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Report of the Workshop on Age estimation of Norwegian Spring Spawning Herring between, Norway, Denmark, Iceland and the Faroe Islands (WKNSSAGE)

9-10 November 2015

Charlottenlund, Denmark

ICES CIEM

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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Executive summary

The workshop on age reading of Norwegian Spring Spawning herring (WKNSSAGE), chaired by Jane Aanestad Godiksen, met 9–10 November 2015 in Charlottenlund, Denmark. The meeting was attended by 12 experts from four countries. WKNSSAGE was a request from WGWIDE to WGBIOP to review any technical problems regarding age-reading of Norwegian spring spawning herring between Norway, Denmark, Iceland and the Faroe Islands. In 2015 Norway sampled the same areas as the three other countries during the IESNS-survey, and during the post-cruise meeting the differences between the age distributions from trawl samples taken in the same area and period became apparent. For example, from one stratum the Norwegian samples were dominated by three age groups, while the age distribution from Denmark looked much more uni-modal. Also comparison of samples taken by Norway, the Faroe Islands and Iceland show age distributions, which are not comparable, and thus, ageing or sampling issues are likely to exist among all of the participating nations. As a result, WGWIDE approached WGBIOP with a request for a fast-track workshop between age readers to address any potential issues related to the age determination of NSS herring.

Denmark and the Faroe Islands are ageing NSS herring by reading their otoliths, while Norway and Iceland use scales. To establish a common understanding of how both structures appear and are interpreted the workshop was initiated by examining some pre-annotated scales and otoliths. Concerns over the interpretation of the edge were addressed and there appeared to be very little disagreement in the interpretation of the growth zones in either structure. Thereafter an exercise containing otoliths and scales from the same fish was prepared in WebGR, the actual structures were also available to the readers. The results showed a low level of agreement (52%) between age readings and a general trend appeared where the scales were estimated to be one year older than the otoliths. This lead to an apparent loss of the strong year class of 2004. After reviewing the structures in plenary, it was clear that it was most often the first winter ring in the scale which was not clearly visible in the otolith. In order to review the problem in more detail a numerical analysis was attempted utilizing the measurements extracted from WebGR. A number of shortcomings were noticed when using this approach to identify potential problem areas in the age interpretation. The problems could be associated with mixing of subpopulations and/or stocks.

WKNSSAGE concluded that the different ages obtained from scale and otolith readings could be due to a number of issues relating to identification of the first winter ring and age interpretation of older fish, confounded by stock mixing issues. Final conclusions cannot be reached based on the samples from this workshop. We believe the sampling and stock mixing issues should be addressed separately by WGWIDE.

Overall, WKNSSAGE recommend that the Workshop on Age estimation of Norwegian Spring Spawning Herring (*Clupea harengus*) (WKARNSSH) 2017 consider the shortcomings of the present workshop and develops an ageing protocol that contain robust procedures for a quality check. The above mentioned ageing issues should be addressed in full based on a larger sample set of good quality scales and otoliths and defined instructions for annotation. Prior to WKARNSSH within country disagreements need to be resolved. Also, stock mixing issues need to be addressed (potentially by genetics combined with otolith shape analysis) and sampling protocols need to ensure that both otoliths and scales are sampled from the same fish (at least subsamples).

1 Terms of reference

A Workshop on Age estimation of Norwegian Spring Spawning Herring between Norway, Denmark, Iceland and the Faroe Islands (WKNSSAGE), chaired by Jane A Godiksen, Norway will be established and will meet in Copenhagen, Denmark, 9–10th November 2015, to:

a) Review the technical problems regarding age-reading of Norwegian spring spawning herring between Denmark and Norway regarding the extra growth added in May-sample.

b) Analyse the problematic structures (otoliths/scales) from the IESNS-surveys (May-surveys) described by WGWIDE.

c) Clarify the interpretation of annual rings in particular during spring/summer.

d) Improve the protocol of the guideline on age estimation and the applied structure (otolith or scale).

WKNSSAGE will report by 1 December 2015 for the attention of WGWIDE and WGBIOP.

Supporting Information

PRIORITY:	Age determination is an essential feature in fish stock assessment to estimate the rates of mortality and growth. In order to arrive at appropriate management advice ageing procedures must be reliable. Otolith processing methods and age reading methods might differ considerably between countries. Therefore, otolith exchanges should be carried out on a regular basis, and if serious problems exist age reading workshops should be organized to solve these problems.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	The aim of the workshop is to review the technical problems regarding age-reading of Norwegian Spring Spawning herring between Denmark and Norway regarding the extra growth added in May-samples. Otoliths and scales from the May-surveys will be brought to the WK and discussed.
RESOURCE REQUIREMENTS:	No specific resource requirements beyond the need for members to prepare for and participate in the meeting. Otoliths and scales from the May-surveys should be available.
PARTICIPANTS:	Age readers working on Norwegian spring spawning herring.
SECRETARIAT FACILITIES:	None.
FINANCIAL:	None
LINKAGES TO ADVISORY COMMITTEES:	ACOM, SCICOM
LINKAGES TO OTHER COMMITTEES OR GROUPS:	WGBIOP, WGWIDE, ACOM, SCICOM, SSGIEOM, RCMs.
LINKAGES TO OTHER ORGANIZATIONS:	There is a direct link with the EU Data Collection Framework

2 Agenda and participation

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

3 Introduction

In the otoliths and scales of fish contrasting zones of opaque and translucent material are laid down on a yearly basis and the resulting growth pattern observed in the structures is a reflection of the growth experienced by the fish. Differences in preparation techniques and interpretation of the growth structures, including the position of the first winter ring, the annual structure of growth rings and the interpretation of when to include a structure on the edge of the otolith were the primary sources of bias in age estimation outlined by WKNARC2 (ICES 2013). Ageing laboratories provide age data on an annual basis for use in stock assessment. The ages, the proportions of each age class and the inferred parameters (growth and mortality) are of primary importance and are used to infer stock status and population dynamics. One of the main objectives for the age reader community is to achieve consistency between groups of age readers estimating the age of a certain species or stock and to minimize the amount of bias in the age data which is used in stock assessment and can have serious consequences for the resulting scientific advice which is used for the management of fish stocks. The aim of this workshop was to address age reading issues apparent with the NSS herring stock and thus minimize the bias associated with the age data provided to WGWIDE for assessment purposes.

In addition to the age reading issues, the following points are likely to be confounding the age structures seen by the different countries during the 2015 survey:

The potential representation of several herring stocks in the surveyed area was discussed during the WKNSSAGE. During on-screen discussion of otolith structures brought by all participating nations, readers were able to identify otoliths from stocks other than the NSS herring. Given the highly migratory nature of this species there is a very high likelihood that a mixture of Icelandic summer spawners, local fjord populations and NSS herring could mix on the summer feeding grounds (which is supported by the literature, e.g. Libungan *et al.* 2015). Observations were based on the differences in distance to the first winter-ring, size of larval centre and overall shape of the otoliths. Some laboratories already take such issues into account when age-reading herring from the area and others combine visual identification of the otoliths with the maturity index and gonad weight to help identify summer spawners. This stock mixing issue and how these data are passed on to the stock coordinators needs to be explored further given the serious implications for the quality of the assessment both in terms of age and stock structure.

There are also issues related to sampling procedures and vessel capabilities utilized by the different countries taking part in the IESNS surveys which need to be considered by the assessment group. These were not addressed in depth at the Workshop as they are not specifically age related issues but are mentioned here. The type of sonar used to detect the schools combined with the gear type plus depth and duration of the hauls are likely to affect the size of the fish being captured. This issue is already included in the upcoming benchmark on NSS herring and will, alongside with the WKNSSAGE report, provide clarity on the discrepancies observed in the age-distributions from the May survey (ICES 2015a, ICES 2015b).

4 Review the technical problems regarding age-reading of Norwegian spring spawning herring between Denmark, Norway, Iceland and the Faroe Islands regarding the extra growth added in May-sample (ToR a)

There has for several years been concern that observed differences in NSS herring age distribution in samples from the different participating vessels reflect bias in age readings among involved institutes. In 2015 the Norwegian vessel overlapped with areas covered by Danish, Faroese and Icelandic vessels during the IESNS survey (also known as the "May survey") (Figure 4.1). During the post-cruise meeting after the May survey, age distributions of NSS herring from trawl samples from the different participating countries were compared.

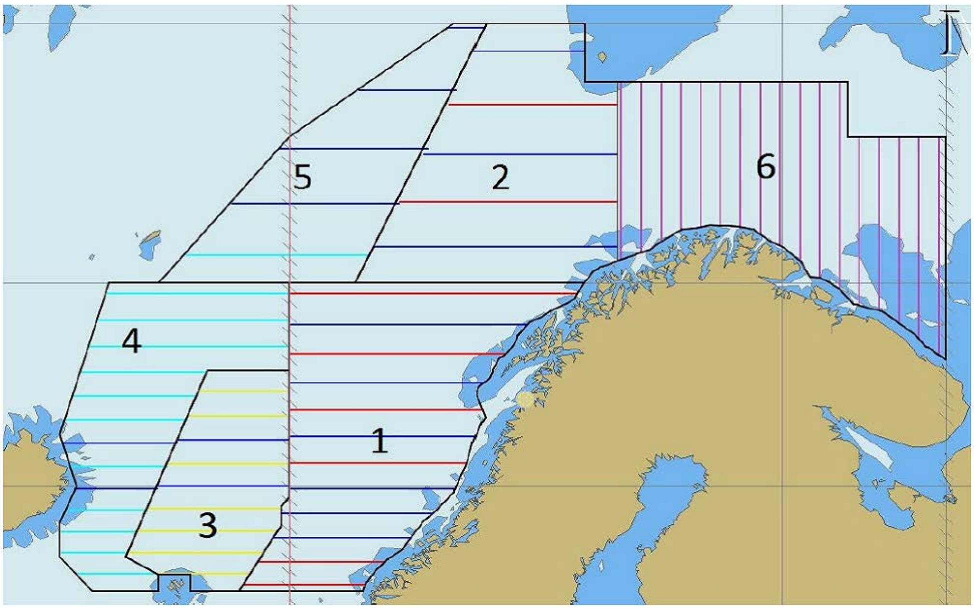

Figure 4. The strata and transects used in the IESNS survey 2015.

Figure 4.1. The strata and transects used in the IESNS survey 2015. Blue = Norway, Red = Denmark, Yellow = Faroese, Turquoise = Iceland. From the IESNS survey report.

It is stated in the 2015 Post Cruise Meeting IESNS report: "In the last years there have been concerns regarding age reading of herring, because the age distributions from the different participants have showed differences". This is also the case in 2015 (Figure 4.1.1). These age distributions were quite different, even for samples taken in the same area and period e.g. the age distribution from the Norwegian and the Danish vessels from stratum 1 where the Norwegian samples are dominated by three age groups while the age distribution from Denmark looks much more uniform. Also, there was an apparent difference in the age distribution in Stratum 4 between the Icelandic and the Norwegian vessel with respect to age groups 10–12 years, which might be a consequence of a "drift" of 2004 year class into the 2003 and 2005 year classes during the ageing. However, the differences may also reflect differing spatial distribution of age groups, and partly, they may reflect variable growth conditions for the stock, and consequently growth rate as seen on the fish scales and otoliths. In spring 2014 an otolith and scale exchange was conducted, initiated by PGCCDBS (Godiksen, 2014). The report stated that the agreement among readers was low (67%) and it was recommended to conduct a larger scale exchange where both scales and otoliths are sampled from the same fish. Thus, the survey group emphasized the need for an age-reading workshop for the primary herring age readers prior to the 2016 May survey. And consequently WGWIDE approached WGBIOP with a request for a fast-track workshop between age readers to resolve any potential issues related to the age determination of NSS herring:

4.1 Interpretation of the season and age dependent growth at the edge of the structure

The initial concern was that the interpretation of the edge of either otolith or scale (depending of which is used for age determination) in May differed between laboratories. Typically, if the age reader decides that there is opaque growth on the edge following the translucent winter ring, then they do not count the opaque edge but count the previous winter ring as the final winter ring used in the age estimation. When there is no opaque growth yet at the edge (during the present year) they count the edge as a winter ring, thereby adding one more year to the age estimate. What confounds the issue is that when the fish becomes older it becomes more difficult to interpret the edge of the otolith as the growth zones become so narrow. Depending on the distance from the beginning of the last winter ring to the edge of the structure some readers will add an extra year and some will not.

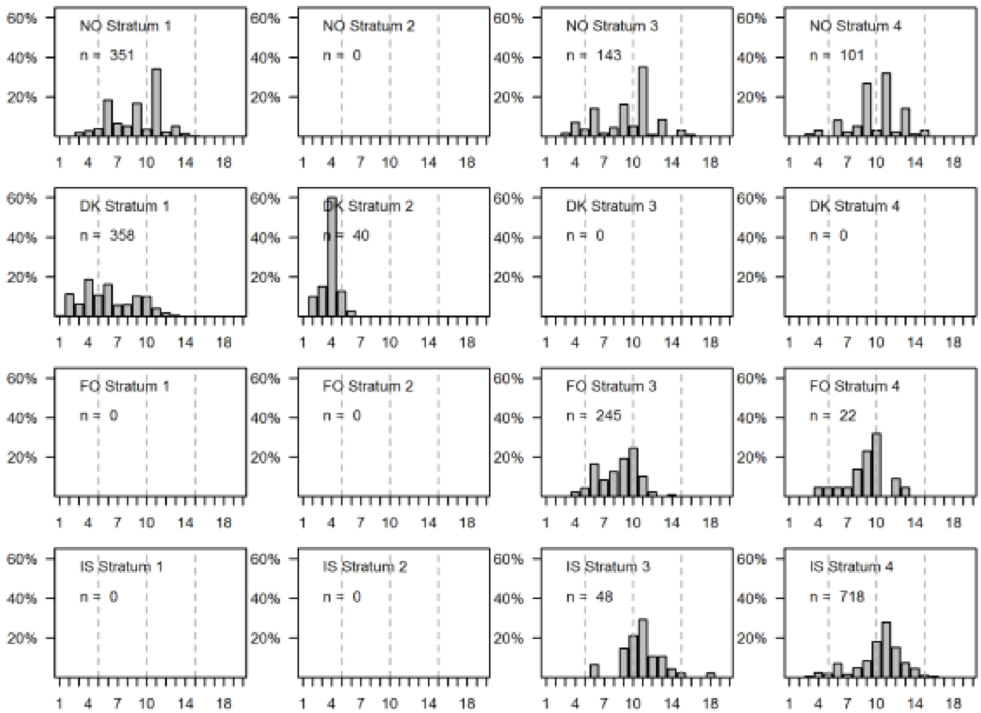

Figure 4.1.1. Comparison of the age distributions of NSS-herring by stratum and vessel in IESNS 2015. From the IESNS survey report.

The age distribution of NSS herring is characterized by having a very spiky appearance, this is due to a few very large year classes occurring with long time-intervals in between. These year classes dominate the stock and can typically be followed for at least 15 years throughout the time-series. If readers mis-interpret the edge of the ageing structure, the risk to transfer fish from a large year class like 2004 down to a smaller year class like 2005 or vice versa is increased (Figure 4.1.2). The problem will increase as a year class gets older, and growth ceases. The older the fish get, the narrower the distance between the winter rings, and the more difficult it is to decide if there is growth added to scales and otoliths in May.

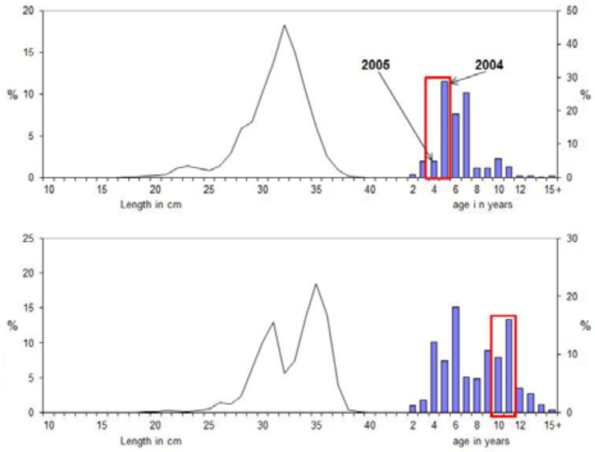

Figure 4.1.2. Comparison of the relationship between the large 2004 year class and the following 2005 year class by length in the spawning sample and by age in the May survey in 2009 (upper panel) and 2015 (lower panel). The age-distribution in the lower panel does not match the length distribution observed during spawning and additionally as the relationship between the 2004/2005 year class changed from 5.853 (2009, upper panel) to 1.578 (2015, lower panel). This indicates a transfer from the 2004 year class to the 2005 year class in the age-readings of the older individuals from those year classes in 2015. From Stenevik, Folkvord and Slotte, presentation at the WKNSSAGE.

As a general rule it is seldom that NSS herring have added growth to the edge of the scales in May, which means that the translucent edge should be included in the age determination of individuals caught in May. In otoliths, opaque material is visible at the edge (most clearly seen in fish up to 4 years of age) and thus the winter ring before this outer opaque zone should be included in the age determination of individuals caught in May. NSS herring show very little somatic growth during the first months of the year, starting the growth season from May and continuing through to August. The growth pattern observed in the structures reflects somatic growth, thus when interpreting the structure edge from fish caught prior to the growth season, it should be kept in mind that potentially little growth has happened at this point in the season. There are, however, indications that individuals displaying faster growth will have opaque material on the edge of the otoliths. It is also a possibility that growth may be visible on the edge of otoliths/scales from fish sampled in certain areas and/or in years where food and temperature have been suitable for growth. The workshop focused on this particular issue in order to produce a guideline for interpretation of the edge of either otolith or scale depending on age of the fish and season of capture (see Annex 4).

4.2 Potential differences related to the use of otolith or scale for age determination

The general problems of reduced quality of scales and difficulties of ageing old fish using otoliths were points for discussion at the WKNSSAGE. Scales are often easier to interpret than otoliths for old individuals provided that the scales are in a good state. However, in May it is difficult to get good quality scales from herring samples as they are often 'washed off' during the trawling process. This makes visual identification of the edge even more difficult. If the sampling is done from pelagic trawls with long haul duration, it is almost impossible to get good quality scale samples, thus some laboratories, who only receive samples from trawls will only sample otoliths. This is discussed further in Section 5.

4.3 Recommended future work

The aim of the current analysis was to address the age reading problems associated with the identification of the first winter ring and edge interpretation numerically by utilizing the measurement output from the WebGR annotations. If strict guidelines are given for how the otoliths should be annotated and these are followed methodically then a complete analysis could be applied to a more defined and larger sample set of better quality images as part of the WKARNSSH 2017.

Otolith shape analysis has been used to discriminate between populations for a variety of species and for herring this approach has had increasing success with development of imaging techniques and statistical methods. Environmental differences and geographical separation of populations has shown to give rise to variation in the shape of otoliths for various populations of herring (Bird *et al*., 1986; Turan, 2000; Burke *et al*., 2008; Eggers *et al*., 2014). These variations may suggest differences in the spawning area and environment of populations within a species. Both genetic and environmental influences have been reported as important in determining the shape of the otolith and that different genotypes induce important differences in otolith shape (Cardinale *et al*. 2004). For herring in this area a recent study has demonstrated a 94% correct discrimination between Icelandic summer spawners and NSS herring based on a model of shape differences (Libungan *et al*. 2015). Thus in order to approach the potential mixing of herring stocks in the summer feeding area, analyses of otolith shape should be considered. Such analyses could be supported by use of maturity staging and genetic markers if available.

A study reviewing potential validation methods for age estimation of NSS-herring is recommended e.g. possible radiocarbon analysis to confirm age estimates of archived samples (Campana *et al*., 2002) or identifying known occurrences of increased freshwater input in otoliths of overwintering fish in Norwegian fjords by means of micro chemical analysis.

4.4 Conclusions and recommendations

When addressing the age reading issues alone the common issues exist such as narrowing growth zones, split/false zones and difficulties in distinguishing between opaque and translucent zones seen in old fish. In addition it became apparent during the workshop that there is disagreement between otolith and scale readers on the interpretation of the first winter ring. There are however additional issues related to sampling and stock mixing which are confounding the differences in age distributions between countries.

The recommendations from the workshop are therefore:

WGWIDE needs to address differences in the sampling procedures applied by each country which could possibly account for some of the variation in age structure of NSS-herring.

Mixing of stocks is an issue which can cause confusion during age reading. The maturity data for the fish used in the workshop exercise stated that the fish were all spring spawners but several otoliths appeared to belong to summer spawners. It is therefore important to be aware of stock affiliation when aging so as to avoid mis-representation of the stock when the data are supplied for assessment.

The guidelines outlined in the protocol for interpretation of the edge need to be followed by all laboratories. WKARNSSH 2017 will further develop both the guidelines and protocol by expanding the sample set. The sample set should cover a broader range of age groups and months and include images of better quality scales and otoliths from the same fish.

Clear guidelines are to be given to the age readers on how to annotate the otoliths when exchange calibration exercises are to be completed. This will reduce the variability of the measurement output from WebGR.

If the sampling procedure is likely to result in poor quality scales then otoliths should be collected. For quality assurance all institutes should collect a subsample of both structures from the same fish on each survey. This should be included in the survey manual for all institutions collecting NSS-herring.

5 Analyse the problematic structures (otoliths/scales) from the IESNS-surveys (May-surveys) described by WGWIDE (ToR b)

5.1 Pre-workshop samples

30 otoliths samples from Denmark and 30 scales samples from Norway were annotated by the main reader from the respective country. These were made available on WebGR with the intention that everyone should get an impression of how the structures are read prior to the workshop. The samples consisted of otoliths/scales captured in April/May and November-January.

During the workshop and prior to the calibration exercise these structures with annotations were discussed in plenary.

5.1.1 Otoliths

Issues with direction of the reading were revealed. Generally all readers read in direction of rostrum (Figure 5.1.1.1), the Faroese often read younger fish (up to 6-8 years old) in the direction of post-rostrum. However, it was found the general rule applied was that it should be possible to follow a true winter zone all the way around the otolith. In older fish there might be a difference in ring counts towards rostrum and post-rostrum. Usually the age along rostrum (which is usually the oldest age count) is used as the zones are usually clearest at the tip. Towards post-rostrum the zones often get too narrow to distinguish them from each other.

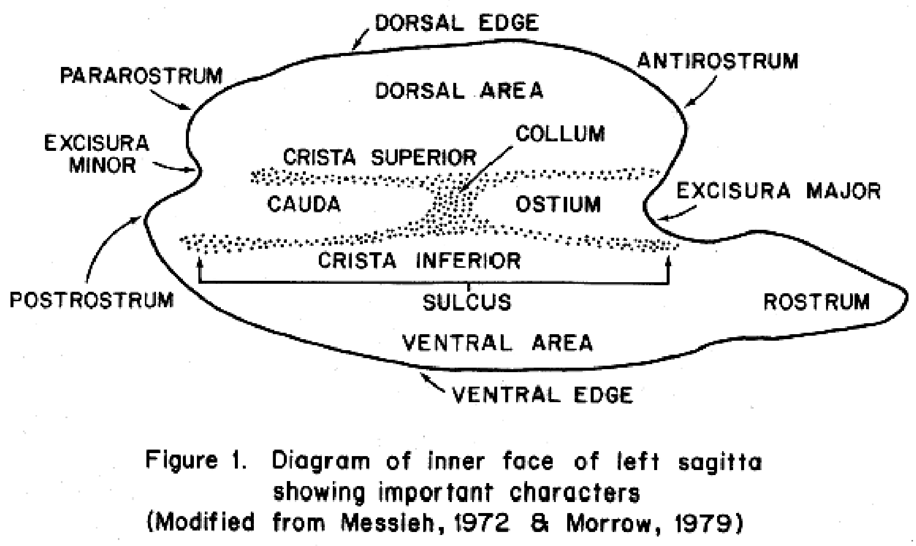

Figure 5.1.1.1 Illustration of a herring sagitta.

In old fish the outermost zone is often difficult to see. The decision whether to count an extra winter ring at the edge depends on the proportion of the last year's summer and winter growth combined, compared to the amount of material which is visible at the edge. If the distance from the beginning of the last visible winter ring to the edge is wide compared with the previous year's full growth then an extra ring is counted at the edge (even if it is not clearly visible) and one adds a year to the age in order to get the correct age.

5.1.2 Scales

The scales were of very good quality and there was little disagreement. In some examples it was a little difficult to see when the edge was an actual winter zone to be counted or when it was a shadow due to the light under the stereomicroscope. Readers explained that at least 3 scales are taken from an individual fish and all are read. This can lead to uncertainty in the final age when the same age is not attained from each scale from an individual fish. Problems can also arise when regenerated scales are used as fewer growth rings will be visible on partly regenerated scales.

5.1.3 Issues regarding both structures

The following important points refer to both the otoliths and the scale readings:

- As the fish increase with age the growth zones become narrower, thus it becomes more and more difficult to distinguish the opaque from translucent zones. This makes it difficult to clearly identify the width of the outermost zone compared with the previous year's full growth and to be able to assign an age with confidence.
- In some institutes there is a tendency to assign a fish to a large year class if the readers are unsure of the age but are aware that one of the possible ages means that it belongs to such a year class. For example if the reader cannot decide if the fish is 10 or 11 years old, and knows that the age class was large for the 11-year olds, then often the fish is aged to be 11.
- Not all countries use a readability scale and thus any uncertainties related to the confidence that the reader has in the actual reading or the quality of the otolith will not be noted anywhere.

5.1.4 Conclusions

There was little disagreement in age reading of the selected otoliths and scales. The main impression was that it is not only the interpretation of the edge which is causing the problems in differing age distributions among countries reading NSS herring. The otoliths and scales annotated prior to the workshop were from different fish and thus a meaningful comparison of methods was not possible. Each structure was discussed individually and the agreement was generally high. In order to get a better impression of the issues noted by WGWIDE a comparison of otoliths and scales from the same individuals were included in a calibration exercises completed at WKNSSAGE the results of which are presented in section 5.2.

5.2 Workshop exercise

During the workshop an exercise was conducted where otoliths and scales from the same 48 fish captured during spring and summer were annotated in WebGR. Given that readers often prefer to age read using the actual structures as opposed to images the structures were available for the readers to study under a stereomicroscope as well. The readers only read the structures they normally read at their institute.

5.2.1 Material and methods

48 otoliths and scales from May and July 2014 collected in ICES area IIa (Figure 5.2.1.1) were used in the exercise (Table 5.2.1.1).

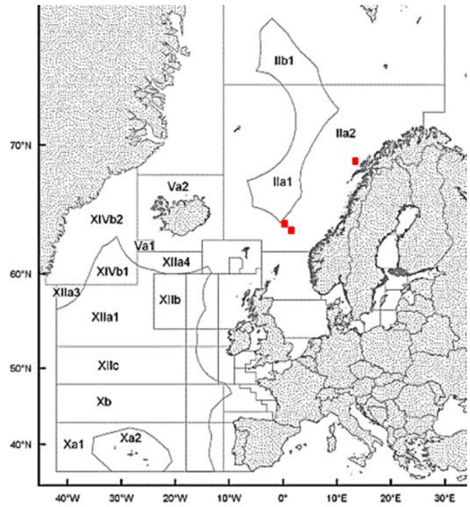

Figure 5.2.1.1 Approximate sample locations of NSS herring for comparison of scale and otolith annual growth structures.

The scales were prepared on glass slides, with approximately 5 scales available from each fish. Photographs of one of the scales were taken at IMR. Images were taken using x0.63 magnification under a reflected light source. The quality of the scales were unfortunately not very good, which is a common problem during spring and summer surveys.

Otoliths were embedded in Entelan in black otolith trays at IMR and sent to DTU-Aqua for photographing. Images were taken using x2.0 magnification under a circular reflected light source.

	May		July
ICES rectangle	55F1	57E9	66G4
Number	18	20	10
Fish length (mm)	270-350	295-360	250-320

Table 5.2.1.1. Overview of structures used for the exercise.

5.2.2 Results

Three readers (1, 3 and 4) were reading the scales; one from Norway and two from Iceland. Four readers (2, 5, 6 and 7) were reading otoliths; one from Denmark and three from the Faroe Islands. The results of each reading were added to an EFAN-sheet (Eltink, 2000) and the ages compared for each structure. Modal age of each structure was then included in an ATAQCS-sheet (Etherton, 2015) and the differences determined.

5.2.2.1 Scales

The scale results showed an overall agreement of 80.6% (ranging between 33% and 100%) with a precision of 5.9% CV (ranging from 0 to 20%). Of the 48 scales, 22 (44.9%) were read with 100% agreement. All age readings and selected figures can be found in Annex 3.

For age readers combined, the relative bias was found to be minimal (0.04), however, for individual age readers the relative bias varied from -0.02 to +0.15. There was no clear tendency among readers towards either over- or underestimation of ages. But individually some trends could be seen; reader 1 and 3 had little over- and underestimation, but reader 4 generally overestimated most ages. This shows that there was some miss-interpretation of growth structures within the scale, though the Wilcoxon inter-reader bias test showed no sign of bias between readers or with modal age.

Comparisons of areas were difficult because of the age difference between areas. In the most northern area (off Lofoten) only modal ages 3–5 were found, while ages up to 11 and 12 were found in the two southern areas (SE of the international EEZ).

5.2.2.2 Otoliths

The percentage agreement with modal age ranged from 50% to 100% with an average percentage of 75.0%. 16 otoliths out of 48 were read with 100% agreement (32.6%). The precision CV ranged from 0% (corresponding to 100% agreement in readings) to 24% with an average of 7.6%. The otoliths read ranged 3–12 years old according to modal age. Their range of variation between the readings was quite large. All age readings and selected figures can be found in Annex 3.

For age readers combined, the relative bias was found to be minimal (-0.00), however, for individual age readers the relative bias varied from -0.33 to +0.17. There was no clear general tendency among readers towards either over- or underestimation of ages. But individually some trends could be seen; reader 2 generally underestimated younger ages and overestimated older ages. Reader 6 overestimated most ages, but underestimated some of the very oldest. Reader 7 underestimated fish over 4 years of age. This shows that there is a general mis-interpretation of growth structures within the otolith – both between and within institutes. The Wilcoxon inter-reader bias test showed possibilities of bias or no sign of bias between readers 2, 5 and 6 and with modal age, but reader 7 showed possible or certain signs of bias between the other readers and modal age.

Comparisons of areas were difficult because of the age difference between areas. In the most northern area only modal ages 2–5 were found, while ages up to 11 and 12 were found in the two southern areas.

5.2.2.3 Combined

The agreement between modal age of the two structures was as low as 52%, only 25 of 48 herring got the same age. When studying the comparison matrix (Figure 5.2.2.3.1 and 5.2.2.3.2) it is clear that the otolith age often is at least one year lower than the scale age. In a number of cases the ages estimated differ by more than one year. Among older fish this is because of a general difficulty in interpretation of the zones, but among younger fish it seems rather unlikely that this would be the cause of a two/three years difference in the estimated age. This could possibly be due to some errors when collecting the structures. All age readings and selected figures can be found in Annex 3.

Annotations of both scale and otolith were discussed in plenary, and one reoccurring issue was the count of an inner ring in scales which in otoliths was not always visible. In only a few situations, the scale readers would say that after seeing the otoliths and scales next to each other, they would not have included the inner ring in the count of age but would choose to ignore it and not include it in the estimation of age. In these situations the distance from the nucleus to the first winter ring in of the otolith was often rather large, and some otolith readers would claim it was a summer spawner and not a spring spawner. However, the stadium specification made from looking at the gonads at the survey said it was a spring spawner.

		Otolith														
	Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Scale	0															
	1															
	2															
	3				7											
	4			1	1	1										
	5					2	9									
	6						5	1								
	7						2		1							
	8						1	1		1	1					
	9									2	2	1				
	10									4		2		1		
	11												1			
	12												1			
	13															
	14															

Figure 5.2.2.3.1. Matrix comparing modal age readings of otoliths and scales (derived from ATAQCS sheet by Cefas).

	Otolith														
Scale Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0															
1															
2															
3				1.000											
4			0.333	0.333	0.333										
5					0.182	0.818									
6						0.833	0.167								
7						0.667		0.333							
8						0.250	0.250		0.250	0.250					
9									0.400	0.400	0.200				
10									0.571		0.286		0.143		
11												1.000			
12												1.000			
13															
14															

Figure 5.2.2.3.2. Error matrix of modal age readings of otoliths and scales (derived from ATAQCS sheet by Cefas).

5.2.3 Conclusions and evaluation of the exercise

There was some disagreement in aging among age readers of both the scales and otoliths. An agreement of 75% among otolith readers and 81% among scale readers is not satisfactory. However, few people participated in this small workshop exercise, and the "within structure" differences will not be discussed further here. This subject will be analysed thoroughly during the upcoming large exchange prior to WKARNSSH 2017.

Comparison of age readings of otolith vs. scale showed rather poor results. An agreement of 52% is very low and much time was spent during the workshop comparing the annotations of the two structures in order to find the problems. It was clear that otoliths were often aged to be younger than scales. It would appear that this may be a combination of both a difference in the interpretation of the first winter (which may be more visible in the scales) and difficulties in the interpretation of the edge in the older fish. Measurements of the zones were extracted from WebGR and analysed in order to see if this gives a solution. This was, however, not possible (see section 6 for details).

6 Clarify the interpretation of annual rings in particular during spring/summer (ToR c)

6.1 Introduction

In general, age reading errors may emerge due to a number of different causes with two different characteristics - precision and accuracy - the age readings may then score in two dimensions how reproducible and how close to the true age they are. Age may thus be highly reproducible but wrong (biased but with high precision) or poorly reproducible but on average accurate (unbiased but with low precision) or both inaccurate and imprecise.

Estimation of bias essentially needs known age material, but indications may be derived indirectly from deviations from expected modal progression of age distributions over a number of years. This type of deviation is exactly what is observed in the comparison of Norwegian and Faroe readings from 2015 (Figure 4.1.1) where there is correspondence in the peak of 6 year old herring but a shift from the Norwegian peak of 11 year olds to Faroe peak of 10 year olds. It would have been optimal with a more systematic comparison between the age distributions from the different laboratories over a number of years to identify at what age the deviation likely start to develop. Instead the overall age stratified abundance estimate per year over all survey strata was plotted in Figure 6.1.1. Here the domineering 2004 and 2009 year classes are clear, without any other strong year class signals.

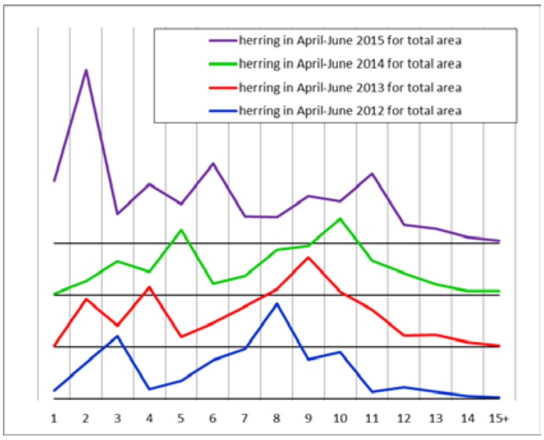

Figure 6.1.1. Age stratified abundance estimates of Norwegian spring-spawning herring in April-June 2012-2015 for total area from WGIPS and IESNS reports

Precision is more easily addressed by the lack of reproducibility within or among readers and is typically what is in focus during reading "calibration" exercises. To avoid confounding errors when comparing different samples as in the May survey, structures (scales and/or otoliths) from the same fish should be read by the readers to be compared.

Age reading is a holistic discipline where many factors are considered more or less consciously, and the protocols should reflect that. However to disentangle reading errors, different levels of information could be available for the readers, from full information of the individual fish as well sampling time and location to double blind selection and reading.

In both otoliths and scales age is interpreted as the number of reoccurring annually formed growth structures, in herring they are most easily distinguished as the number of discernible winter rings. In many age reading calibration exercises growth structures are electronically annotated on images and discussed in the group to identify problematic interpretations. To add further to this process and fully explore diversities of interpretation in the group a numerical analysis of the annotations was attempted for the present workshop. Age estimation protocols are different between the different laboratories. Further, the protocol for annotated readings did not compare to any of the standard routine protocols applied in the laboratories. Caution should therefore be taken when interpreting the results of the present analysis of the workshop age readings.

6.2 Methods

Age readings were comparable between all seven readers for 49 specimens with three scale readings and four otolith readings on each. When plotted vs. modal age over all readings a general impression is that scale readings overestimated modal age for young ages with high variability especially on age 5, whereas otolith readings underestimated modal age for old ages with high variability especially at age 9 and 10 (Figure 6.2.1). A deviation of more than one year from modal age would indicate problems at more than one location like the edge or the first winter ring in a growth structure. 9.4% of all readings deviated more than one year from modal age.

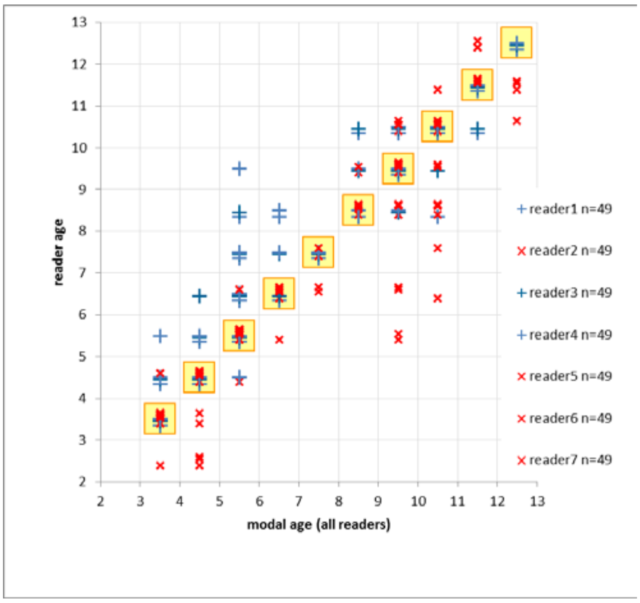

Figure 6.2.1. All age reading plotted against modal age. Blue crosses represent the scale readings and red crosses represent the otolith readings.

These readings were then compared with the measurements between the reader's annotated positions for each successive annual growth mark.

Correct measurements from WebGR are dependent a systematic annotation of successive growth structures from the centre to the edge. Unfortunately no x-y coordinates are registered in WebGR to identify and correct erroneous sequences of annotations. The sum of increments from the centre to the edge should reflect the radial distance in direction of annotation. Deviating sums of increments were identified and checked for the sequence of annotations, and in case of incorrect numbering of the growth structures the reading was dismissed from numerical analysis.

After screening for incorrect annotation and dismissing the corresponding measurements, the structure radius from the centre to the edge was analysed vs. herring total length (Figure 6.2.2). The selected scales exhibited low correlation with total length (R^2=0.59) whereas otoliths were more closely correlated to total length (R^2=0.74), however the measurement unit had to be multiplied with a factor of ca. 3.5 to be converted to mm.

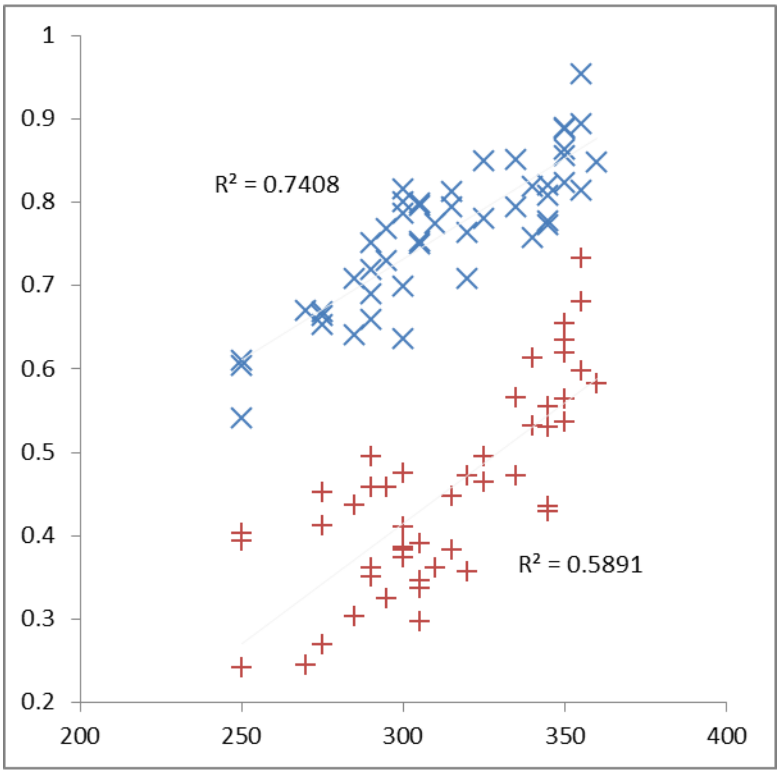

Figure 6.2.2. Shows the correlations between herring total length and otolith radius length (blue crosses) and herring total length and scale radius length (red crosses).

This implied that measurements of scales growth in a number of analyses would have to be carried out relative to the individual maximum scale radius, adjusted for fish size.

The annotation of the otolith and the scale centre exhibited more variability than the annotation of the edge. Therefore to avoid added noise from annotation of the centre position, the first year's growth from the centre to the first winter ring in the rostrum/mid-scale (C<->R_1) was calculated as the mean (over readers) of the centre to the rostrum/mid-scale edge (C<->RE) minus the individual reader's measurement of the distance from the first winter ring (R_1) to the rostrum/mid-scale edge (R_1<->RE).

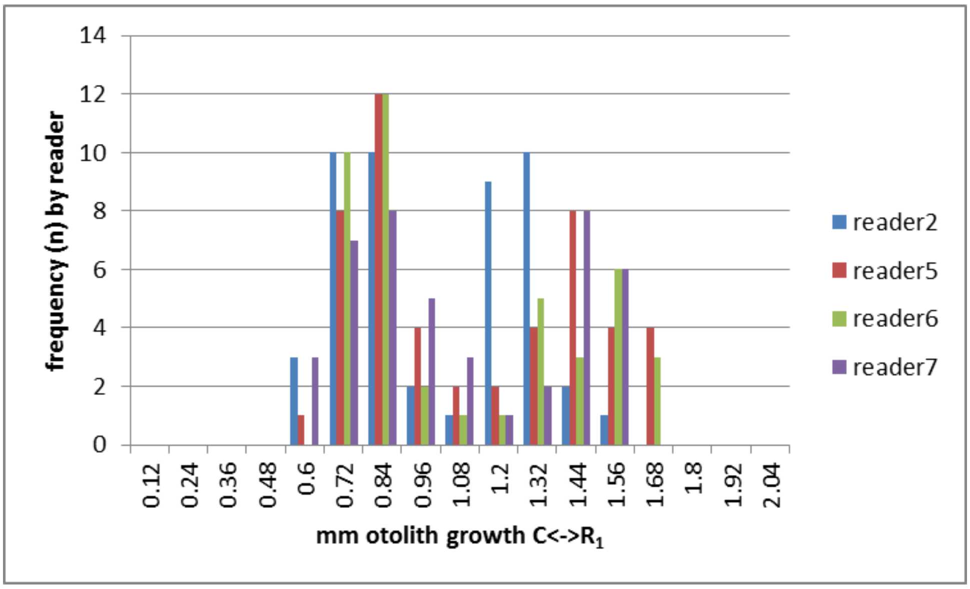

Figure 6.2.3. Frequency plot of the mean distance from the centre to the first winter ring observed in otoliths.

The first year otolith growth exhibited a clear bimodal distribution (see fig 6.2.3). Since this could be interpreted as an undetected winter ring for the otoliths with a large C<->R_1 this was scrutinised further by the available data as well as by including other information. First year scale growth exhibited a uni-modal right skewed distribution (see fig 6.2.4).

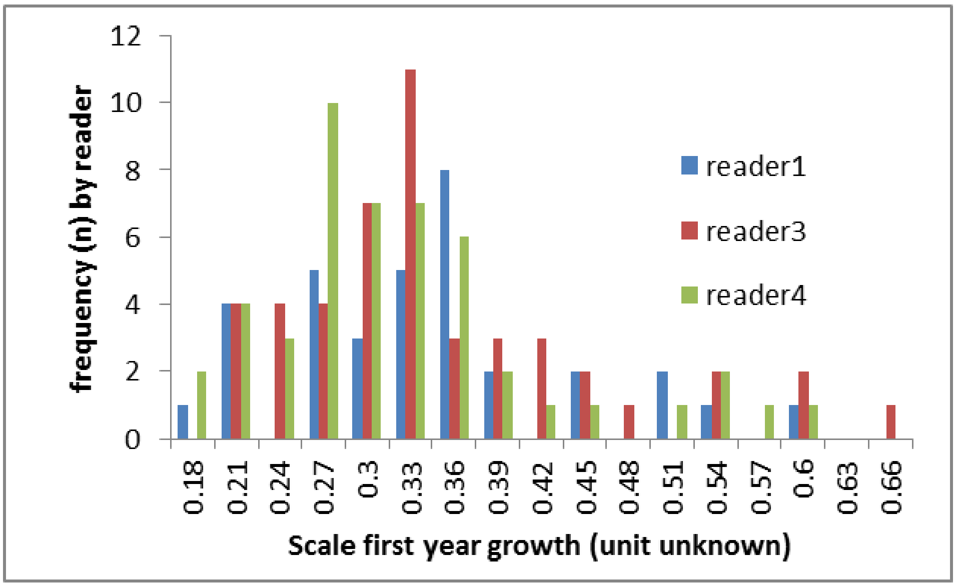

Figure 6.2.4. Frequency plot of the mean distance from the centre to the first winter ring observed in scales.

Scale first year growth was then compared to the low and high mode otolith first year growth (Figure 6.2.5).

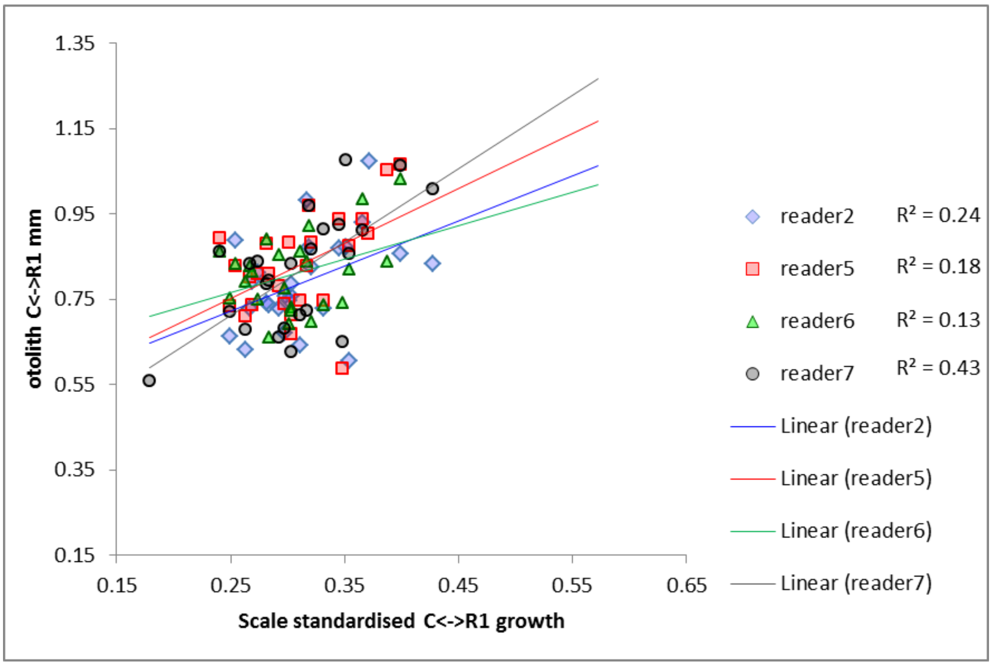

Figure 6.2.5. The correlation between the low mode of the otolith first year growth and scale first year growth.

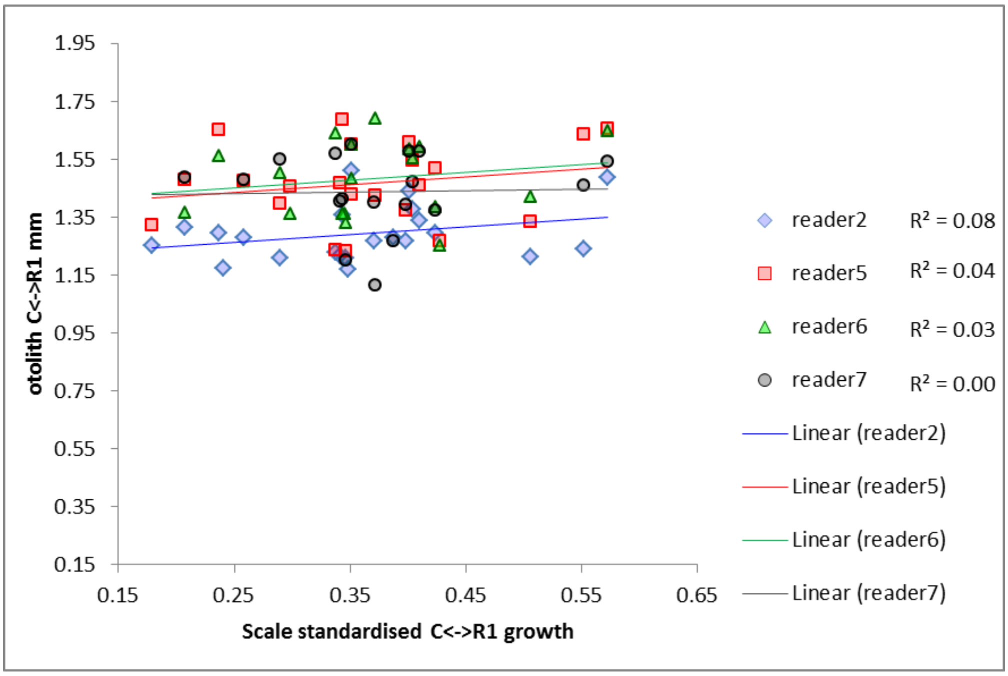

Figure 6.2.6. The correlation between the high mode of the otolith first year growth and scale first year growth.

The correlation plots seen in Figures 6.2.5 and 6.2.6 show that there is a stronger relationship between the low mode of the otolith first year growth and the first year growth observed in the scales compared to that seen with the high mode of the otolith first year growth and the first year growth observed in the scales. During the plenary discussion of the scales and otoliths used during the workshop, concerns were raised over what the scale readers were observing to be the first winter ring in examples where the distance from the nucleus to the first winter ring in the otolith was rather large. As mentioned in section 5.2.2.3 it was discussed whether a not so well defined scale winter ring close to the centre should be counted or not. The comparisons above shows that it is possible that the innermost ring seen in the scales could in fact be a true winter ring. The variability observed in the growth patterns of the otoliths could indicate the presence of subpopulations and/or stock mixing. It is known that stock mixing occurs in these areas and during the May survey it is difficult to distinguish between these stocks using maturity staging and scale readings. Otoliths appear to provide more information and could possibly be used to distinguish between stocks and subpopulations. It is possible that otoliths with a very opaque centre and/or a large distance to the first clearly visible winter ring belong to another stock (North Sea herring or summer spawners). Otoliths from these fish are aged differently than NSS-herring (see section 7.2.4.2). This could explain why the ages obtained from the otoliths are often one year less than those obtained from the scales. What was also observed was a consistent pattern of higher growth rate in the herring with smallest first winter ring with the initially smallest otoliths turning out be to the largest at the oldest ages.

The width of the edge after the formation of last winter ring and the width of the two last increments were studied to identify potential misinterpretations of otoliths as well as scales. Scrutinising annotations of reader 6 revealed that the otolith edge was not annotated, and subsequently analysis of edge interpretation by reader 6 was left out.

In general there was reasonable correlation between growth of successive increments in both structures (Figure 6.2.7). Further the slope of the edge width vs. last annual increment (1vs.2) was lower than the slope of the last annual increment vs. the second last (2vs.3) indicating that growth of the edge was interpreted as less than a full year's growth. As the annotation of the last winter rings is apparently difficult and the narrow zones in old fish however could not be distinguished in this analysis as seen from the large variation in ratio of width between successive zones in the older fish.

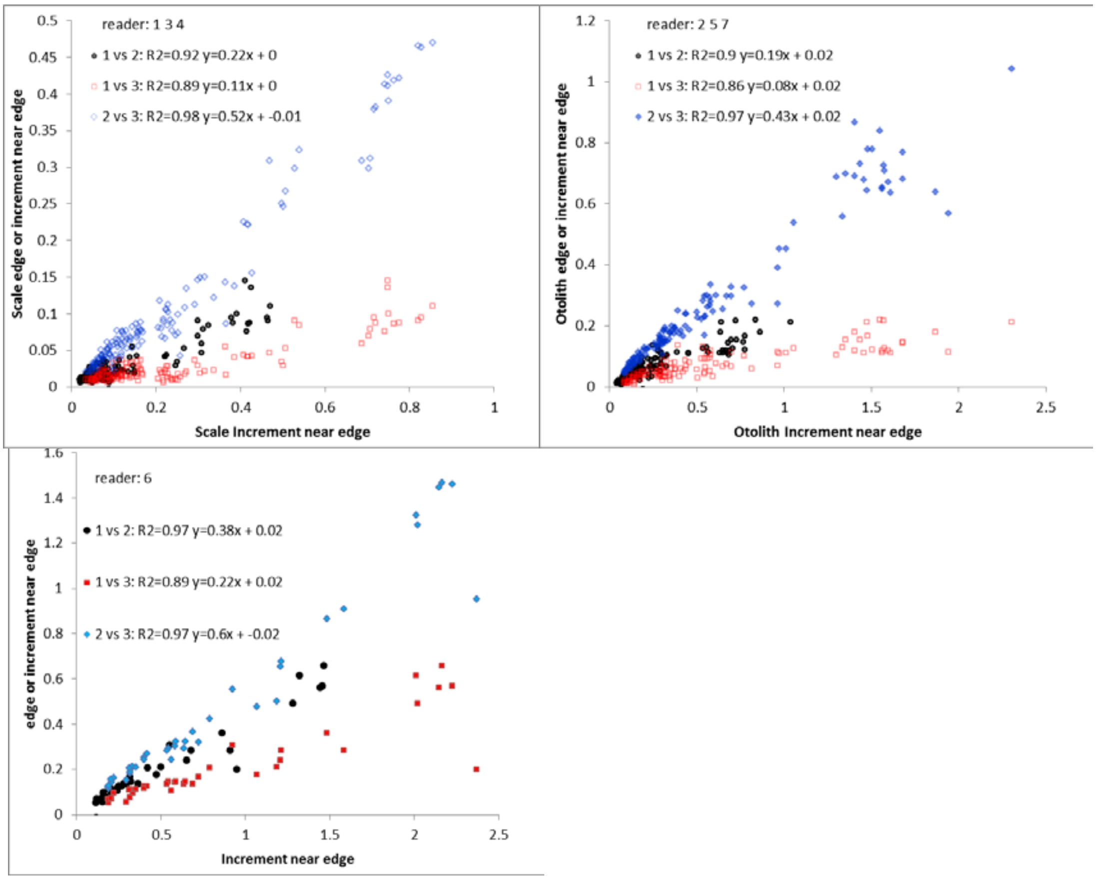

Figure 6.2.7. Correlation between growth of successive increments. Measurements of the readers are combined.

6.3 Conclusions and recommendations

The otolith analysis shows there to be a bi-modal distribution in the distance from the centre to the first winter ring. This indicates that the otoliths used for the exchange display different growth patterns, which are most likely due to mixing of stocks and/or subpopulations with varying growth patterns, which was not taken into account during the reading process.

A rather large proportion of the age readings deviations (9.4%, see Figure 6.2.1) from the modal age are greater than one year, indicating that more than one reading issue is leading to the error. A repeated analysis of growth zone measurements from a larger collection of otoliths and scales from fish taken throughout the year should be conducted. This analysis should be carried out with strict annotation instructions to the readers, to make sure the measurements are comparable.

7 Improve the protocol for age estimation of the applied structure (otolith or scale) (ToR d)

7.1 Introduction

Prior to the workshop no agreed protocol existed for NSS herring age estimation. Having the primary readers for this stock at a workshop allowed for the development of a protocol which combines the methods used when determining ages based on both scales and otoliths. This agreed protocol can be applied in the future and aid the standardization of growth zone interpretation among institutes.

On the first day of the Workshop much time was spent looking through the WebGR example exercises of annotated otoliths and scales in plenary. It was not possible to use the scales from the calibration exercise as the quality was poor and there were few otoliths available from young fish captured in the winter. Every effort was made to include samples from as many age classes and times of the year as possible and only good quality images were used. The otolith samples were from December 2014 and May 2015. The scale samples were from November 2014, January 2015 and April 2015. The reason for the exercise was twofold; first, for the readers who are not familiar with one type of structure to see how the structure is interpreted and secondly, to look at the progression of the edge appearance throughout the year on both the scales and the otoliths.

A library of images was compiled (see Annex 4) to illustrate the progression of the edge appearance throughout the year in both otoliths and scales. The table below is based on these images and outlines the "Edge type" throughout the year and indicates when the outermost translucent zone should be included in the count of age. There will be cases where the distance from the start of the final visible winter ring to the edge is so wide that there must be another winter zone hidden at the edge, and should be counted although it is not visibly clear. See images from the IESNS May 2015 ST.50 sample numbers 55, 57 and 59 (Annex 4). This can occur from approximately 7/8 years of age and onwards.

Table 7.1.1. Overview of development of the edge over the year. The tables have been divided in younger and older fish, as the edge type varies with age.

Otoliths			
Month	Age	Edge type	Is the outermost translucent zone included in the count of age?
May	3	Narrow opaque zone	Yes
May	6	Narrow opaque zone	Yes
May	7	Narrow opaque zone	Yes
December	3	Broad translucent zone	No
December	5	Broad translucent zone	No
May	9	Narrow opaque zone	Yes
May	10	Narrow opaque zone	Yes
May	11	Narrow opaque zone	Yes
December	8	Narrow translucent zone	No
December	9	Narrow translucent zone	No

SCALES			
Month	Age	Edge type	Is the outermost translucent zone included in the count of age?
January	5	Broad translucent zone	Yes
April	6	Broad translucent zone	Yes
November	8	Narrow translucent zone	No
January	9	Narrow translucent zone	Yes
January	11	Narrow translucent zone	Yes
April	9	Narrow translucent zone	Yes
April	10	Narrow translucent zone	Yes
April	11	Narrow translucent zone	Yes
April	13	Narrow translucent zone	Yes
November	10	Narrow translucent zone	No

7.2 Preparation and aging methods

7.2.1 Preparation

Otoliths:

Generally otoliths should be clean from blood and tissue. They should be in one piece and not broken. Crystallized otoliths should not be used. When you read it is important that the otolith lies with sulcus down.

The Faroe Islands and Norway: The otoliths are embedded in Entellan/Histokit in black plastic trays numbered from 1-25. The otoliths have to be completely dry before they are embedded, and each tray hole needs to be completely filled. The otoliths are kept in the plastic tray for storage.

Denmark: The otoliths are placed in black plastic trays numbered from 1-25. For storage the otoliths are placed in cardboard pre-holed sheets and then laminated.

Scales:

Generally scales should be cleaned from blood, tissue and the fat membrane. It's very important that the scale comes from the specific herring. Before you take the scales you have to wipe the herring with the knife in order to remove scales from other herring. It's important to have "good" scales and several to choose from. The "good" scales are located in the area a bit behind the gills and towards the dorsal fin along the sideline.

Norway: At least three scales from each herring are put onto a glass slide. The slides are prepared with gelatin solution. It is important that the scales are laid on the glass convex side up to prevent the scales from falling off and to ensure that there are no air bubbles under the scales.

The scales are stored on the slides packed in the slide boxes.

Iceland: At least three scales from each herring are prepared and put onto a glass slide. It is important that the scales are placed on the glass with the convex side up to prevent the scales from falling off and to ensure that there are no air bubbles under the scales when they are put on the glass. To read the scales transmitted light is used, and the scales are usually read at 1.0 and up to x1.6 magnification.

The scales are stored on the slides packed in the slide boxes.

7.2.2 Viewing of structures and images

Otoliths

Denmark: Otoliths are read in ethanol, on a black background, using reflected light and usually read at 2.5x magnification.

Faroes: For the reading it's very important that the holes are completely filled up with Entellan/Histokit. Reflected light is used when reading the otolith and they are usually read at x1.6 magnification.

Scales

Norway: To read the scales transmitted light is used and the scales are usually read at x1.6 magnification. The distances from the center to each winter zone and the total length from the center to the edge are measured.

Iceland: Usually start at lower magnification, and then gradually increase as reaching the outermost rings.

Otoliths and scales are read at a range of magnifications from x1 to x2.5.

7.2.2.1 Images

The use of images as an alternative to the actual structures is practical in the sense that the interpretation of the zones can be preserved, measured, analysed and discussed afterwards. However, it is important to note that images do not always hold all the information and details that the real structures do. Especially in older individuals or when the structures are of poor quality, it is an advantage – and sometimes a necessity – to have the actual structure under a stereomicroscope with the possibility to zoom in and out and change light to what suits the reader best.

7.2.3 Age determination criteria

1) The date of birth is assumed to be 1st January and the fish is assigned to a year class on this basis. Therefore, the date of capture of the sample should always be available.

2) One opaque zone and one translucent (hyaline) zone constitutes one year of growth (annulus).

3) The timing of the formation of the opaque zone on the edge of the otolith depends on the age of fish. Young fish (< age 4) may form an opaque zone earlier in spring than older fish.

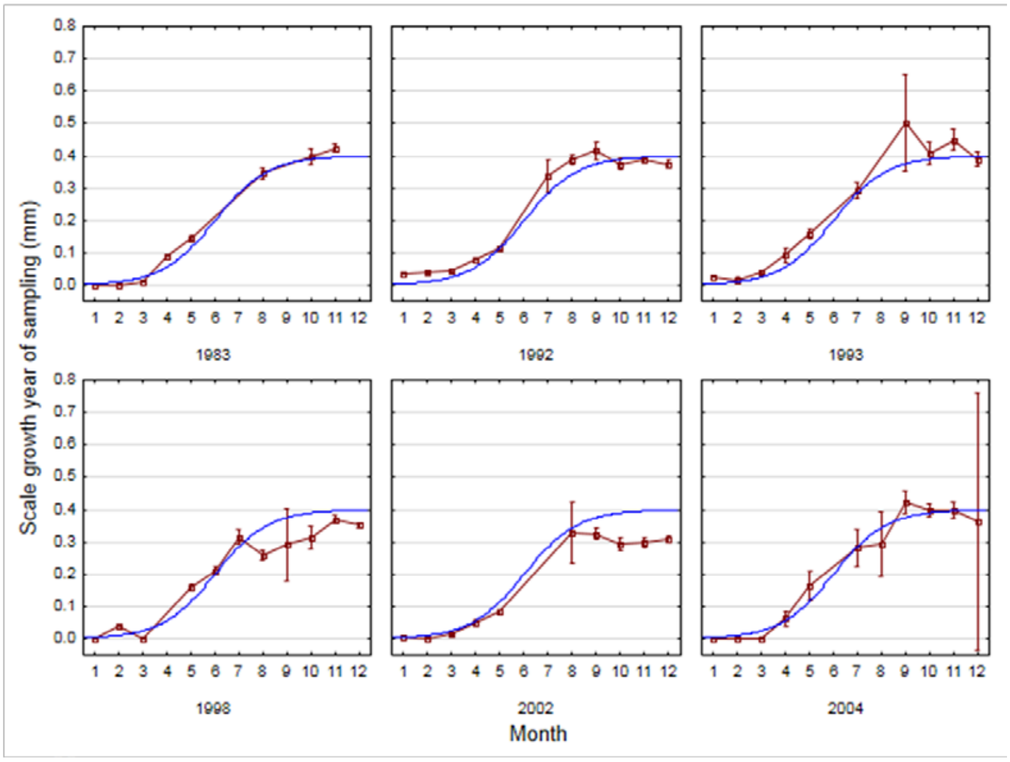

Figure 7.2.3.1. Scale growth of 5 year old NSS herring from the 6 largest year classes after the recovery of the herring stock. The red line represents the monthly cumulative distribution of scale growth (five year old fish). Error bars are confidence intervals. There is a large variation in some months due to low n. The blue line is a reference logistic curve with y= 0.4/(1+exp(-0.9*(x-6))). From Stenevik, Folkvord and Slotte, presentation at the WKNSSAGE.

4) In general, for a zone to be a "true" growth zone it should be continuous around the otolith or scale so that it is possible to "follow the ring" in more than one part of the structure.

5) As a guideline, exponentially decreasing ring-widths should be seen from the centre towards the edge. It is important always to be aware of the distances between the rings. Usually when the distance from the centre to the first growth zone is narrow then you see fast growth in the subsequent years and vice versa.

6) Normally, for herring captured from January 1st until the formation of a narrow opaque band (including herring caught in May), all hyaline (winter) rings and the hyaline edge laid down in the previous winter are always counted, even if no hyaline edge or very little is visible. However, sometimes in young fish, the new hyaline growth zone may not be counted as it depends on how much opaque growth is present (the outermost narrow hyaline zone may not be a real winter zone). The decision to include the edge or not should be based on time of year, but is also affected by age of fish. Based on Figure 7.2.3.1, for scales from fish caught from 1st January to September the reader should count all translucent (hyaline) rings. For structures from late September to the end of December the reader should assume that the last hyaline ring is not fully formed and is from the in-year winter and therefore not count it.

7) It is a recommendation of this workshop that readers register the confidence level they have in their age readings based on the following 3 point readability scale proposed by WKNARC (ICES 2011) and thus, reflecting the quality of the data.

AQ1: Easy to age with high precision.

If a scale of 1-100 is applied, where 100 is when the reader has the highest possible confidence in the age reading and 1 is when the reader has no confidence in the age reading, age quality 1 (AQ1), will apply to approximately the top 25 % of the possible quality ratings. AQ1 is an indication that the age data are considered reliable for stock assessment.

AQ2: Difficult to age with acceptable precision.

Age quality 2 (AQ2), will apply approximately to age readings within 25 and 75 percentiles of the possible quality ratings. AQ2 is an indication that the age data are sufficiently reliable to be used for stock assessment purposes but improvement is required.

AQ3: Unreadable or very difficult to age with acceptable precision.

Age quality 3 (AQ3), will apply to approximately the lowest 25 % of the possible quality ratings. 3 AQ3 is an indication that there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs.

7.2.3.1 Otolith specific criteria

Having both otoliths is preferable as it is possible that the growth zones may be clearer on one of the two sagitae.

It should be possible to follow the growth zones around the otoliths; this is especially true with otoliths from younger fish. For older fish, it should be possible to follow the growth zones in more than one area of the otolith.

As the fish grow older the amount they grow on a yearly basis decreases and this can be seen in the otoliths when the distance between increments decreases towards the edge of the otolith. This needs to be taken into consideration when interpreting the edge. When deciding whether to include the outermost winter zone or not the width of the outmost winter zone should be compared to the width of the last year’s winter and summer growth combined. In older individuals you would count the edge as a winter zone from January until May and in some cases winter growth may be visible at the edge up until September.

Annotations for zone measurement should be made consecutively from the centre towards the edge and in the direction of rostrum. This is where the growth zones are most visible in the otoliths of the older fish. Conflicting ages may be achieved if several parts of the otoliths are examined (usually in older fish). Looking at the continuity of the width of the zones may help determining whether a zone is real or false.

7.2.3.2 Scale specific criteria

Good quality scales are important for correct age determination. Therefore several scales should be sampled from behind gills. Confounding elements are: scales from suboptimal areas of the skin, scales from a different fish, and regenerated scales.

In good quality scales age is usually easy to interpret, however, in trawl catches it may be difficult to get good scales. This makes aging rather difficult especially in old herring.

Another reason for collecting several scales is that sometimes scales from other individuals are sticking to a fish, and if only one scale is collected, it may be from another fish. Cleaning of a fish before collection is therefore of very high importance.

Furthermore, regeneration of scales can happen and it is important that the scale used for age reading is as old as the fish.

Annotations should be done along the axis perpendicular to the attachment point. In some cases the winter zones are very indistinct and thin and the scale has to be studied very carefully to detect them.

It is important that the base of the stereomicroscope used for interpreting scales has good possibilities to adjust the lighting and shading of the scales using transmitted light.

7.2.3.3 False or Split Rings

False rings are seen both in scales and otoliths. False or split rings are usually considered to be those rings that are not as well defined as annual rings. The reason for the deposition of false or split rings is not certain, but they might be caused by aberrant temperature, feeding or spawning conditions, stress or disease.

False rings did not seem to be much of an issue during the workshop, but it is important to be aware of them when aging herring.

7.2.4 Factors affecting annual ring formation

7.2.4.1 Reduced growth in old fish

In young fish the growth pattern is well defined as the zones are broad and often easy to count. Also the edge is clear and it is easy to see during all seasons what should be counted as a zone. Following the onset of maturity the annual rings become narrower. In older individuals (>7 years old) the zones become very narrow and it may be difficult to distinguish opaque from translucent zones, especially at otolith post-rostrum but also towards the rostrum, where the calcium carbonate may be too thick for zone identification.

7.2.4.2 Mix of populations

It is possible that among the NSS herring samples, otoliths which look like North Sea autumn spawners may be found, these have a hyaline centre which should not be counted as a ring and thus the first winter ring will represent 1½ years of age. Therefore the real age is determined as count of winter rings +1. NSS herring are not expected to spawn in summer or autumn.

Otoliths of herring spawned in summer or autumn have a hyaline centre. This centre should not be counted as a ring. NSS-herring are not expected to spawn in summer or autumn.

It is also possible that Icelandic summer spawners may be found among the NSS herring samples. These can be identified by a clearly dark centre and long distance to the first winter ring compared to what is found in NSS herring.

It is also likely that NSS herring mix with the local populations of herring in the fjords along the Norwegian coast during spawning migrations (e.g. Johannessen *et al*. 2009). The zone pattern observed may be different in the fjord populations compared to the open ocean and also based on latitudinal differences. Therefore, one has to be aware of a possible variation in zone size or time of formation when aging these fish.

It appears that the differentiation in stocks is only visible in the otoliths. Thus reinforcing the need to sample both structures.

7.2.4.3 Formation of the first winter ring

The NSS herring spawn mainly from February-March. Therefore life starts with a feeding season. This is seen in the otoliths as an opaque centre. The distance from the centre to the beginning of the first winter ring is generally narrower for juvenile herring in the Barents Sea than for juvenile fish in fjords further south. For some of the otoliths discussed at the workshop, the distance to the first visible winter ring was unreasonably wide for being the first winter ring. No exact measure was decided upon at the workshop for when an extra year should be added as first annulus. This did not appear to be the same problem with the scales. The first winter ring in the scales from juvenile herring in the Barents Sea is quite sharp (like the edge of a knife), which is characteristic for the Northern type. The first winter ring in the scales from juvenile herring in fjords farther south is usually more diffuse, which is characteristic for the Southern type.

7.2.4.4 Age at maturity

The zones of younger fish are wide, but after sexual maturation somatic growth ceases, which is also seen in the age-reading structures as narrow zones after sexual maturation. At the workshop, the attendants did not work with the exact age span when NSS-herring reaches sexual maturity.

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Annex 1: Agenda

Monday 9th		
09:00 – 09:30	Welcome and ToRs	Jane Godiksen
09:30 – 10:30	Presentation of issues regarding age readings	Erling Kåre Stenevik
10.30 – 11.00	Discussion in plenary	Plenary
11:00 – 11:30	Presentation of edge development in scales of Norwegian samples over a year	Bjørn Vidar Svendsen
11:30 – 12:00	Presentation of edge development in otoliths	Henrik Mosegaard
12:00 – 13:00	Lunch	
13:00 – 14:30	Discussion of the prepared otoliths and scales using WebGR	Plenary
14:30 – 15:00	Break	
15:00 – 17:00	Look at and discuss the growth of the otoliths and scales brought by everyone	Plenary
Tuesday 10th		
09:00 – 12:00	Calibration exercise of summer samples of otoliths and scales	*Individually*
12:00 – 13:00	Lunch	
13:00 – 14:45	Conclusion on age reading?	*Plenary*
14:45 – 15:15	Break	
15:15 – 16:00	Construct a “Translation key” between otoliths and scales	*Plenary*
16:00 – 16:30	Going trough the draft report	*Plenary*
16:30 – 17:00	Sum up and goodbye	

Annex 2: List of participants

Name	Country	e-mail	Expertise	Assessment	Structure
Jane A Godiksen (chair)	Norway	jane.godiksen@imr.no	Coordinator		
Julie Coad Davies	Denmark	joco@aqua.dtu.dk	Age-reading coordinator		
Jostein Røttingen	Norway	jostein@imr.no	Expert	X	Scale
Stina Bjørk Stenersen Hansen	Denmark	sb@aqua.dtu.dk	Expert	X	Otolith
Guðrún Finnbogadóttir	Iceland	gunna@hafro.is	Intermediate		Scale
Ragnhildur Ólafsdóttir	Iceland	raddy@hafro.is	Expert	X	Scale
Poul Vestergaard	Faroe Islands	poulv@hav.fo	Expert	X	Otolith
Jens Arni Thomassen	Faroe Islands	jensarni@hav.fo	Expert	X	Otolith
Eydna í Homrum	Faroe Islands	eydnap@hav.fo	Intermediate		Otolith
Lotte W Clausen	Denmark	law@aqua.dtu.dk	Scientist		
Henrik Mosegaard	Denmark	hm@aqua.dtu.dk	Scientist		
Erling Kåre Stenevik	Norway	erlings@imr.no	Stock coordinator		

Annex 3: Results of the workshop exercise

Otolith exercise

Table 1 **NSS-herring otolith exercise during workshop**

Stratum	year	Sample no	Fish no	Fish length	Sex	Landing month	Stina Reader 2	Eydna Reader 5	Poul Reader 6	Jens Arni Reader 7	RANGE MODAL (R1-R5)	Prosent enighet	Presisjon CV
55F1	2014	Sild__23102_02	1	300	female	5	5	5	6	5	5	75 %	10 %
55F1	2014	Sild__23102_04	2	300	female	5	9	8	8	7	8	50 %	10 %
55F1	2014	Sild__23102_06	3	345	female	5	11	11	11	10	11	75 %	5 %
55F1	2014	Sild__23102_08	4	290	male	5	4	5	6	5	5	50 %	16 %
55F1	2014	Sild__23102_09	5	270	male	5	3	3	4	3	3	75 %	15 %
55F1	2014	Sild__23102_11	6	325	female	5	9	9	9	8	9	75 %	6 %
55F1	2014	Sild__23102_12	7	300	female	5	5	5	5	5	5	100 %	0 %
55F1	2014	Sild__23102_13	8	310	male	5	5	5	7	4	5	50 %	24 %
55F1	2014	Sild__23102_14	9	335	female	5	8	9	7	8	8	50 %	10 %
55F1	2014	Sild__23102_15	10	275	female	5	2	3	3	3	3	75 %	18 %
55F1	2014	Sild__23102_20	11	305	female	5	5	5	6	5	5	75 %	10 %
55F1	2014	Sild__23102_23	12	305	female	5	5	5	6	5	5	75 %	10 %
55F1	2014	Sild__23102_24	13	290	female	5	4	4	5	4	4	75 %	12 %
55F1	2014	Sild__23102_25	14	285	female	5	5	5	5	4	5	75 %	11 %
55F1	2014	Sild__23102_27	15	350	male	5	9	10	9	8	9	50 %	9 %
55F1	2014	Sild__23102_28	16	300	female	5	4	4	4	4	4	100 %	0 %
55F1	2014	Sild__23102_29	17	295	male	5	5	5	5	5	5	100 %	0 %
55F1	2014	Sild__23102_30	18	305	female	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_02	19	320	female	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_03	20	355	female	5	8	9	9	8	8	50 %	7 %
57E9	2014	Sild__23103_06	21	350	female	5	12	11	11	11	11	75 %	4 %
57E9	2014	Sild__23103_07	22	360	female	5	10	10	9	8	10	50 %	10 %
57E9	2014	Sild__23103_08	23	355	male	5	10	10	8	8	10	50 %	13 %
57E9	2014	Sild__23103_11	24	335	female	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_12	25	345	female	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_13	26	325	male	5	6	9	8	8	8	50 %	16 %
57E9	2014	Sild__23103_14	27	340	male	5	6	6	6	6	6	100 %	0 %
57E9	2014	Sild__23103_15	28	350	female	5	10	10	10	8	10	75 %	11 %
57E9	2014	Sild__23103_16	29	340	female	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_18	30	350	male	5	7	6	7	6	7	50 %	9 %
57E9	2014	Sild__23103_19	31	295	female	5	5	6	6	6	6	75 %	9 %
57E9	2014	Sild__23103_20	32	345	female	5	5	5	5	4	5	75 %	11 %
57E9	2014	Sild__23103_21	33	355	male	5	8	9	9	8	8	50 %	7 %
57E9	2014	Sild__23103_22	34	350	male	5	12	12	11	11	12	50 %	5 %
57E9	2014	Sild__23103_25	35	350	female	5	9	10	9	8	9	50 %	9 %
57E9	2014	Sild__23103_27	37	315	male	5	11	10	8	8	8	50 %	16 %
57E9	2014	Sild__23103_28	38	305	male	5	5	5	5	5	5	100 %	0 %
57E9	2014	Sild__23103_30	39	345	female	5	8	10	9	8	8	50 %	11 %
66G4	2014	Sild__37546_01	40	300	female	7	3	4	4	4	4	75 %	13 %
66G4	2014	Sild__37546_04	41	290	female	7	3	3	4	3	3	75 %	15 %
66G4	2014	Sild__37546_05	42	275	male	7	3	3	3	3	3	100 %	0 %
66G4	2014	Sild__37546_06	43	275	female	7	3	3	3	3	3	100 %	0 %
66G4	2014	Sild__37546_08	44	250	male	7	2	2	3	3	2	50 %	23 %
66G4	2014	Sild__37546_09	45	285	female	7	3	3	3	3	3	100 %	0 %
66G4	2014	Sild__37546_10	46	320	male	7	5	5	5	5	5	100 %	0 %
66G4	2014	Sild__37546_12	47	250	female	7	3	3	3	3	3	100 %	0 %
66G4	2014	Sild__37546_16	48	250	female	7	3	3	3	3	3	100 %	0 %
66G4	2014	Sild__37546_18	50	290	female	7	5	5	5	4	5	75 %	11 %
						5	48	48	48	48		75.0%	7.6%
						5	0	0	0	0			

Table 11.1.1. Individual age readings and modal age calculated from all four readers.

COEFFICIENT OF VARIATION (CV)

	MODAL (R1-R5)	Stina Reader 2	Eydna Reader 5	Poul Reader 6	Jens Arni Reader 7	ALL Readers
	0	-	-	-	-	-
	1	-	-	-	-	-
	2	-	-	-	-	-
	3	12 %	0 %	14 %	0 %	6.1%
	4	16 %	0 %	13 %	0 %	8.4%
	5	5 %	0 %	11 %	9 %	5.9%
	6	13 %	0 %	0 %	0 %	4.3%
	7	-	-	-	-	-
	8	18 %	8 %	9 %	5 %	11.1%
	9	0 %	6 %	0 %	0 %	8.0%
	10	0 %	0 %	11 %	0 %	11.2%
	11	6 %	0 %	0 %	7 %	4.5%
	12	-	-	-	-	-
	13	-	-	-	-	-
	14	-	-	-	-	-
	15	-	-	-	-	-
Weighted mean	0-15	8.2%	1.5%	9.2%	4.2%	7.6%
	RANKING	3	1	4	2	

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	MODAL (R1-R5)	Stina Reader 2	Eydna Reader 5	Poul Reader 6	Jens Arni Reader 7	ALL
	0	-	-	-	-	-
	1	-	-	-	-	-
	2	100 %	100 %	0 %	0 %	50 %
	3	88 %	100 %	75 %	100 %	91 %
	4	67 %	100 %	67 %	100 %	83 %
	5	94 %	100 %	71 %	76 %	85 %
	6	50 %	100 %	100 %	100 %	88 %
	7	100 %	0 %	100 %	0 %	50 %
	8	57 %	14 %	43 %	86 %	50 %
	9	100 %	33 %	100 %	0 %	58 %
	10	100 %	100 %	33 %	0 %	58 %
	11	50 %	100 %	100 %	50 %	75 %
	12	100 %	100 %	0 %	0 %	50 %
	13	-	-	-	-	-
	14	-	-	-	-	-
	15	-	-	-	-	-
Weighted mean	0-15	83.3%	81.3%	66.7%	68.8%	75.0%
	RANKING	1	2	4	3	

RELATIVE BIAS

	MODAL (R1-R5)	Stina Reader 2	Eydna Reader 5	Poul Reader 6	Jens Arni Reader 7	ALL
	0	-	-	-	-	-
	1	-	-	-	-	-
	2	0.00	0.00	1.00	1.00	0.50
	3	-0.13	0.00	0.25	0.00	0.03
	4	-0.33	0.00	0.33	0.00	0.00
	5	-0.06	0.00	0.35	-0.24	0.01
	6	-0.50	0.00	0.00	0.00	-0.13
	7	0.00	-1.00	0.00	-1.00	-0.50
	8	0.29	1.14	0.29	-0.14	0.39
	9	0.00	0.67	0.00	-1.00	-0.08
	10	0.00	0.00	-1.00	-2.00	-0.75
	11	0.50	0.00	0.00	-0.50	0.00
	12	0.00	0.00	-1.00	-1.00	-0.50
	13	-	-	-	-	-
	14	-	-	-	-	-
	15	-	-	-	-	-
Weighted mean	0-15	-0.02	0.19	0.17	-0.33	-0.00
	RANKING	1	3	2	4	

Table 11.1.2. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

Inter-reader bias test and reader against MODAL age bias test

	Stina Reader 2	Eydna Reader 5	Poul Reader 6	Jens Arni Reader 7
Reader 2				
Reader 5	∗			
Reader 6	–	–		
Reader 7	∗	∗∗	∗∗	
MODAL (R1-R5)	–	∗	–	∗∗

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

Table 11.1.3. The Inter-reader bias test gives probability of bias between readers and with modal age.

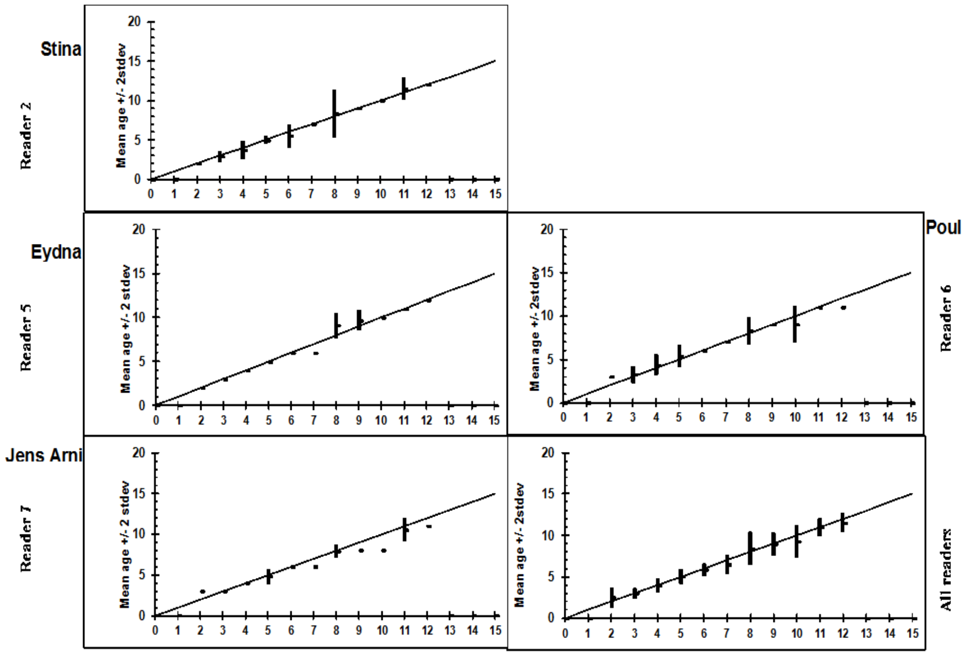

Figure 11.1.4. In the age bias plots the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

Scale exercise

Table 1 **NSS-herring scale exercise during workshop** RANGE

Stratum	year	Sample no	Fish no	Fish length	Sex	Landing month	Jostein Reader 1	Gudrun Reader 3	Ragnhildur Reader 4	MODAL (R1-R5)	Prosent enighet	Presisjon CV
55F1	2014	Sild_23102_02	1	300	female	5	6	6	6	6	100 %	0 %
55F1	2014	Sild_23102_04	2	300	female	5	10	10	9	10	67 %	6 %
55F1	2014	Sild_23102_06	3	345	female	5	12	12	12	12	100 %	0 %
55F1	2014	Sild_23102_08	4	290	male	5	5	5	6	5	67 %	11 %
55F1	2014	Sild_23102_09	5	270	male	5	4	4	5	4	67 %	13 %
55F1	2014	Sild_23102_11	6	325	female	5	10	8	8	8	67 %	13 %
55F1	2014	Sild_23102_12	7	300	female	5	5	5	5	5	100 %	0 %
55F1	2014	Sild_23102_13	8	310	male	5	5	5	4	5	67 %	12 %
55F1	2014	Sild_23102_14	9	335	female	5	10	10	10	10	100 %	0 %
55F1	2014	Sild_23102_15	10	275	female	5	3	3	4	3	67 %	17 %
55F1	2014	Sild_23102_20	11	305	female	5	5	5	4	5	67 %	12 %
55F1	2014	Sild_23102_23	12	305	female	5	5	6	6	6	67 %	10 %
55F1	2014	Sild_23102_24	13	290	female	5	5	6	5	5	67 %	11 %
55F1	2014	Sild_23102_25	14	285	female	5	5	5	7	5	67 %	20 %
55F1	2014	Sild_23102_27	15	350	male	5	9	9	10	9	67 %	6 %
55F1	2014	Sild_23102_28	16	300	female	5	4	5	5	5	67 %	12 %
55F1	2014	Sild_23102_29	17	295	male	5	6	6	7	6	67 %	9 %
55F1	2014	Sild_23102_30	18	305	female	5	6	7	7	7	67 %	9 %
57E9	2014	Sild_23103_02	19	320	female	5	7	7	9	7	67 %	15 %
57E9	2014	Sild_23103_03	20	355	female	5	10	10	9	10	67 %	6 %
57E9	2014	Sild_23103_06	21	350	female	5	11	11	11	11	100 %	0 %
57E9	2014	Sild_23103_07	22	360	female	5	10	10	10	10	100 %	0 %
57E9	2014	Sild_23103_08	23	355	male	5	9	9	9	9	100 %	0 %
57E9	2014	Sild_23103_11	24	335	female	5	5	6	6	6	67 %	10 %
57E9	2014	Sild_23103_12	25	345	female	5	5	5	5	5	100 %	0 %
57E9	2014	Sild_23103_13	26	325	male	5	10	10	10	10	100 %	0 %
57E9	2014	Sild_23103_14	27	340	male	5	6	6	7	6	67 %	9 %
57E9	2014	Sild_23103_15	28	350	female	5	10	10	10	10	100 %	0 %
57E9	2014	Sild_23103_16	29	340	female	5	6	6	6	6	100 %	0 %
57E9	2014	Sild_23103_18	30	350	male	5	7	7	7	7	100 %	0 %
57E9	2014	Sild_23103_19	31	295	female	5	8	7	8	8	67 %	8 %
57E9	2014	Sild_23103_20	32	345	female	5	5	5	5	5	100 %	0 %
57E9	2014	Sild_23103_21	33	355	male	5	8	9	8	8	67 %	7 %
57E9	2014	Sild_23103_22	34	350	male	5	10	10	11	10	67 %	6 %
57E9	2014	Sild_23103_25	35	350	female	5	10	9	9	9	67 %	6 %
57E9	2014	Sild_23103_27	37	315	male	5	8	9	10	9	33 %	11 %
57E9	2014	Sild_23103_28	38	305	male	5	8	8	6	8	67 %	16 %
57E9	2014	Sild_23103_30	39	345	female	5	9	8	10	9	33 %	11 %
66G4	2014	Sild_37546_01	40	300	female	7	4	4	4	4	100 %	0 %
66G4	2014	Sild_37546_04	41	290	female	7	3	3	3	3	100 %	0 %
66G4	2014	Sild_37546_05	42	275	male	7	3	3	3	3	100 %	0 %
66G4	2014	Sild_37546_06	43	275	female	7	3	3	3	3	100 %	0 %
66G4	2014	Sild_37546_08	44	250	male	7	4	4	4	4	100 %	0 %
66G4	2014	Sild_37546_09	45	285	female	7	3	3	3	3	100 %	0 %
66G4	2014	Sild_37546_10	46	320	male	7	5	5	5	5	100 %	0 %
66G4	2014	Sild_37546_12	47	250	female	7	4	3	3	3	67 %	17 %
66G4	2014	Sild_37546_16	48	250	female	7	3	3	3	3	100 %	0 %
66G4	2014	Sild_37546_18	50	290	female	7	5	5	5	5	100 %	0 %
							48 0	48 0	48 0		**80.6%**	**5.9%**

Table 11.2.1. Individual age readings and modal age calculated from all three readers.

COEFFICIENT OF VARIATION (CV)

	MODAL (R1-R5)	Jostein Reader 1	Gudrun Reader 3	Ragnhildur Reader 4	ALL Readers
	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	12 %	0 %	12 %	4.9%
	4	0 %	0 %	13 %	4.4%
	5	6 %	6 %	16 %	7.2%
	6	9 %	0 %	8 %	6.4%
	7	9 %	0 %	15 %	7.9%
	8	12 %	10 %	13 %	10.9%
	9	8 %	5 %	6 %	6.9%
	10	0 %	0 %	7 %	2.5%
	11	-	-	-	-
	12	-	-	-	-
	13	-	-	-	-
	14	-	-	-	-
	15	-	-	-	-
Weighted mean	0-15	6.6%	2.7%	11.0%	5.9%
	RANKING	2	1	3	

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	MODAL (R1-R5)	Jostein Reader 1	Gudrun Reader 3	Ragnhildur Reader 4	ALL
	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	86 %	100 %	86 %	90 %
	4	100 %	100 %	67 %	89 %
	5	91 %	91 %	64 %	82 %
	6	67 %	100 %	67 %	78 %
	7	67 %	100 %	67 %	78 %
	8	75 %	50 %	75 %	67 %
	9	60 %	80 %	40 %	60 %
	10	100 %	100 %	57 %	86 %
	11	100 %	100 %	100 %	100 %
	12	100 %	100 %	100 %	100 %
	13	-	-	-	-
	14	-	-	-	-
	15	-	-	-	-
Weighted mean	0-15	83.3%	91.7%	66.7%	80.6%
	RANKING	2	1	3	

RELATIVE BIAS

	MODAL (R1-R5)	Jostein Reader 1	Gudrun Reader 3	Ragnhildur Reader 4	ALL
	0	-	-	-	-
	1	-	-	-	-
	2	-	-	-	-
	3	0.14	0.00	0.14	0.10
	4	0.00	0.00	0.33	0.11
	5	-0.09	0.09	0.09	0.03
	6	-0.33	0.00	0.33	0.00
	7	-0.33	0.00	0.67	0.11
	8	0.50	0.00	-0.50	0.00
	9	0.00	-0.20	0.60	0.13
	10	0.00	0.00	-0.14	-0.05
	11	0.00	0.00	0.00	0.00
	12	0.00	0.00	0.00	0.00
	13	-	-	-	-
	14	-	-	-	-
	15	-	-	-	-
Weighted mean	0-15	-0.02	0.00	0.15	0.04
	RANKING	2	1	3	

Table 11.2.2. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

Inter-reader bias test and reader against ACTUAL age bias test

	Jostein Reader 1	Gudrun Reader 3	Ragnhildur Reader 4
Reader 1			
Reader 3	–		
Reader 4	–	–	
MODAL (R1-R5)	–	–	–

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

Table 11.2.3. The Inter-reader bias test gives probability of bias between readers and with modal age.

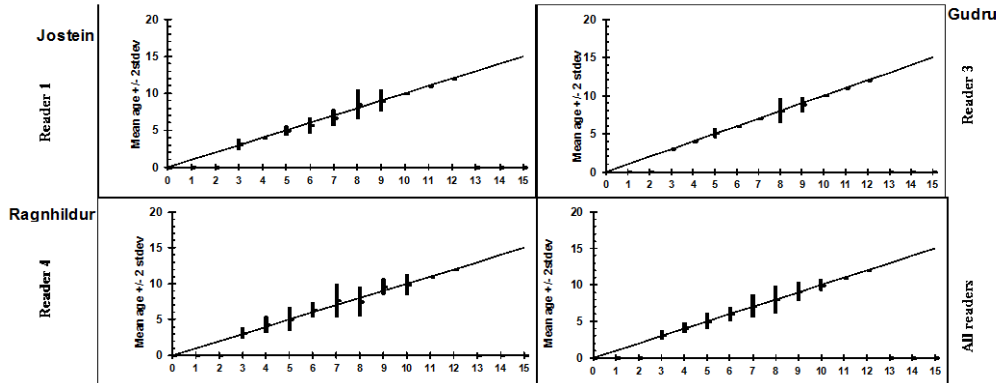
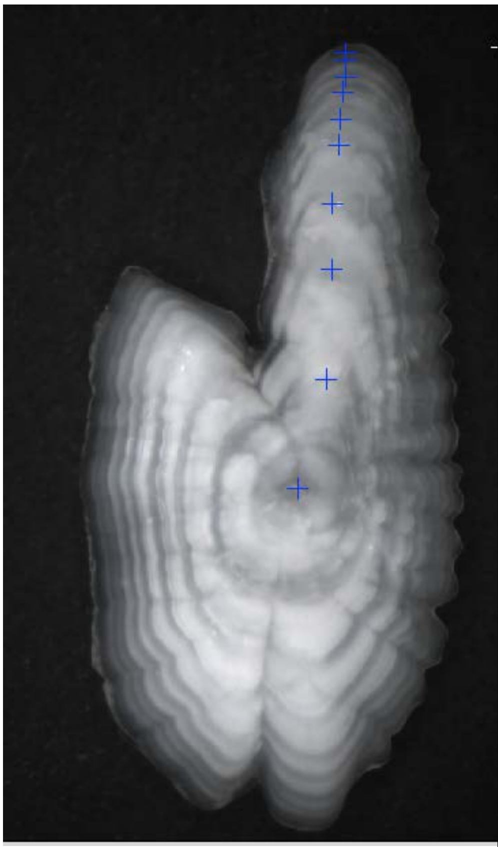


Figure 11.2.4. In the age bias plots the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

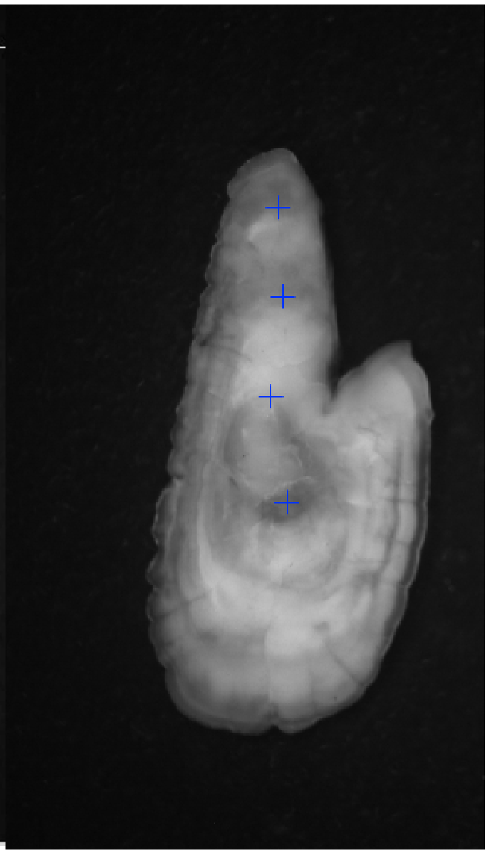
Annex 4: Edge development

The images below have been used to compile the tables in section 7.1, which outlines the edge development throughout the year for different age classes, and whether the outermost translucent zone should be included in the age.

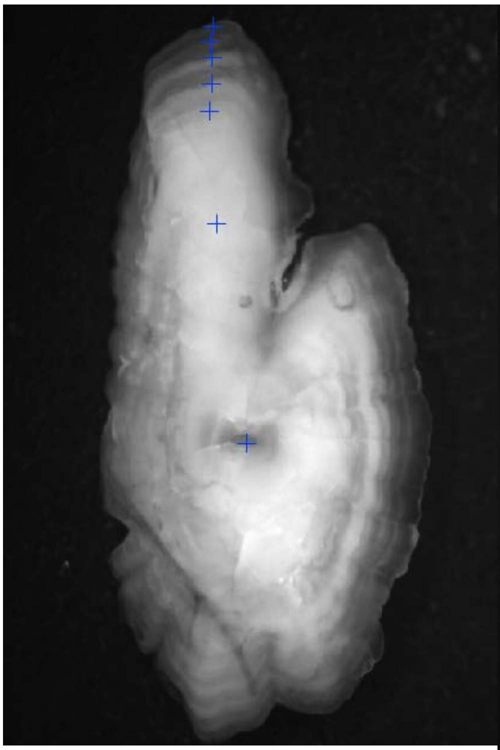
EDGE DEVELOPMENT OF OTOLITHS



Capture date: Dec 2014 Jnr. 467_25 Agreed age 9

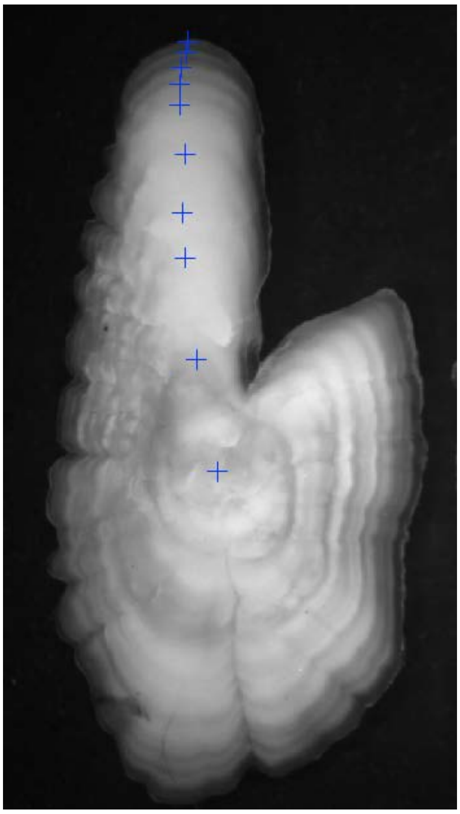


Capture date: May 2015 IESNS ST. 50_14 Agreed age 3

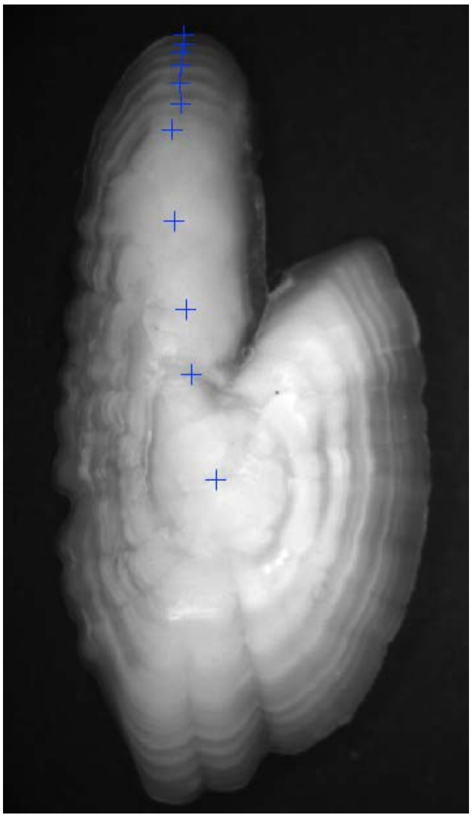


Capture date: May 2015 IESNS ST 50_35 Agreed age 6 agreed

Capture date: May 2015 IESNS ST 50_58 Agreed age 7



Capture date: May 2015 IESNS ST.50_59 Agreed age 9



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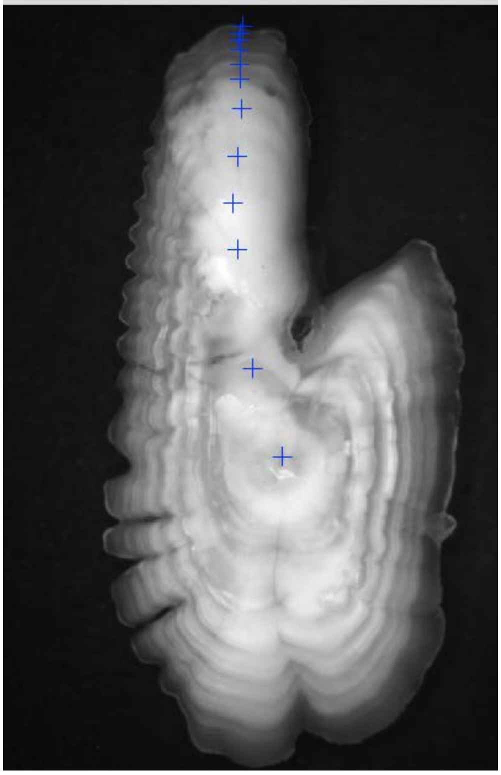
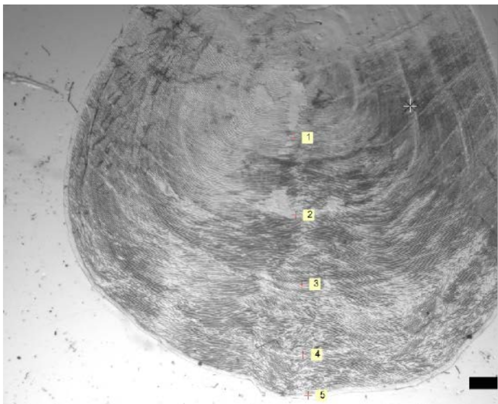
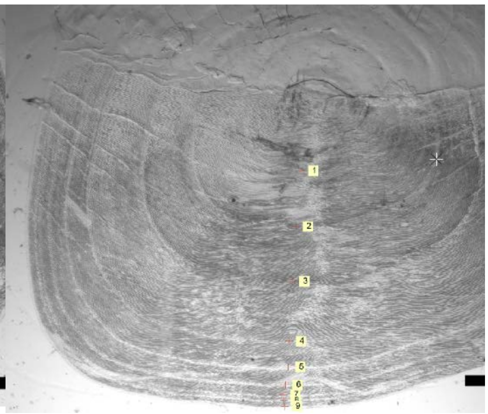


Capture date: May 2015 May IESNS ST 50_57
Agreed age 11

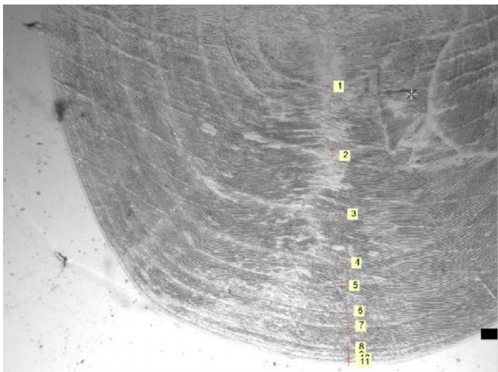
Edge deveoplment in scales



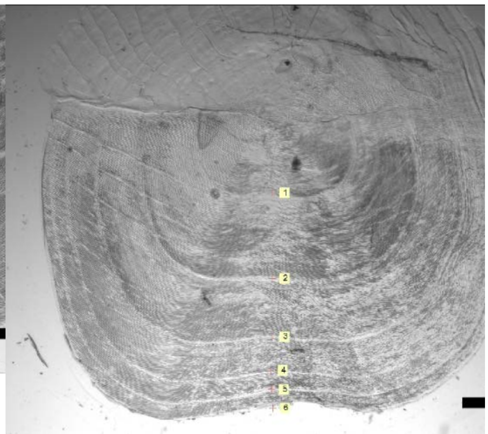
Capture date: 05/01/15 Agreed age 5 (320)



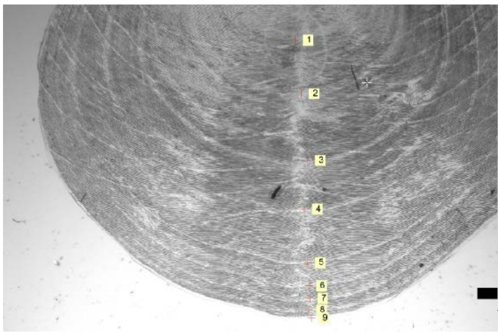
Capture date: 15/01/15 Agreed age 9



Capture date: 15/01/15 Agreed age 11



Capture date: 30/04/15 Agreed age 6



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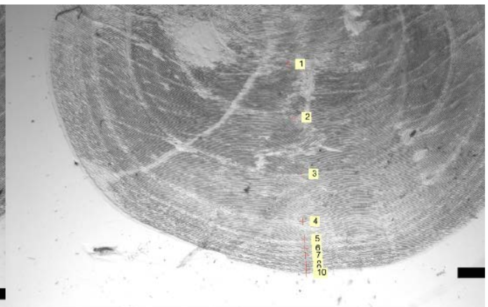
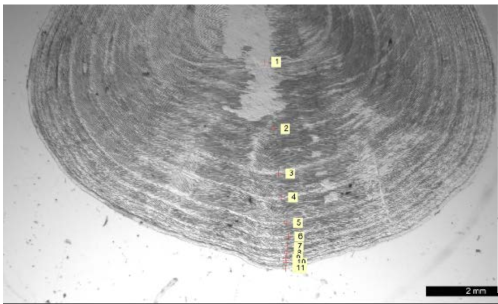


Capture date: 30/04/15 Agreed age10



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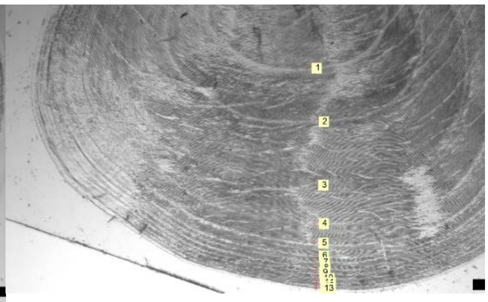
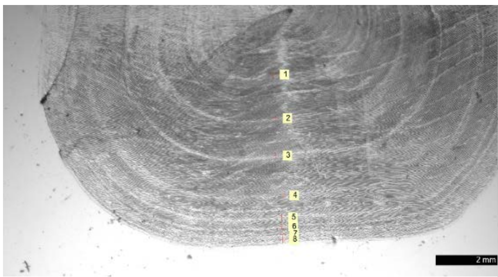
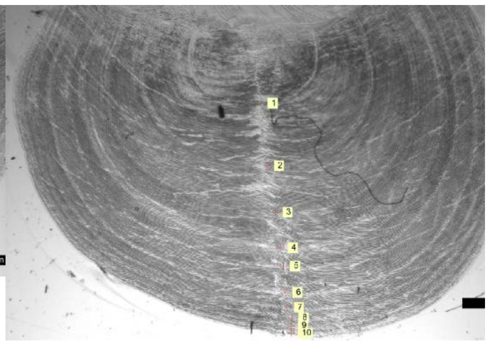


Capture date: 30/04/15 Agreed age 13



Capture date: 23/11/14 Agreed age 8



Capture date: 23/11/14 Agreed age 10

Annex 5: Recommendations

Recommendation	To:
Workshop (WKARNSSH) and pre-workshop exchange	WGBIOP, ACOM
Stock mixing issues during the May survey needs to be addressed	WGBIOP, WGIPS, WGWIDE, ACOM
Sampling of both structures from the same fish	WGBIOP, WGIPS
Standardization / calibration of sampling procedures	WGWIDE, WGIPS, ACOM
Implementation of the agreed guidelines by all laboratories	All NSS-herring laboratories