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PRELIMINARY RESULTS OF A COMPARATIVE COD OTOLITH AGE READING EXPERIMENT CARRIED OUT BY SCIENTISTS FROM SWEDEN AND GERMANY IN 1994

by

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ABSTRACT

Baltic cod growth rates, life cycle, and general population characteristics have been frequently examined. But age reading of Baltic cod is still difficult and therefore uncertain, primarily due to the absence of reliable age indicator structures on cod otoliths. These structures herein defined as check zones (narrow, translucent (hyaline) zones in reflected light and growth zones (broad opaque regions)) that can be on the surface of a broken otolith hold promise as a technique for ageing cod. The question is: What can otolith structures tell us about seasonal events in the life history of cod ? To improve the skill of readers and the conformity of interpretations of otolith structures, two workshops and an otolith exchange programme were conducted by Swedish and German institutes from 1992 to 1994. The objective of these activities was to mimimize between-reader bias and variability in the accuracy of age determinations, either between or within cod age reading laboratories. Results from these excercises are analyzed in this paper. The method used is a comparison of the results of for two or more readers for individual otolith. The distribution of differeces in age readings were compared with demanded distributions for the agreement for the readers. The variability of age determination between readers was expected to increase with increasing fish length due to interpretation errors. But as results show increasing fish length and age , respectively have no effect on the variability of age determination. Typically, subgroups of readers had acceptable agreement in ageing, but grouping was not consistent concerning the different sets of otoliths. This indicates a rather instable ageing also by readers concerned to be experts.
Among others it is recommended to introduce a digitized Baltic cod otolith catalogue, available to all institutes interested in using it as a training and comparing tool. Using this catalogue a greater degree of conformity in ageing cod otoliths may be achieved.

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Introduction

In 1992 and 1993 a cod age determination workshop was held in Karlskrona and Lysekil respectively.
Of the seven readers having taken part in the 1992 workshop. Two were considered to be experts. The other readers were biased among themselves and with the experts, except for one trainee. A second workshop on the same topic was held in 1993 with six readers. Four of them and the trainee have also participated at the 1992 workshop (list of participants, see Appendix A).
Workshops were held because new responsibilities were assigned in Swedish as well as in German institutes. The staff seems to be not experienced enough to solve inconsistencies in cod age determination data.

The main goals of the meetings were to

- i develop appropriate ageing criteria for cod
- ii compare the age determination techniques
- iii provide inexperienced investigators with adequate training in order to become able to age cod independently
- iv read a certain number of otoliths
- v made a statistical analysis of ageing results

As a conclusion of these two meetings it was agreed to carry out an otolith exchange programme between those reader who routinely read cod otoliths in there national laboratories. The participants of this exchange programme are shown in Appendix A

A fairly common problem of stock assessment procedures ("Virtual Population Analysis") regards combining obviously different weight at age and catch at age data of different nations. The main reasons are supposed to be different age interpretation of cod otolith structures by investigators involved in ageing procedures.
A better statistical investigation on cod age determination data is needed to ensure that our knowledge leads to a higher degree of conformity rather than the divergence, and to allow reliable calculations of the population stock size and age composition of Baltic cod stocks.

Material and Method

Altogether 1203 cod otoliths are included in these analyses in its final version. Otoliths were from different areas of the Baltic and from cod of different length, 10 cm to the maximum length collected (important size groups for the VPA are more frequent in the otolith sets).

Method of fish age determination

Different pattern of otolith growth zones made it possible to distinguish between cod of various origin (Bagge and Steffensen, 1980). The cod otoliths seem to be of three types:

1 Belt Sea cod:
The otolith has broad and well-defined annual growth zones.

2 Kattegat cod:
The otolith has also well-defined annual growth zones, but these zones are more narrow.

3 Baltic cod:
The otolith shows a more or less diffused growth pattern. It is therefore difficult to distinguish the annual rings.

The growth of fish and their hard tissues, such as otoliths are periodic processes (Bingel, 1980). In general, it is assumed that the otolith grows most rapidly in the anterior and posterior section and the growth patterns are best expressed on the dorso-ventral sagittal plane (Panella,1974).
On the other hand, it is also well known that the periods of summer and winter correspond to two different production rates of organic and inorganic materials. The rate of production of inorganic and organic material during the fast growing period is high. During the slow growing period less organic material is produced and the rate of $CaCO_2$-production is low (Panella, 1971; Liew, 1974). Furthermore it is noticed that in summer large but in winter small aragonite crystals were formed (Liew, 1974).
The first indication of otolith formation was the appearence of electron-dense granules in the anterior and posterior parts of the otocysts at about the 30-somite stage of the embryos. A less condensed organic matrix was deposited around the otolith core. The organic matrix consists of filamentous material (Dale,1984). The formation of growth rings in the otolith is probably caused by differential production and/or deposition of growth material, as reflected by differential density of the deposited material in the otoliths. The activity rhythm is the decisive factor of the formation of growth rings in teleost otoliths. The mechanism may be explained as follows: During activity, the otoliths are more "excited", causing the otolith growth material to be more densely packed than during inactivity, when the otoliths are "quit" and there is no turbulence in the endolymph (Dale, 1984).
The otolith of cod may have incorporated within their structural and chemical components a large amount of live history information. External and internal examinations of all three otoliths (sagitta, asteriscus, and lapillus) for otolith macrostructure and microstructure by light and Scanning Electron Microskopy indicate that yearly, daily and population rhytmic pattern exist. Many ecological facets of cod may be interpreted from the structural and chemical patterns of otoliths. Otoliths are calcium carbonate concretions situated in the membraneous labyrinth of teleost fishes.

There are three otoliths (the sagitta, asteriscus, and the lapillus) on each side of the brain area, but only the largest otolith, usually the sagitta, has been utilized for most studies. (Radtke, 1984). Otoliths of most teleost fishes are mineralogically composed of aragonitic calcium carbonate (Carlstrom, 1963). The sagitta otoliths are from 200 to 300 times larger by weight than the asteriscus and the lapillus.

The sagittae of adult cod displayed zones which have been utilized to provide annual records of age. These yearly zones are present in most cod sagittae and the annual nature of these zones is widely accepted by most fish biologists (Radtke, 1984).
It is suggested that visual differences in asteriscus antirostrum shape may be indicative of stock or population entities.
Lapilli were larger than sagittae at hatching and remained so for approximately 25 days. Sagittae became significantly larger than lapilli at the end of the larval period. The comparison of otolith diameter and the diameter of the first, usually very distinct, growth increment indicated that zone formation started somewhat before hatching. All larvae hatched with planoconvex lapilli and sagittae, whereas the asterisci were either very small or not formed at this stage (Bergstad, 1984).

Comparison of different techniques

One of the visible periodicities in otoliths is the alternation of opaque and translucent (hyaline) zones, commonly interpreted to determine the age of fish (Williams and Bedford, 1974). In temperate regions, intensive growth is reflected by the formation of the opaque spring-summer zone. Probably due to much reduced growth rate in winter, the translucent zone is being formed (Immerman, 1908). Although originally narrow translucent zones were interpreted as "false" or "check rings" (Jensen, 1965) we should agree that translucent (hyaline) zones correspond to the annually, occuring decreases in somatic growth in winter times. Gauldie (1988) stated that on the cristal level there are no criteria to differentiate between annual check rings and additional (false) check rings. If this statement is correct, it would follow that contrast-enhancing techniques, i.e. staining or burning, which are specific for annual checks are bound to fail (Welleman, 1990).
The annual otolith growth is expressed by two different rings. A opaque zone of white colour and a hyaline (transparent, translucent) zone using reflected light (Holten and Raitt, 1974). One opaque and one hyaline zone were determined as one year.
There are two well distinguished cod stocks in the Baltic with different growth pattern as reflected by otolith structures. In otoliths from cod east of Bornholm the structure of the opaque zone is not yet finished at the end of the calender year. The hyaline rings are created in February to July time .Cod west of Bornholm develop hyaline structers in late autumn, early winter respectively.
Examination of growth increments in hard parts such as otoliths requires validation of their periodicity to estimate true age (Beamish and Mc Farlane, 1983)

Besides the description of false and annual rings, there is a greater disagreement on the terms hyaline and opaque, especially in respect with the zone with the richest organic matter. According to Dannevig (1956), Molander (1947), and Irie (1960), the organic material is present only in the opaque spring-summer zone, while the hyaline winter zone consists entirely of aragonite (calcium-carbonate). This seems to be wrong, because after burning (Møller Christensen, 1964; Blacker, 1974) the optical dark parts (rich on organic material) correspond to the parts of the hyaline zone. Therefore Wellman (1990) gives a terminological distinction in the otolith features depending on the aspects that are being considered above (reflected light).

general interpretation	optical feature	after burning	organic material	inorganic material
check zone	translucent (hyaline)	dark	protein-rich	short-cristals
growth zone	opaque	light	protein-poor	long-cristals

The annual growth (ring) pattern is often very hard to notice objectively and a lot experience is needed to recognize the contrast in ring features. The macrostructural ring pattern is supposed to be a result of the change in width of daily increments and the length of crystals would then be the major difference between translucent and opaque zones (Wellman, 1990).

The result of burning techniques is explained by the differences in protein content between the translucent and opaque zone. The reason for the dark features of hyaline rings after burning might be explained as follows. Because that protein covers the crystal structure of otoliths and smaller aragonite crystals reflect less light, the dark burning zone is optically translucent (more light will be "swallowed" by this structure).
Structural differences between the zones are often shown by treating them with acid and by staining or burning of otoliths. A main disadvantage of burning is that it affects also the structure of aragonite that is not the most stable form of calcium carbonate.
According to Wellman (1990) one must have some insights in the chemical and physical processes in the formation of hyaline and opaque zones before starting to develop an automatic ageing method by means of image analysis. In principle, a video camera fitted to a stereomicroscope and combined with a computer can be used in ageing of fish otoliths (Fawell, 1974). Besides this, the sawing of otoliths as mechanical aid for otolith reading needs some preparation effort and is therefore still seldom used (Rauck, 1975).

Description of the method used

Although it could be expected that computer enhanced video image analysing systems can reduce the amount of subjective interpretation in the ageing process most cod biologists still use only a stereomicroscope (binoculare) to count the annual rings of cod otoliths. When using otoliths for ageing cod fishes, the otoliths should show clear ring formations before an interpretation of rings and determination of age is possible.

Therefore cod otoliths were transvers cutted through the otolith core. In some cases the surface of the broken halves needs grinding on wet grinding paper (220 Grit) in order to get a surface close to the nucleus.
The halves of the otoliths were held horizontally immersed in water or alcohol and viewed under a stereomicroscope. The beam from the microscope lamp should be directed horizontally to the ventral or dorsal side of the otoliths in order to produce as much as possible contrast between the hyaline and opaque zones of the otoliths. Annuli on otoliths were identified and counted using reflected light under a binocular microscope at the magnification of 16 x.

It is possible to use also transmitted light.

The opaque <Latin> non-transparent zones appear in reflected light clear and in transmitted light dark whereas the hyaline <Greeke> transparent zones appear in reflected light dark and in transmitted light clear.
One should have in mind that the 1 January means the "birthday" of fish i.e. the change of age-group. That means a cod born in June 1993 is of age zero on the 31 December 1993 and of age 1 on the 1 January 1994.

Exchange programme

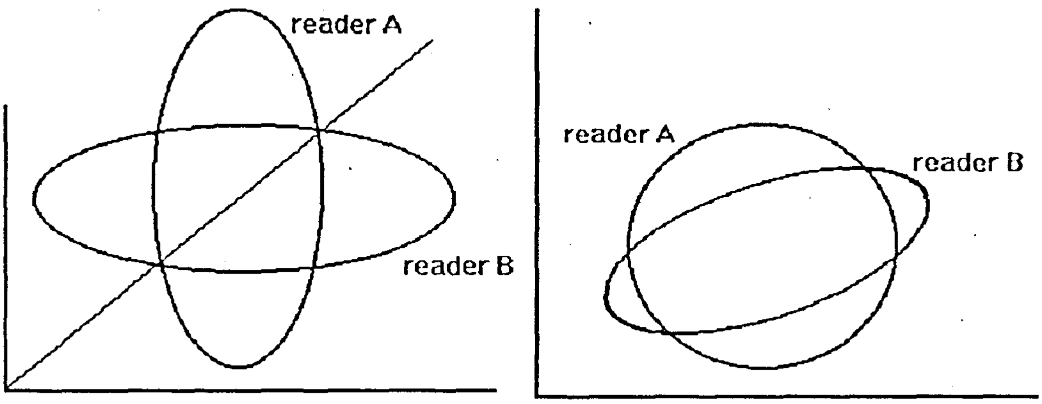
The results of our investigations based on two comparative age reading experiments carried out in Karlskrona in 1992 and in Lysekil in 1993. Routinely cod otolith ageing scientists from Lysekil, Karlskrona, and Rostock participated at the experiments. To get more basic information cod otoliths from different parts of the Baltic were circulated among the institutes in 1994. Otoliths were examined in random order. These examinations are the background for this presentation. The number of otoliths investigated is presented in Appendix B

Statistical methods

In contrast to Lassen (1985) who assessed the tendency of results of age determination of reader x compared to reader y,z , our investigations based on the comparison of reader by reader results. The method used by Lassen (1985) gives information about the general trend, for example reader x read on average a higher age than reader y,z , or vice versa.
This method contains two disadvantages:

First: The calculated trend is strongly influenced by the age of the oldest fishes.
Second: The trend is not a reliable clue to the exacttitude of the age determinations.

See the following drawings.



The results of the two readers are substantial different but the trend is nearly the same. Therefore we think that these method gives only little information about the quality of age determination results.

The main goal of an age comparing experiment is to get as much as possible conformity for every individual otolith.

Our aim was to develop a method that not only allows to compare pairwise the ageing results of readers but also the results of N readers. Only a great accuracy of ageing results of individual otoliths from all age-groups and areas can minimize the occurrence of systematical errors from different samples. Basis consideration for pairwise agreement of age determinations of every individual reader was to use a distribution function. By means of this simulation on can assess the distribution of the following parameters (for example the sum of pairwise differences). The comparison of theoretical distribution of ageing results with realy counted ages can be used as a criteria for the accuracy of the age determinations.

As minimum agreement with the true age between the results of reader *x* the following distribution should be agreed on.

Table 1.1

frequency (%)	1	9	80	9	1
difference of ageing	-2	-1	0	1	2

If this exacness will be demanded from all readers, the distribution of age determination differences should be as follows

Table 1.2

frequency (%)	0.01	0.18	2.4	14.6	65.6	14.6	2.4	0.18	0.01
difference of ageing	-4	-3	-2	-1	0	1	2	3	4

By simulation of data we can analyse the effect of this basic demand on the results of age determination gained by a certain number of reader.

For the data analysis we propose to use the the sum of the absolute deviations of ageing results of all readers to the true age (standard age or original age).

As result one get a frequency distribution of the possible individual sums

$$SUM = \sum_{i=1}^{N} |\text{true age} - \text{result of reader } i|$$

The result is depending from the number of participating readers N. If there are other data ,for example fish length, avarage fish length, the correspondig sums of deviations may also be used (mean length for SUM= 0, SUM= 1...,see results in table 1.8).
This data are helpfull if the demand in Table 1.1 is not realizable.
The following tables show the result of this simulation for 10.000 age results between true ages 3 and 6. It was supposed that all reader have a degree of conformity as in Table 1.1 mentioned. We also suppose that the true age is unknown and no bias (reader *x* to reader *y,z*) occured.
Table 1.3 shows the simulated age distribution and the corresponding age distribution from reader A to reader D.

Table 1.3

age group	true age	reader A	reader B	reader C	reader D
1	0	16	21	20	29
2	0	241	291	226	253
3	2439	2228	2172	2240	2159
4	2447	2367	2419	2432	2419
5	2538	2538	2504	2513	2557
6	2576	2325	2312	2308	2281
7	0	259	252	226	286
8	0	26	29	35	16

In Table 1.4 the reader by reader differences between the original age and the result of the reader are presented.

Table 1.4

Table 1.4				
difference of the age to the original data	reader A	reader B	reader C	reader D
-2	93	106	101	111
-1	868	939	935	888
0	8019	7977	7995	7950
1	926	873	857	954
2	94	105	112	97
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0

Table 1.5			
difference of the reader to reader A	reader B	reader C	reader D
-5	0	0	0
-4	2	2	0
-3	15	20	18
-2	233	233	237
-1	1437	1401	1474
0	6506	6576	6530
1	1546	1504	1477
2	246	247	245
3	14	15	19
4	1	2	0

In praxis the so-called original age is unknown. Therefore the result of one reader must be taken as a standard. For the simulation the results of reader A were choosen to be the standard values. Table 1.5 shows the differences of reader B, C, and D to reader A (so-called paired differences).

Despite the relative large differences of reader B, C, and D compared to reader A (Table 1.5.) the produced age distribution as shown in Table 1.3 has a high degree of correspondence.
A χ^2 test based on results of table 1.3 shows no significant difference between the readers. The reason is the supposed exactitude of the ageing results and that the readers are unbiased.

The reason for a significant difference between the age frequency distributions of two reader may be:

a) Differences in the exactness of the aging process.

b) A systematical interpretation error.

The judgement whether reason a) or b) occured is possible by means of the symmetry of the differences (for example reader x read in allmost all cases 1 or 2 years more than reader y,z means a systematical error).

For testing the results of different readers the sum of the absolute deviations (differences) can be used.
Table 1.6 shows these sums of deviations from the simulated age distribution (original data), starting with the SUM for reader N=1 (reader A) to N=4 (reader A, B, C, and D).

Table 1.6

sum of absolute differences to the original data	reader A	reader A +reader B	reader A +reader B +reader C	reader A +reader B +reader C +reader D
0	8019	6363	5086	4034
1	1794	2946	3503	3754
2	187	621	1156	1636
3	0	66	216	466
4	0	4	38	98
5	0	0	1	10
6	0	0	0	2
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0

Table 1.7

sum of absolute differences to the reader A	reader B	reader B +reader C	reader B +reader C +reader D
0	6569	5169	4097
1	2905	2516	2770
2	482	1746	1283
3	41	337	1200
4	3	189	366
5	0	35	121
6	0	7	117
7	0	0	33
8	0	1	11
9	0	0	2

Because in praxis the original data, the true age, is unknown, the results of reader A are again taken to be the standard. The sum of deviations from the results of reader A were calculated for N= 1 (reader B) to N = 3 (reader B, C, and D) and presented in Table 1.7.

An other simulation example with a reduced degree of conformity of age determinations with the so-called true age (instead of 80% (Table 1.1) only 60% (Table 2.1.) is presented in Tables C.1 to C.7 of Appendix C.

The described method can be used to estimate the agreement of two or more age readers. If the agreement for the individual otolith is demanded like in Table 1.1, theoretical distribution of different values (sum of the absolute differences) can be calculated. A comparison between these theoretical distributions and the resulte of age readings can be used to estimate the quality of the agreement.

The effect of precision of catch at age data on VPA output is addressed in Pope (1972). Together with the known number of participants on a comparative age reading experiment it is possible to predict a demand of exactness for the agreement of ageing results for the participants.

Results and Discussion

Analyses are based on cod age determinations from samples collected at different Sub-divisions of the Baltic. In this paper we only discuss the results for Sub-division 24 (Arkona Basin, western stock).
Appendix A listed the participants and their abbreviations used for the statistical tests.
Appendix B gives an overview of number of compared cod otoliths by Sub-divisions.
Tables 2.1. and 2.2. show the statistical terms and the calculated statistical values for Sub-division 24.

Table 2.1 Statistical terms

	Difference
EA	E - A
EB	E - B
EC	E - C
ED	E - D
EF	E - F
EG	E - G
EH	E - H

Table 2.2. Calculated values

	Value
AB	A - B
AH	A - H
SUME	abs(EA)+abs(EB)+...+abs(EH)
SUME2	$EA^2+EB^2+EC^2+...+EH^2$
SUM1	$EA^2+EB^2+EC^2+...+EG^2$
SUM2	$EC^2+ED^2+EF^2+EG^2$
SUM3	$EB^2+EF^2+EG^2$
SUME3	abs(EC)+abs(ED)+abs(EF)

Table 2.3 contains the independent reading results

Table 2.3

reader\age	2	3	4	5	6	7	8	9	10	unknown	sum
A	69	22	6	1						2	100
B	63	31	5			1					100
C	37	52	10			1					100
D	41	53	5		1						100
E	36	56	6		1					1	100
F	38	53	6		1					2	100
G	29	57	12	1				1			100
H	6	78	13	1	1				1		100

The results of the $\chi2$ test are shown in Table 2.4. The table contains the compared readers, the test value, the degree of freedom and the quantil of the $\chi2$ distribution. The asterix * marks results with a significant difference in age distribution.

Table 2.4

compared readers	value of χ^2 test	degree of freedom	control limit
E - G	8.523 *	3	5.99
B - E	34.40 *	3	5.99
B - F	24.19 *	3	5.99
E - F	0.397	3	5.99

Table 2.5. shows the pairwise differences of all participating readers to reader.E.

Table 2.5

	difference in age readings to E								
difference	-4	-3	-2	-1	0	1	2	3	sum
EA				8	49	39	1		97
EB				9	54	35	1		99
EC			1	22	56	19	1		99
ED			1	15	61	21	1		99
EF			2	17	55	22	1		97
EG		1		28	58	11	1		99
EH	1	1	1	40	53	3			99
AB			2	17	67	12			98
AH			2	69	22	2			98

The readers C, D, E, and F have nearly the same age distributions determined. The distributions of readers B and G were significantly different in comparison to reader E. The distribution of readers A and H were extremely different.

Comparing the age distributions of readers EC, ED and EF with the demands postulated in Table 1.2 one can conclude that the readers C, D, E, and F have a good agreement with the true age of the fishes (Table 1.1).

The results show that all readers (except reader A) have more than 50% total agreement with reader E. In spite of the large number of differences (nearly 50%), the readers C, D, E, and F estimated approximately the same age distribution. This surprising result can be explained in that way that the variable age readings are uniform distributed in all age groups and a systematical error in the age reading does not exist, even if only 55% of age readings corresponded (readers E and F). If we compare the difference of readers EB we see also 54 otoliths with the same age , but the value of the $\chi 2$ test shows a significant difference in the estimated age distribution. This results emphasizes a systematical difference existing in the age readings between the reader E and B.

In the following text we describe whether the fish length is influencing the difference in aging or not.

Table 2.6 shows the mean length of fish according to the difference in age reading compared to reader E.

Table 2.6

	sum of the absolute differnces of all readers to reader E									
SUME	0	1	2	3	4	5	6	7	9	9+
number	3	24	12	22	8	12	8	2	0	3
mean length (cm)	35.00	37.54	36.42	39.50	43.88	35.50	36.50	35.50		57.00

for example:

	reader						
otolith	A	B	C	D	E	F	SUM
age 1	2	2	3	2	3	1	
absolute difference	1	1	2	1	2	0	7

This means one fish with absolute difference 7.

Table 2.7 contains the absolute differences of all age data of the readers C, D, and F and all otoliths to the standard reader E. Additionally the average length of fish with the same SUM differences were calculated and reported in Table 2.7.

Table 2.7

	sum of the absolute differnces of readers C, D and F to reader E									
SumE3	0	1	2	3	4	5	6	7	9	9+
number	34	23	22	16			2			
mean length (cm)	38.12	41.18	38.22	35.69			35.50			

The result presented there is that no length dependent difference in aging comparing to standard reader E exists.

Preliminary comparison of the ageing results of samples from different ICES Sub-divisions in the Baltic

Table 3.1 shows the participants of the different readers at the samples in the ICES Sub-divisions.
The following notations were used in the Table 3.1

- \- participation
- *, ** age distributions do not differ significantly between the readers

reader	ICES 21	ICES 22 A	ICES 22 B	ICES 24 A	ICES 24 B	ICES 25 A	ICES 25 B	ICES 27	ICES 28	ICES 30
A	*	*	-	*	-	*	*	*	*	-
B	*		-	*	-	**	-	-	-	
C	*	*	-	-	-	*	-	-	-	-
D		*	*	**	-		-	*	*	-
E	*	*	*	**	-		*	**	*	-
F	-		-	-	-	-	-	-	-	-
G	-	*	-	-	-	**	*	**	*	
H			-		-	-	-	-	-	
I			-		-		-	-	-	

ICES 21

It can be assumed that the readers C, D, E, F and G have an agreement required with the original age (Tablel 1.1)

ICES 22 A

The data contain only the age groups 0 and 1. Some fishes may have the age group 2.

ICES 22 C

Only the readers A and B have not a significant difference in the age distribution. There are many differences in the age groups 1, 2 and 3. The readers G and H have systematically lower age estimations.

ICES 24 A

It can be assumed that the readers C, D, E and F have an agreement with the original age (Table 1.1). The reader H estimates a higher age than the reader E. This is different to the results of ICES22 Sub-division 22.

ICES 24 B

There are strong differences in the estimation of the age groups 1 to 4. The readers F, G and H have systematically different results to the readers A to D.

ICES 25 A

There are two groups of readers that have a good agreement, reader C with reader F and reader B with reader D. Between the groups are systematical differences. The results of SUMC show that the sum of absolute differences to the reader C is correlated with the total length.

ICES 25 B

The readers C, A, and D have an aggrement with the true age (Table 1.1). The readers F, G and H estimated systematically lower.

ICES 27

ICES 28

The readers C, A, B and D have a good agreement in age distributions. The results of the χ^2 test indicate no significant differences. The readers F, G and H are an other group with higher ageing results.

ICES 30

The number of 30 otolith is to low to test significant differences in the age readings.

Comments, Conclusions, and Recommendations

1. There is a group of readers with high agreement, but not in all samples. Significant differences in the age distributions are also possible.
   The difference of a second group of readers to this group is rather small.
   A third group has systematically other results.

2. There is not one pair of readers without significant differences comparing all samples. This result indicates a rather instable ageing by all readers.This results clearly emphasize the need for further worhshops or exchange programmes between this institutes or betwen all institutes involved in Baltic cod age reading.

Traditional workshops on age-determination techniques using annual growth increments visible on broken or sliced pieces of otoliths are often unsuitable to explain the reason of differences in ageing. It only can document the actual level ot agreement or disagreement. Progress is needed and for example possibl by using computer enhanced video image analysing systems.

We suggest:

1. To produce a digitized cod otolith catalogue. For every photograph the age should be mentioned and critical structures on the otolith surface empasized.
   Starting with otoliths with clear structures also otoliths with rather small difficulties and especially otoliths with confusing structures should be presented in the catalogue.
   An exchange of the catalogue on discettes should also be possible.

2. A training and comparing catalogue with the so-called true age will be prepared available for all institutes. In that way that all institutes can use the same otolith catalogue a higher degree of conformity might be possible.

3. Exchange of otoliths is necessarry every third year to reach or maintain adequate levels of consistency between readers.

4. To use statistical tools presentet in Anon, 1994 (2) to compare between-reader bias (t-test, Wilcoxon, Bowkers symetry test).

As a second step we will analyse our basic material using other methods than supplied in this report: Average percent age error (APE) (Beamish and Fournier, 1981); coefficient of variation (Chang, 1982); and the chance-corrected observer-agreement measure (kappa) (O'Connell and Dobson, 1984, Schouten, 1982).

References

Anon, 1994 (1). Report of the workshop on Baltic cod age reading Gdynia, Poland 1994, ICES C.M. 1994/J:5.

Anon, 1994 (2) Report of the workshop on sampling strategies for age and maturity, Copenhagen, Denmark 1994, ICES C.M.1994/D:1.

Bagge,O. and E. Steffensen , 1980. Growth pattern in cod otoliths as indicator for mixing of stocks. ICES C.M. 1980/J:12, Baltic Fish Committee.

Beamish, R.J. and A.D. Fournier, 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aqua. Sci. 38: 982-983

Beamish, R.J. and G.A. McFarlane, 1983. Validation of age determination estimates: the forgotten requirement. NOAA natn.mar.Fish.Serv.Tech.Rep. (: 29-34

Bergstad, O.A., 1984. A relationship between the number of growth increments on the otoliths and age of larval and juvenil cod *Gadus morhua* L. In : E. Dahl, D.S. Danielssen, E.Moksness and P. Solemdal (Editors), The propagation of cod *Gadus morhua* L. Flodevigen rapportser., 1, 1984: 251-272.

Blacker, R.W., 1974.Recent advances in otolith studies. In: F.R.H. Jones (ed.). Sea fisheries research. Wiley New York. pp.67-90.

Bingel,F. 1980. Growth of cod otolith on the crystal level. Meeresforschung-Reports on Marine Research, Bd. 28(1981),H. 4, S. 212-215.

Carlstrom, D., 1963. A crystallographic study of vertebrate otoliths. Biol. Bull. (Woods Hole) 125: 441-463.

Chang, W. Y. B., 1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aqua. Sci. 39: 1208-1210.

Chilton, D.E. and R.J. Beamish, 1982. Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. Can. Spec. Publ. Fish. Aquat. Sci. 60:120p

Dale, T. 1984. Embryogenesis and growth of otoliths in the cod (*Gadus morhua* L.) In : E. Dahl, D.S. Danielssen, E.Moksness and P. Solemdal (Editors), The propagation of cod *Gadus morhua* L. Flodevigen rapportser., 1, 1984: 231-250.

Dannevig, E.H., 1956. Chemical composition of the zones in cod otoliths. J.Cons. Int. Explor. Mer 21: 156-159.

Fawell, J.K., 1974. The use of image analysis in the ageing of fish. In: T.B. Bagenal (ed.). The ageing of fish. Unwin Brothers Limited,England. pp. 103-107.

Härkönen, T., 1986. Guide to the otoliths of the bony fishes. Danbiu ApS. Biological Consultants, Henninggsens Alle 58, DK-2900 Hellerup, Denmark.

Holden, D.E. and D.F.S. Raitt, 1974. Manual of Fisheries Science, part 2 - Methods of Resource Investigation and Their Application. FAO Fish. Tech. Pap., (115) Rev. 1: 2/4p.

Immerman,F., 1908. Beiträge zur Altersbestimmung der Fische. II. Die innere Struktur der Schollenotolithen. Wiss. Meeresuntersuch. Abt.Helg.NF, 8: 129-176.

Irie, T., 1960. The growth of the fish otolith. J. Fac. Fish. Anim. Husb., Hiroshima Univ. 3: 203-221.

Jensen, A.C., 1965. A standard terminology and notation for otolith readers. ICNAF Res. Bull 2: 5-7.

Lassen, H. 1985. Results from comparative age-readings of Baltic cod and flounder otoliths. ICES C.M. 1985/J:12.

Liew,P.K.L., 1974. Age determination of American eels based on the structure of their otoliths. In: Bagenal,T.B.(ed): Proceedings of the international symposium on the ageing of fish, held at Reading University, UK, on 19-20.July 1973, 124-138. Unwin Bros.,Old Woking (UK).

Meyer, A.., 1965. Sawing of otoliths as mechanical aid for otolith reading. ICNAF Research Bulletin Nr.2, Canada 1965.

Molander, A.R., 1947. Observations of the growth of the plaice and on the formation of annual rings in its otoliths. Svenska hydrogr.-biol. Komma. Skr. NY serie. Biol. 2(8): 3-11.

Moller Christensen, J., 1964. Burning of otoliths, a technique for age determination of soles and other fish. J. Cons. Int .Explor. Mer 29: 73-81.

O'Connell, D. L. and A. J. Dobson, 1984. General observer-agreement measures on individual subjects and groups of subjects. Biometrics 40: 973-983.

Panella,G. 1974. Otolith growth pattern: an aid in age determination in temperate and tropical fishes. In: Bagenal,T.B.(ed): Proceedings of the international symposium on the ageing of fish, held at Reading University, UK, on 19-20.July 1973, 28-39. Unwin Bros.,Old Woking (UK).-1971. Fish Otoliths: Daily growth layers and periodical patterns. Science 173: 1124-1127.

Penttila, J. and C.M. Dery, 1988. Age Determination Methods for Nortwest Atlantic species. NOAA Techn. Rep. NMFS 72:135p

Pope, J.G., 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. ICNAF Res. Bull. 9: 65-74.

Radtke, R.L., 1984. Cod fish otoliths: information storage structures. In : E. Dahl, D.S. Danielssen. E.Moksness and P. Solemdal (Editors), The propagation of cod *Gadus morhua* L. , Flodevigen rapportser., 1, 1984: 273-298.

Rauck, G., 1975. A new technique of sawing otoliths. ICES C.M. 1975/ F: 23 Demersal Fish Comm.

Schouten, H. J. A., 1982. Measuring pairwise interobserver agreement when all subjects are judged by the same observer. Statistica Neerlandica 36: 45-61.

Welleman,H.C., 1990. Annual check formation in otoliths as a starting point for ageing by means of computer analysis. ICES C.M. 1990/G: 29. Demersal Fish Committee.

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

Appendix A:
Participants:

Karlskrona 1992:

Name	Place of Institution
Anderson, A.-L.	Lysekil
Bratt, A.-M.	Lysekil
Lundahl, L.	Karlskrona
Nielsson, R.	Karlskrona
Sellerberg, G.	Karlskrona
Sjöberg, R.	Lysekil
Walter, Y.	Karlskrona
Schulz, N.	Rostock

Lysekil 1993:

Anderson, A.-L.	Lysekil
Bratt, A.-M.	Lysekil
Lundahl, L.	Karlskrona
Hjelmberg, M.	Riksmuseet Stockholm
Sjöberg, R.	Lysekil
Svedäng, H.	Kustlaboratoriet Öregrund
Schulz, N.	Rostock

Exchange programme 1994:

Rajlie Sjöberg	HfL Lysekil
Anne-Lie Anderson	Hfl Lysekil
Ann-Marie Bratt	HfL Lysekil
Lars Lundahl	Östersjölab.Karlskrona
Yvonne Walther	Östersjölab.Karlskrona
Ruthild Hoffmann	Ostseefisch. Rostock
Günter Ulrich	Ostseefisch. Rostock

Appendix B:

Overview of all data available for analysis

ICES SD	Number of age reading	Number of readers	Comments
21	100	7	B, C
22 A	50	7	A
22 B	144	10	
24 A	100	8	B, C
24 B	145	10	
25 A	100	7	B, C
25 B	207	7	A
27	101	10	
28	78	10	
30	30	7	A

Comments refer to other working reports.

A Report of the cod age determination workshop.
Lysekil, 06 - 09 December 1993 (internal)

B Preliminary results, presented in Lysekil.
Lysekil, 06 - 09 December 1993 (internal)

C Report of the cod age determination workshop.
Lysekil, 20 - 29 October 1992 (internal)

Appendix C:

Table C.1

frequence in %	5	15	60	15	5
difference of aging	-2	-1	0	1	2

Table C.2

frequence in %	0.25	1.5	8.25	19.5	41.0	19.5	8.25	1.5	0.25
difference of aging	-4	-3	-2	-1	0	1	2	3	4

Table C.3

age group	true age	reader A	reader B	reader C	reader D
1	0	142	115	127	121
2	0	490	444	494	479
3	2468	1980	1980	1988	2024
4	2483	2313	2432	2341	2287
5	2487	2381	2335	2360	2412
6	2562	2056	2066	2058	2039
7	0	513	508	521	491
8	0	125	120	111	147

Table C.4

difference of the age to the original data	reader A	reader B	reader C	reader D
-2	545	477	488	462
-1	1439	1487	1512	1491
0	5985	6016	6019	6081
1	1530	1484	1491	1454
2	501	536	490	512
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0

Table C.5

difference of the reader to reader A	reader B	reader C	reader D
-5	0	0	0
-4	37	24	37
-3	171	145	129
-2	852	862	850
-1	1904	1944	1907
0	4045	4033	4180
1	2009	1963	1899
2	822	857	832
3	136	146	145
4	24	26	21

Table C.6

sum of absolute differences to the original data	reader A	reader A +reader B	reader A +reader B +reader C	reader A +reader B +reader C +reader D
0	5985	3585	2142	1335
1	2969	3614	3233	2571
2	1046	2077	2759	2763
3	0	606	1319	2013
4	0	118	443	916
5	0	0	87	312
6	0	0	17	81
7	0	0	0	8
8	0	0	0	1
9	0	0	0	0

Table C.7

sum of absolute differences to the reader A	reader B	reader B +reader C	reader B +reader C +reader D
0	4093	2276	1359
1	3859	2782	2122
2	1660	2714	2202
3	339	1171	1881
4	49	677	1027
5	0	259	602
6	0	98	381
7	0	21	256
8	0	2	124
9	0	0	32