

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

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

1.4 APPLICATIONS OF THE DAILY INCREMENTS ANALYSIS

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

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

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

2 COD OTOLITH INTERPRETATION

2.1 TERMINOLOGY

Age estimation (age determination): The process of assigning an age to a fish. Sometimes also called ageing that can be confused with the alteration of the organism over time. Age estimation is the preferred term.

Age group: The cohort of fish that has a given age, not to be mixed up with year class. The change of age group is by determination the 1st of January.

Annulus: one of a series of concentric zones, either opaque or hyaline that may be interpreted in terms of age.

Annual increment: one opaque zone and one translucent zone constituting the growth during one year.

Checks: Discontinuity in a zone (annulus) or in a pattern of opaque and hyaline zones often referred to as "false rings or zones". Not to be included in the age estimation. Checks are assumed to be stress induced and can occur more than once in one year, especially in the first opaque zone.

Cohort: group of fish of the same age that were spawned in the same time interval, if the time interval referred to is year the cohort is called a year class.

Core: the area or areas surrounding one or more primordia

Edge of otolith: (Zone at the edge of the otolith): Either opaque or hyaline zone. A problem area for age estimation of Baltic Cod, since the formation of hyaline zones occurs at different times depending on surrounding factors in the life cycle of the fish. In some cases one year is added to the age estimation on the assumption that the last zone on the edge of the otolith is under formation and not visible to the eye.

Juvenile zone: one ore more narrow translucent zones surrounding the centre of the otolith, assumed to be formed in connection with the settling of the larvae but are not found in all otoliths.

Metamorphic ring: a circular zone in the centre for the otolith. The formation is connected the initiation of the secondary growth centre. Usually not visible.

Translucent (hyaline) zone: a zone that allows the passage of greater quantities of light than an opaque zone. The term hyaline zone should be avoided; the preferred term is translucent zone.

Nucleus: indicate the primordia and core of the otolith, the preferred terms are primordium and core.

Primordia: the initial complex structure of an otolith, it consists of granular or fibrillar material surrounding one or more optically dense nuclei from 0.5 micron to 1.0 micron in diameter. In the early stages of otolith growth, if several primordia are present, they generally fuse to form the otolith core.

Opaque zone: a zone that disperses the penetration of light compared with a translucent zone. The term is a relative one because a zone is determined to be opaque on the basis of the appearance of adjacent zones in the otolith (see translucent zone). On the surface of broken otoliths and slices under reflected light, the opaque zone appears bright, in transmitted light it appears dark. The opaque zones in otoliths from cod in the Eastern Baltic are formed in the period of intensive feeding.

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

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

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

Settling (=juvenile) ring: See juvenile zone.

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

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

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

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

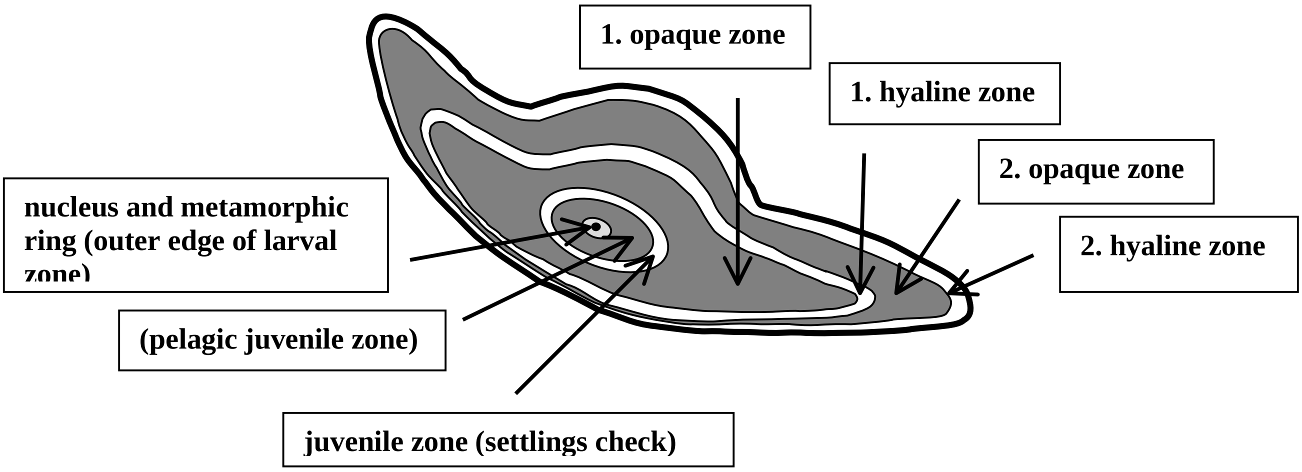


Figure 2.1 Schematic drawing of the transversal section of a cod otolith as seen in transmitted light.

2.2 DESCRIPTION OF AGE ESTIMATION PROCEDURES

The purpose of fish age reading is the estimation of the age structure of a fish stock. The method for Baltic cod is based upon area specific characteristics of the otolith zone formation.

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

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

2.2.1 Interpretation of cod otoliths from SD 22

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

2.2.2 Interpretation of cod otoliths from SD 26 and 28.

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

2.2.3 The formation of zones on young cod otoliths

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

	Month	Otolith zone	Designation	Age
The first year (Year of hatching)	XII	One opaque	0+	0
The second year	I-IV	One opaque	0+)1	1

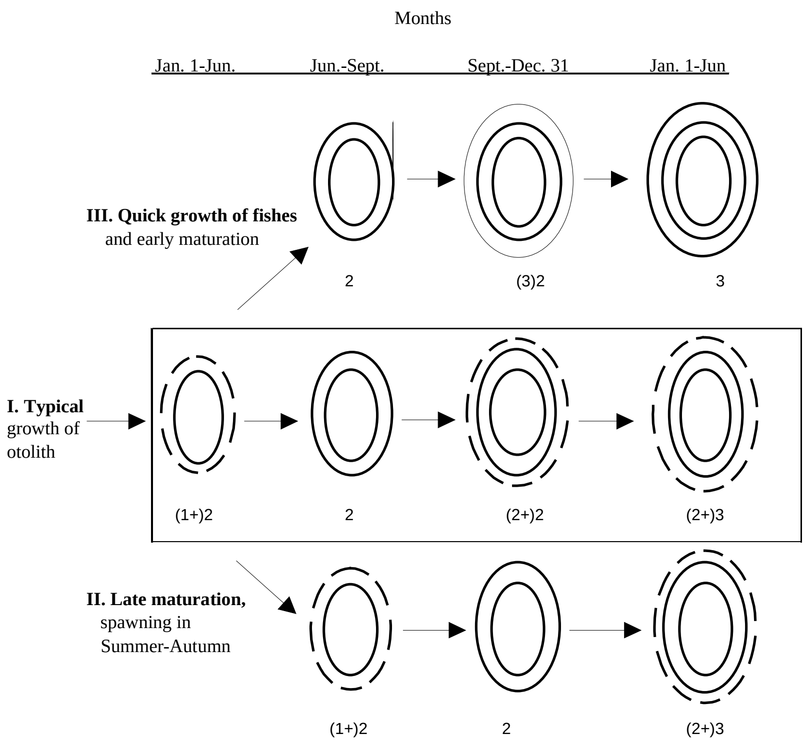
	V-VI	one opaque + one hyaline	1	1
	VII-XII	one annual zone (one opaque + one hyaline) + opaque growth zone at the edge of otolith	1+	1

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

2.2.4 The formation of zones on otoliths of adult cod

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

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



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

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

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

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

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

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

At the meeting it was not possible to come to an agreement of a common interpretation of otoliths from all Baltic Sub-divisions. It was therefore decided that a more theoretically satisfactory approach should be taken. A simplified hypothesis for the formation of cod otolith structure was discussed. It was thereby agreed upon that if a common hypothesis for otolith structure formation could be applied to both cod stocks the interpretation following this hypothesis would build the basis for age determination. The hypothesis should be so specific that it would lead to a series operative decision rules for assigning age to a given specimen, given information on time of catch, geographical area and depth distribution, size, maturity and past otolith growth.

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

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

The following examples will lead to sub-optimal growth:

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

Examples:

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

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

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

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

The different environmental conditions and their influence on otolith structure appearance are schematically visualised in the Figure 2.2.5 below.

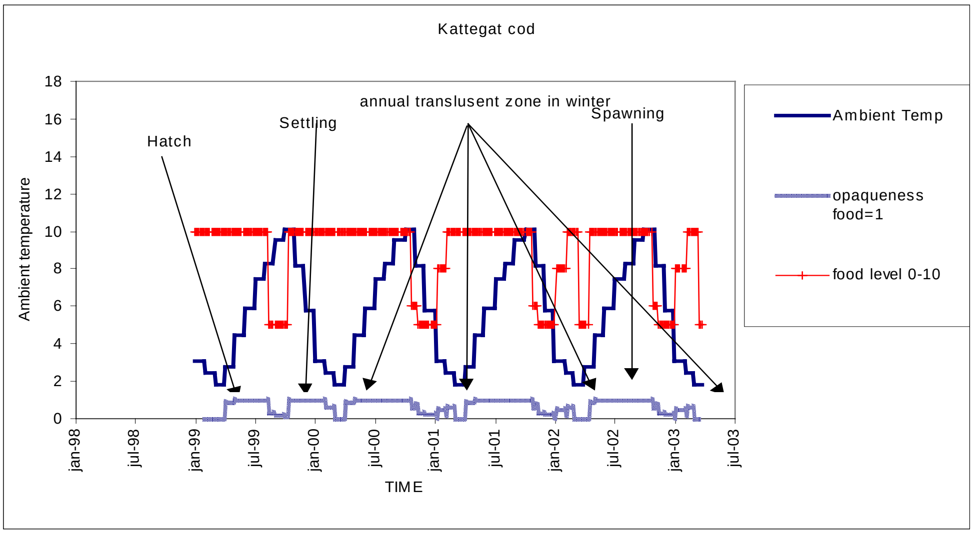
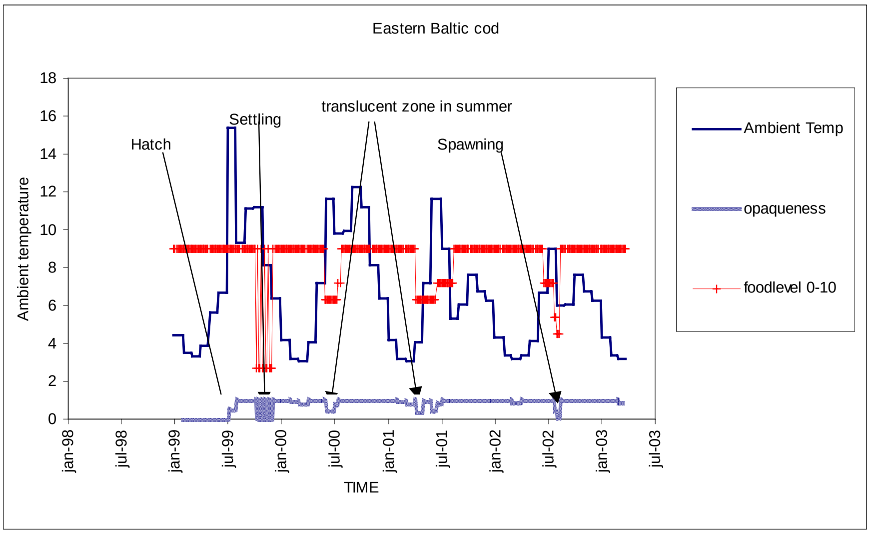


Figure 2.2.5. Illustration of how different levels of environmental factor (food and ambient temperature) in the Western (upper panel) and the Eastern Baltic (lower panel could influence formation of translucent zones in cod otoliths. The example is illustrated with two mature 4 years old cod from 1999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



cm.

Figure 2.2.5 continued

Recommendations: More research is needed to understand the mechanisms behind the formation of optically different structures in cod otoliths. Ongoing experimental should be combined with field observations from different areas. Extensive studies on the seasonal formation of the outer edge and the apparent otolith growth rate of optically different zones would be of great help for age validation. Therefore, publication of such studies from all areas should be encouraged.

3 REFERENCE COLLECTION

3.1 COMPILING THE REFERENCE COLLECTION

The Reference collection was compiled in 1995–96 by the members of the SGBCAR. Each country selected one pair of otoliths per 5 cm length class of the fish by season and Sub-division from surveys in 1995, to form the basic material for extraction of the reference collection. In some Sub-divisions where there were no surveys in 1995 the otoliths were taken from earlier trips (Table 3.1). The seasons where chosen to cover pre-spawning, spawning and feeding periods of the fish. It was recommended that the material provided for the reference collection should contain both typical and atypical otoliths. Information about the samples such as fish length, sex, maturity stage, ICES rectangle, catch year and month were registered. A Form for age reading analysis and Codes for the Age Reading Form was created for the readers notes about the characteristic of the otolith (Appendix 1 and 2) The otoliths were sent to co-ordinators assigned to the specific Sub-divisions. The co-ordinator then selected the otoliths to be included in the reference collection.

Table 3.1. Reference collection compiled by SGBCAR

SD	Catch Year	Catch months	No fish (pairs of otoliths)	No digitised images	Age range	Otoliths for collection provided by
21	1995	2,5,11	61	61	1–7	DK, SWE
22	1995	3,5,6,9,11	34	23	1–5	DK, SWE
23	1995	2,5,9	47	47	1–6	SWE
24	1995	2–5, 8–12	49	17	1–5	DK, D, SWE
25	1995		0*	0*		DK, D, PL, SWE
26	1995	2,3,5,8,9,11,12	38	38	1–10	PL, LV, RU, SWE
27	1995	3,8,12	33	26	1–8	SWE
28	1995	3,8,11,12	42	21	1–9	LV, SWE
29,3032	1986	1,4,12	50	50	2–10	LV

* otoliths are missing after exchange programmes

3.2 STORAGE OF THE REFERENCE COLLECTION

The Reference collection is stored in the Danish Institute of Fisheries (DIFRES) to have a better overview of the otoliths and make it more accessible to the presumable users.

Besides storing the otoliths the Danish Institute of Fisheries Research has taken digitised images of the otoliths. These images have been handed out to each participating laboratory on CD-ROM. In future DIFRES will store the images on a Database that will be possible to view from the WWW.

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

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

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

3.3 PURPOSE WITH THE REFERENCE COLLECTION

The original purpose with the reference collection was to have a set of otoliths as reference material for calibration and training of new and established readers, to reach consensus of the interpretation of the characteristics of the otoliths. In this case circulation of the otoliths have been made. The term reference collection is often confused and therefore are two different types of collections described below, with terms adopted from a paper called "Guidelines for Collections" (Eltink, 2000)

3.3.1 Control collection

The reference collection could be used as a control collection for rereading by the same reader to estimate changes in precision a relative bias at age over time. This is however difficult due to the logistics connected with circulation of the otoliths. The otoliths will also gradually deteriorate by the constant rereading. It is possible to increase durability by imbedding the otoliths in resin and make slices that are glued to glass slides, but since this is not the generally used method this might cause differences in interpretation. The control collection is therefore recommended to be read by the same method as the annual otolith samples. Preferably each age reading laboratory should have their own control collection for usage mainly by experienced readers. It could also be used by new readers but they will probably benefit more from an agreed collection

3.3.2 Agreed collection

The age of the otoliths on 89 digitised images from Sub-divisions 21, 24, 26, 28 and 29–32 were agreed on. These 89 samples are considered to be an agreed collection. On the images the Study Group inserted comments about age, ring formation and other structures important for age estimation. The information is outlined on the images as 3 different layers: nucleus, translucent zones and text. The layers can be switched on and off in the original image. The program used to view the digitised images were Paint Shop Pro. The images, without comments, may also be displayed in the Microsoft program Power Point.

The quality of the images is sometimes poor. With a digital camera, it is preferable to have a very even surface to focus on. Therefore, it is preferable if the broken otolith is polished to the nucleus. In addition, if it is going to be burned it has to be polished first, since the surface is very delicate after the burning process and material often breaks off along the edge.

In future otoliths that reach at least 80 % agreement can be included in the agreed collection (Eltink, 2000.) This will particularly be useful for training of new readers. In case it is difficult to obtain and read such a collection production of digitised images is a helpful tool and therefore it is recommended to continue producing such images.

3.4 UPDATING OF THE REFERENCE COLLECTION

The reference collection should be large enough to make necessary comparative readings by Sub-division.

Renewal of the reference collection should be made on an annual basis from an agreed method. The otoliths should be extracted from the national otolith samples for assessment, and the method should be expeditious and well defined. The sub-sample should reflect the variability of the otolith characteristics. After selecting and working up new otoliths for the reference collection (with e.g., otolith length, weight and width) they should be sent together with the Age Reading Form to the Danish Institute of Fisheries Research for storage. The thus updated reference collection should be examined at a regular basis for example at Work Shops to be included in the agreed collection.

The agreed collection should be stored on digitised images and supplied to the institutes by CD-ROM. In future it will be possible to view the images at DIFRES through the WorldWideWeb.

4 STATISTICAL ANALYSIS OF AGEING

4.1 STATISTICAL METHODS USED

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

4.2 BIAS AND PRECISION CALCULATIONS, QUALITY ASSURANCE AS WELL AS DESIGN AND USE OF A REFERENCE COLLECTION

Some general remarks:

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

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

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

- the precision is varying with age.

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

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

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

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

$$read\ age = true\ age + systematic\ effects + non-systematic\ effects$$

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

In principle, it is possible to correct biased readings if the sources of the systematic effects can be detected quantitatively as well as the precision is high and does not vary strongly with age. Following the theory of Gröger

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

with

$$VAR(non-systematic) > 0\ .$$

(1996a,b) as well bias as precision can be measured within the framework of a simple or (for more than one influencing systematic effects) a multiple regression approach. For the simple case this is:

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

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

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

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

with

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

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

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

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

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

4.3 SOME SUMMARISING GUIDELINES

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

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

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

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

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

5 GUIDELINES FOR BALTIC COD AGE READERS

- To estimate the population age distribution, first of all a representative sample of otoliths should be taken.
- To omit different obstacles in age determination the procedure of otolith preparation and conditions of readings should be as for as possible standardised. The group is of the opinion, that:
- One of the two otoliths should be broken through its centre (nucleus).
- The second otolith should not be used provided the first otolith is readable. Otherwise it could be burned or cut to a thin slice (0.2 – 0.5 mm).
- The otolith surface should be moistened with water, ethanol or water with soap and mounted in black wax or clay under the binocular microscope.
- Usually one microscope lamp should be used when reading in reflected light, but if it is necessary to read some of the otoliths in transmitted light then a second lamp could be used.
- Magnification should be in the range of X: (5 – 10) but it is not recommended to use larger magnification then 12 X.

6 REFERENCES

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APPENDIX 1

FORM FOR AGE READING ANALYSIS

Page of

SPECIES:

ICES SUB-DIVISION:

READER Name: Nation: Experience:

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

Catch date				Fish data			Characteristics of the otolith										
No	Year	Month	Rectangle	Total length of fish	Sex	Maturity stage	Weight	Length	Width	Edge	Nucleus	Zones	Readability	Age	Year class

APPENDIX 2 – CODES FOR THE AGE READING FORM

1. Reader experience

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

2. Sex

   1 male
   2 female

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

4. Edge of the otolith

   1 small hyaline zone
   2 full hyaline zone
   3 small opaque zone
   4 wide opaque zone

5. Nucleus

   1 nucleus present
   2 nucleus not present
   3 juvenile zone
   4 no juvenile zone

6. Zones

   1 not variable
   2 variable
      1 ... No of the ring
      H hyaline
      O opaque
      D double ring
      S slim zone
      B broad zone
      N normal zone

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

7. Readability

   1 sure
   2 not sure
   3 not readable