ICES WKARSPRAT REPORT 2016

SCICOM/ACOM STEERING GROUP ON INTEGRATED ECOSYSTEM OBSERVATION AND MONITORING

ICES CM 2016/SSGIEOM:19

REF. ACOM AND SCICOM

Report of the Workshop on age estimation of sprat (*Sprattus sprattus*) (WKARSPRAT)

15–18 November 2016

Galway, Ireland

ICES
International Council for the Exploration of the Sea
CIEM
Conseil International pour l'Exploration de la Mer

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Recommended format for purposes of citation:

ICES. 2017. Report of the Workshop on age estimation of sprat (*Sprattus sprattus*) (WKARSPRAT), 15-18 November 2016, Galway, Ireland. ICES CM 2016/SSGIEOM:19. 129 pp.

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Executive summary

The Workshop on Age Estimation of Sprat (*Sprattus sprattus*) (WKARSPRAT) met in Galway, Ireland on 15–18 November 2016. The meeting was chaired by Julie Coad Davies of DTU Aqua, Denmark and Claire Moore of MI, Ireland and included eight age readers from five national laboratories (Ireland, Northern Ireland, Germany, Norway, Sweden, and Denmark). The samples included in the workshop represented three stocks; North Sea (4), Skagerrak and Kattegat (3.a), and the Celtic Seas Ecoregion (Divisions 7 (excluding 7.d, e) and 6). The objectives of the workshop were to standardize procedures and provide guidelines for reliable age interpretation, to complete an age reading exercise for each stock, to provide age error matrices for the stock assessment working group, and to create an agreed age reference collection of otoliths. The age reading of sprat is confounded by some features of the otoliths which make correct age determination difficult, such as the presence of faint translucent rings and bands of alternating opaque and translucent zones. These problems were addressed for each stock separately and much time was spent discussing image examples of otolith in plenary. Age estimation and age validation studies were used to support the compilation of age interpretation guidelines which are an outcome of the workshop, an agreed age reading protocol and a reference collection of agreed age otoliths. The results of the age reading exercises and the plenary discussion of otoliths at the workshop indicate that there are regional differences in the readability of the otoliths which is reflected in the levels of agreement and precision seen in the results of the age reading exercises. Otoliths from the Celtic Seas Ecoregion are much easier to interpret compared with those from the North Sea, while those from the Skagerrak and Kattegat are complex and very difficult to age reliably. For stock assessment purposes, the age error matrices can be used as an indication of the quality of the age data used directly in the models e.g. North Sea, or used for exploratory assessments e.g. Skagerrak and Kattegat and the Celtic Seas Ecoregion. The low levels of accuracy and agreement for the Skagerrak and Kattegat need to be improved and the exercise will be completed and reanalysed. Otolith microstructure analysis will be carried out in participating laboratories to further reduce the uncertainties in the age estimates of fish from each stock.

1 Opening of the meeting

1.1 Introduction

Age determination of fish is based on the interpretation of annual growth patterns seen within the calcified structures (otoliths, bones, scales, and vertebrae) of the individual. In otoliths, bands of opaque and translucent material are laid down on a yearly basis and the resulting patterns reflect the growth experienced by the fish and is a consequence of the environmental conditions in which it has been living. Age reading laboratories employ various methods and techniques to estimate the ages of fish species on a yearly basis. This information underlies the parameterization of vital input into fish stock assessment (growth rates, catch-at-age, mortality, etc.) which ultimately form the basis of fish stock management decisions. Thus, when bias exists in the age data it can have serious consequences in regards to over or under exploitation of fish stocks which may already be in jeopardy or alternatively not being harvested to their full potential.

In order to identify errors resulting from incorrect ageing practices an otolith exchange will be carried out where age readers from different age reading laboratories estimate the ages of a sample of representative otoliths. Based on the results, an age reading workshop may be recommended with the main objective being to decrease the relative/absolute bias and to improve the precision or reproducibility of age determinations across the different age reading laboratories.

In 2012, the PGCCDBS (Planning Group on Commercial Catches, Discards and Biological Sampling) identified the need for a full-scale otolith exchange for North Sea sprat to take place in 2013 (ICES, 2012)). In the same year HAWG (Herring Assessment Working Group) recommended that sprat in the Celtic Seas Ecoregion be included in this exchange (ICES, 2013).

Following these recommendations a full-scale otolith exchange was initiated and coordinated by DTU Aqua Denmark in 2013 which included samples from the North Sea and Celtic Seas Ecoregion. The objectives of this exchange were to assess the quality of age determination by examining the level of agreement between the age readers and to identify problematic areas in the age determination. Numerous technical problems were encountered during the exchange, including the crash of WebGR (available at http://webgr.azti.es/). As a result all data, including age readings, were lost. Due to time constraints only the otoliths from the North Sea were analysed. It was decided to include samples from the Celtic Seas Ecoregion at a later date. Overall, the results of the exchange were poor, with a high CV (coefficient of variation) and a low level of agreement (Coad Davies *et al.*, 2014). The quality of the results were further hampered as many of the "expert" readers were not experienced in reading otoliths from the North Sea, therefore the results of the exchange could not be improved by basing the results on the age readings of the expert readers only. A number of problems in the age estimation of sprat were identified from the results of this exchange, first, difficulty in the identification of the first winter ring and second the identification of the subsequent annuli. The exchange group recommended that a calibration workshop be held; validation of the first annulus; application of microstructure data to provide guidelines for identification of subsequent annuli and an expansion of the workshop to include samples from other ecoregions.

1.2 Previous exchanges and workshops for age determination of Sprat

Previous workshops on age estimation of sprat in the North Sea and Skagerrak-Kattegat have been held in 1994, 1996, 2002 (Torstensen), and 2004 (Torstensen *et al.*). The national representation and level of expertise of the participants of these exchanges and workshops has varied overtime but the results were similar; the level of agreement and precision was low and a number of reoccurring issues in relation to the age estimation of sprat have been identified as the reasons for the poor results; first, the difficulty in the interpretation of the first translucent ring and second, the interpretation of narrow opaque zones or fragments (false rings) within in the reading area of the otolith (Torstensen, 2002). Studies based on the otolith microstructure of sprat from the Baltic Sea have demonstrated structural differences between what are defined as true and false translucent rings (Mosegaard and Baron, 1999) and for sprat populations of Skagerrak-Kattegat (3.a) preliminary studies have been conducted using marginal increment analysis to validate the first winter ring (Torstensen, 2002). See Section 3 for an overview of validation studies done to date on sagittal otoliths of sprat from different regions of their spatial distribution.

Regardless of these validation studies there is still disagreement between age readers of sprat within the different areas. The results from the 2014 exchange showed a low level of agreement (62% based on all readers) and a low level of precision (CV of 44% based on all readers), based on expert readers alone the % agreement increased to 78% but with little change in the CV of 45%. An additional analysis was included in this exchange based on the distances between the annotated winter rings made by the readers on images of the otoliths. The advantage of this being twofold; there exists a visual reference of which structures the readers are interpreting as the winter rings and it also allows for a comparison of the growth curves complied for each reader and each fish. Based on the annotated images it was concluded that the first winter ring was not clearly identified by all readers.

Based on the results of the 2014 exchange a recommendation was made to the Working Group on Biological Parameters (WGBIOP) that an age calibration workshop be held and which should be expanded to include samples from other ecoregions (Coad Davies *et al.*, 2014).

1.3 Objectives of this workshop

Age determination is an essential feature of stock assessment. Information on the age structure of a population is required to obtain reliable estimates of morality and growth from stock assessment models. Therefore, to ensure appropriate management practices the age determination procedures for a species must be deemed reproducible and reliable. The results of previous exchanges and workshops outline a number of reoccurring issues associated with age estimation of sprat. This workshop aims to address these issues; provide guidelines for reliable age interpretation and to improve the accuracy and precision of sprat age determination by standardization of procedures. The application of such guidelines should permit the provision of reliable age estimates to be used in stock assessment models.

The following stock divisions are based on those decided upon by the Benchmark Workshop on Sprat Stocks (WKSPRAT, 2013) and all stocks are managed and assessed separately:

- North Sea (4);
- 3.a (Skagerrak and Kattegat);

- English Channel (7.d, e);
- Celtic Seas Ecoregion (Divisions 7 (excluding 7.d, e) and 6).

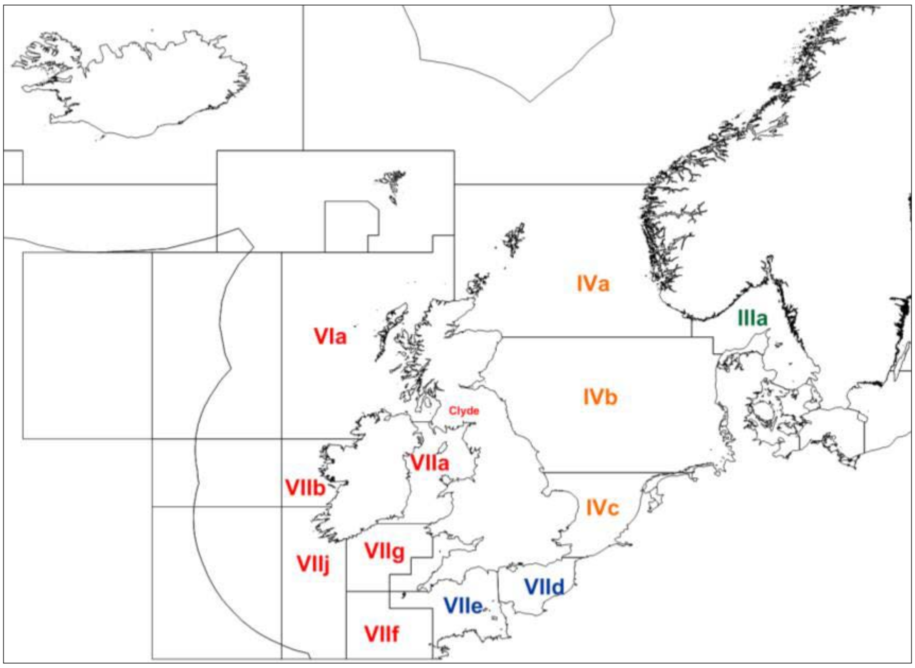


Figure 1.3.1. Sprat stock divisions (taken from the Benchmark Workshop on Sprat Stocks (WKSPRAT, 2013)

Based on the above divisions (Figure 1.3.1) three sets of otoliths were compiled; North Sea (4), Skagerrak and Kattegat (3.a) and Celtic Seas Ecoregion (Divisions 7 (excluding 7.d, e) and 6), no samples were available for English Channel (7.d, e). The analysis and discussion of age estimation error and bias is discussed separately for each stock.

The objectives of the workshop were achieved by addressing the following ToRs:

1.4 Terms of Reference (ToRs)

A **Workshop on Age estimation of Sprat (***Sprattus sprattus)* (WKARSPRAT), chaired by Julie Coad Davies, Denmark and Claire Moore, Ireland, will be established and will meet in Galway, Ireland, 15–18 November 2016 to:

a) Analyse the results of the rerun of the WebGR North and Celtic Sea Sprat Exchange;

b) Follow the development of age estimation and validation studies being undertaken;

c) Analyse growth increment patterns in sprat and compile guidelines for the interpretation of sprat otoliths;

d) Create a reference collection of agreed aged otoliths;

e) Address the generic ToRs adopted for workshops on age calibration (see WGBIOP's Guidelines for Workshops on Age Calibration).

WKARSPRAT will report by 13 January 2017 for the attention of ACOM, SCICOM and WGBIOP.

The Terms of Reference are answered in Sections 2, 3, 4, 5, and 6.

2 ToR a) Analyse the results of the rerun of the WebGR North and Celtic Sea Sprat Exchange;

From April to September 2016 a sprat otolith exchange took place for the North Sea (4) and Celtic Seas Ecoregion (Divisions 7 (excluding 7.d, e) and 6). 18 readers from 8 institutes took part. Images were made available on WebGR for annotation and the otoliths were provided to all readers for visual examination.

2.1 List of participants

Table 2.1.1. List of participants of the pre-workshop exchange

READER	FIRST NAME	LAST NAME	INSTITUTE	PROVIDES AGES FOR ASSESSMENT
1	Ian	McCausland	AFBI Northern (Ireland)	Yes
2	Maria	Jarnum	DTU Aqua (Denmark)	Yes
3	Stina	Bilstrup Hansen	DTU Aqua (Denmark)	Yes
4	Marianne	Johansson	SLU Aqua (Sweden)	Yes
5	Marie	Leiditz	SLU Aqua (Sweden)	No
6	Olof	Lovgren	SLU Aqua (Sweden)	Yes
7	Annelie	Hilvarsson	SLU Aqua (Sweden)	No
8	Eilert	Hermansen	IMR (Norway)	Yes
9	Anne Liv	Johnsen	IMR (Norway)	Yes
10	Bjarn Vidar	Svendsen	IMR (Norway)	Yes
11	Jostein	Røttingen	IMR (Norway)	Yes
12	Jan de	Lange	IMR (Norway)	Yes
13	Inger	Henriksen	IMR (Norway)	Yes
14	Jean Louis	Dufour	Ifremer (France)	Yes
15	Jan	Beintema	IMARES (The Netherlands)	No
16	Andre	Dijkman	IMARES (The Netherlands)	No
17	Claire	Moore	MI (Ireland)	No
19	Gertrud	Delfs	Thünen Institute (Germany)	Yes

2.2 Samples

It was originally intended that the exchange would consist of 3 sample sets representing each of the stocks; North Sea (Table 2.2.1), Celtic Seas Ecoregion (Table 2.2.2) and Skagerrak and Kattegat (3.a). Due to technical difficulties it was not possible to upload the 3.a images to WebGR and thus this sample set was read during the workshop instead.

Samples were provided by AFBI Northern Ireland, MI Ireland, MARLAB Scotland, DTU Aqua Denmark, and SLU Aqua Sweden. Otolith images were digitized at DTU Aqua using a standard set up. Images were taken of the otoliths on a black background, soaked in alcohol, under reflected light, at x 3.2 magnification, using a Leica stereomicroscope MZ6, Leica camera DFC320 and Leica Imaging software (LAS V.4.2). Otolith images where then uploaded to WebGR and two exercises made available for annotation by the readers, one for the North Sea area and one for the Celtic Seas Ecoregion.

Table 2.2.1 Overview of samples from the North Sea

Area	Year	Quarter	Length range	n
4.b	2016	1	70–140 mm	18
	2015	2	85–110 mm	16
	2015	3	70–130 mm	18
	2015	4	60–120 mm	19
	2014	3	75–130 mm	25
4.c	2016	1	125–140 mm	4
Total				**100**

Table 2.2.2 Overview of samples from the Celtic Seas Ecoregion

Area	Year	Quarter	Length range	n
6.a	2015	1	80–140 mm	18
	2014	4	70–130 mm	14
	2010	2	95–115 mm	8
	2010	3	95–110 mm	16
	2009	4	125–155 mm	8
7.b	2016	4	80–125 mm	10
7.g	2011	3	120–140 mm	8
	2010	2	70–90 mm	2
	2009	4	85–120 mm	6
7.j	2010	4	100–115 mm	3
	2009	4	95–125 mm	7
Total				**100**

2.3 Methods

In April 2016, a Skype meeting was held where all of the age readers and age reader coordinators were invited. The purpose of this meeting was to first, agree on the timetable for reading (based on readers time preferences) and the logistics in sending the otolith samples to all laboratories; second, to give a WebGR demonstration of the annotation guidelines and third; to explain the rational for these guidelines.

Readers were asked to place their annotations along a defined and marked axis, placing the first annotation on the centre point of the otolith and then to place the following annotations in the correct sequential order towards the edge of the otolith with a final annotation placed on the edge. Readers were asked to place their annotations at the start of the winter rings and to give a final estimate of age. In addition, readers were asked to provide a level of readability based on the ICES scale of AQ1, AQ2, and AQ3 (WKNARC, 2011); and to define the edge type based on a predefined scale (TW: wide transparent zone at the edge, TN: narrow transparent zone at the edge, OW: wide opaque zone at the edge and ON: narrow opaque zone at the edge). An excel file for recording the readability and edge type for each sample was presented and provided for each reader. This file also gave definitions of aforementioned parameters.

2.4 Analysis

2.4.1 Age data

An R script (R v.3.2.1 available at http://www.R-project.org) was developed which follows the traditional analyses of agreement between readers as used in the Guus Eltink spreadsheet (Eltink, A.T.G.W. 2000):

- % agreement (nmodal age/ntotal*100)
- coefficient of variation (CV) (standard deviation/average*100)
- bias tests and plots

In addition an index of average percentage error (APE) was calculated based on the method outlined by Beamish and Fournier (1981). This method is not independent of fish age and thus provides a better estimate of precision. As the calculations of both CV and APE pose problems if the mean age is close to 0, all observations for which modal age was 0 were omitted from the CV and APE calculations.

Age error matrices (AEM) were produced following procedures outlined by WKSABCAL (2014) where the matrix shows the proportion of each modal age mis-aged as other ages. The sum of each row is 1, which equals 100%.

The age data were analysed twice, the first time all readers were included and the second time only the "expert" readers were included. If a reader is an expert then they are considered well trained and they provide ages for use in stock assessment. When the AEM is compiled for assessment purposes it uses only those readers who provide age data for the stock assessment in that specific area. This was only possible for the North Sea (4). For 3.a (Skagerrak and Kattegat), only 3 experts provide ages for assessment and for the Celtic Seas Ecoregion (Divisions 7 (excluding 7.d, e) and 6) where there is currently no age based assessment conducted for sprat, the AEMS were compiled using all expert readers.

2.4.2 Growth data

WebGR provides a measure of distance between the annotations made by the readers and thus provides a measure of growth increment width. The "alldistances" dataset from WebGR was used to establish growth curves for each fish and for each reader. For each set of annotations belonging to a single fish and reader, the distance between two consecutive annotations was added to the sum of the previous distances and the distances were cumulated from centre point to the outermost annotated winter ring, thus a growth curve for each individual image and reader was compiled. These growth curves were analysed using Linear Mixed Effects Models (LMEM) to examine whether there are consistent differences in growth curves estimated by the readers.

For both exchanges the model that best fits the data is a model with winter ring (log) and reader as fixed effects and individual images as random effects.

Age data provide information on whether the age readers agree in age estimates and the growth data can identify specifically where the problems are, i.e. differences in intercept only are attributable to problems with the first winter ring, while differences in slope indicate a general inconsistency in structures used for age estimation.

2.5 Results

2.5.1 Age data from the North Sea pre-workshop exchange

The age estimations of all readers combined shows a percentage agreement of 88.1% (Table 2.5.1.1) and an overall CV of 16.3% (Table 2.5.1.2). At age 0 there are only 2 readers who are not in 100% agreement with modal age. From ages 0–3 the individual percentage agreement is above 75% with the exception of readers 15, 16, and 17 but as modal age increases the percentage agreement decreases, it should be noted that for modal age 4 there are only 2 fish.

The overall CV is high but it should be taken into consideration that CV in this case is calculated based on a sample set of fish with an age range of 0–4 years. The overall average percentage error (APE) is 10.7% and this is calculated taking the age of the fish into consideration.

The lowest percentage agreement and the highest CV were attributed to reader 16 and 17. The relative bias values (Table 2.5.3) indicate that reader 16 is underestimating compared with modal age while reader 17 is overestimating compared with modal age. In the inter-reader bias test (Table 2.5.1.4) it is also these 2 readers who show the most bias in their readings, both compared with the other readers and also compared with modal age. Readers 5 and 14 also show certainty of bias compared with modal age while 11 and 19 both show a possibility of bias compared with modal age (Table 2.5.1.4). The age bias plots in Annex 3 confirm these results.

Table 2.5.1.1 Percentage agreement based on modal age for all readers in the North Sea pre-workshop exchange

Modal Age	2 DNK 1	3 DNK 2	4 SWE 1	5 SWE 2	7 SWE 3	8 NOR 4	9 NOR 5	11 NOR 1	12 NOR 2	13 NOR 3	14 FRA 1	15 NLD 1	16 NLD 2	17 IRL 1	19 DEU 1	ALL
0	100	100	100	100	100	100	60	100	100	100	100	100	80	100	100	96
1	97	98	98	92	95	98	98	76	97	93	88	90	84	61	100	91
2	95	86	81	90	90	86	80	86	81	95	76	76	70	86	80	84
3	86	86	79	79	79	86	93	93	86	79	93	50	64	50	100	80
4	100	100	0	100	100	100	100	100	100	100	100	0	0	100	0	73
Weighted Mean	95.2	93.9	90.9	90.2	92	93.9	91.8	81.9	92.3	91.9	86.9	81.1	77.2	67	94.9	88.1
Rank	1	3	9	10	6	3	8	12	5	7	11	13	14	15	2	-

Table 2.5.1.2. Coefficient of variation (CV) between the modal age and the age estimation of all readers in the North Sea pre-workshop exchange

Modal Age	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	18	13	13	32	21	13	13	35	18	24	29	28	42	46	0	16.8
2	11	19	22	22	14	19	23	20	22	11	22	26	28	19	23	16.2
3	12	12	13	19	13	13	9	9	13	32	9	21	25	15	0	14.9
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.3
Weighted Mean	14.6	13.3	14.1	26.2	17.1	13.5	20.6	26.1	17.1	20.9	23	24.9	45.6	33.2	4.6	16.3
Rank	5	2	4	13	6	3	8	12	6	9	10	11	15	14	1	-

Table 2.5.1.3. Relative bias values based on modal age for all readers in the North Sea pre-workshop exchange (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1	ALL
0	0	0	0	0	0	0	0.4	0	0	0	0	0	0.2	0	0	0.04
1	0.03	0.02	0.02	0.1	0.05	0.02	0.02	0.24	0.03	0.07	0.12	0.1	-0.09	0.49	0	0.08
2	-0.05	0.05	0	0.14	0.1	-0.14	-0.2	-0.05	-0.19	-0.05	0.14	-0.14	-0.3	0.05	-0.2	-0.06
3	0.14	0.14	0.21	0.29	0.21	-0.14	-0.07	-0.07	-0.14	-0.36	0.07	-0.5	-0.43	0.5	0	-0.01
4	0	0	-1	0	0	0	0	0	0	0	0	-1	-1	0	-1	-0.27
Weighted Mean	0.03	0.04	0.03	0.13	0.08	-0.04	-0.02	0.12	-0.04	-0.02	0.11	-0.05	-0.18	0.37	-0.05	0.03
Rank	3	5	3	13	10	5	1	12	5	1	11	8	14	15	8	-

Table 2.5.1.4. Inter-reader bias tests for all readers in the North Sea pre-workshop exchange ("-"= no sign of bias (p>0.05); "*" = possibility of bias (0.01<p<0.05); "" = certainty of bias (p < 0.01))**

	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1
2 DNK1	NA	-	-	*	-	*	-	-	-	-	*	-	**	**	*
3 DNK2	-	NA	-	*	-	*	-	-	-	-	-	-	**	**	**
4 SWE1	-	-	NA	*	-	-	-	-	-	-	*	-	**	**	*
5 SWE2	*	*	*	NA	-	**	**	-	**	*	-	**	**	**	**
7 SWE3	-	-	-	-	NA	**	*	-	*	-	-	*	**	**	**
8 NOR4	*	*	-	**	**	NA	-	**	-	-	**	-	*	**	-
9 NOR5	-	-	-	**	*	-	NA	**	-	-	*	-	*	**	-
11 NOR1	-	-	-	-	-	**	**	NA	**	*	-	**	**	**	**
12 NOR2	-	-	-	**	*	-	-	**	NA	-	**	-	*	**	-
13 NOR3	-	-	-	*	-	-	-	*	-	NA	*	-	**	**	-
14 FRA1	*	-	*	-	-	**	*	-	**	*	NA	*	**	**	**
15 NLD1	-	-	-	**	*	-	-	**	-	-	*	NA	*	**	-
16 NLD2	**	**	**	**	**	*	*	**	*	**	**	*	NA	**	*
17 IRL1	**	**	**	**	**	**	**	**	**	**	**	**	**	NA	**
19 DEU1	*	**	*	**	**	-	-	**	-	-	**	-	*	**	NA
Modal Age	-	-	-	**	*	-	-	*	-	-	**	-	**	**	*

Results based on expert readers only

When the results are based on only those readers who provide age data for use in stock assessment the percentage agreement increases to 91.4% (Table 2.5.1.5), the CV (Table 2.5.1.6) decreases to 10% and the APE decreases to 7.6% indicating that there is an overall improvement in the results. The overall relative bias value of 0.01 (Table 2.5.1.7) indicates a slight overestimation of the ages compared with modal age.

The age error matrix (Table 2.5.1.8) shows that the proportions of fish aged in agreement with modal age for each age group is high (from 95% at age 0 to 88% at age 4). At modal age 0 to 3 the tendency is to overestimate but for modal ages 2-4 there is also a tendency for the readers to underestimate the age. In most cases there is only a difference of one year compared with modal age.

Table 2.5.1.5. Percentage agreement based on modal age for only expert readers in the North Sea pre-workshop exchange

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	100	100	100	100	60	100	100	100	100	100	96
1	98	98	98	100	98	78	98	93	90	100	95
2	95	82	77	86	76	86	82	91	77	76	83
3	86	86	79	86	93	93	86	79	93	100	88
4	100	100	0	100	100	100	100	100	100	0	80
Weighted mean	95.8	92.9	89.8	95	90.7	83.2	92.9	91	88.2	93.9	91.4
Rank	**1**	**4**	**8**	**2**	**7**	**10**	**4**	**6**	**9**	**3**	**-**

Table 2.5.1.6. Coefficient of variation (CV) between the modal age and the age estimation of expert readers in the North Sea pre-workshop.

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	-	-	-	-	-	-	-	-	-	-	-
1	13	13	13	0	13	34	13	24	28	0	8.5
2	11	22	25	19	25	19	22	15	22	25	14.2
3	12	12	13	13	9	9	13	32	9	0	9.7
4	0	0	0	0	0	0	0	0	0	0	11.1
Weighted Mean	11.6	14.1	14.9	6	21.1	25.2	14.2	21.7	22.3	5.3	10
Rank	**3**	**4**	**6**	**2**	**7**	**10**	**5**	**8**	**9**	**1**	-

Table 2.5.1.7. Relative bias values based on modal age for only expert readers in the North Sea pre-workshop exchange (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	0	0	0	0	0.4	0	0	0	0	0	0.04
1	0.02	0.02	0.02	0	0.02	0.22	0.02	0.07	0.1	0	0.05
2	-0.05	0	-0.05	-0.14	-0.24	-0.05	-0.18	-0.09	0.14	-0.24	-0.09
3	0.14	0.14	0.21	-0.14	-0.07	-0.07	-0.14	-0.36	0.07	0	-0.02
4	0	0	-1	0	0	0	0	0	0	-1	-0.2
Weighted Mean	0.02	0.03	0.02	-0.05	-0.03	0.11	-0.05	-0.03	0.1	-0.06	0.01
Rank	**1**	**3**	**1**	**6**	**3**	**10**	**6**	**3**	**9**	**8**	**-**

Table 2.5.1.8. Age error matrix on expert readers, who provide ages for stock assessment in the North Sea (numbers in bold indicate the proportion in agreement with modal age, those in red indicates underestimation compared with modal age while those in black indicate overestimation compared with modal age)

Age | Modal Age	0	1	2	3	4
0	0.95	0	0	0.01	0
1	0.05	0.94	0.11	0	0
2	0	0.06	0.86	0.07	0
3	0	0	0.03	0.89	0.12
4	0	0	0	0.03	0.88

2.5.2 Growth data from the North Sea pre-workshop exchange

Figure 2.5.2.1 shows the combined growth curves for all fish and all readers in the North Sea pre workshop exchange. Analysis of the LMEM showed a significant reader effect for both the intercept and slope (LMEM, $p < 0.05$) demonstrating inter reader differences in the interpretation of the first winter ring and the subsequent winter rings. However, there is little overlap between the average distances from the centre to the first and second winter ring and this indicates that the readers are in general agreement as to where to identify winter rings 1 and 2. In some examples there is disagreement between readers with the result being a significant reader effect in the model analysis. These examples were discussed during the workshop. Notes on this discussion can be found in the Table 2.6.1 in Section 2.6. As the average distance from the centre to the subsequent winter rings increases an overlap becomes more apparent and this indicates that the readers are less in agreement as to which structures are in fact the true winter rings.

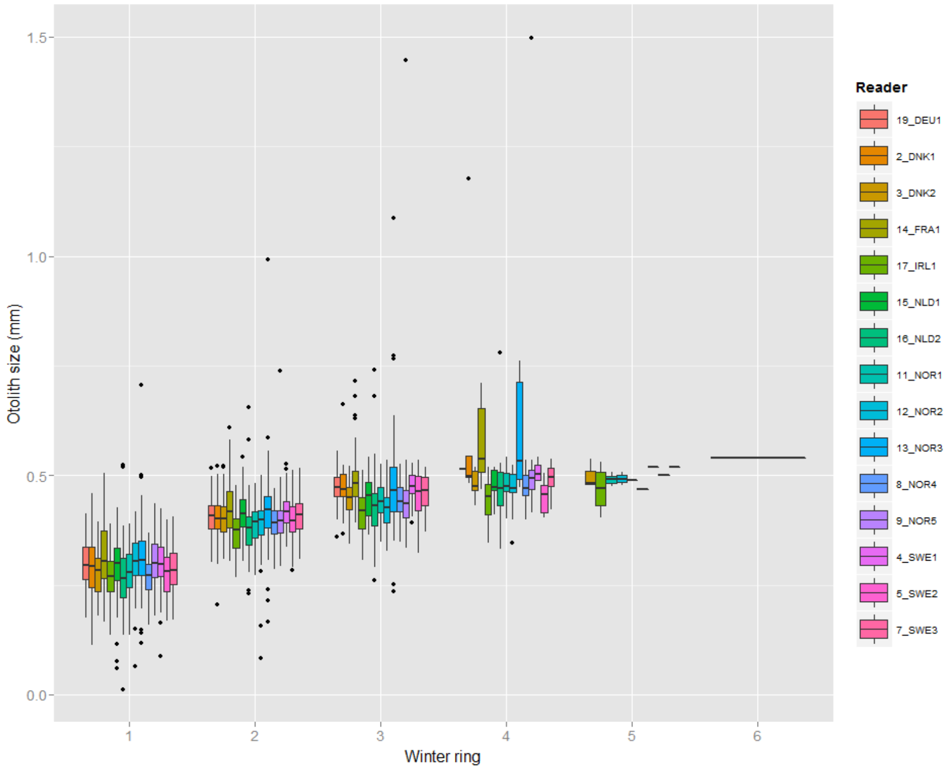


Figure 2.5.2.1. Plot of average distance from the centre to the beginning of winter rings 1-4 and the edge for all readers in the North Sea pre-workshop exchange. The boxes represent the mean, upper and lower box boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

2.5.3 Age data from the Celtic Seas Ecoregion pre-workshop exchange

When the age estimations from all readers are included in the analysis the overall percentage agreement is 94.3% (Table 5.5.3.1) with an overall CV of 12.7% (Table 2.5.3.2). At age 0 it is only readers 16 and 17 who are not in 100% agreement with modal age. From ages 0–2 the individual percentage agreement is above 90% with the exception of readers 15, 16, and 17. It should be noted that at modal age 3 there are only three fish.

The overall CV is high but it should be taken into consideration that CV in this case is calculated based on a sample set of fish with an age range of 0–3 years. The overall APE is 7.4% and this is calculated taking the age of the fish into consideration.

Readers 15, 16, and 17 showed the lowest overall percentage agreement with modal age and the highest CV (reader 12 included) in relation to the modal age. The relative bias values (Table 2.5.3.3) show that readers 15 and 16 are underestimating while reader 17 is overestimating compared with modal age. In the inter reader bias test (Table 2.5.3.4) readers 15 and 17 show certainty of bias against modal age while reader 16 falls just outside the significance level but should be considered to be biased based on the above mentioned results. The age bias plots in Annex 3 confirm these results.

Table 2.5.3.1. Percentage agreement based on modal age for all readers in the Celtic Seas Ecoregion pre-workshop exchange

Modal Age	2 DNK 1	3 DNK 2	4 SWE 1	5 SWE 2	7 SWE 3	8 NOR 4	9 NOR 5	11 NOR 1	12 NOR 2	13 NOR 3	14 FRA 1	15 NLD 1	16 NLD 2	17 IRL 1	19 DEU 1	All
0	100	100	100	100	100	100	100	100	100	100	100	100	85	85	100	98
1	98	97	97	100	98	97	97	92	91	93	97	95	97	63	93	94
2	100	100	100	96	100	100	100	96	92	100	100	80	84	96	96	96
3	100	100	100	100	100	100	33	100	33	100	67	33	33	67	100	78
Weighted Mean	98.8	98.2	98.2	99	98.8	98.2	96.2	94.3	90.7	95.9	97.2	90	90.3	74	94.9	94.3
Rank	**2**	**4**	**4**	**1**	**2**	**4**	**8**	**11**	**12**	**9**	**7**	**14**	**13**	**15**	**10**	**-**

Table 2.5.3.2. Coefficient of variation (CV) between the modal age and the age estimation of all readers in the Celtic Seas Ecoregion pre-workshop exchange

Modal Age	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1	All
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	13	18	18	0	13	18	18	26	29	25	18	23	19	36	24	15.9
2	0	0	0	10	0	0	0	10	15	0	0	23	20	10	10	5.2
3	0	0	0	0	0	0	25	0	25	0	22	25	25	22	0	12.1
Weighted Mean	7.7	10.6	10.6	2.5	7.7	10.6	11.4	17.8	21.6	14.8	11.3	20.1	50.8	56.6	16.7	12.7
Rank	**2**	**4**	**4**	**1**	**2**	**4**	**8**	**11**	**13**	**9**	**7**	**12**	**14**	**15**	**10**	**-**

Table 2.5.3.4. Relative bias values based on modal age for all readers in the Celtic Seas Ecoregion pre-workshop exchange (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1	All
0	0	0	0	0	0	0	0	0	0	0	0	0	0.23	0.15	0	0.03
1	0.02	0.03	0.03	0	0.02	0.03	0.03	0.08	0.02	0.03	0.03	-0.02	-0.03	0.37	0.07	0.05
2	0	0	0	-0.04	0	0	0	-0.04	-0.08	0	0	-0.2	-0.16	0.04	-0.04	-0.03
3	0	0	0	0	0	0	-0.67	0	-0.67	0	-0.33	-0.67	-0.67	-0.33	0	-0.22
Weighted Mean	0.01	0.02	0.02	-0.01	0.01	0.02	0	0.04	-0.03	0.02	0.01	-0.08	-0.05	0.24	0.03	0.02
Rank	**2**	**6**	**6**	**2**	**2**	**6**	**1**	**12**	**10**	**6**	**2**	**14**	**13**	**15**	**10**	**-**

Table 2.5.3.5. Inter-reader bias tests for all readers in the Celtic Seas Ecoregion pre-workshop exchange ("-"= no sign of bias (p > 0.05); "*" = possibility of bias (0.01 < p < 0.05); "" = certainty of bias (p < 0.01))**

	2 DNK1	3 DNK2	4 SWE1	5 SWE2	7 SWE3	8 NOR4	9 NOR5	11 NOR1	12 NOR2	13 NOR3	14 FRA1	15 NLD1	16 NLD2	17 IRL1	19 DEU1
2_DNK1	NA	-	-	-	-	-	-	-	-	-	-	*	-	**	-
3_DNK2	-	NA	-	-	-	-	-	-	-	-	-	*	-	**	-
4_SWE1	-	-	NA	-	-	-	-	-	-	-	-	*	-	**	-
5_SWE2	-	-	-	NA	-	-	-	-	-	-	-	*	-	**	-
7_SWE3	-	-	-	-	NA	-	-	-	-	-	-	*	-	**	-
8_NOR4	-	-	-	-	-	NA	-	-	-	-	-	*	-	**	-
9_NOR5	-	-	-	-	-	-	NA	-	-	-	-	*	-	**	-
11_NOR1	-	-	-	-	-	-	-	NA	-	-	-	**	*	**	-
12_NOR2	-	-	-	-	-	-	-	-	NA	-	-	-	-	**	-
13_NOR3	-	-	-	-	-	-	-	-	-	NA	-	*	-	**	-
14_FRA1	-	-	-	-	-	-	-	-	-	-	NA	*	-	**	-
15_NLD1	*	*	*	*	*	*	*	**	-	*	*	NA	-	**	**
16_NLD2	-	-	-	-	-	-	-	*	-	-	-	-	NA	**	-
17_IRL1	**	**	**	**	**	**	**	**	**	**	**	**	**	NA	**
19_DEU1	-	-	-	-	-	-	-	-	-	-	-	**	-	**	**NA**
Modal Age	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	*	**-**	**	**-**

Results based on expert readers only

When the results are based on only those readers who provide age data for use in stock assessment the percentage agreement increases to 96.6% (Table 2.5.3.6), the CV (Table 2.5.3.7) decreases to 5.5% and APE decreases to 3.8% indicating that there is an overall improvement in the results. The overall relative bias value of -0.02 (Table 2.5.3.8) indicates a slight underestimation of the ages compared with modal age.

As there is no stock assessment carried out for sprat in the Celtic Seas Ecoregion an age error matrix (AEM) based on only readers providing ages for assessment was not possible. In order to give an indication of the possible errors around the ages of sprat in this area an AEM has been compiled based on all of the expert readers who attended the workshop.

The AEM (Table 2.5.3.9) shows that the proportions of fish aged in agreement with modal age for each age group is high (from 100% at age 0 to 83% at age 3). At modal age 1 a small proportion of the samples are both underestimated and overestimated by 1 year while at modal ages 2 and 3 there is a tendency is to underestimate. In all cases there is only a difference of one year compared with modal age.

Table 2.5.3.6. Percentage agreement based on modal age for only expert readers in the Celtic Seas Ecoregion pre-workshop exchange

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	100	100	100	100	100	100	100	100	100	100	100
1	98	100	100	100	100	96	93	96	96	98	98
2	89	96	96	96	96	96	85	96	89	96	94
3	100	100	100	100	33	100	33	100	67	100	83
Weighted mean	95.8	98.9	98.9	98.9	96.9	96.6	89.9	96.6	93.7	97.8	96.6
Rank	**8**	**1**	**1**	**1**	**5**	**6**	**10**	**6**	**9**	**4**	**-**

Table 2.5.3.7. Coefficient of variation (CV) between the modal age and the age estimation of expert readers in the Celtic Seas Ecoregion pre-workshop exchange.

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	-	-	-	-	-	-	-	-	-	-	-
1	13	0	0	0	0	18	27	19	18	13	5.1
2	17	10	10	10	10	10	20	10	17	10	5.6
3	0	0	0	0	25	0	25	0	22	0	11
Weighted Mean	12	2.8	2.8	2.8	3.5	12.9	21.4	13.4	15.5	10.1	5.5
Rank	**6**	**1**	**1**	**1**	**4**	**7**	**10**	**8**	**9**	**5**	**-**

Table 2.5.3.8. Relative bias values based on modal age for only expert readers in the Celtic Seas Ecoregion pre-workshop exchange (values in red indicate a negative bias, those in black indicate a positive bias).

Modal Age	2_DNK1	3_DNK2	4_SWE1	8_NOR4	9_NOR5	11_NOR1	12_NOR2	13_NOR3	14_FRA1	19_DEU1	All
0	0	0	0	0	0	0	0	0	0	0	0
1	0.02	0	0	0	0	0.04	0	0	0.04	0.02	0.01
2	-0.11	-0.04	-0.04	-0.04	-0.04	-0.04	-0.15	-0.04	-0.11	-0.04	-0.06
3	0	0	0	0	-0.67	0	-0.67	0	-0.33	0	-0.17
Weighted Mean	-0.02	-0.01	-0.01	-0.01	-0.03	0.01	-0.06	-0.01	-0.02	0	-0.02
Rank	**7**	**2**	**2**	**2**	**9**	**2**	**10**	**2**	**7**	**1**	**-**

Table 2.5.3.9. Age Error Matrix based on only expert readers for the Celtic Seas Ecoregion exercise (numbers in bold indicate the proportion in agreement with modal age, those in red indicates underestimation compared with modal age while those in black indicate overestimation compared with modal age).Error! Not a valid link.Growth data from the Celtic Sea pre-workshop exchange.

Age | Modal Age	0	1	2	3
0	1	0.01	0	0
1	0	0.98	0.06	0
2	0	0.02	0.94	0.17
3	0	0	0	0.83

Figure 2.5.3.1 shows the combined growth curves for all fish and all readers in the Celtic Seas Ecoregion pre-workshop exchange. The LMEM analysis showed a significant reader effect on both the intercept and slope of the LMEM (LMEM, $p < 0.05$) meaning that there are differences between readers in their interpretation of the first winter ring and the following winter rings. At winter ring 1 and 2 there is very little overlap between the average distances from the centre to the beginning of these 2 rings and even less compared with results from the North Sea. Overall this can be interpreted as the readers are in general agreement as to where to identify winter rings 1 and 2. There are examples where this is not the case, with the result being a significant reader effect in the model analysis. Problematic otoliths were discussed in plenary and notes from this discussion can be found in Table 2.6.1 in Section 2.6. As the average distance from the centre to the winter rings 3 and 4 increases an overlap becomes more apparent and this indicates that the readers are less in agreement as to which structures are in fact the true winter rings.

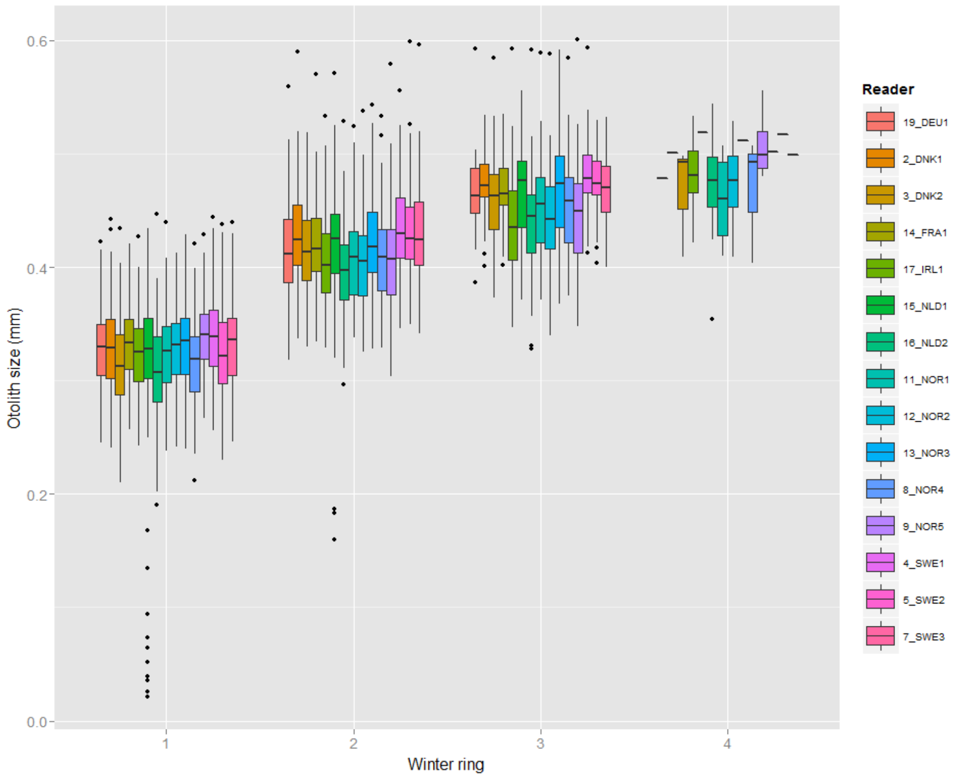


Figure 2.5.3.1. Plot of average distance to the centre for winter rings 1–3 and the edge for all readers in the Celtic Seas Ecoregion pre-workshop exchange. The boxes represent the mean, upper and lower box boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

2.6 Discussion of age determination results of the pre-workshop exchange

During the workshop some example images, where there was both agreement and disagreement among the readers, from the pre-workshop exchange were shown to the group in plenary and the estimated ages and positioning of the annotations were discussed. Age reading issues which became apparent from the analysis of the samples included the identification of the edge type; when to include the transparent outer most edge in the count of age and the interpretation of false translucent zones.

The details of these images and the conclusions formed by the group in plenary can be found in Table 2.6.1. For most images it was possible, after the group discussion, to agree on how the otoliths should be interpreted and to make an agreed age otolith collection (Annex 5). The group decided to include a selection of otolith images where agreement could not be reached in this section as they represent the issues which were not possible to resolve at the workshop but which may be resolved by examination of the otolith microstructure.

The plenary discussion identified a number of regionally specific issues. In the North Sea samples there are many otoliths where the first winter ring will often appear to be more like a translucent band as opposed to a ring which is punctuated by narrow opaque zones. The group agreed to refer to this as a "band" as opposed to a ring, see otolith 6953077 (Figure 2.6.1). As this band varies in tones of grey readers felt it was easier to mark the end of this winter ring as opposed to the beginning and suggested that this be set as an annotation procedure for future exchanges. In the Celtic Seas Ecoregions samples there was discussion around how the edge should appear at different times of the year and when a translucent edge should be included in the count of age. OSEP1117_VIIg (Figure 2.6.2) shows an example of where this can be an issue as readers could not conclude if this was age 2 or 3 but suggested that viewing the otolith under the microscope would help.

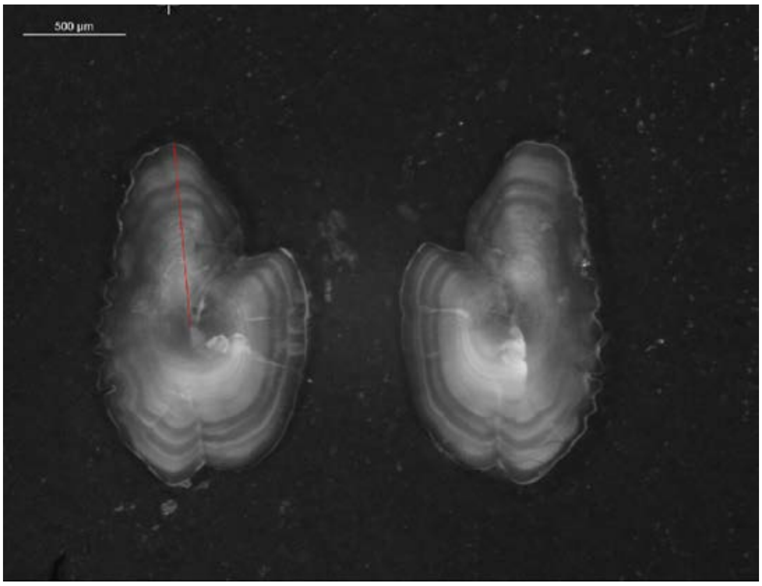


Figure 2.6.1. 6953077_4B, capture date 19/08/2014, North Sea.

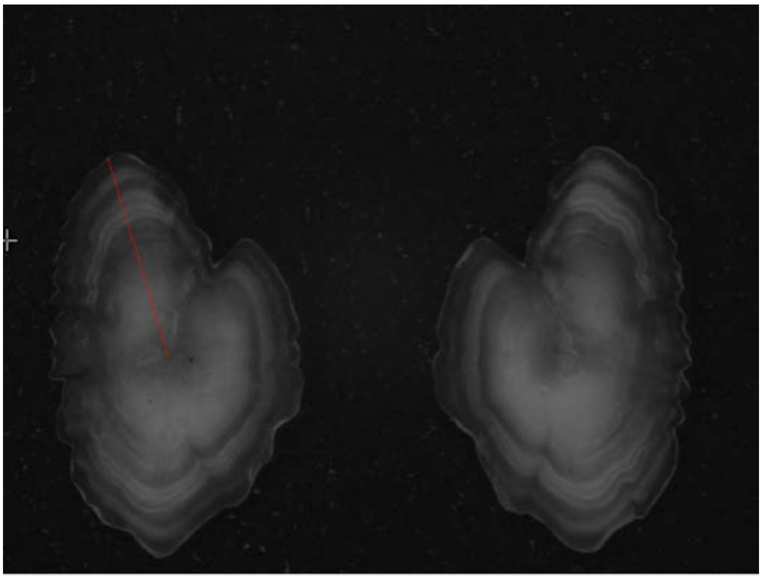


Figure 2.6.2. OSEP1117_VIIg, capture date ??/09/2011, Celtic Seas Ecoregion

Table 2.6.1. Notes made on individual otoliths during the plenary discussion of the pre-workshop exchange grouped by area

	Otolith image	Capture date	Notes	Discussion	Conclusion
North Sea	6953228_4B.jpg	14/09/2014	Modal age 0 but 2 readers assigning age 1	During the pre-workshop exercise only two readers assigned an age 1. After some discussion the group agreed on age 0 and to include it in age reference collection.	- Unanimous agreement of age 0 - Suitable for inclusion in the reference collection
	6953077_4B.jpg	19/08/2014	Only 67% agreement Modal age 1,but many readers assigning age 2	The group concluded that the two inner bands should be considered one zone. This zone formation has not previously been described in sprat and group concluded it must be described for new readers. MIA would need to be conducted to see if there is a sharp or gradual reduction in the width of increments leading up to and between this split band. If there is a sharp and not gradual transition then it is clear that these are false rings, however it is much more biologically likely that these are just punctuated periods of feeding during the winter period. For both aging exercises, pre-workshop and workshop, we have marked the beginning of the winter ring for the purposes of measuring the size of an annulus and counting the number of annuli. However it has been noted by this workshop that it is too difficult to identify the beginning of the first winter ring, therefore we suggest that for annotation purposes we should mark the end of the winter zone as it is clearer. It has been suggested that this could be an age two and that it would be important to consider the over-	- No agreed age - There is a need to define the structure of the first winter ring on a regional basis. - Within the protocol we need to specify that the annulus should be marked at the end and not at the beginning. - There is a need to discuss the biological relevance of the size and structure of the first winter rings.

				all "signal" from the original sample from which it came. For the workshop exercise I think that for the Celtic Sea ecoregion it may be possible to too use the proportions of the zones (T and O) to ID the start of the rings, however that does not seem consistent in the North Sea and 3.a where the propositions seem even sometimes and not others.	
	7116379_4B.jpg	23/06/2015	Modal age 1, some age 2 and one age 3 because of the edge Similar problem with double ring structure	This is another example of a double ring. It was discussed by the group and decided to be an age 1, it was noted that the very thin edge may only be a brief cessation in feeding and may not be a true winter ring.	- Agreed age of 1 - Suitable for inclusion in the reference collection
	7116356_4B.jpg	23/06/2015	Modal age 1, one age 2	The group agreed an age of 1	- Unanimous age 1- Suitable for inclusion in the reference collection
	7116359_4B.jpg	23/06/2015	Modal age 1, one age 2	The group agreed an age of 1, this otolith was described as a good example of an age 1	- Unanimous age of 1yr old - Suitable for inclusion in the reference collection
	7116382_4B.jpg	23/6/2015	Modal age 1 but age 1, 2 and 3 also given	Group concluded it was an age 1, with a slight translucence at the edge, however they felt to be sure they need to see it under the microscope.	- Inconclusive
	7116383_4B.jpg	23/6/2015	Modal age 1 one reader age 2	Group agreed an age of 1, quite good growth, thin transparent edge may be just because it is thin so they called it an opaque edge. One reader forgot to annotate the ring.	- Agreed age 1 - Suitable for inclusion in the reference collection

	7190397_4B.jpg	24/08/2015	Modal age 1 % agreement of 53%	Group agreed an age of 1, the first ring was described as very thin and vague. The edge was described as transparent as the winter ring is starting to appear. It was described as a transparent zone as you can follow it in all three sides.	- Agreed age 1 - Suitable for inclusion in the reference collection
	7216460_4C.jpg	09/12/15	Modal age 1 but age 1,2, and 3 also given	Age is 1, clear winter ring at edge.	- Agreed age of 1 - Suitable for inclusion in the reference collection
	6953092_4B.jpg	19/08/2014	Modal age 2ages 1 2 3 % agreement of 47%	Initially the group decided it was an age 1 as the shape does not look like a 0 group and the fish is 12 cm. Has this fish experienced a really difficult life? It has many thin transparent lines It could possibly be the third year of its growth and a very thin otolith. Need to identify why this one is very different. Later in the discussion the group decided not to set an age for this.	- Inconclusive
	7235987_4B.jpg	14/02/2016	Modal age 3,	The group decided that this must be an age 2 fish. It was concluded that the inner ring was false as it is not the shape of a juvenile otolith. The size of this fish is 11.5	- Agreed age of 2yr - Suitable for inclusion in the reference collection
Celtic	IGFS06111320 _VIIg.jpg	05/11/2009	Modal age 0 2 readers (16 and 17) age 1	We conclude that this is age 0	- Agreed age of 0 - Suitable for inclusion in the reference collection

	IGFS1317840 _VIIb.jpg	28/11/2009	Modal age 0 Reader (16) age 2	We conclude that this is age 0	- Agreed age of 0 - Suitable for inclusion in the reference collection
	01814S_446_6 _VIa.jpg	27/11/2014	Modal age 1 But a few reading age 2;	Age 1	- Agreed age of 1 - Suitable for inclusion in the reference collection
	IGFS134523_ VIa.jpg	03/10/09	Modal age 1 some age 2 % agreement at 60%,	Age 1, unanimous	- Agreed age of 1 - Suitable for inclusion in the reference collection
	NWHAS01071401 _VIa.jpg	01/07/2010	Modal age 1 1 reader age 2 due to inclusion of edge	Age 1, with good year growth, edge type is slight but not officious Transparent so marked as Opaque as this transparency may be due to the thin nature of the growing otolith	- Agreed age of 1 - Suitable for inclusion in the reference collection
	NWHAS01071413 _VIa.jpg	01/07/2010	Modal age 2 But readers also marking age 1 2 3 % agreement 73%.	The group concluded an age of 2 based on broken otolith, the two otoliths present look different but are in fact the same	- Agreed age of 1 - Suitable for inclusion in the reference collection
	OSEP1117_VIIg.jpg	??/09/2011	Modal age 3 But a lot reading age 2 due to inclusion of edge	There was no agreement on the age, the group decided it was probably an age 2 but possibly a 3 and they would have to see it under the microscope to be sure.	- Inconclusive

2.7 Conclusion

Overall the results from the 2 exercises in the pre-workshop exchange were good with an overall high level of agreement between readers. The higher agreement and lower CV on samples from the Celtic Seas Ecoregion indicates otoliths from this area are easier to read compared with those from the North Sea. These results were further improved by excluding trainee readers and therefore basing the results on expert readers alone. The AEMs compiled for both areas show errors of only 1 year around modal ages 1 and 2 with only a small proportion of the otoliths being incorrectly aged. No stock assessment is carried out in the Celtic Seas Ecoregion however in the North Sea the age data are used for assessment purposes. Although the AEM for this area cannot be directly incorporated into the assessment models it gives an indication of the error around the ages and whether or not some adjustments should be made to the data. The age reading issues which were most apparent from the exercises were; the interpretation of translucent band which has some opaque zones within it, misinterpretation of the edge type and when to include a translucent zone at the edge in the count of age. The agreed age reading protocol (Section 4.6) and agreed age reference collection (Annex 5) were compiled in plenary and readers should refer to these when in doubt about any of these issues.

3 ToR b) Follow the development of age estimation and validation studies being undertaken

During the Working Group on Biological Parameters (WGBIOP, 2016) the importance of age validation studies in supporting the quality assurance of age data, which is an important biological parameter used in fish stock assessment was noted. The group concluded that future otolith exchanges and workshops should include input from validation studies and/or techniques and that the recently submitted CRR 'Handbook of fish age estimation protocols and validation methods' be referred to as part of the preparatory work.

Time was allocated on the WKARSPRAT agenda to conduct a tutorial on microincrement analysis as a validation tool. However due to time constraints, and effort being focused on discussion of problematic otoliths, the full tutorial was not possible. The participants were given a tour of the laboratory where the mounting, polishing, and photographing of images for microincrement analysis is conducted and a demonstration of the software used for counting and measuring daily rings was given.

A validated and reproducible description of age structure forms an important building block by which to define and assess a fish stock. Error in age determination introduces systematic bias to stock models and can lead to over/underestimation of the stock size and can even result in stock collapse (Campana, 2001). In order to eliminate error in an aging process two steps must be taken; first to estimate accuracy and reproducibility of the process and second to validate the process itself across all ages. For sprat, the estimation of accuracy and reproducibility of the ageing process is conducted at an international level at the ICES workshop on the age estimation of sprat (WKARSPRAT) where age reading exercises are conducted to calibrate age readers. This process was reinforced during this workshop with the development of a generally accepted protocol (Section 4.6) and the composition of a reference collection of agreed age otoliths (Annex 5). Accuracy and reproducibility are also estimated on a national level with institutes conducting their own inter-calibration exercises.

A validated ageing method confirms the frequency of formation of growth increments in a given structure, and confirms that they have been interpreted correctly by the age readers (Campana, 2001). Additionally, many of the issues encountered by sprat age readers, such as the identification of false rings (Torstensen, Eltink, Casini, McCurdy, and Clausen, 2004) can be resolved using these validation techniques. Although the validation of absolute age would be ideal this is often not possible due to the cost and time required (Campana, 2001). Therefore two steps must be taken to validate this aging process; first to validate the formation of the first winter ring and second to verify increment periodicity across the entire age range of interest, as growth of immature fish seldom resemble those of mature fish (Campana, 2001). This information not only validates the process of aging but acts as a guide by which future age readers can develop their skills. An essential prerequisite to the above mentioned method is to validate the production of daily increments which was successfully done for sprat under laboratory conditions by Alshuth (1988). To date four validation studies have been completed on the age estimation of sprat, all of which are summarized below.

Mosegaard and Baron (1999) used the pattern of daily increment (DI) widths to identify the difference between true and false winter rings. Using 0-group sprat otoliths caught in the Danish Belts and the Bornholm Basin of the Eastern Baltic (ICES Subdivisions 22 and 25) they demonstrated that prior to the winter ring being deposited the width of the daily increments gradually reduces (Figures 3.1 and 3.2). If there is no gradual reduction in the width of the daily increments formed prior to the translucent ring then the translucent ring is not a true winter ring, perhaps an unexpected punctuated period of bad feeding or poor growth conditions. Therefore, in otoliths where the age reader is in doubt as to whether a translucent zone is true or false the validity of the ring can be examined using the otolith microstructure.

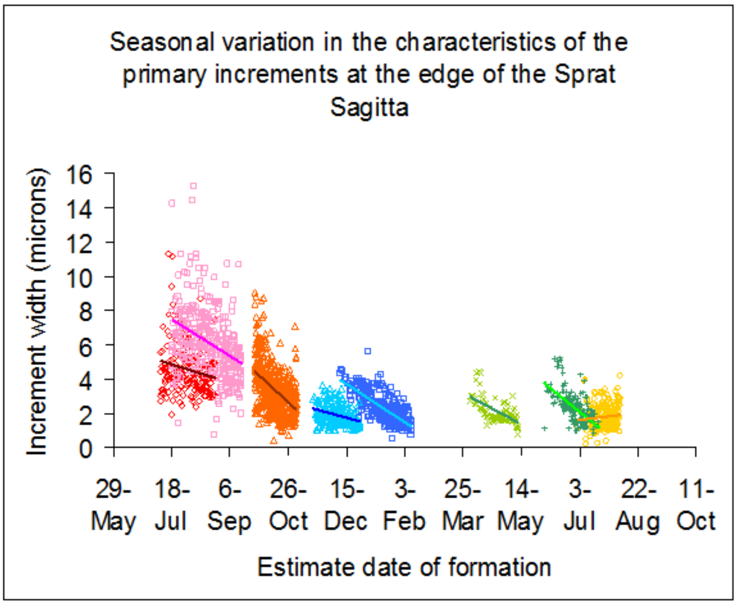


Figure 3.1. Development of daily increment width on the edge of sprat otoliths from individuals caught during winter, demonstrating the decrease in increment width prior to the formation of the true winter ring (Mosegaard and Baron, 1999).

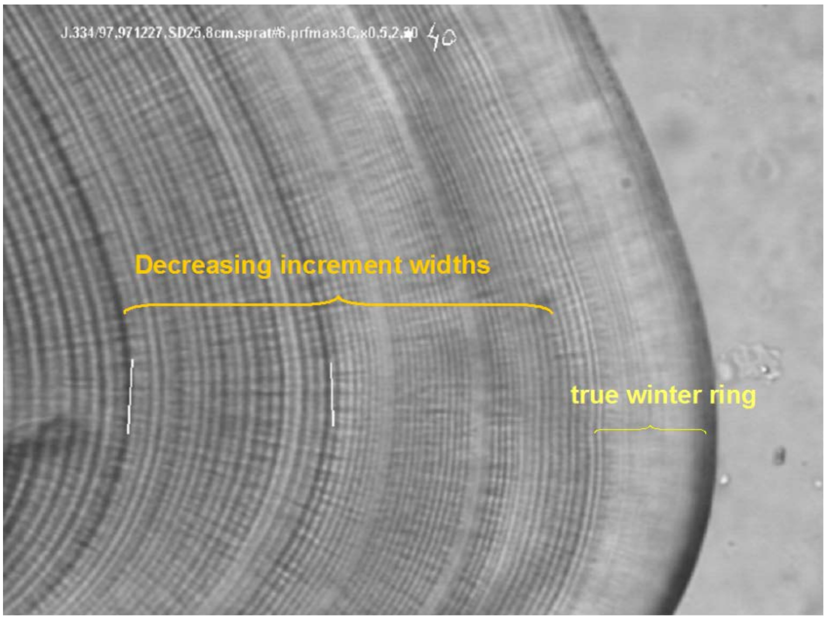


Figure 3.2. Development daily increments at the edge of sprat otolith from an individual caught in December (Mosegaard and Baron, 1999).

Torstensen *et al.*, (2004) used marginal increment analysis (MIA) to explore the trends in cyclical deposition of annuli and the age readers precision in identifying the completion of these annuli. Sprat used in this study were caught in the Skagerrak and Kattegat between February 2003 and January 2004. MIA is based on the premise that if a growth increment is formed on a yearly cycle then the average state of completion of the outermost increment should display a yearly sinusoidal cycle when plotted against time (Campana, 2001). Using this technique Torstensen *et al.,* (2004) found evidence that a translucent zone (winter ring) was laid down once a year (Figure 3.3). The width of the outermost increment decreased slowly between February and May. The deposition of the outermost translucent ring was completed during summer, June and July, and is corroborated by the sudden drop in the increment width of the outermost ring between May and July. The 0-group sprat did not follow this sinusoidal pattern, which the authors attribute to the long spawning period (March to July) and consequently the late development of the first winter ring. The results of the MIA showed that the growth rate of sprat otoliths decreases with increasing age of the fish (Figure 3.4). This study is highly informative about the growth and progression of zone development within sprat otoliths caught in the Skagerrak and Kattegat. However, it is important to note that this is just a snapshot, both spatially and temporally, and the varied growth rates characteristic of sprat (Peck *et al.*, 2012) means that these results may not be directly applicable to other areas. Additionally, only one tool (MIA) was used to elucidate the pattern in growth, therefore to consider this a complete validation study more work needs to be done to corroborate this development across all ages of sprat within the region.

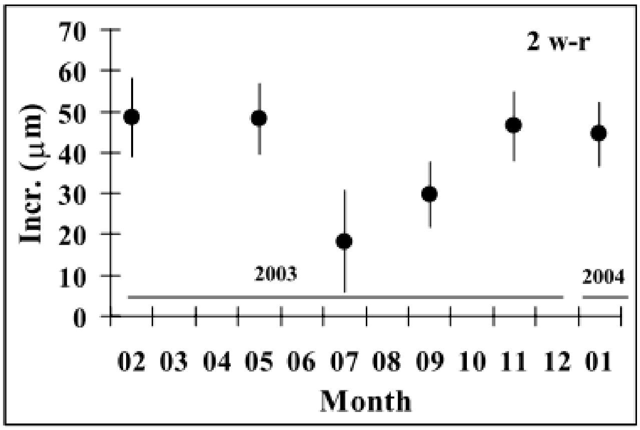


Figure 3.3. Marginal increment analysis of age 2 (2 w-r) sprat caught in the Skagerrak Torstensen *et al.* (2004).

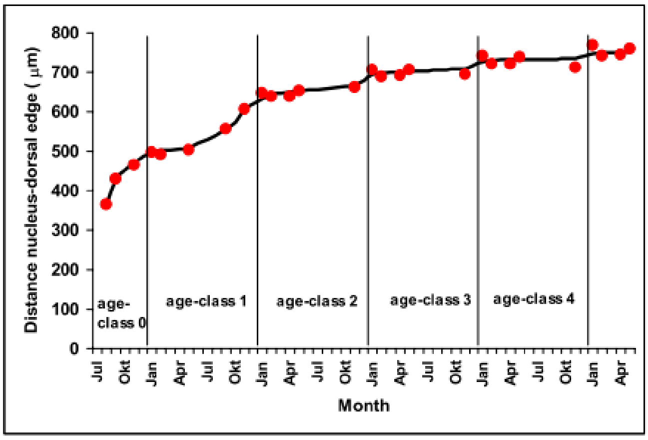


Figure 3.4. Otolith growth in Skagerrak, measured from the nucleus to the dorsal edge, across all available age ranges Torstensen *et al.* (2004).

Another validation study on the age estimation of sprat was conducted in preparation for the benchmark workshop on sprat stocks (WKSPRAT, 2013). This study used 61 0-group sprat caught in the North Sea during 2010 and 2011. Counts of DI were used to validate the formation of the first winter ring. A measurement from the core to the beginning of the winter ring was recorded. This measurement was then compared to the markings made by an experienced reader who identified the formation of the first winter zone during three separate readings of the imaged whole otolith. The variation in the appearance of the first winter-ring as identified by the age reader and validated using the otolith microstructure analysis is described below (Figure 3.5). The variation in width of the first identified winter ring did not differ significantly between otoliths; however, this analysis was based on one reader only.

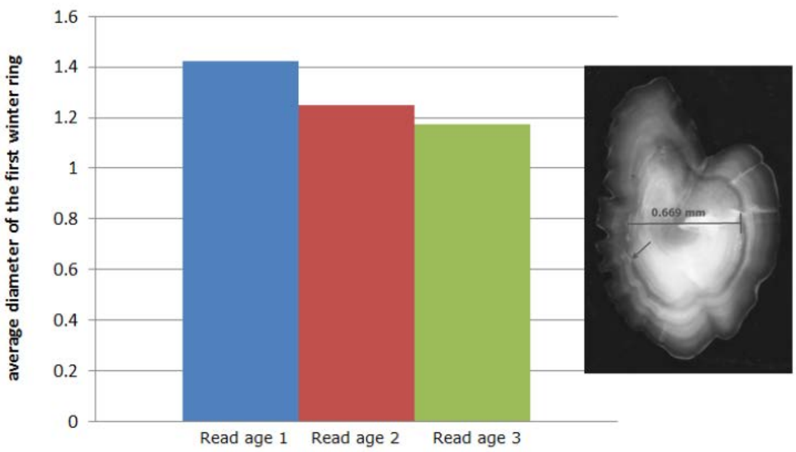


Figure 3.5. Average width of the growth during the first season until the first true winter ring (mm) in sprat aged 1–3 (Taken from WKSPRAT, 2013).

Moore *et al.* (in press) validated the age estimation of sprat in the Celtic Seas Ecoregion, a process which included the validation of the first winter ring, corroboration of age estimation in later annuli and description of general patterns of otolith development across all available ages in the region. The deposition of the first winter ring was validated on six 0-group fish. Counts of DI revealed a consistent pattern, with clearly defined increments from the nucleus to the edge, increasing steadily to maximum width of 0.3 microns, thereafter decreasing and finally fading away at an average of 175 days (±3 SE). Three readings were made for each otolith and were found to have an overall ACV (average coefficient of variation) of 5.34%.

The results of this validation study were supported and related to subsequent annuli formed in age 1, 2 and 3 fish using two accepted and commonly used validation tools (Campana, 2001); marginal increment ratio (MIR) and edge type analysis. The MIR of 127 otoliths demonstrated a movement from a low value in June to a high value in September and showing a transition from a growth phase (opaque) in June, July, to a phase of slow or no growth (transparent) in September (Figure 3.6). These findings were further corroborated with the results of the edge type analysis (n = 551) where the proportion of opaque edge present in June was 60% which swiftly reduced to 15% in July and eventually disappeared completely in September (Figure 3.7), demonstrating the transition between the period of growth and winter.

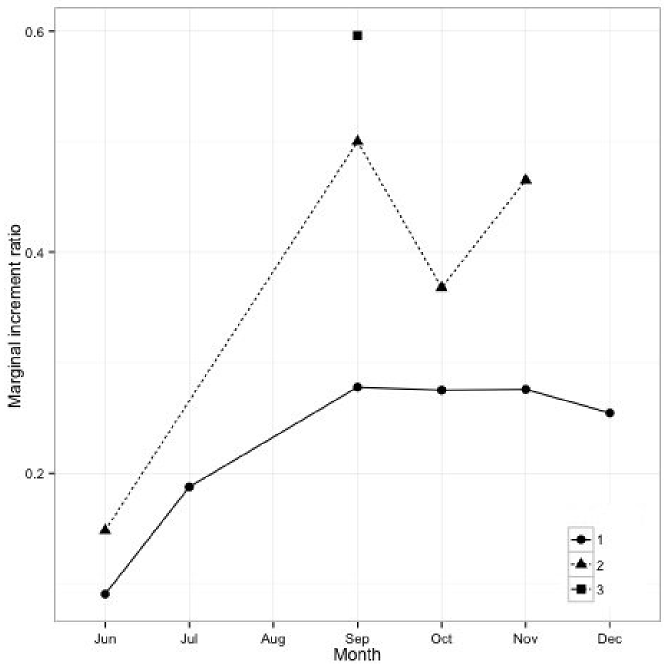


Figure 3.6. Marginal Increment ratio in relation to month of capture, plotted separately per age class. Note only 1 age three fish was included in this analysis due to small numbers in sample Moore *et al.* (in press)

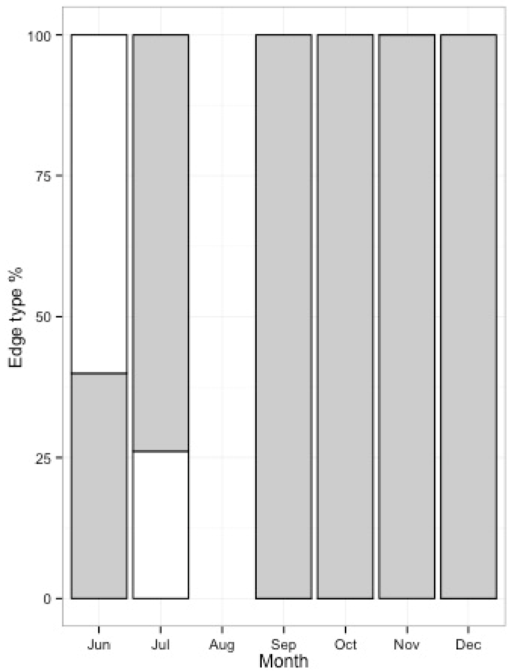


Figure 3.7. Visual appearance of the otolith edge as a percentage of the whole number of otoliths analysed each month (white, opaque, black, transparent). The proportion of edge type present in the sprat otoliths by month of capture. Moore *et al.* (in press)

The general pattern of otolith development was described and analysed for trends in the characteristics of the annuli. The first and the second opaque zone differed significantly in size ($F(1,158) = 735.6$, *p_value* = < 0.001, $R^2 = 0.823$), with the first opaque zone being three times the size of the second opaque zone. No significant difference was found in the size of the first and second transparent zone (($F(1,156) = 12.74$, *p_value* = < 0.001, $R^2 = 0.076$) (Figure 3.8). This description of growth was discussed during the workshop and was found to be very useful when describing the aging process of sprat in the Celtic Seas Ecoregion to new readers and helped to inform readers on how to identify false winter rings.

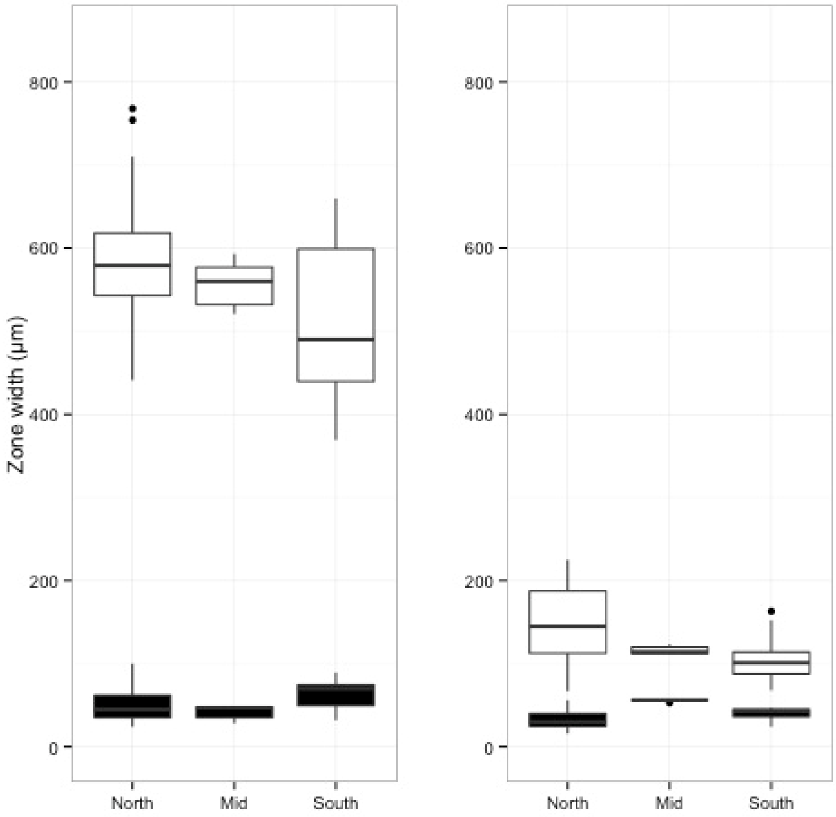


Figure 3.8. Plot of the average width of the opaque (white) and transparent (black) zones of the first (left panel) and second (right panel) annulus of sprat within the Celtic Seas Ecoregion Moore *et al.* (in press).

As with previous sprat age reading workshops and exchanges (Torstensen *et al.*, 2004; Korniovs *et al.*, 2006; Davies *et al.*, 2014) the importance of validation studies on the age estimation of sprat was noted by WKARSPRAT 2016. It was evident during this workshop that there is a wide variation in sprat otolith development between study regions and therefore individual validation studies are an essential requirement for each of these study regions.

To encourage the implementation of validation studies a presentation on the validation of sprat within the Celtic Seas Ecoregion and a short tutorial on the analysis of otolith microstructure using ObjectJ (Vischer and Nastase, 2016) were given during the workshop.

4 ToR c) Analyse growth increment patterns in sprat and compile guidelines for the interpretation of sprat otoliths;

The objective of this section is to document the various growth patterns seen in the otoliths from the different sprat stocks and to standardize how the readers interpret these structures when estimating the age of the fish from these stocks. It includes the discussion of otoliths which were brought to the workshop by the readers from Norway (Section 4.1), the age reading exercises which were completed at the workshop for each stock (Section 4.2), the national age reading protocols (Section 4.5) and finally the agreed age reading protocol (Section 4.6) compiled at the workshop and based on the findings of the workshop.

4.1 Examination of North Sea otoliths brought by readers to the workshop

On the first day of the workshop time was spent looking at samples which readers had brought with them for examination by the group. The sample images which were displayed on the large screen and discussed in plenary were those brought by the Norwegian readers, caught in the North Sea in January 2016. The main age reading issues which were discussed by the group based on the samples were 1) the occurrence of a wide translucent "band" composed of thin areas of opaque and translucent material and 2) the occurrence of a faint translucent ring which may possibly be the first winter ring. Both the date of capture and fish length were considered when discussing these images. The discussion was very useful as both issues are common problems when age reading sprat from the North Sea area.

Figure 4.1.1 is representative of the issue 1. From the discussion the group decided that this fish should be estimated to be 2 years old as it is a common feature of North Sea sprat otoliths that the first winter ring is often a band of translucent material punctuated by narrow opaque zones which should be counted as just 1 winter ring (the second winter ring can be seen on the otolith outermost edge).

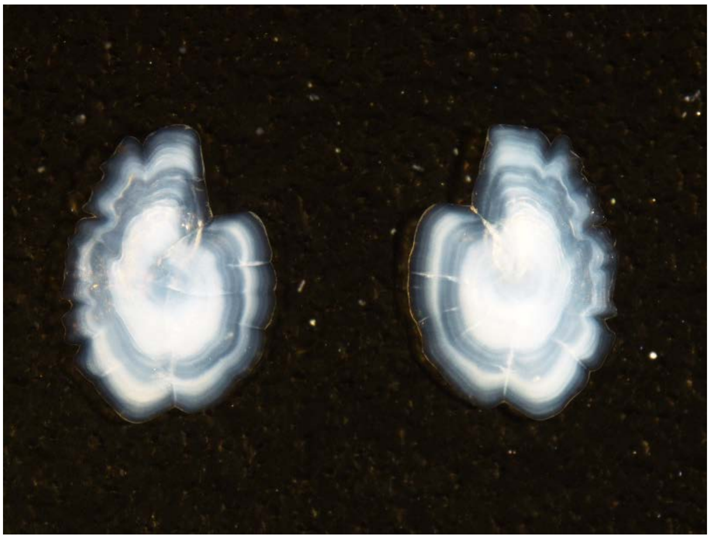


Figure 4.1.1. 86456_28, Capture date 10/01/2016, North Sea

Figure 4.1.2 is representative of the issue 2, where it is unclear whether or not the faint translucent ring should be considered a true winter ring and included in the count of age. It was decided by the group that this otolith is just 1 year old and the faint ring is not a true winter ring. It could be a false winter ring or it could be part of the so called "band" like first winter ring referred to in issue 1 above. At IMR Norway otolith microstructure examination is underway, in order to distinguish between true and false winter rings in otoliths with the abovementioned appearance, the preliminary findings are documented in Annex 6.

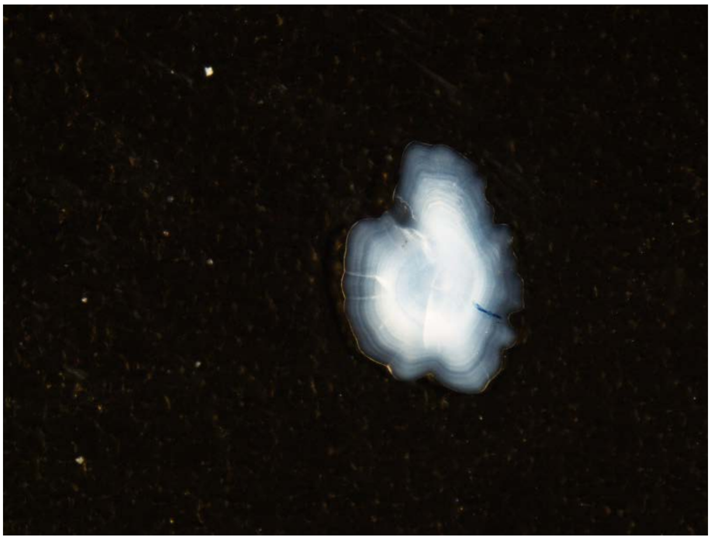


Figure 4.1.2. 86456_34, Capture date 10/01/2016, North Sea

Information which was considered important by the readers during the discussion was that a) a decrease in the width of the opaque zones should be observed from the centre towards the outer edge b) transparent zones are generally wide and maybe composed of bands of alternating translucent and opaque material, c) the visual characteristics of other otoliths from the same sample should be considered when interpreting the pattern of the annuli, d) the biology of the species in the area should be considered and e) the fish length should not be used as guideline for interpreting age.

4.2 Age reading exercises at WKARSPRAT

On day two of the workshop all readers took part in an age reading exercise. Three exercises were provided for the readers, one for each stock; Skagerrak and Kattegat (3.a), North Sea (4) and Celtic Seas Ecoregion (Divisions 6 and 7), (excluding 7d, e).

4.2.1 List of participants

Table 4.2.1.1. List of participants of the age reading exercises at WKARSPRAT

Reader	First name	Last name	Institute	Reads for assessment
1	Ian	McCausland	AFBI Northern (Ireland)	Yes
2	Maria	Jarnum	DTU Aqua (Denmark)	Yes
3	Marianne	Johansson	SLU Aqua (Sweden)	Yes
4	Annelie	Hilvarsson	SLU Aqua (Sweden)	No
5	Eilert	Hermansen	IMR Aqua (Norway)	Yes
6	Jostein	Røttingen	IMR (Norway)	Yes
7	Claire	Moore	MI (Ireland)	No
8	Gertrud	Delfs	Thünen Institute (Germany)	Yes

4.2.2 Samples

Samples were provided by MI Ireland, MARLAB Scotland, DTU Aqua Denmark, and SLU Aqua Sweden. Otolith images were digitized at DTU Aqua using the same standard set up used for the pre-workshop exchange image sets (Section 2.2). Otolith images where then uploaded to WebGR and 3 exercises made available for annotation by the readers; 3.a (Table 4.2.2.1), North Sea (Table 4.2.2.2) and Celtic Sea ecoregion areas (Table 4.2.2.3). The full sample set for 3.a was composed of 100 images but given the time restraints at the workshop only 45 were included in the workshop exercise.

Table 4.2.2.1. Overview of samples from 3.a

Area	Year	Quarter	Length range	n
3.aN	2015	4	100–120mm	8
3.aS	2015	3 and 4	70–150 mm	14
	2014	2 and 3	75–105 mm	9
	2013	1	125–145mm	14
Total				**45**

Table 4.2.2.2 Overview of samples from the North Sea

Area	Year	Quarter	Length range	n
4.b	2016	1	125–130 mm	4
	2015	2 and 3	90–115 mm	10
4.c	2016	3	80–140 mm	16
Total				**30**

Table 4.2.2.3 Overview of samples from the Celtic Seas Ecoregion

Area	Year	Quarter	Length range	n
6.a	2015	1	105–125 mm	3
	2014	2, 3, and 4	85–130 mm	6
	2013	4	140 mm	1
7.b	2014	4	95–115 mm	4
	2013	4	85–110 mm	3
7.g	2014	4	80 mm	1
	2013	4	95–100 mm	2
	2011	3	115–130 mm	3
7.j	2014	4	100–115 mm	4
	2013	4	110–115 mm	3
Total				**30**

4.2.3 Methods

Three exercises were made available on WebGR, one for each area; 3.a (Skagerrak and Kattegat), North Sea, and Celtic Seas Ecoregion and the corresponding otoliths were available for the readers to view under microscopes. As the 3.a set had not been included in the pre-workshop exchange this was the first time that the majority of the readers looked at otoliths from this region. The readers were asked to give an estimate of age and to follow the same guideline for annotation outlined in the pre-workshop exchange; where the first annotation is placed on the centre point, and then the beginning of each winter ring is marked in succession towards the otolith edge, with a final annotation placed on the otolith edge. Each image had a defined line along which the readers should place their annotations. Readers were provided with an excel sheet on which to record the readability scale for each otolith. They were also asked to record the edge type based on an examination of the otoliths under the microscope. As the readers had found it difficult to define the edge type precisely using the scale from the pre-workshop exchange they were asked to define the edge type as either “T” for transparent or “O” for opaque based on a visual examination of the otoliths under the microscope.

4.2.4 Analysis

The same methods were applied as to those used in the pre-workshop exchange (see section 2.4). Each area was analysed separately.

4.2.5 Results

4.2.5.1 Age data from the 3.a (Skagerrak and Kattegat) workshop exercise

Only Denmark and Sweden routinely read sprat otoliths from this area with reader 3_SWE1 being the most experienced of the three readers present at the workshop from these two laboratories. When analysing the results of this exercise and given the difficulties in age estimation of sprat from this area the readers’ level of experience was taken into consideration and it was decided to use the age estimations of 3_SWE1 as modal age. It should also be taken into consideration that the sample set consists of just 45 otoliths as there was not enough time for the readers to read the full set of 100.

When comparing all of the other readers against the age estimations of reader 3_SWE1 the overall percentage agreement was just 68.6% (Table 4.2.5.1.1) with an overall CV of 22.8% (Table 4.2.5.1.2). With just 3 fish at modal age 0 the percentage agreement was low at 79%, one of these otoliths had a percentage agreement of just 50% due to the presence of a faint translucent zone which some of the readers counted as a ring. The microstructure of this otolith will be studied to identify if this is in fact a true winter ring. The percentage agreement increases for modal age 1 to 94% but drops to just 33% at modal age 2 and remains low. At modal age 4 there are just 4 fish and at modal age 5 just 1 fish.

Table 4.2.5.1.1. Percentage agreement using the age estimations of 3_SWE1 as modal age, for all readers in the 3.a workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	67	100	100	33	67	67	100	100	79
1	95	86	100	95	95	95	91	95	94
2	33	33	100	33	33	0	0	33	33
3	8	42	100	75	33	0	75	50	48
4	0	0	100	25	0	0	25	25	22
5	0	0	100	0	0	0	0	0	13
Weighted mean	55.2	62.1	100	73.1	61.9	50.9	73.4	70.9	**68.6**
Rank	7	5	1	3	6	8	2	4	-

Table 4.2.5.1.2. Coefficient of Variation (CV) between the age estimations of reader 3_SWE1 as modal age and the age estimations for all readers in 3.a workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	-	-	-	-	-	-	-	-	-
1	20	31	0	20	20	20	31	20	12.1
2	43	50	0	43	43	0	0	43	36.5
3	36	43	0	31	33	35	24	28	31.5
4	0	29	0	35	18	40	35	35	39.3
5	0	0	0	0	0	0	0	0	46.6
Weighted Mean	33.8	32.5	0	29.8	34.6	34.2	24.7	23.2	**22.8**
Rank	6	5	1	4	8	7	3	2	-

The high overall CV of 22.8% can be partly attributed to the large number of fish with a modal age range of 1–3 years. The APE which takes the age of the fish into consideration is lower at 16.9%.

The relative bias values (Table 4.2.5.1.3) show that the readers are overestimating at age 0 and 1 compared with the age estimations of the most experienced reader of 3.a sprat, whereas at ages 2–5 there is a large negative bias indicating that the readers are underestimating compared with this expert reader. The inter reader bias tests (Table 4.2.5.1.4) and the age bias plots in Annex 5 confirm these results.

Table 4.2.5.1.3. Relative bias values between the ages of 3_SWE1 as modal age and the age estimations of all readers in the 3.a workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	0.33	0	0	0.67	0.33	0.33	0	0	0.21
1	0.05	0.14	0	0.05	0.05	0.05	0	0.05	0.05
2	-0.67	0	0	-0.67	-0.67	-1	-1	-0.67	-0.58
3	-1.25	-0.92	0	-0.42	-0.83	-1.5	-0.33	-0.58	-0.73
4	-2	-2.25	0	-1.25	-1.25	-2.75	-1.25	-1.25	-1.5
5	-3	-1	0	-3	-2	-4	-3	-2	-2.25
Weighted Mean	-0.58	-0.4	0	-0.27	-0.38	-0.75	-0.33	-0.33	**-0.38**
Rank	**7**	**6**	**1**	**2**	**5**	**8**	**3**	**3**	**-**

Table 4.2.5.1.4. Inter-reader bias tests for all readers in the 3.a workshop exercise, with the age estimations of 3_SWE1 used as modal age ("-"= no sign of bias (p>0.05); "*" = possibility of bias (0.01 < p < 0.05); "" = certainty of bias (p < 0.01))**

	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1
1_GBR1	NA	-	**	**	-	-	*	*
2_DNK1	-	NA	**	-	-	**	-	-
3_SWE1	**	**	NA	*	**	**	**	**
4_SWE2	**	-	*	NA	-	**	-	-
5_NOR1	-	-	**	-	NA	**	-	-
6_NOR2	-	**	**	**	**	NA	**	**
7_IRL1	*	-	**	-	-	**	NA	-
8_DEU1	*	-	**	-	-	**	-	NA
Modal Age	**	**	**-**	*	**	**	**	**

Results based only on the readers who provide ages for stock assessment

When the results are based on only those readers who provide age data for stock assessment the percentage agreement decreases slightly to 67.8% (Table 4.2.5.1.5) and the CV decreases to 22.3% (Table 4.2.5.1.6). The APE is unchanged at 16.9%. These results indicate that there are no improvements in the results.

Table 4.2.5.1.4. Percentage agreement using the age estimations of 3_SWE1 as modal age, for only expert readers in the 3.a workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	67	100	100	67	67	100	83
1	96	87	100	96	96	96	95
2	33	33	100	33	0	33	39
3	8	42	100	33	0	50	39
4	0	0	100	0	0	25	21
5	0	0	100	0	0	0	17
Weighted mean	56.6	63.1	100	63.1	52.4	71.9	67.8
Rank	**5**	**3**	**1**	**3**	**6**	**2**	**-**

Table 4.2.5.1.6. Coefficient of Variation (CV) between the age estimations of reader 3_SWE1 as modal age and the age estimations of only expert readers in the 3.a workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	-	-	-	-	-	-	-
1	20	30	0	20	20	20	9.3
2	43	50	0	43	0	43	37.5
3	36	43	0	33	35	28	34.4
4	0	29	0	18	40	35	43.8
5	0	0	0	0	0	0	47.1
Weighted Mean	33.5	32	0	34.3	33.9	23.2	22.3
Rank	**4**	**3**	**1**	**6**	**5**	**2**	**-**

Table 4.2.5.1.7. Relative bias values between the age estimation of 3_SWE1 as modal age and the age estimations of only expert readers in the 3.a workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	0.33	0	0	0.33	0.33	0	0.17
1	0.04	0.13	0	0.04	0.04	0.04	0.05
2	-0.67	0	0	-0.67	-1	-0.67	-0.5
3	-1.25	-0.92	0	-0.83	-1.5	-0.58	-0.85
4	-2	-2.25	0	-1.25	-2.75	-1.25	-1.58
5	-3	-1	0	-2	-4	-2	-2
Weighted Mean	-0.57	-0.39	0	-0.37	-0.74	-0.33	**-0.4**
Rank	**5**	**4**	**1**	**3**	**6**	**2**	**-**

As there were only two expert readers providing ages for the 3.a stock assessment it was not possible to make an age error matrix (AEM) for this area. In order to give an indication of the possible errors around the ages of sprat in this area an AEM (Table 4.2.5.1.8) has been compiled based on all of the expert readers who attended the workshop.

Table 4.2.5.1.8. Age Error Matrix based on only expert readers for the 3.a workshop exercise

Age | Modal Age	0	1	2	3	4	5
0	**0.83**	0	0	0	0	0
1	0.17	**0.95**	0.56	0.24	0.17	0.17
2	0	0.05	**0.39**	0.38	0.46	0.17
3	0	0	0.06	**0.39**	0.17	0.33
4	0	0	0	0	**0.21**	0.17
5	0	0	0	0	0	**0.17**

4.2.5.2 Growth data from the 3.a (Skagerrak and Kattegat) workshop exercise

Figure 4.2.5.2.1 shows the combined growth curves for all fish and all readers in the 3.a workshop exercise. The Linear Mixed Effects Model analysis showed a significant reader effect on both the intercept and slope of the LMEM (LMEM, $p < 0.05$) meaning that there are differences between readers in their interpretation of the first and subsequent winter rings. In general there is little overlap between the average distances from the centre to winter ring 1 and 2 and thus these 2 winter rings can be identified as 2 separate rings. However at winter ring 1 there is variation between where the readers annotate this ring to be and this indicates that it is unclear where the first winter ring begins. Reader 1 is annotating the first winter ring to be slightly further from the centre than the other readers but not enough to overlap winter ring 2. There is an overlap between the subsequent rings and this is reflected in the disagreement between readers on the ages and the general difficulties expressed by the group in aging otoliths from this area. Example images were discussed within the group and notes from this discussion can be found in Table 4.3.1. As the average distance from the centre to the subsequent winter rings increases an overlap becomes more apparent and this indicates that the readers are less in agreement as to which structures are in fact the true winter rings.

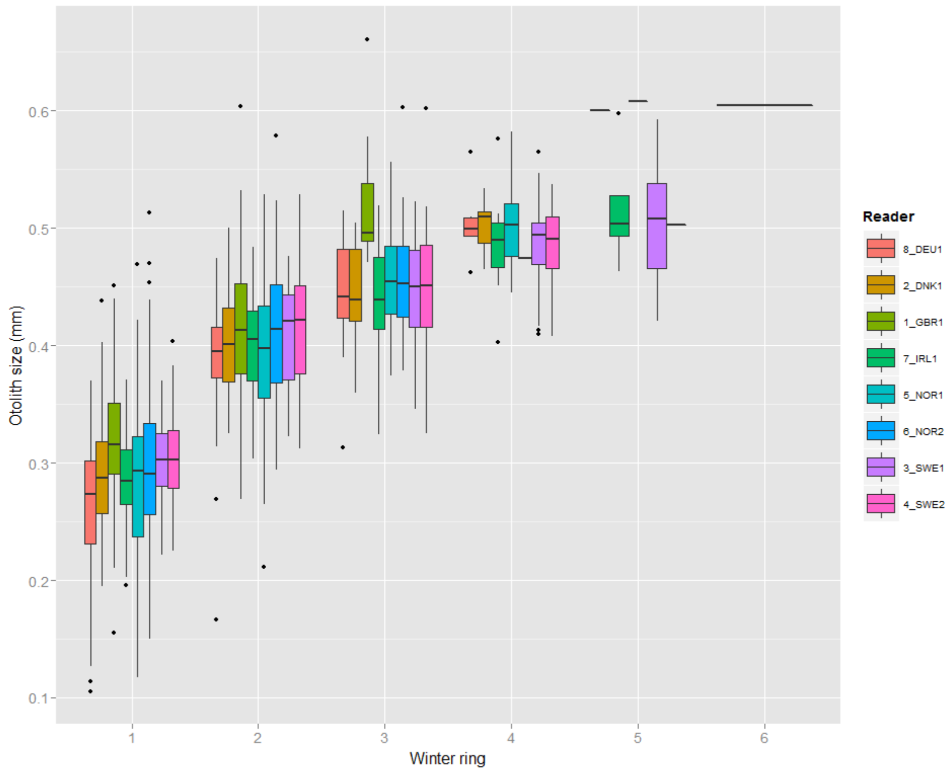


Figure 4.2.5.2.1. Plot of average distance to the centre for winter rings 1–4 and the edge for all readers in the 3.a workshop exercise. The boxes represent the mean, upper and lower box boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

4.2.5.3 Age data from the North Sea workshop exercise

When all of the readers are included in the analysis the overall percentage agreement is 81.5% (Table 4.2.5.3.1) with an overall CV of 20.4% (Table 4.2.5.3.2). Only one reader does not reach 100% agreement at age 0 (based on 1 otolith), however after a group discussion agreement was reached. At modal age 1 the percentage agreement is much lower and mostly due to reader 1 who is overestimating by 1 year. At modal ages 2 and 3 the percentage agreement decreases again (Table 4. 2.5.3.1).

The overall CV is high (20.4%) but it should be taken into consideration that CV in this case is calculated based on a sample set of fish with a young age range. The overall APE is 15.4% and this is calculated taking the age of the fish into consideration.

The relative bias values (Table 4.2.5.3.3) show that at ages 0 and 1 the tendency is to overestimate compared with modal age while at ages 2 and 3 the tendency is to underestimate. The inter reader bias tests (Table 4.2.5.3.4) shows that it is only reader 2 who shows a possibility of bias towards modal age. The age bias plots in Annex 4 confirm these results.

Table 4.2.5.3.1. Percentage agreement based on modal age for all readers in the North Sea workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	100	100	50	100	100	100	100	100	94
1	55	91	100	91	100	82	91	100	89
2	92	62	77	69	92	77	100	77	81
3	0	75	100	75	50	50	25	100	58
Weighted mean	66.7	76.9	86.2	79.9	89.9	76.8	86.2	90	**81.5**
Rank	**8**	**6**	**3**	**5**	**2**	**7**	**3**	**1**	**-**

Table 4.2.5.3.2. Coefficient of Variation (CV) between the modal age and the age estimation of all readers in the North Sea workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	ALL
0	-	-	-	-	-	-	-	-	-
1	36	33	0	28	0	45	28	0	21
2	14	31	26	28	14	25	0	24	19.2
3	0	18	0	18	43	23	22	0	22.6
Weighted mean	19.3	27.9	21.4	24.8	11.8	30.4	13.7	10.4	**20.4**
Rank	**4**	**7**	**5**	**6**	**2**	**8**	**3**	**1**	**-**

Table 4.2.5.3.3. Relative bias values between the modal age and the age estimation of all readers in the North Sea workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	0	0	0.5	0	0	0	0	0	0.06
1	0.45	-0.09	0	0.09	0	0	0.09	0	0.07
2	-0.08	-0.38	-0.08	-0.31	-0.08	-0.23	0	0.08	-0.13
3	-1	-0.25	0	-0.25	-0.75	-0.5	-0.75	0	-0.44
Weighted mean	0	-0.23	0	-0.13	-0.13	-0.17	-0.07	0.03	**-0.08**
Rank	**1**	**8**	**1**	**5**	**5**	**7**	**4**	**3**	**-**

Table 4.2.5.3.4. Inter-reader bias tests for all readers in the North Sea workshop exercise ("-"= no sign of bias (p>0.05); "*" = possibility of bias (0.01<p<0.05); "" = certainty of bias (p<0.01))**

	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1
1_GBR1	NA	-	-	-	-	-	-	-
2_DNK1	-	NA	-	-	-	-	-	*
3_SWE1	-	-	NA	-	-	-	-	-
4_SWE2	-	-	-	NA	-	-	-	-
5_NOR1	-	-	-	-	NA	-	-	-
6_NOR2	-	-	-	-	-	NA	-	-
7_IRL1	-	-	-	-	-	-	NA	-
8_DEU1	-	*	-	-	-	-	-	NA
Modal Age	-	*	-	-	-	-	-	-

Results based only on the expert readers

When the results are based on only those expert readers who provide age data for use in stock assessment there is little change in the results. Percentage agreement decreases just slightly from 81.5% to 81% (Table 4.2.5.3.5) and the CV (Table 4.2.5.3.6) increases from to 20.4% to 21.7%. The APE is 16.2% indicating that there is a very slight deterioration the results.

The AEM (Table 4.2.5.3.7) is based on only those expert readers who provide ages for the North Sea stock assessment and it shows that the proportions of fish aged in agreement with modal age for each age group is high for ages 0,1, and 2 (from 100% at age 0 to 81% at age 2). At modal age 1 a small proportion of the samples are both underestimated and overestimated by 1 year while at modal ages 2 the proportion underestimated increases slightly. At modal age 3, 25% are estimated to be 2 years old and a smaller proportion to be just 1 year old.

Table 4.2.5.3.5. Percentage agreement based on modal age for only expert readers in the North Sea workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	100	100	50	100	100	100	92
1	55	91	100	100	82	100	88
2	92	62	77	92	77	77	79
3	0	75	100	50	50	100	61
Weighted mean	66.7	76.9	86.2	89.9	76.8	90	**81**
Rank	**6**	**4**	**3**	**2**	**5**	**1**	**-**

Table 4.2.5.3.6. Coefficient of Variation (CV) between the modal age and the age estimation of only expert readers the in North Sea workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	-	-	-	-	-	-	**-**
1	36	33	0	0	45	0	23.3
2	14	31	26	14	25	24	20
3	0	18	0	43	23	0	23.1
Weighted Mean	19.3	27.9	21.4	11.8	30.4	10.4	**21.7**
Rank	**3**	**5**	**4**	**2**	**6**	**1**	**-**

Table 4.2.5.3.7. Relative bias values between the modal age and the age estimation of only expert readers in the North Sea workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	0	0	0.5	0	0	0	0.08
1	0.45	-0.09	0	0	0	0	0.06
2	-0.08	-0.38	-0.08	-0.08	-0.23	0.08	-0.13
3	-1	-0.25	0	-0.75	-0.5	0	-0.42
Weighted Mean	0	-0.23	0	-0.13	-0.17	0.03	**-0.08**
Rank	**1**	**6**	**1**	**4**	**5**	**3**	**-**

Table 4.2.5.3.8 Age Error Matrix based on only those readers (2, 5, 6 and 8) who provide ages for stock assessment in the North Sea

Age | Modal Age	0	1	2	3
0	**1**	0.04	0	0
1	0	**0.92**	0.15	0.06
2	0	0.04	**0.81**	0.25
3	0	0	0.04	**0.69**

4.2.5.4 Growth data from the North Sea workshop exercise

Figure 4.2.5.4.1 shows the combined growth curves for all fish and all readers in the North Sea workshop exercise. The Linear Mixed Effects Model analysis showed a significant reader effect on both the intercept and slope of the LMEM (LMEM, $p < 0.05$) meaning that there are differences between readers in their interpretation of the first winter ring and the following winter rings. The significant reader effect from the model is partly due to reader 8 who in some examples placed the outermost mark past the otoliths edge. At winter ring 1 there is variation between where the readers annotate this ring to be and this indicates that it is unclear where the first winter ring begin. In general, there is little overlap between the average distances from the centre to winter rings 1 and 2 and overall this can be interpreted as the readers are in general agreement as to where to identify these winter rings. As the average distance from the centre to the subsequent winter rings increases an overlap becomes more apparent and this indicates that the readers are less in agreement as to which structures are in fact the true winter rings. These examples were discussed within the group and notes from this discussion can be found in Table 4.3.1.

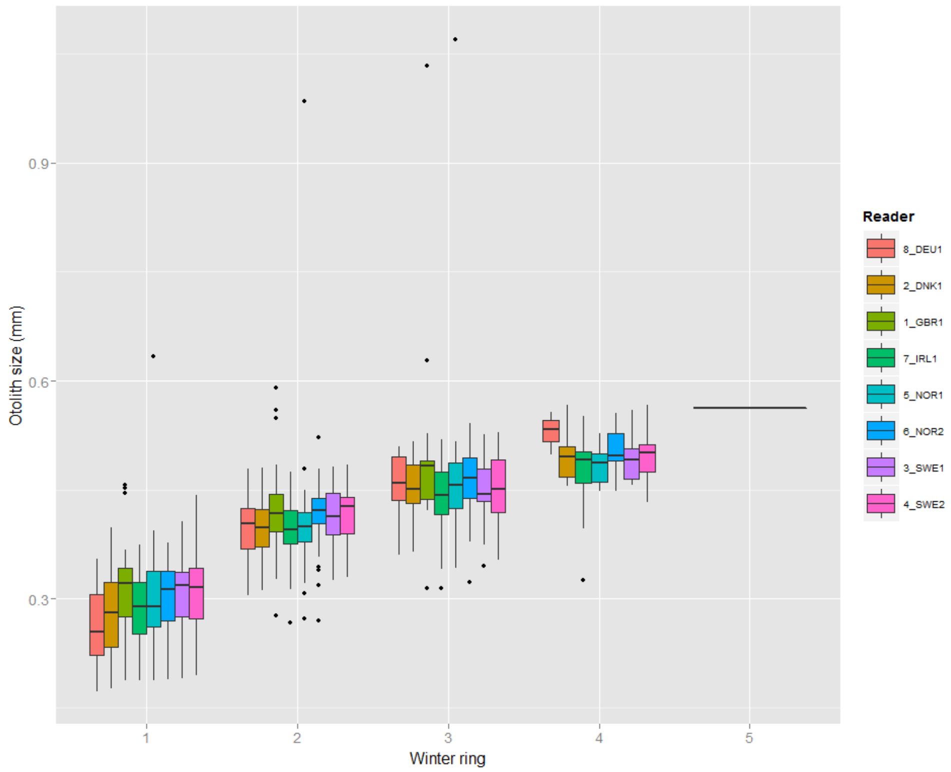


Figure 4.2.5.4.1. Plot of average distance to the centre for winter rings 1–3 and the edge for all readers in the North Sea workshop exercise. The boxes represent the mean, upper and lower box boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

4.2.5.5 Age data from the Celtic Seas Ecoregion (7 and 6, excluding 7.d and 7.e) workshop exercise

When all of the readers are included in the analysis the overall percentage agreement is 94.9% (Table 4.2.5.5.1) with an overall CV of 12.1% (Table 4.2.5.5.2). Only reader 8 did not reach 100% agreement at age 0 and this was due to just one fish bring incorrectly aged. At modal age 1 the percentage agreement is only just lower with all readers reaching a percentage agreement above 90%. At modal age 2 the lowered percentage agreement is mainly due to the poor agreement of reader 1_GBR1 who sometimes omitted to include the outermost ring in the count of age.

It should be taken into consideration that CV in this case is calculated based on a sample set of fish with a young and narrow age range. The overall APE is 7.9% and this is calculated taking the age of the fish into consideration.

The relative bias values (Table 4.2.5.5.3) show that readers are both underestimating and overestimating ages compared with the modal age. The inter reader bias tests (Table 4.2.5.5.4) shows that there is little bias compared with modal age by all readers. The age bias plots in Annex 4 confirm these results.

Table 4.2.5.5.1. Percentage agreement based on modal age for all readers in the Celtic Seas Ecoregion workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	100	100	100	100	100	100	100	83	98
1	95	95	100	95	100	100	95	90	96
2	25	100	75	100	100	75	100	100	84
Weighted mean	86.7	96.7	96.7	96.7	100	96.4	96.7	89.9	**94.9**
Rank	8	2	2	2	1	6	2	7	-

Table 4.2.5.5.2. Coefficient of Variation (CV) between the modal age and the age estimation of all readers in the Celtic Seas Ecoregion workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	ALL
0	-	-	-	-	-	-	-	-	-
1	24	24	0	21	0	0	21	34	9.3
2	40	0	67	0	0	29	0	0	26.1
Weighted mean	21.3	16	8.9	14	0	4.1	14	71.7	**12.1**
Rank	7	6	3	4	1	2	4	8	-

Table 4.2.5.5.3. Relative bias values between the modal age and the age estimation of all readers in the Celtic Seas Ecoregion workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1	All
0	0	0	0	0	0	0	0	0.17	0.02
1	-0.05	-0.05	0	0.05	0	0	0.05	-0.1	-0.01
2	-0.75	0	-0.5	0	0	-0.25	0	0	-0.19
Weighted mean	-0.13	-0.03	-0.07	0.03	0	-0.04	0.03	-0.03	**-0.03**
Rank	8	2	7	2	1	6	2	2	-

Table 4.2.5.5.4. Inter-reader bias tests for all readers in the Celtic Seas Ecoregion workshop exercise ("-"= no sign of bias (p > 0.05); "*" = possibility of bias (0.01 < p < 0.05); "" = certainty of bias (p < 0.01))**

	1_GBR1	2_DNK1	3_SWE1	4_SWE2	5_NOR1	6_NOR2	7_IRL1	8_DEU1
1_GBR1	NA	-	-	*	-	-	*	-
2_DNK1	-	NA	-	-	-	-	-	-
3_SWE1	-	-	NA	-	-	-	-	-
4_SWE2	*	-	-	NA	-	-	-	-
5_NOR1	-	-	-	-	NA	-	-	-
6_NOR2	-	-	-	-	-	NA	-	-
7_IRL1	*	-	-	-	-	-	NA	-
8_DEU1	-	-	-	-	-	-	-	NA
Modal Age	-	-	-	-	-	-	-	-

Results based only on the expert readers

When the results are based on only those readers who provide age data for use in stock assessment the percentage agreement decreases just slightly to 94.4% (Table 4.2.5.5.5) and the CV (Table 4.2.5.5.6) increases to 12.5%. The overall APE is 9.3% indicating that there is very little change in the results.

As there is no stock assessment carried out for sprat in the Celtic Seas Ecoregion an age error matrix (AEM) based on only readers providing ages for this stock assessment was not possible. In order to give an indication of the possible errors around the ages of sprat in this area an AEM has been compiled based on all of the expert readers who attended the workshop (Table 4.2.5.5.8). The proportions of fish aged in agreement with modal age 0 and 1 are high at 97%. At age 2 the proportion in agreement is 79% with the remaining fish being aged either 1 or 2 years old.

Table 4.2.5.5.5. Percentage agreement based on modal age for only expert readers in the Celtic Seas Ecoregion workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	100	100	100	100	100	83	97
1	95	95	100	100	100	90	97
2	25	100	75	100	75	100	79
Weighted mean	86.7	96.7	96.7	100	96.4	89.9	**94.4**
Rank	6	2	2	1	4	5	-

Table 4.2.5.5.6. Coefficient of Variation (CV) between the modal age and the age estimation of expert readers in the Celtic Seas Ecoregion workshop exercise

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	-	-	-	-	-	-	-
1	24	24	0	0	0	34	8.8
2	40	0	67	0	29	0	31.1
Weighted Mean	21.3	16	8.9	0	4.1	71.7	**12.5**
Rank	5	4	3	1	2	6	-

Table 4.2.5.5.7. Relative bias values between the modal age and the age estimation of only expert readers in the Celtic Seas Ecoregion workshop exercise (values in red indicate a negative bias, those in black indicate a positive bias)

Modal Age	1_GBR1	2_DNK1	3_SWE1	5_NOR1	6_NOR2	8_DEU1	All
0	0	0	0	0	0	0.17	0.03
1	-0.05	-0.05	0	0	0	-0.1	-0.03
2	-0.75	0	-0.5	0	-0.25	0	-0.25
Weighted Mean	-0.13	-0.03	-0.07	0	-0.04	-0.03	**-0.05**
Rank	6	2	5	1	4	2	-

Table 4.2.5.5.8. Age Error Matrix based on only expert readers for the Celtic Seas Ecoregion exercise (numbers in bold indicate the proportion in agreement with modal age, those in red indicates underestimation compared with modal age while those in black indicate overestimation compared with modal age).

Age | Modal Age	0	1	2
0	**0.97**	0.03	0.04
1	0.03	**0.97**	0.17
2	0	0	**0.79**

4.2.5.6 Growth data from the Celtic Seas Ecoregion (7 and 6, excluding 7.d and 7.e) workshop exercise

Figure 4.2.5.6.1 shows the combined growth curves for all fish and all readers in the Celtic Seas Ecoregion workshop exercise. The Linear Mixed Effects Model analysis showed a significant reader effect on both the intercept and slope of the LMEM (LMEM, $p < 0.05$) meaning there are differences between readers in their interpretation of the first winter ring and the following winter rings. These differences are slight and the significant reader effect from the model is due to reader 1 who in some examples placed his annotations towards the end of the winter ring as opposed to the start. In general there is little overlap between the average distances from the centre to the winter rings and thus overall this can be interpreted as the readers are in general agreement as to where to identify these winter rings. Problematic examples were discussed within the group and notes from this discussion can be found in Table 4.3.1.

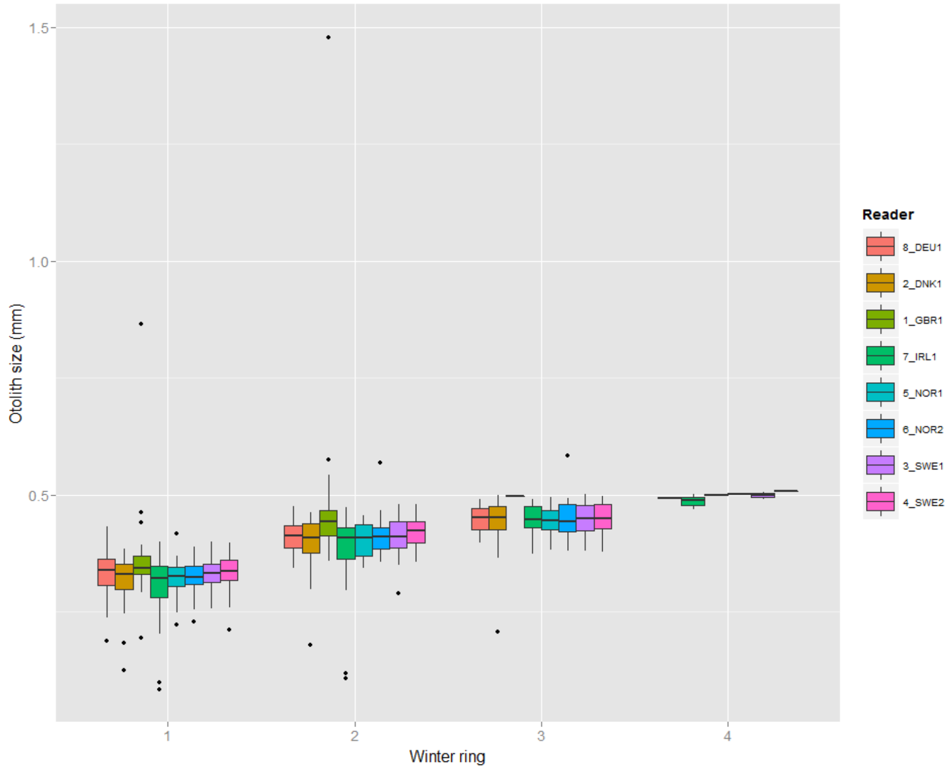


Figure 4.2.5.6.1. Plot of average distance to the centre for winter rings 1–2 and the edge for all readers in the Celtic Seas Ecoregion workshop exercise. The boxes represent the mean, upper and lower box boundaries of the interquartile range, whiskers represent the minimum and maximum values and the dots represent the outliers.

4.3 Discussion of age determination results of the workshop exercise

When the results of the workshop age reading exercise were presented to the group much time was spent looking at both the non-problematic and problematic images in plenary. In many examples it was possible, after the group discussion, to agree on how the otoliths should be interpreted and to make an agreed age otolith collection (Annex 5). The group considered it important to include some images of those otoliths where agreement could not be reached as they are representative of certain issues that were not possible to resolve at the workshop but which may possibly be resolved by examination of the otolith microstructure.

Most time was spent on the Skagerrak and Kattegat (3.a) samples as these are the most problematic when comparing the three sprat stocks. Also, this was the first time that an exercise was conducted for this stock, both in preparation for or as part of this workshop. The table in the following section describes the images which were discussed and the conclusions formed by the group in plenary for the three areas.

A discussion of the 3.a samples lead to the conclusion that it is often difficult to distinguish between opaque and translucent zones because there are many grey areas in the otoliths. Samples 6698256 and 6932318 (Figures 4.3.1 and 4.3.2) are good examples of this, where some readers found it difficult to determine if a true winter ring had been laid down. It was decided that these otoliths would be polished and the daily ring structure examined to confirm if the faint greys zones are in fact true winter rings.

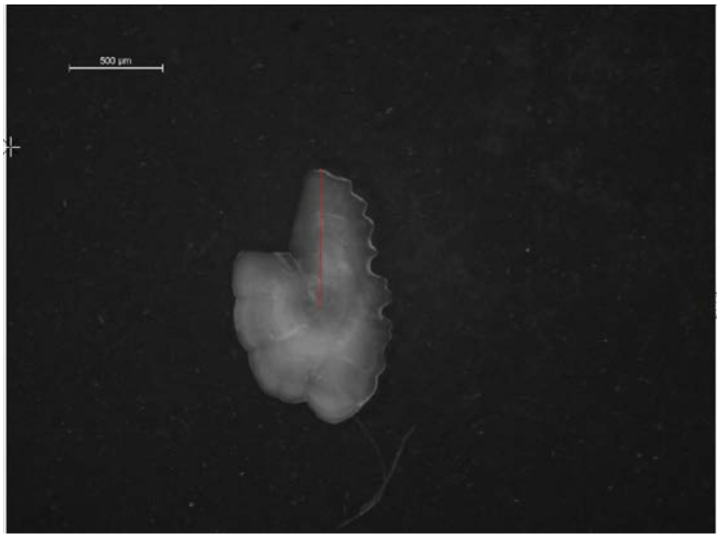


Figure 4.3.1. 6698256, Capture date 18/01/13, 3.a

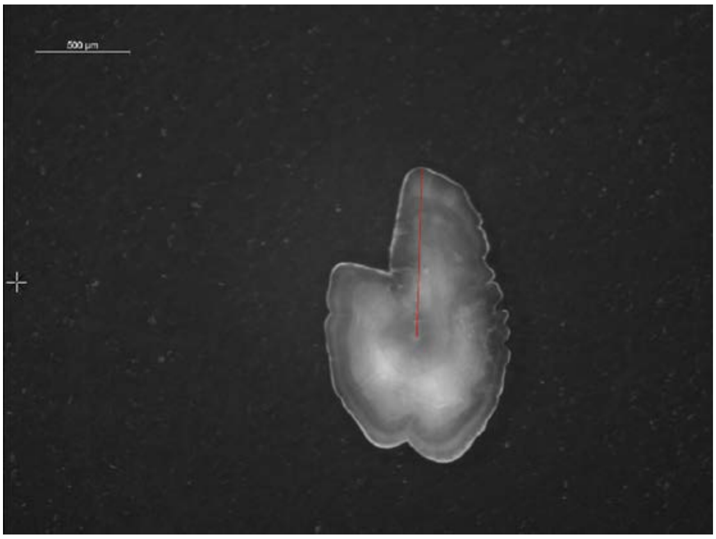


Figure 4.3.2. 6932318, Capture date 10/06/2014, 3.a

Overall, the samples in the 3.a exercise were from larger fish and readers found it difficult to determine the ages without the use of a microscope, see 6698269 (Figure 4.3.3) as an example. This image also illustrates the above mentioned issue of it being difficult to distinguish between opaque and translucent zones due to the overall grey appearance of the structures. But considering this the readers were still in agreement as to the location of the first winter ring in these older samples.

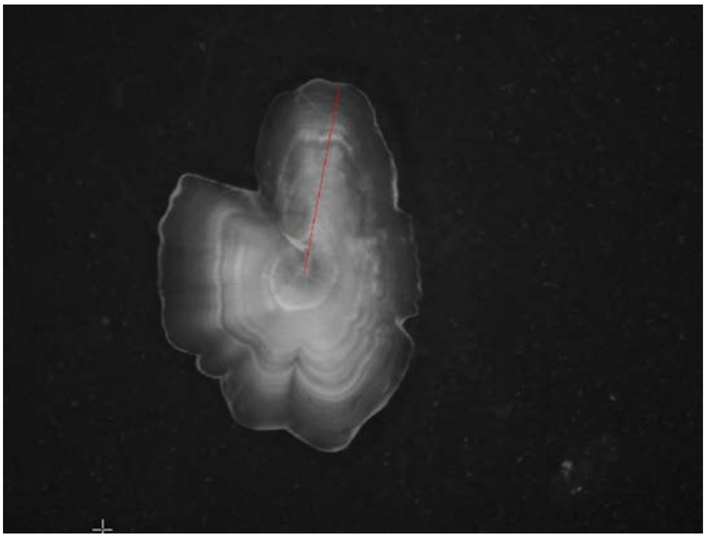


Figure 4.3.3. 6698269, Capture date 18/01/2013, 3.a

There was also much discussion on samples from the North Sea exercise. 7116368_4B (Figure 4.3.4) was given a modal age of 1 in the exercise, but when the group looked at it in plenary they felt that the pattern seen in the translucent zones meant that it was likey that the first winter ring was a band of opaque and translucent material, with the second winter ring at the outermost edge of the otolith, therfore it might not be a 1 year old but no conclusion was reached. The discussion around 7116393_4B (Figure 4.3.5) was the issue of whether or not the innermost faint and narrow translucent zone is infact a true winter ring. The readers said that this ring should only be counted if the shape of it matches the outline shape of an otolith and it can be followed all the way around the otolith. It was decided that the microstructure of this otoltih should be examined. A similar discussion was had for sample 7351935_4C (Figure 4.3.6) and no conclusion could be reached except that the microstructure should also be examined.

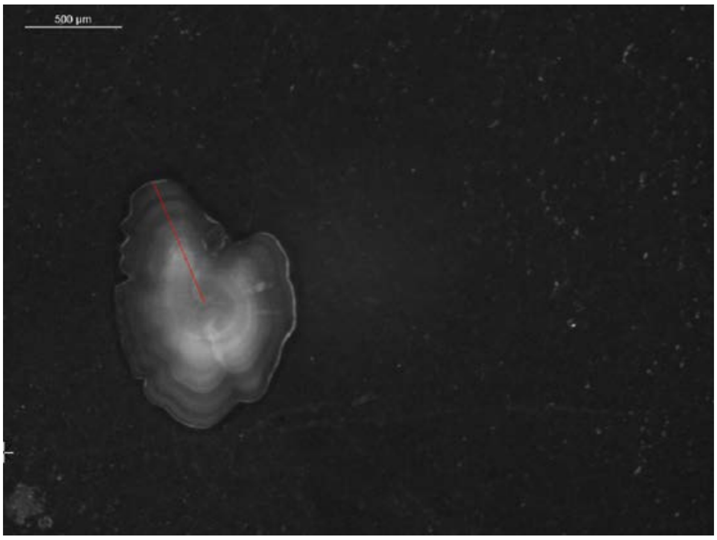


Figure 4.3.4. 7116368_4B, Capture date 23/06/2015, North Sea

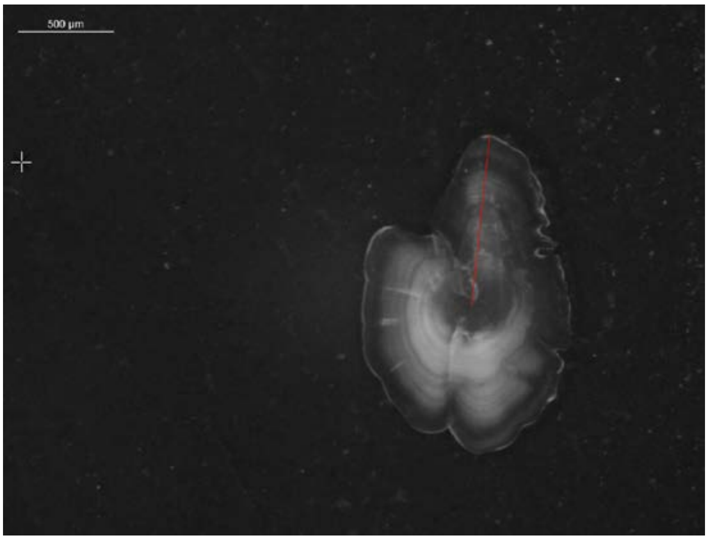


Figure 4.3.5. 7116393_4B., Capture date 23/06/2015, North Sea

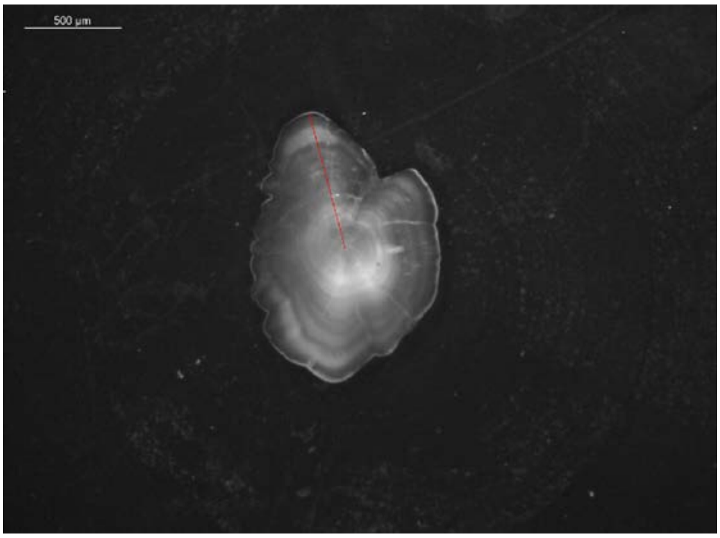


Figure 4.3.6 7351935_4C, Capture date 06/08/2016, North Sea

Table 4.3.1 Notes made on individual otoliths during the plenary discussion of the workshop exercises, grouped by area

	Otolith image	Date of capture	Notes	Discussion	Conclusion
Skagerrak Kattegat	6698256.jpg	18/01/2013	Modal age 1	Only one (trainee) reader gave age 2 as they thought the L1 region is too large.	- Inconclusive - Microstructure should be studied
	6932318.jpg	10/06/2014	Modal age 1	Only one reader gave age 2 as they thought the L1 region is too large. The group described this otolith as impossible to age. There is a translucent band at the edge.	- Inconclusive - Microstructure should be studied
	6932321.jpg	10/06/2014	Modal age 1 But three readers gave age 2 62% agreement	During the discussion everyone agreed on the first band and described it as a split band. The edge was counted. Age could not be agreed and the group agreed there appears to be no proportionality between zones in 3.a	- Inconclusive - Microstructure should be studied
	6932322.jpg	10/06/2014	Modal age 1	The group described the growth of this otolith as "very confusing" due to the fact that the first translucent zone does not have the characteristic shape of a sprat otolith. As a result some of the readers marked both rings. The only way to discount this inner ring as a false ring is to analyse the microstructure of the otolith. The group concluded that we should not count the edge. However it is a very large fish for But it is very large for June, perhaps an example where knowledge of the size of the fish Everyone has now agreed it is one year old, based on size of fish and otolith And the edge defined as transparent by the group.	- Inconclusive - However group acknowledges that the microstructure should be studied
	6932324.jpg	10/06/2014	Modal age 1	The group agreed that they can see vague shadow in the edge, however they felt it should not be counted, it was s that if it was an age of 2 in June then the transparent zone should be bigger and must follow an opaque zone.	Agreed as 1 year. But microstructure should be studied
	7190488.jpg	24/09/2015	Modal age 1	Full agreement. Clear opaque at edge	- Agreed age 1
	6698269.jpg	18/01/2013	Modal age 2	Group felt it is an age 3 or 4 but very difficult to say for sure,	- Inconclusive

				therefore it needs to be looked at under the microscope.	- Agreed age of age 3 +
	6698280.jpg	18/01/2013	Modal age 2	Difficult to say. Need to look at microscope. Its large size and its wide, age 2 but possibly 3	- Inconclusive - Age 2 +
	6698282.jpg	15/10/2015	Modal age 3 Length is 138mm	Discussion and conclusion was age 3/4/5.	- Inconclusive - Age 3 +
	6698258.jpg	18/01/2013	Modal age 3	Discussion this is a really good example of a three year old fish	- Agreed age 3
	6698260.jpg	18/01/2013	Modal age 3 With two readers giving age 2	With discussion we have concluded it as an age 3.	- Agreed age 3
	6698268.jpg	18/01/2013	Modal age 3 Ages 2, 3 and 4	With discussion we have concluded that it is age 4	- Agreed age 4
North Sea	7351932.jpg	06/08/2016	Modal age 0 1 person age of 1	1 reader differed from modal age; this was due to a typographic error when entering age into WebGR. Group agrees on thin translucent zone on edge.	- Agreed age 0
	7116368.jpg	23/06/2015	Modal age 1 Lowest % agreement	From the discussion it was concluded that the distance between the rings is too regular, so readers conclude an age of 1. The first translucent ring is not so clear along the third axis of the otolith; therefore readers can't follow it the whole way around. Readers felt that if the middle translucent ring is the first true ring then the third one cannot be real as it is too close. No agreed age was found for this otolith.	- Inconclusive - Microstructure should be studied
	7116393.jpg	23/06/2015	Modal age 1 Two readers gave age of 2	After discussion the two readers now agree with the modal age of 1. There is a thin inner ring that the readers discussed and suggested not to include in the count as it is not a clear band and it is not the characteristic shape of an otolith. However this was not unanimous	- Inconclusive - Microstructure should be studied
	7116403.jpg	23/06/2015	Modal age 1 1 reader overestimated	Only 1 reader had a an age of 2 but now he agrees with modal age	- Agreed age 1
	7351935.jpg	06/08/2016	Modal age 1 Some reading 0	Discussed but no convulsion reached. Translucence at the edge and overall growth described as very poor.	- Inconclusive - Microstructure should be studied

			Length of 95mm		
	7116365.jpg	23/06/2015	Modal age 2 Length 95mm	First winter being described as a split band. After discussion there was agreed age of age 1. Original modal age of age 2 was due to an error of counting the wide opaque zone.	- Agreed age 1 - With some uncertainty
Celtic Sea	IGFS1317832.jpg	28/11/2009	Modal age 0	Only 1 reader (Gertrud) had a an age of 1 but now she agrees with modal age	- Agreed age 0
	ALBAA143164.jpg	08/11/2010	Modal age 1	Some evidence of a split first ring.	- Agreed age 1
	CA09121310.jpg	08/12/2009	Modal age 1 100% agreement Length 95mm	Discussion about thin second opaque. Validation study in the Celtic Seas Ecoregion indicates that the thin second opaque zone is common for the region.	- Agreed age 1
	OSEP1112.jpg	??/09/2011	Modal age 1 Reader 7 gave age 2	Group agrees on age 1. There is a small opaque zone at the edge of the otolith is thought to be an artefact of the way the photo was taken, it was suggested that baby oil would get rid of this issue.	- Agreed age 1
	0315S932.jpg	24/02/2011	Modal age 2 1 reader not in agreement	Only I reader differed from modal as they did not count the edge, on discussion they agree it is an age 1	- Agreed age 2
	0315S9733.jpg	26/02/2011	Modal age 2 1 reader not in agreement	Only I reader gave an age 1 as they did not count the edge, on discussion they agree it is an age 2	- Agreed age 2
	OSEP1120.jpg	??/09/2011	Modal age 2	No disagreement	- Agreed age 2

4.4 Conclusions

In the North Sea exercise the overall percentage agreement was 81.5% with a CV of 20.4% and APE was 15.4%. Following a group discussion of the results and the samples used all readers were in 100% agreement at modal age 0. At modal age 1 the poor agreement was mostly contributed to the misinterpretation of the edge by reader 1_GBR1, which was also rectified by the discussion. However at modal ages 2 and 3 there is still a tendency for the readers to underestimate the age of the fish compared with modal age, most notably readers 2_DNK1 and 5_NOR1. When only the expert readers are included in the analysis there is very little difference in the overall results. The AEM was calculated using only the expert readers who provide ages for the North Sea stock assessment and the proportions underestimated at modal ages 2 and 3 are again mostly due to the tendency of readers 2_DNK1 and 5_NOR1 to underestimate compared with modal age.

When comparing the results from the North Sea age reading exercises completed at the workshop with those from the pre-workshop exchange there is a deterioration in the results. It should be noted that the number of participants at the workshop was much lower than in the exchange and the sample set much smaller. In general, the same overall trend of overestimation at ages 0 and 1 and underestimation of the older ages exist. Readers are recommended to follow the standardized guidelines outlined in the agreed age reading protocol (Section 4.6) compiled at the workshop.

In the Celtic Seas Ecoregion exercise the overall percentage agreement was 94.9% with an overall CV of 12.1% and APE was 7.9% Again, following the group discussion there was 100% agreement at modal age 0. Time did not allow for all otoliths to be discussed but the overall agreement at ages 1 and 2 also improved following the plenary discussion. The general tendency is to underestimate compared with modal age with the exceptions of readers 4_SWE2 and 7_IRL1 who are overestimating compared with modal age and who are trainees. When only the expert readers are included in the analysis there is very little difference in the overall results. The AEM shows the general underestimation of ages compared with modal age and following the plenary discussion the proportion in agreement with modal age 0 should be 1.

When comparing the results from the Celtic Seas Ecoregion age reading exercises completed at the workshop with those from the pre-workshop exchange there is very little change in the results. Again, the number of participants at the workshop was much lower than in the exchange and the sample set much smaller.

For the 3.a exercise, 5 of the 8 readers had no experience in reading otoliths from this stock as it had not been possible to run the pre-workshop exchange for this area. Taking this into consideration and given the difficulties in interpreting the structures in these otoliths it was decided to use the age estimations of the most experienced reader for this stock as modal age. When comparing all of the other readers against the age estimations of reader 3_SWE1 the overall percentage agreement was just 68.6%, with an overall CV of 22.8% and APE was 16.9%. Of the 100 images available only 45 otoliths were read by all readers and thus the analysis was based only on these samples. In general, there is an overestimation of ages at ages 0 and 1 and an underestimation of ages at ages 2–5 when compared to the age estimations of reader 3_SWE1. It is recommended that the 3.a exercise be completed with the participation of another experienced reader so that a more thorough analysis can be carried out based on a new calculation of modal age.

The readers were provided with an excel sheet to record the readability scale and the edge type for each otolith. The latter should have been completed based on an examination of the otoliths under the microscope but the readers did not have the time for this. They would also have liked more time examining the otoliths under the microscope, especially for those otoliths where the structures were difficult to interpret.

The initial edge type classification outlined for the readers in the pre-workshop exchange was simplified for the workshop exercise as the readers felt it was difficult to define the edge type precisely. The intention had been to use this information to compile guidelines on edge interpretation for each stock but this was not possible given the unreliability of the data.

Ideally all age readers who participate in an exchange should also participate in the workshop. This was not the case at WKARSPRAT and therefore discussion of individual readers' issues was not possible for all readers who participated in the pre-workshop exchange.

It should be mentioned that the results are based on modal age which is the age most frequently estimated by the group but which may not necessarily be the true age of the fish.

It is recommended by the group that for future exchanges the readers annotate the end of the winter rings as opposed to the beginning as the transition from translucent zone to opaque zone is much clearer in sprat. This should allow for a more precise analysis of the growth data which is sensitive to the precision with which the readers annotate the winter rings.

In the following sections the protocols from the individual participating laboratories (Section 4.5) and an agreed age reading protocol (Section 4.6) are described, WKARSPRAT recommends that these are referred to by readers when processing the otoliths and estimating the ages of sprat.

4.5 Compilation of national protocols

Norway

For preparation of the sprat otoliths we use two different techniques depending on the size of the sprat. In order to take the otolith from big sprat we break the fish "neck "and then it is easy to take the otolith out with a tweezer. The otoliths are then placed in water in numbered trays. We rinse the otoliths well to remove tissue and then we use a brush to transfer them to black, special designed numerated otolith trays. When the otoliths are completely dry they are mounted with three drops of Entellan®, we leave them in the fume hood for drying for at least 24 hours before age reading.

When removing the otoliths from tiny sprat, we use a stereomicroscope. The fish is held in place with a tweezers and we use a scalpel to take the otoliths out, after which the same procedure as for the big sized fish is followed.

When we are ageing the fish, we use readability 1 if we are sure about the age, 2 if we age the fish but are unsure, and 3 if the otolith is unreadable.

Denmark

A pair of otoliths are removed from the fish, cleaned and placed in a black otolith tray. For reading purposes the whole otoliths are placed sulcus down in the black trays. Otoliths are covered in alcohol and viewed under a reflected light source using a stereomicroscope at magnification 2.5 x 10.

Winter rings are counted to determine the age of the individual fish. The birthday is defined as 1 January so it is important to be aware of the date of capture and the edge type. The winter ring (translucent zone) is usually laid down between the start of November and the start of June. The summer ring (opaque zone) may not be visible until later in the season. Due to the long spawning season of sprat the distance from the centre to the first winter ring is variable and this can make it difficult to identify the first winter ring. Sprat spawned very late in the season can overwinter as larvae and in these fish a winter ring may not be formed. False winter rings can occur in sprat and these are visible as well defined translucent rings. Examination of the daily ring microstructure on either side of this zone can confirm whether a ring is true or false (Figure A).

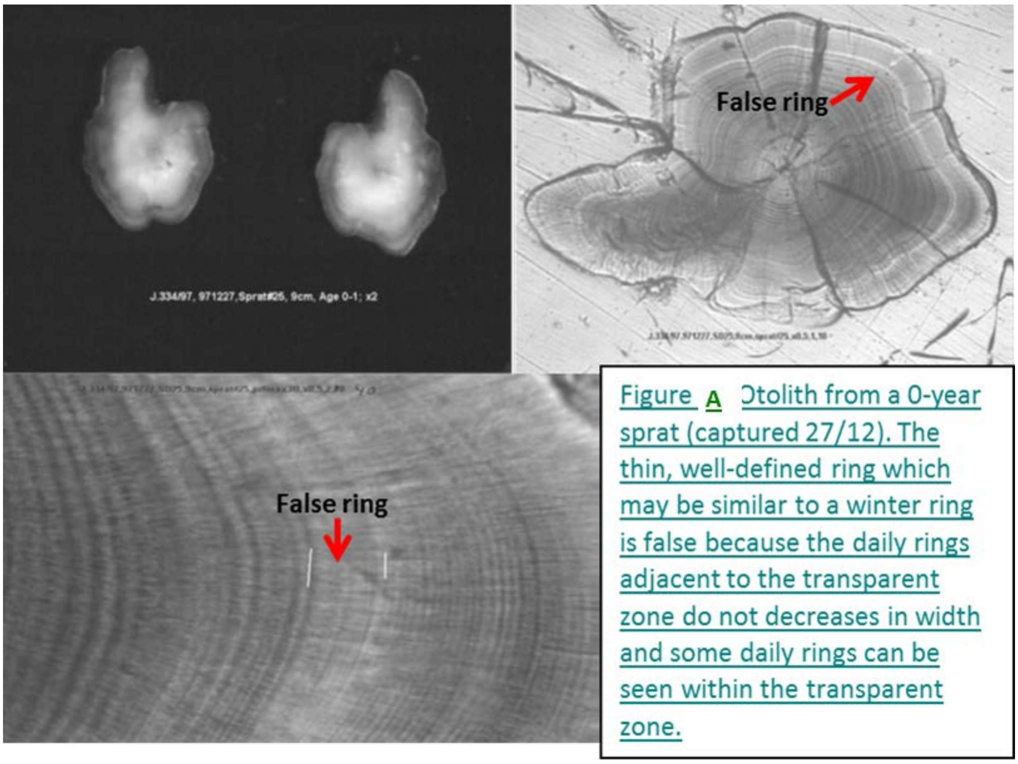


Figure A Otolith from a 0-year sprat (captured 27/12). The thin, well-defined ring which may be similar to a winter ring is false because the daily rings adjacent to the transparent zone do not decreases in width and some daily rings can be seen within the transparent zone.

Germany

The sprat otolith is removed from the fish, washed with water, and then dried. The otolith is then fixed to a glass slide using a drop of polyester resin that does not contain hardener. These otoliths are then viewed under a stereoscopic microscope to ensure that the correct position with the sulcus facing up. Polyester resin with hardener is then added and a glass coverslip placed over it, ensuing that no air bubbles are trapped inside. Weight is then put on top and it is left to dry for one or two days, after which it is cleaned with alcohol. For aging purposes these mounted otoliths are then read using a microscope with reflected light against a black background. To improve contrast water can be placed between the otolith and the black background.

Northern Ireland

96 well micro-titre plates are used to age sprat otoliths. Pairs of otoliths are cleaned in water and placed in the wells for storage using a fine paint brush. For aging purposes

each well is filled with water and the otoliths pushed to the bottom of the well and separated. Using the lowest magnification on the microscope the otoliths are viewed using reflected light against a black Perspex card. Age is determined by ageing points on the otolith. Then the plate is allowed to air dry and the lid secured back on it for storage. For quality assurance purposes, when we had two agers, a subset of otoliths were aged by both to see if there was agreement on aging, if not then both readers aged all of the sprat. However, as there is only ager now, this reader ages about fifty sprat otoliths from previous years, to see if there is agreement with the previous ages, to avoid drift in aging. This is usually done twice.

Sweden

- Preparation

Otoliths from sprat are used whole and untreated. They are placed on slides and are fixated using nail polish or varnish (PERTEX® mounting medium for light microscopy). The otoliths are read using stereomicroscope with glaring illumination and also in microscope with transmitted light.

- Age analysis

The age of sprat otoliths are generally hard to assess. Otoliths from Skagerrak/Kattegat can be hard to read because of vague borders between translucent and opaque zones. It is also common with false zones, seen as weak translucent zones, which for an unexperienced reader easily can be interpreted as a real translucent zone (Figure 4.5.1). In older fish, both the first and last translucent zone can be hard to identify. In contrast to otoliths from Skagerrak/Kattegat, otoliths from the Baltic Sea are clearer in respect to borders between translucent and opaque zones (Figure 4.5.2).

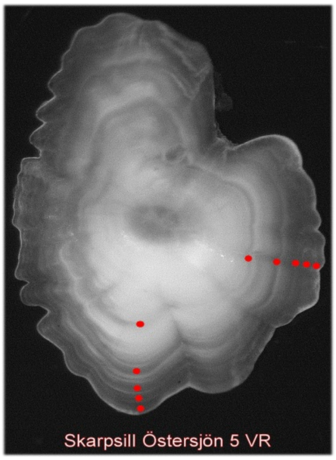
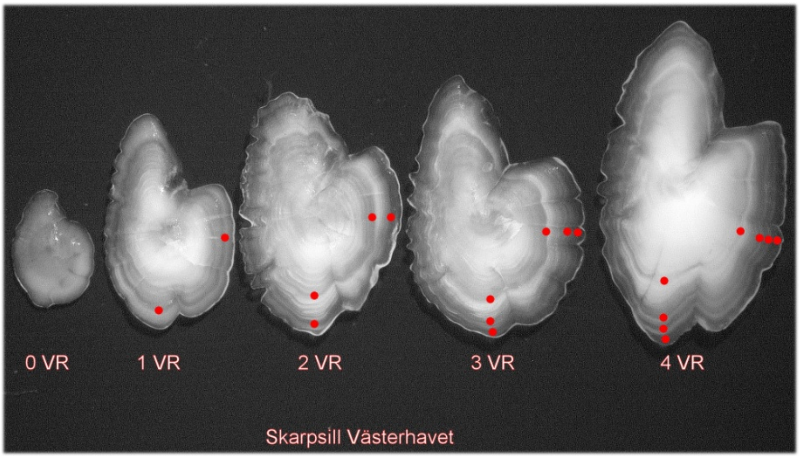


Figure 4.5.1 Otolith from sprat, 1 year, caught in Skagerrak, length 11.5 cm, one winter ring (VR) visible.

Figure 4.5.2 Otolith from sprat, 5 years, caught in the Baltic Sea, length 11.5 cm, 5 winter rings (VR) are visible.

- Translucent zone / Winter zone

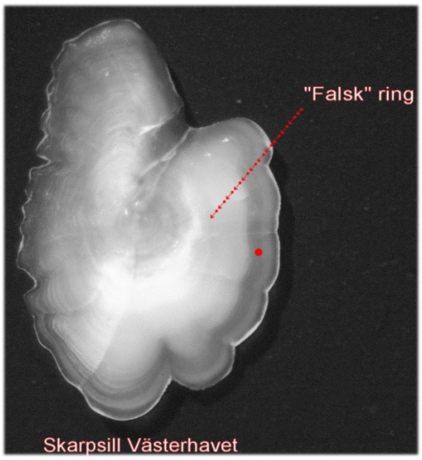
Generally, a real winter zone follows the shape of the otolith around the whole otolith. In older fish, the outermost zone may be hard to follow the whole way around the otolith, but it is almost always visible on the postrostrum and on the dorsal side. The first winter zone is the first clear zone which follows the otoliths more oval shape. It can be diffuse in otoliths from older fish. Inside the first winter zone, the round-shaped core is seen. Sometimes diffuse, false zones are visible inside the first winter zone (Figure 4.5.1 and 4.5.4). In fish older than one year, the construction of the growth zone starts in early spring. The first and second growth zones are usually quite wide, however in older fish, the following growth zones are of more similar width (Figure 4.5.3). The outermost growth zones in older fish can sometimes be very close to each other (Figure 4.5.3), which makes age assessment harder. When interpreting, it is important to remember that the latest formed winter zone and the same year's growth zone is not visible in fish caught during the first half of the year (January–June). The edge of the otoliths should then be counted as a winter zone. In young fish, the same year's winter zone and growth zone can be visible as early as in April–May. The date when the fish is caught must be taken into consideration.

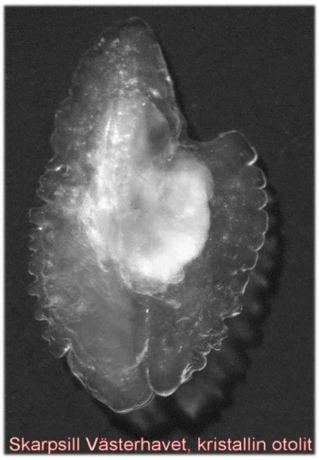


Picture 4.5.3. Growth pattern in sprat otoliths caught in Skagerrak/Kattegat, ages 0 to 4 years (VR=winter zone)

- Difficult otoliths

Difficulties in age assessment can for instance be caused by false rings (Picture 4), or crystallised otoliths (Figure 4.5.5).





Picture 4.5.4. Example of a false winter zone (arrow) in a one year old otolith. The true winter zone is shown with a red dot.

Picture 4.5.5. Example of a crystallised otolith.

As Ireland do not routinely age sprat no protocol is provided.

4.6 Agreed age reading protocol

1. Preparation of otoliths

The otoliths (sagittae) must be cleaned and dried before placing them in trays or slides for reading, this can be done using water or alcohol. A pair of otoliths should be placed side by side with the sulcus acusticus facing down. Sagittae of sprat are small and often difficult to handle with forceps, care should be taken and a moistened fine paintbrush can be a useful tool for handling small otoliths.

Samples should be clearly labelled with catch dates, sample number and fish numbers. If multiple samples are placed on the same slide the plates/slides should be clearly labelled. It is important that the plates/slides are clean, especially if the otoliths are to be photographed. The numbering of fish should not be confusing regarding the arrangement of otoliths (fish numbers) on the plates. This is especially important if the samples are to be sent among laboratories.

2. Viewing of otoliths

First, you need information on the catch date of the fish and the area in which it is caught.

Using a stereomicroscope, the otoliths are viewed on a black background and under reflected light. If the otoliths are viewed on slides under a microscope, transmitted light is used. The whole otolith including its nucleus, the growth zones and the edge structures should be visible to begin with. Otoliths are usually soaked in alcohol, water or covered in baby oil to improve clarity of the growth structures.

Special attention should be given to the edge. The practise of recording of the edge type varies between laboratories but it is advisable to record this information based on the classification of “T” for translucent and “O” for opaque. This should be done using a microscope and otoliths should not be soaking in alcohol for long beforehand.

It is very important that the following area specific characteristics of the annuli are considered.

For the Celtic Seas Ecoregion the otoliths are the easiest to read as the proportionality of the annuli structure is consistent (Figure 3.8).

For the North Sea otoliths the first winter “band “can be split and fragmented into many different translucent rings which makes it wider and can also be wrongly interpreted as several winter rings.

For the 3.a otoliths the level of fragmentation increases again and it is more difficult to distinguish between opaque and translucent zones (refer to the national protocol from Sweden in section 4.5). As a result of this the distance from the centre to the first winter and the proportions of the following annuli do not appear to be consistent.

3. Reading of otoliths:

a) Birthday defined as January 1
b) The light settings should be adapted to each otolith so to attain the best contrast between opaque and translucent zones.

c) For younger fish, usually a lower magnification is recommended, as to avoid the counting of clearly visible sub-annual rings. For older fish, it is advisable to adjust the magnification accordingly, especially for difficult otoliths. This especially applies to otoliths where the edge is very wide and dark. As the sub-annual increments in sprat can be pronounced caution should be taken.

d) Ideally, the interpretation of the otolith is done primarily in the rostrum and post rostrum area. However, there should always be another area within the otolith where the counting can be repeated and if a pair is available that the pattern observed is visible in both.

e) For age determination only translucent zones (winter rings) should be counted.

f) For fish being caught in the second half of the year, a translucent zone at the edge is not counted as a ring.

g) In older sprat (>4 years old) the onset of translucent and opaque zones will often be visible on the rostrum tip before being visible elsewhere on the otolith edge.

h) After reading a sample it is advisable to double check on some of those you were in doubt about and if reading a sample after a long period without reading, the first sample should be read twice. A second reader should be consulted when in doubt.

i) The length of the fish should not be used when deciding on the age of the fish but can be used as a helpful tool if in doubt about outliers.

j) The readability of the otolith to be noted by a readability scale (WKNARC, 2011). WKARSPRAT suggested an updated readability scale to be reviewed by WGBIOP.

Please note:

- The translucent edge of an otolith with a catch date between January and April/May should be counted as a winter ring.
- For 1 year old fish caught in the early months of the year the translucent edge is often not apparent and you should assume that a translucent zone will follow soon, therefore you count as a translucent zone.
- For older fish, when interpreting a wide and obvious opaque zone at the otolith edge and with a catch date between January and June you assume that a transparent zone will follow soon, therefore you count as a transparent zone.
- When in doubt as to whether or not a narrow opaque or translucent zone should be used in the estimation of age you should always check that the overall pattern of opaque and translucent zones follows what one would expect from the true growth pattern seen in otoliths i.e. a decrease in the width of the opaque and translucent zones when examining the otolith from the centre towards the edge Doubts about narrow opaque and translucent zones should to be checked against the proportions of the true annuli.

5 ToR d) Create a reference collection of agreed aged otoliths

A reference collection was compiled at the workshop based on images which were used in both the pre-workshop exchange and the workshop exercise. In some examples it was possible to use otoliths from the exercises where there was 100% agreement on the age of the fish and where a further discussion at the workshop confirmed the readers interpretations. In other examples, otoliths were chosen from the exercises where only 1 or 2 readers were not initially in agreement with the rest of the group and an agreed age was reached following a discussion of the otolith in plenary at the workshop. Ideally an agreed age collection should cover all age and length groups across the represented stocks. This agreed age collection was compiled by area and only based on the samples provided for the exercises thus limiting the temporal and spatial coverage. In addition, there was the limitation of the amount of time available for discussion of otoliths in plenary at the workshop. As a result the agreed age collection which can be found in Annex 5 is compiled of 10 images from each stock.

The images can found in Annex 5 and can easily be extracted from the report and placed in the ageing manual at any sprat age reading laboratory for reference, they will also be made available on the Age Reader's Forum (ARF) which can be found at https://community.ices.dk/ExternalSites/arf/default.aspx.

In future this agreed age collection should be expanded to include examples from all quarters and age groups for each stock.

6 ToR e) Address the generic ToRs adopted for workshops on age calibration (see WGBIOP's Guidelines for Workshops on Age Calibration)

WGBIOP annually updates tables and documents relevant to age readers and age reading labs. Such information includes: age reader contact lists, materials, techniques, and preparation methods by species and areas for age estimation; an interactive table with a historical overview of workshops and exchanges by species and guidelines for exchanges and workshops on age reading. These files can be found on the ICES Data Quality Assurance Repository at http://ices.dk/community/Pages/PGCCDBS-doc-repository.aspx.

The "Guidelines for Exchanges and Workshops on Age Reading" (WGBIOP, 2016) outline the following generic ToR's for age reading workshops:

a) Provide information on participating laboratory procedures;
b) Resolve interpretation differences between readers and laboratories;
c) Create or update an ageing manual;
d) Collate agreed age reference collection;
e) Formulate follow-up actions;
f) Formulate species (and stock specific) target and threshold statistics;
g) The following section will address these ToR's.

6.1 ToR a) Provide information on participating laboratory procedures

WKARSPRAT ToR c) Analyse growth increment patterns in sprat and compile guidelines for the interpretation of sprat otoliths; refers directly to this ToR. During the workshop the readers were asked to compile their individual laboratory procedures for age reading of sprat and these can be found in Section 4.5. Different quality assurance and control procedures are applied in the different laboratories.

Readers from Denmark, Norway, Sweden, Germany, Northern Ireland, and Ireland participated in the exchange and provided information on their national age reading procedures for sprat (excluding Ireland, who do not routinely age read sprat). In general the methods used were similar in that all laboratories read the otoliths whole. The main difference in the methodologies is that at SLU Aqua in Sweden the otoliths are mounted on transparent glass slides and viewed under transmitted light while in the other laboratories readers view the otoliths on a black background and under reflected light. Different mediums are used to clarify the structures in the otoliths; water, alcohol and baby oil and it was discussed that prolonged immersion in both alcohol and baby oil can lead to an increase in the transparency of the otolith which can make reading difficult. It is standard procedure to count the translucent rings when estimating the age of the fish and birthday is always defined as 1 of January.

6.2 ToR b) Resolve interpretation differences between readers and laboratories

Participants were asked to bring otoliths to the workshop for discussion. On the first day much time was spent in plenary discussing otoliths from the North Sea which had been brought to the workshop from Norway and which were displayed on a large monitor. The was a very useful exercise as the samples chosen were good examples of the two main age interpretation problems apparent with otoliths from this area. First, those where the first translucent zone appears more like a band of opaque and translucent material and which can be problematic for the readers to interpret. Second, those where there is a narrow and sometimes faint translucent band which the readers are unsure if they should count it as the first winter ring or exclude it from the count of age, see Section 4.1 for image examples.

Much time was also spent at the workshop discussing images of otoliths from both the pre-workshop exchange and the exercises undertaken at the workshop itself so that agreement could be reached on problematic otoliths but also to demonstrate where readers were in agreement. The notes from these discussions can be found in Section 2.6 (Table 2.6.1) and Section 4.3 (Table 4.3.1).

It was not possible in all examples to resolve the disagreement. It was decided that the otoliths should be polished and the microstructure analysed to see if such structures are in fact true winter rings. It had been planned to hold a tutorial at the workshop on microincrement analysis, however due to time restraints the full tutorial was not possible. On the final day sometime was spent giving a demonstration on how to use image analysis software to photograph polished otoliths and how to count daily increments. These procedures will be applied as a follow on analysis and will be conducted in the laboratories in Norway, Ireland, Sweden, and Denmark. Annex 6 describes the work which has been undertaken at IMR, Norway following the workshop, images of both the whole and polished otoliths used in this study will be made available to other laboratories for examination. WKARSPRAT recommends that if resources are available, individual labs should conduct microstructure analysis to reduce the uncertainties in the ages estimated from reading whole otoliths.

6.3 ToR c) Create or update an ageing manual

WKARSPRAT ToR c) Analyse growth increment patterns in sprat and compile guidelines for the interpretation of sprat otoliths; refers directly to this ToR. Time was spent in plenary discussing what this age reading manual should include and all readers were actively involved in contributing to its compilation. The 2004 Report of the Workshop on age estimation of sprat (Torstensen, 2004) includes a set of guidelines for the age determination of sprat and this was used as a template which the readers felt required updating. The updated manual can be found in Section 4.6 and WKARSPRAT recommends that individual age reading laboratories apply the agreed age reading protocol when age reading sprat.

6.4 ToR d) Collate agreed age reference collection

An agreed age reference collection was compiled for each area at WKARSPRAT and can be found in Annex 5.

6.5 ToR e) Formulate follow-up actions

The full WKARSPRAT report will be made available in the ICES Data Quality Assurance Repository which can be found at (http://ices.dk/community/Pages/PGCCDBS-doc-repository.aspx). An extended summary will be made available to WGBIOP, HAWG, the stock co-ordinators, any other relevant expert groups and the results will be presented to HAWG in March 2017.

During the plenary discussions at WKARSPRAT otoliths were identified where the group could not conclude on the age of the fish. It was decided that a further examination of the otolith microstructure could clarify whether or not the observed structures were true winter rings or not. Many of the participating laboratories have the facilities to mount, polish and examine otolith microstructure. During the workshop a brief tutorial was given on how to digitize images of polished otoliths and how to count the daily increments which can be made clearly visible by the grinding and polishing process. In 2017 otolith microstructure analysis will be carried out in the age reading laboratories at IMR Norway, DTU Aqua Denmark, SLU Aqua Sweden and GMIT Ireland with the view to identifying true winter rings compared with false rings. This should allow a correct determination of the age of these samples which ultimately can be added to the agreed age collection. In future these data could be used to create guidelines for the readers on how to interpret structures which they find difficult to identify as true winter rings when looking the whole otoliths.

All age reading laboratories are encouraged to collect otoliths from 0 group sprat caught throughout the months of the year, these can be used for MIA analysis with the aim to identifying the seasonal pattern of deposition of the opaque and translucent zones within the otoliths of sprat from the different stocks. Samples should be collected predominately for the North Sea and Skagerrak/Kattegat stocks but also for the Celtic Seas Ecoregion where MIA has already been carried out but where additional samples would strengthen the analysis.

All age reading laboratories who estimate the ages of sprat are requested to implement the age reading protocol outlined in Section 4.6 and refer to the agreed age collection in Annex 5 for reference.

A rerun of the 3.a age reading exercise to be carried out in 2017. All 100 otoliths should be read by the workshop participants and any other readers experienced in age reading sprat from this area who did not take part in the workshop. This will test the age reading protocol outlined in Section 4.6 and ensure that the laboratories are implementing the agreed protocol.

6.6 ToR f) Formulate species (and stock specific) target and threshold statistics

During the workshop a presentation was given via Skype by Anna Rindorf of DTU Aqua who is the stock coordinator and assessor for North Sea and 3.a sprat. The presentation covered topics including; how catch-at-age is used to estimate cohort size and thus stock size, recruitment, catch opportunities and to define stock specific biological reference points; the importance of data quality; how poor quality can lead to uncertainty in the assessment and its effects on maximum sustainable yield and how the AEM's can be applied to the stock assessment. The question of whether or not the uncertainty around the age of the fish increases with fish length was raised and will be investigated in future. This should be included in the presentation of the WKARSPART results to HAWG in March, 2017.

Following the analysis of the WKARSPRAT age reading exercises the results were discussed with the HAWG sprat stock co-ordinator and assessor.

There were no concerns raised about the quality of the age data for the North Sea stock based on the AEM for this area (Table 4.2.5.3.8). For this stock, correct age determination at ages 0 and 1 is important for estimating the number of incoming fish in the following year and ultimately the TAC setting. The subsequent age groups are used for recruitment estimations and the proportions of fish aged in agreement with modal ages 2 and 3 are also acceptable. Again concerns were raised about possible ageing errors with the increase in fish length and the effects on the allocations of age in the length distributions for the ALK's.

For the 3.a stock the 0 and 1 age groups from the surveys are used as indicators of the incoming year classes. Given the poor quality of the age data for this stock, described by the AEM (Table 4.2.5.1.8), it will only be possible to run exploratory tests using the 0, 1 and 2+ age groups. Concerns were also raised about the small number of fish aged for the exercise and it was agreed that the full exercise of 100 otoliths should be rerun in order to check if there are differences in the quality of the age data across quarters.

For the Celtic Seas Ecoregion there is no stock assessment carried out, however given the high quality of the age data described by the AEM (Table 4.2.5.5.8) it would be possible to run some exploratory analysis at HAWG 2017.

In terms of target and threshold statistics for the various stocks, the suggested threshold of a CV of 5% (Campana, 2001), is unrealistic given the narrow and young age range of this species. Instead using the individual readers percentage agreement and bias estimates will allow for a reasonable examination of the quality of the readers age estimations. The varying levels of complexity seen in the otolith structures and the resulting differences in the readability between the different stocks should also be taken into consideration.

When discussing the varying levels of readability of the otoliths at the workshop there was a conversation about the 3 point readability scale recommended by WKNARC (2011) and its applicability to the ageing of short lived species such as sprat. It was suggested that this scale could be adapted to include a degree of uncertainty by number of years as the incorrect ageing of such fish by just one year could have negative consequences for recruitment and SSB estimates. Table 6.6.1 was composed and WKARSPRAT would like WGBIOP to consider the applicability of such a readability scale in improving the quality of age data supplied to the end-users.

Table 6.6.1. Suggested updated readability scale

A – GOOD (AQ1)	Easy to read with very clear structures. No doubt about age determination
B – MODERATE (AQ2)	Not so easy to read with unclear/indistinct annual rings. Age determination is quite certain, but can vary by one year AQ2+ : possibly 1 year older AQ2- : possibly 1 year younger
C – POOR (AQ3)	Not easy to read with indistinct structures. Age determination can vary by more than one year

Annex 1: List of participants

Name	Address	Email
Annelie Hilvarsson	SLU Aqua, Dep. of Aquatic Resources, Inst. of Marine Resources, Turistgatan 5, 453 30 Lysekil, Sweden	annelie.hilvarsson@slu.se
Claire Moore (chair)	MI, Rinville, Oranmore, Co. Galway, Ireland	claire.moore@marine.ie
Eilert Hermansen	Institute of Marine Research, P.O.Box 1870 Nordnes, 5817 Bergen, Norway	eilert.hermansen@imr.no
Gertrud Delfs	Thünen-Institute of Sea Fisheries, Palmaille 9 D- 22767 Hamburg, Germany	gertrud.delfs@thuenen.de
Ian McCausland	Agri Food & Bioscienses Institute (AFBI), 18a Newforge Lane, Belfast BT9 5PX Northern Ireland, UK	ian.mccausland@afbini.gov.uk
Jostein Røttingen	Institute of Marine Research, P.O.Box 1870 Nordnes, 5817 Bergen, Norway	jostein.roettingen@imr.no
Julie Coad Davies (chair)	DTU Aqua, Jægersborg Alle 1, 2920 Denmark	joco@aqua.dtu.dk
Maria Jarnum	DTU Aqua, Postbox 101, 9850 Denmark	mja@aqua.dtu.dk
Marianne Johansson	SLU Aqua, Dep. of Aquatic Resources, Inst. of Marine Resources, Turistgatan 5, 453 30 Lysekil, Sweden	marianne.johansson@slu.se

Annex 2: Agenda

Tuesday 15

9:00 – 10:00 Welcome, introduction and logistics - Chairs

10:00 – 10:30 WKARSPRAT ToRs, outline of workshop - Julie Coad Davies

10:30 – 11:00 Coffee

11:00 – 11:30 "Age validation of sprat in Irish waters using microstructure" – Claire Moore

11.30 – 12:30 Examination of otoliths brought by readers – Group discussion

12:30 – 13:30 Lunch

13:30 – 15:00 Results of pre-workshop exchange; Edge type and readability definitions – Chairs and group discussion

15:00 – 15.30 Coffee

15:30 – 16:30 Tutorial for micro-increment analysis – Claire Moore

Wednesday 16

9:00 – 10:30 Reading exercise - Group

10:30 – 11:00 Coffee

11.30 – 12:30 Reading exercise - Group

12:30 – 13:30 Lunch

13:30 – 15:00 Reading exercise - Group

15:00 – 15.30 Coffee

15:30 – 16:30Reading exercise - Group

Thursday 17

9:00 – 9:30 Results of reading exercise - Julie Coad Davies

9:30 – 10:30 Compilation of agreed age reference collection - Group

10:30 – 11:00 Coffee

11.00 – 12:30 Compilation of agreed age reference collection and age reading protocol - Group

12:30 – 13:30 Lunch

13: 30 – 14:00 "Stock assessment and age related issues" – Anna Rindorf

14:00 – 15:00 Compilation of age reading protocol - Group

15:00 – 15.30 Coffee

15:30 – 16:30 Tutorial for micro-increment analysis – Claire Moore

Friday 18

9:00 – 9:30 “Shape analysis for stock structure and fluctuating asymmetry” – Claire Moore

9:30 – 10:30 Revisit ToR’s and tidying up of report - Julie Coad Davies and group discussion

10:30 – 11:00 Coffee

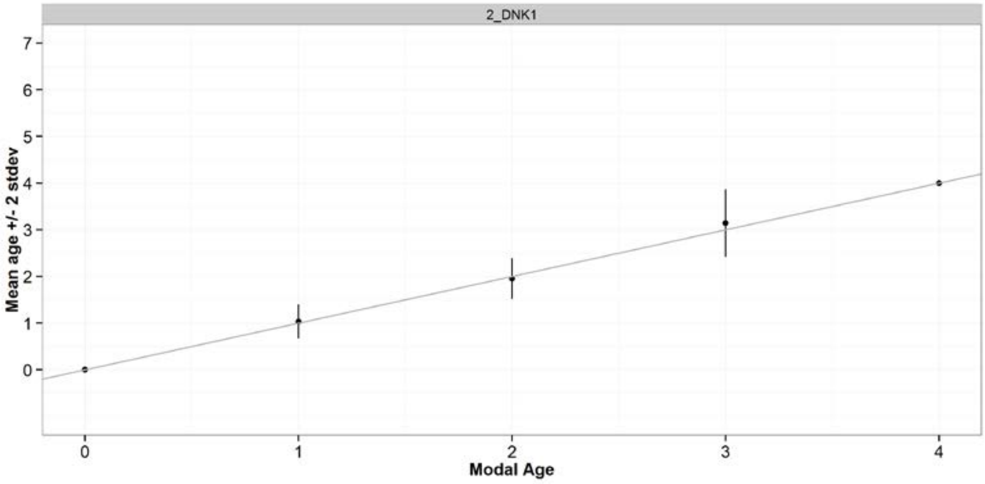
11:00 – 11:30 Tutorial summary – Claire Moore

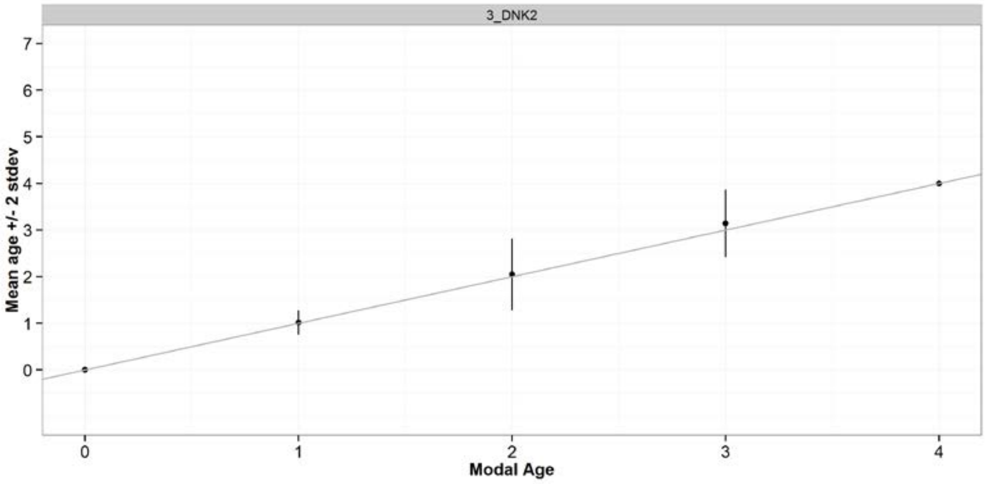
11:30– 12:00 Allocating tasks (if any), Recommendations, Farewell - Chairs

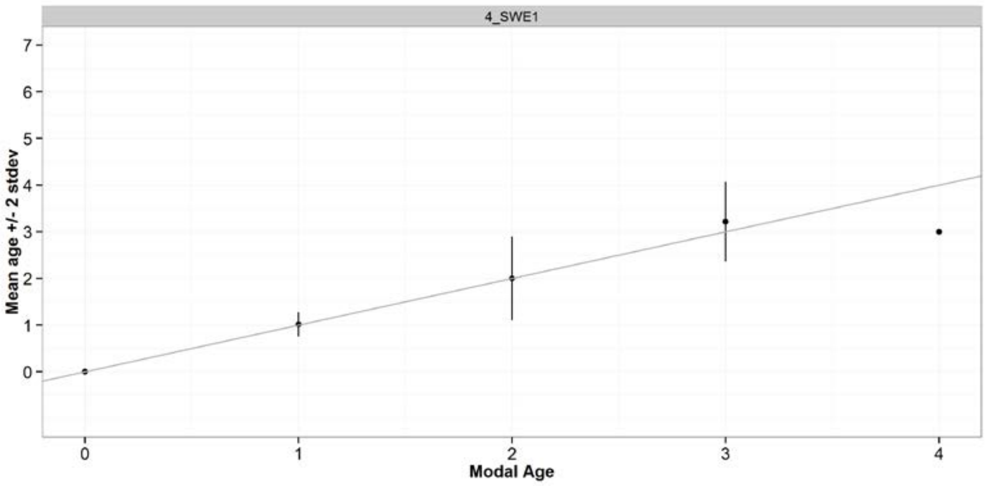
Annex 3: Age bias plots for the pre-workshop exchange

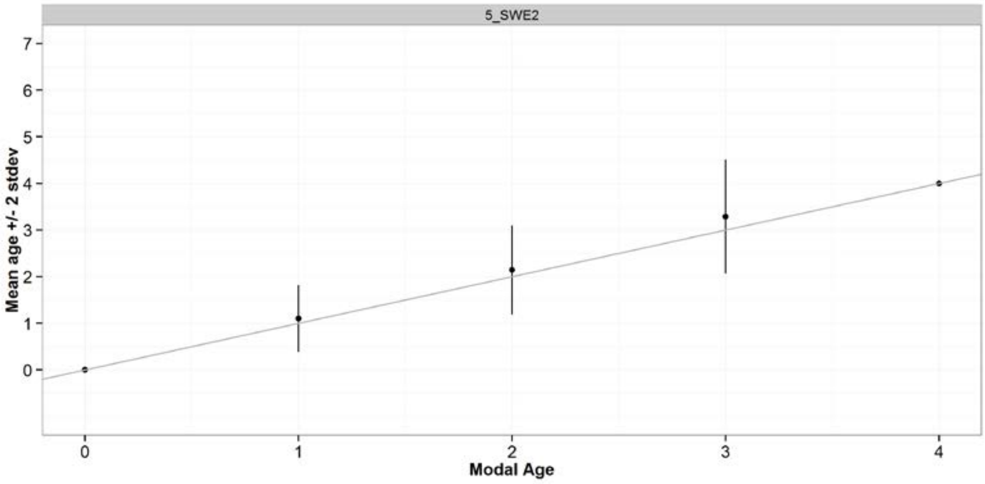
The following graphs are the age bias plots for each reader and for each area/stock. They show the mean age recorded +/- 2stdev of each age reader plotted against the modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line). The reader ID is shown at the top of each graph.

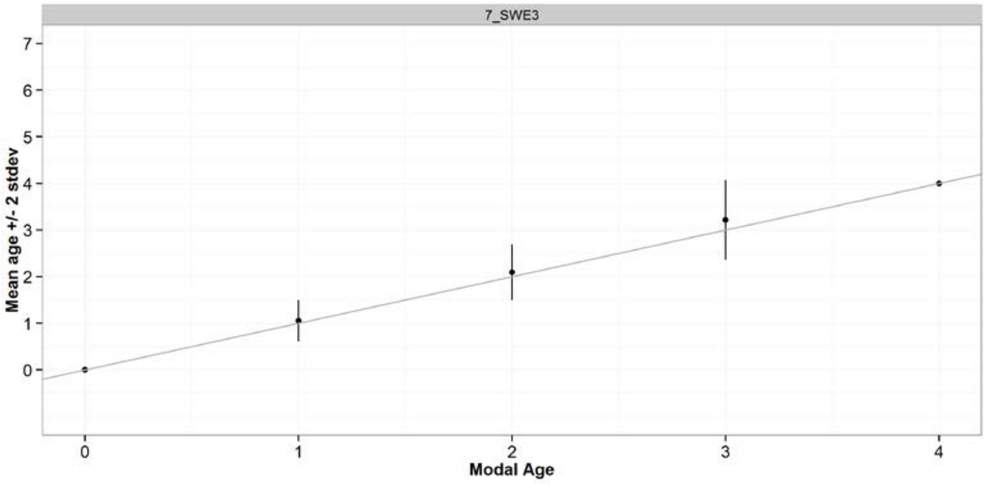
North Sea


2_DNK1
Mean age +/- 2 stdev
Modal Age


3_DNK2
Mean age +/- 2 stdev
Modal Age


4_SWE1
Mean age +/- 2 stdev
Modal Age


5_SWE2
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5
6
7


7_SWE3
Mean age +/- 2 stdev
Modal Age
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1
2
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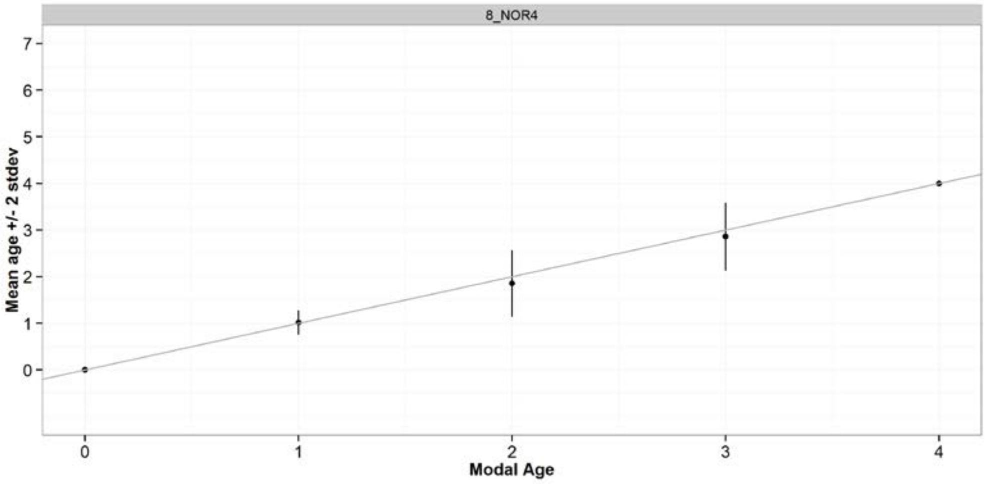
4
5
6
7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Mean age +/- 2 stdev
Modal Age
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4
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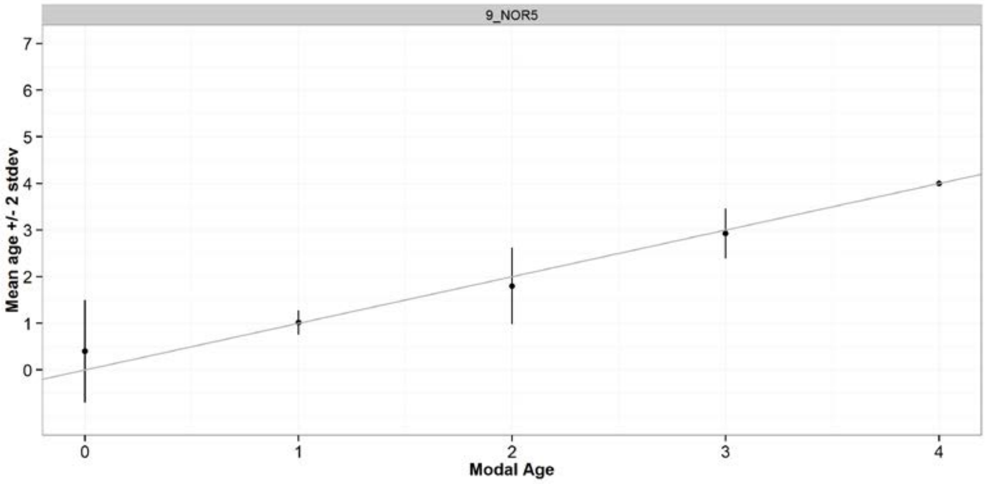
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Mean age +/- 2 stdev
Modal Age
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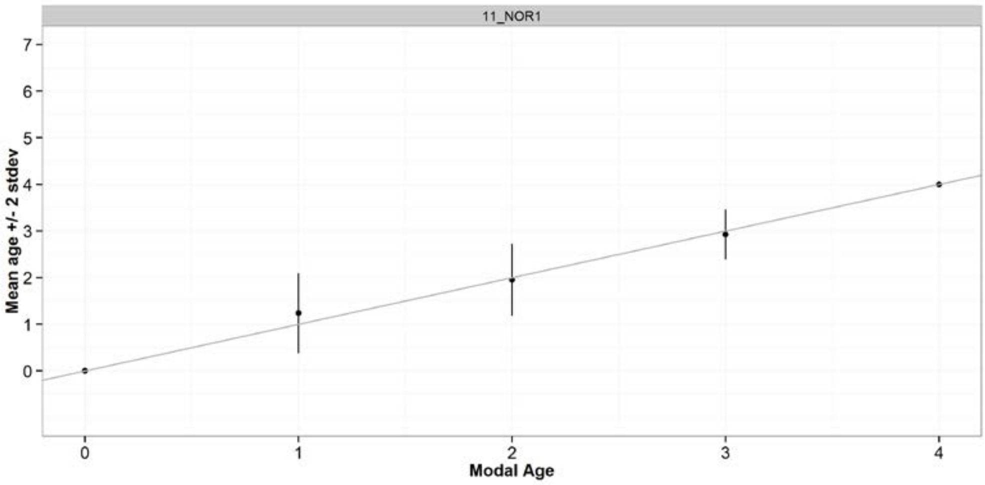
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Mean age +/- 2 stdev
Modal Age
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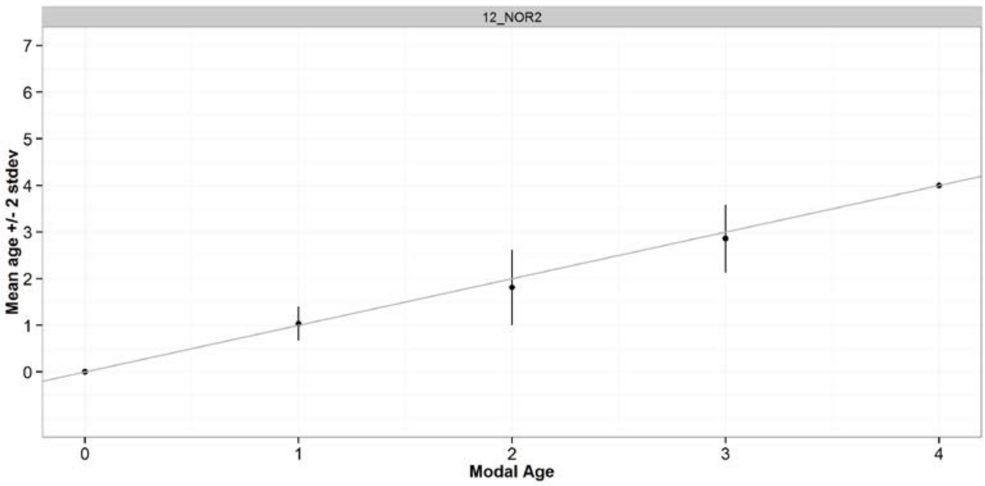
12_NOR2
Mean age +/- 2 stdev
Modal Age
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4
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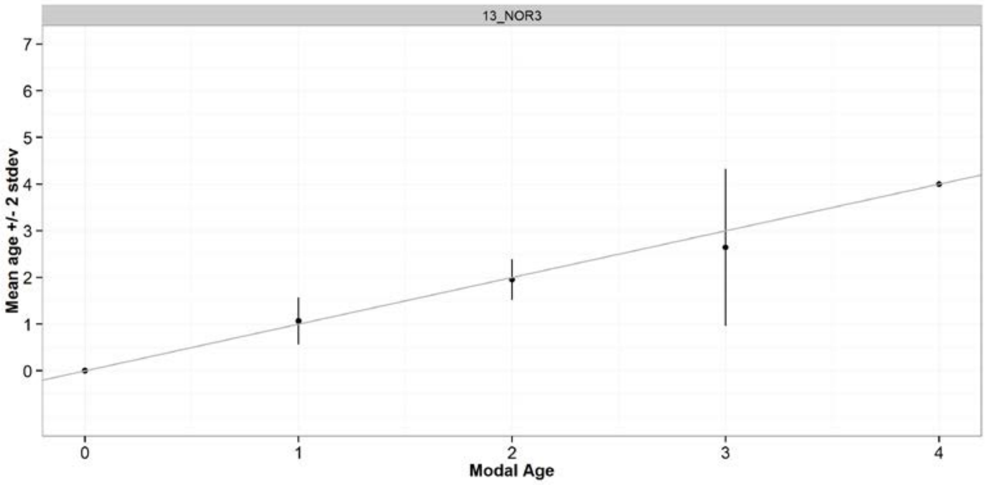
13_NOR3
Mean age +/- 2 stdev
Modal Age
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1
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4
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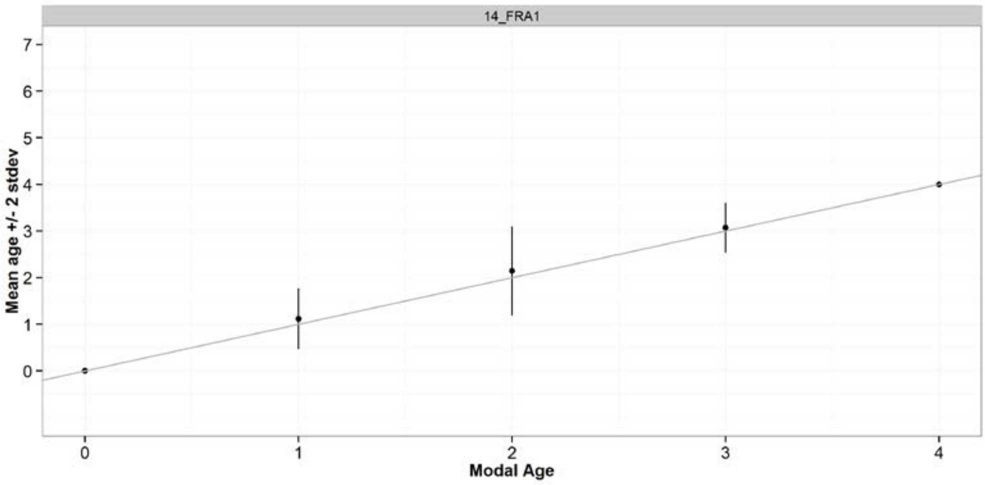
14_FRA1
Mean age +/- 2 stdev
Modal Age
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1
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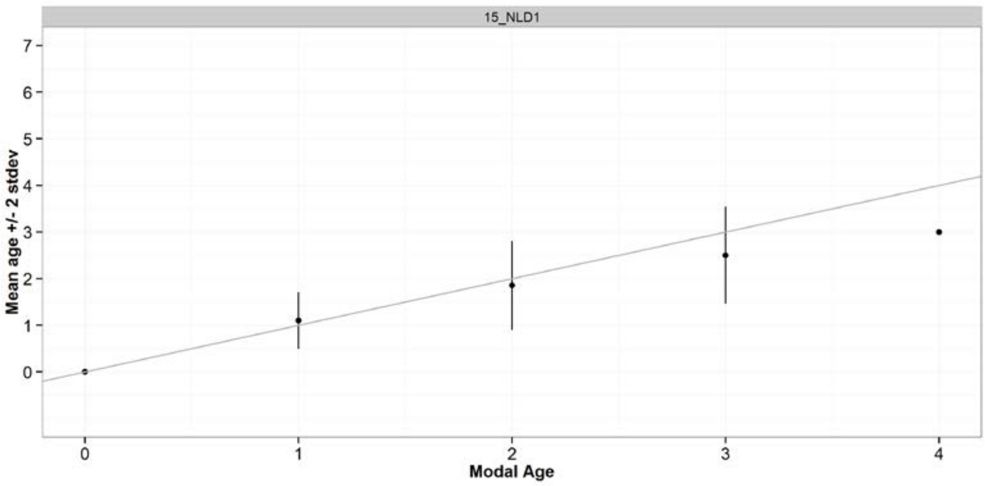
15_NLD1
Mean age +/- 2 stdev
Modal Age
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4
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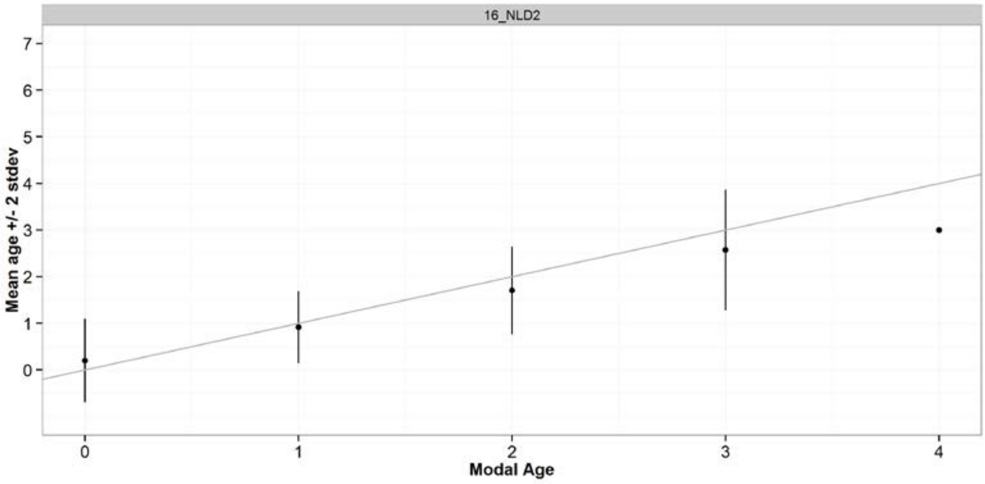
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Mean age +/- 2 stdev
Modal Age
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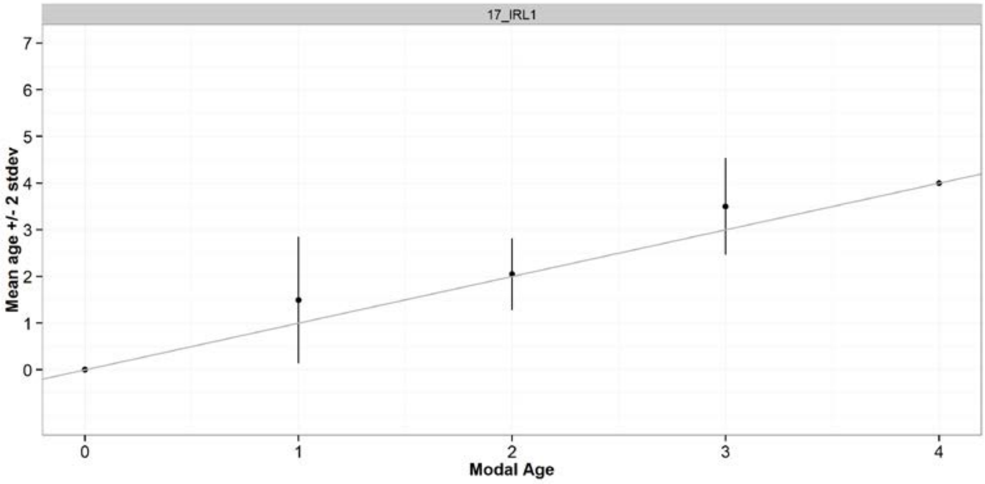
17_IRL1
Mean age +/- 2 stdev
Modal Age
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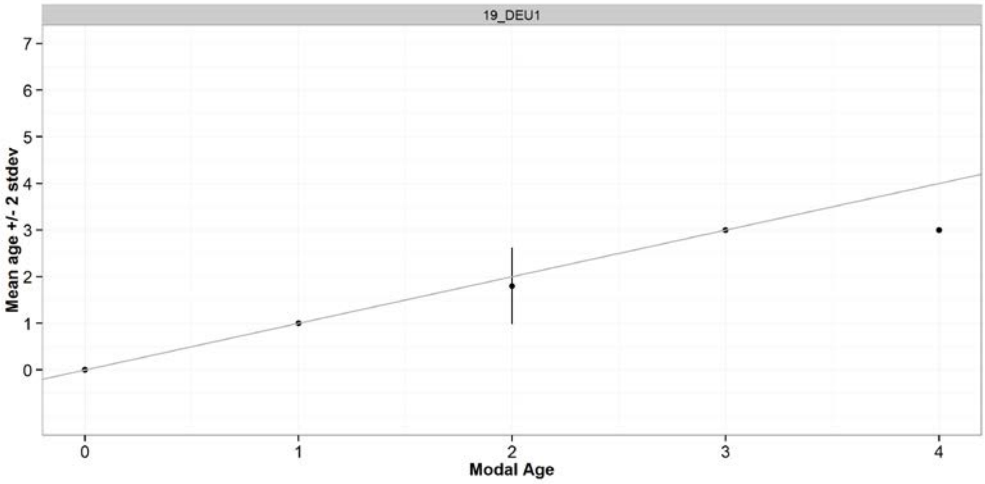
19_DEU1
Mean age +/- 2 stdev
Modal Age
0
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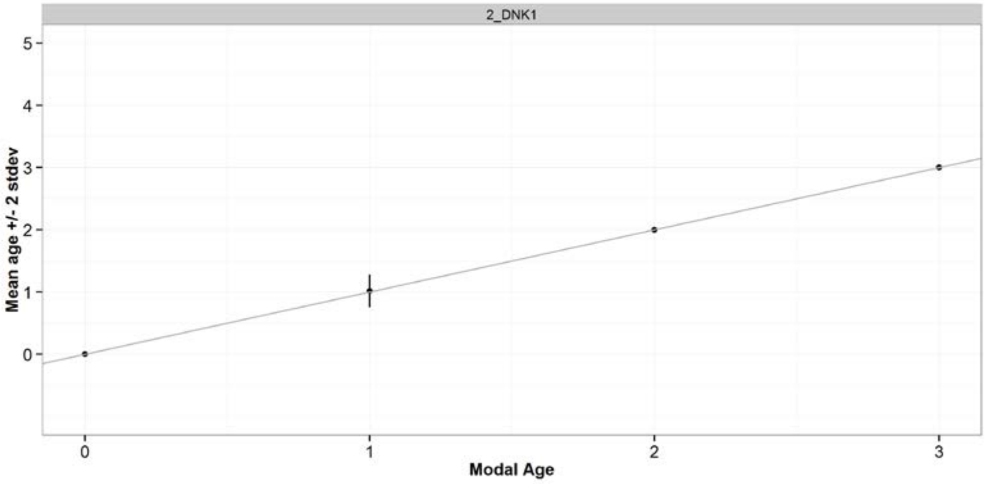
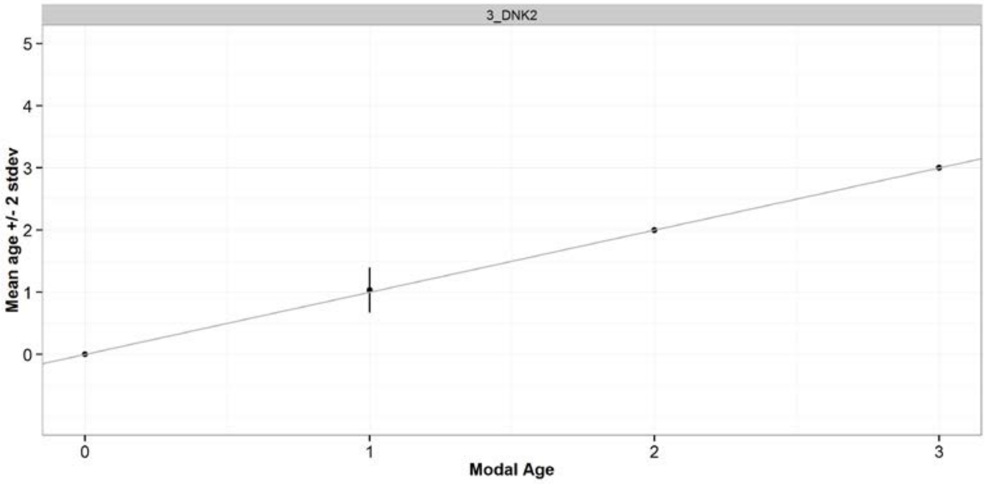
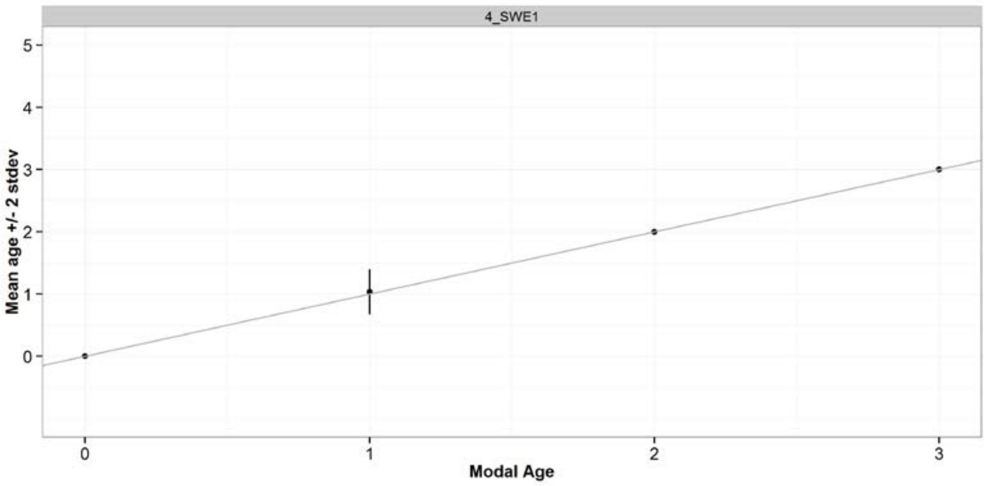
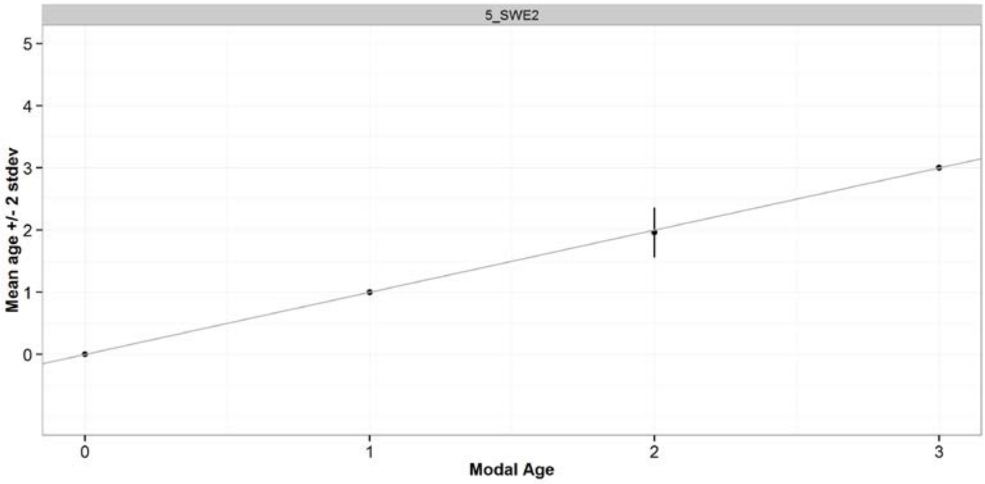
Celtic Seas Ecoregion








5_SWE2
Mean age +/- 2 stdev
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Modal Age
0
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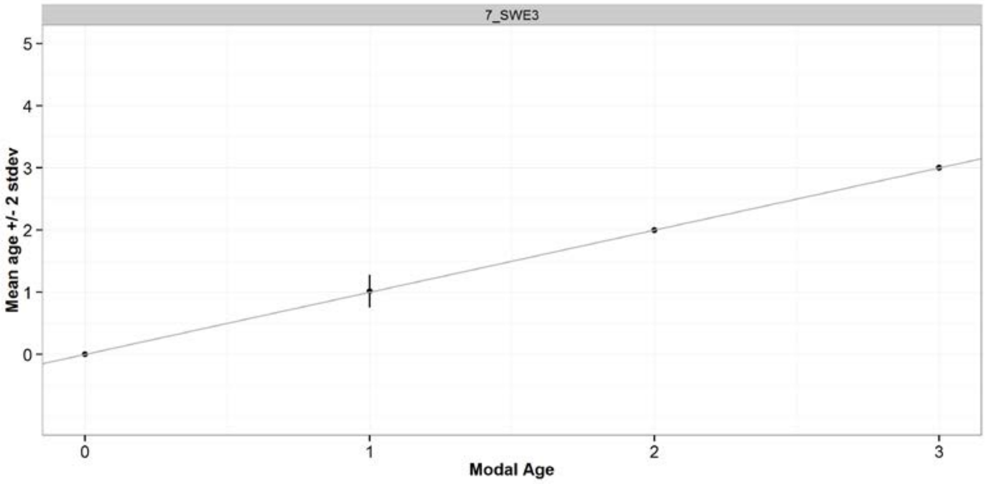
7_SWE3
Mean age +/- 2 stdev
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1
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5
Modal Age
0
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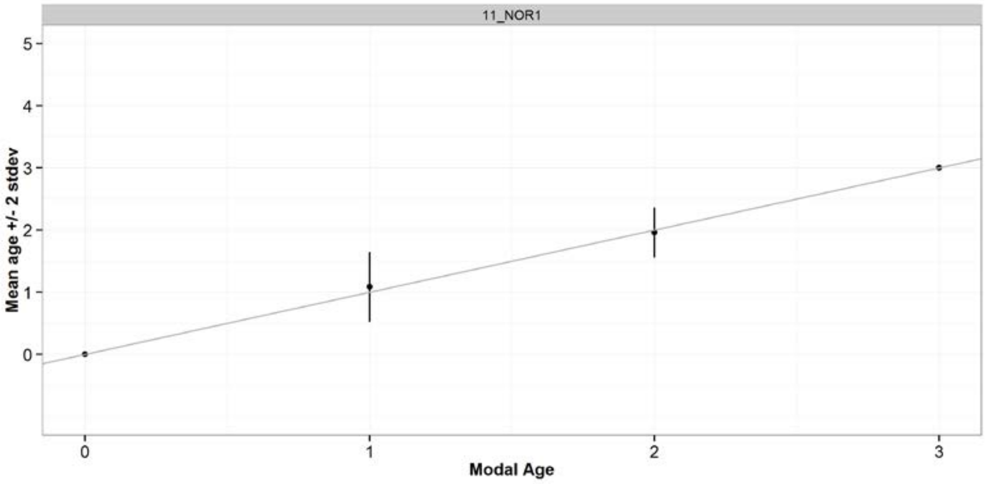
11_NOR1
Mean age +/- 2 stdev
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1
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Modal Age
0
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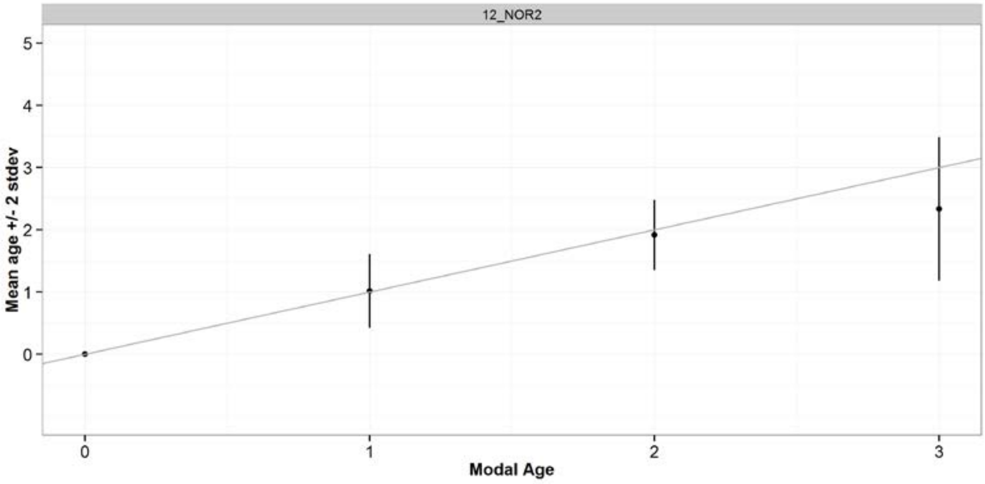
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Mean age +/- 2 stdev
Modal Age
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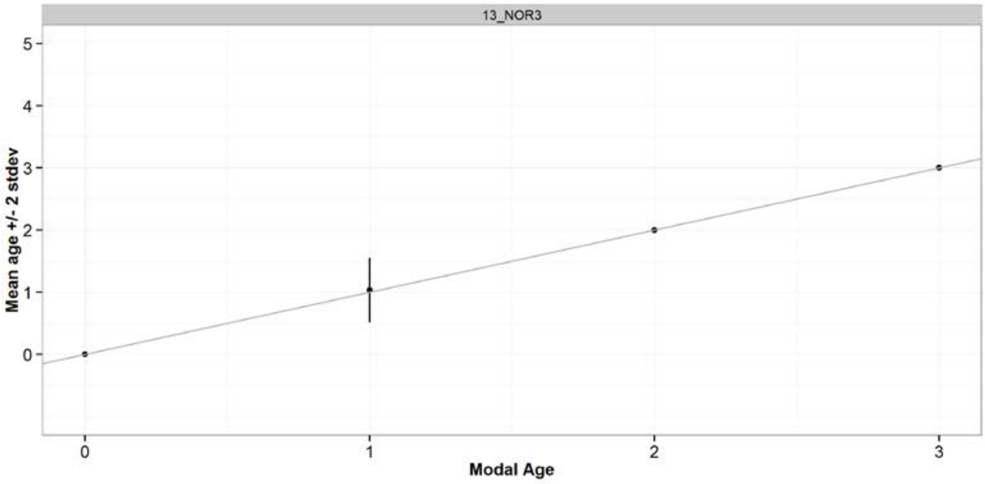
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Mean age +/- 2 stdev
Modal Age
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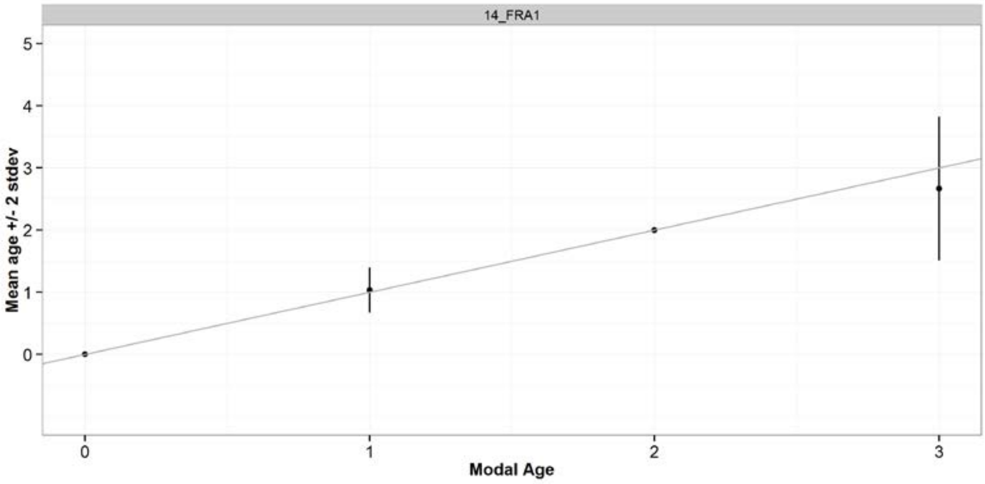
14_FRA1
Mean age +/- 2 stdev
Modal Age
0
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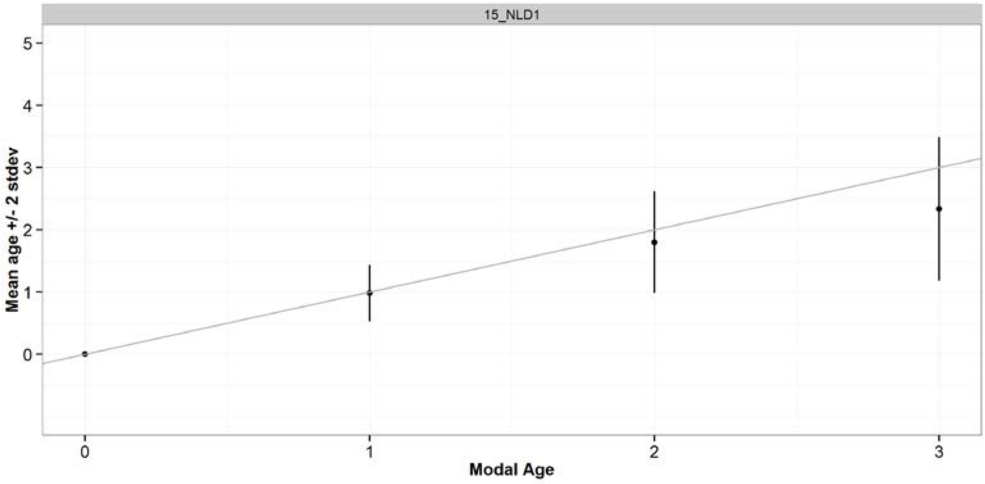
15_NLD1
Mean age +/- 2 stdev
Modal Age
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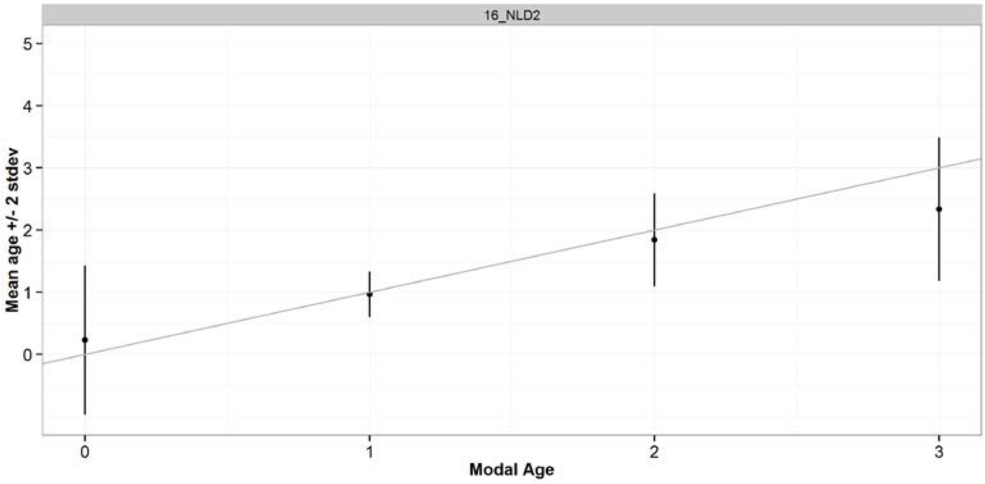
16_NLD2
Mean age +/- 2 stdev
Modal Age
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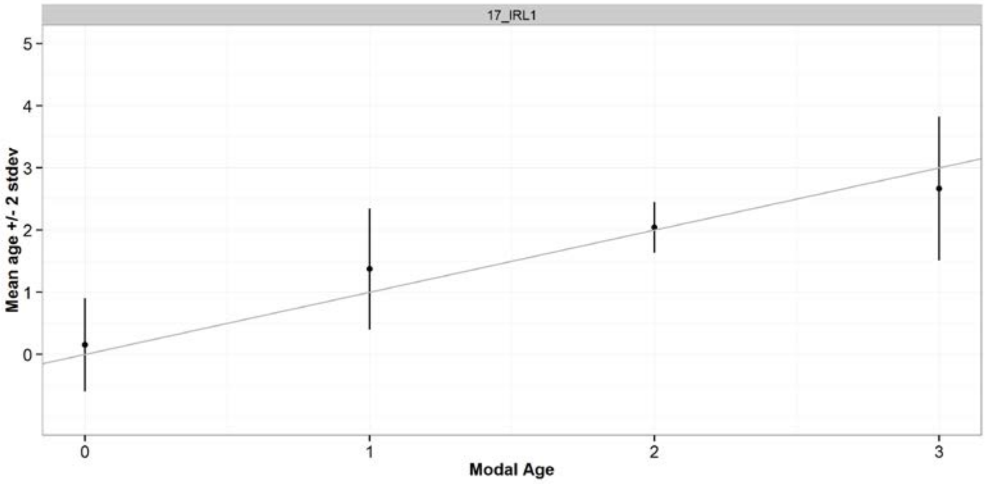
17_IRL1
Mean age +/- 2 stdev
Modal Age
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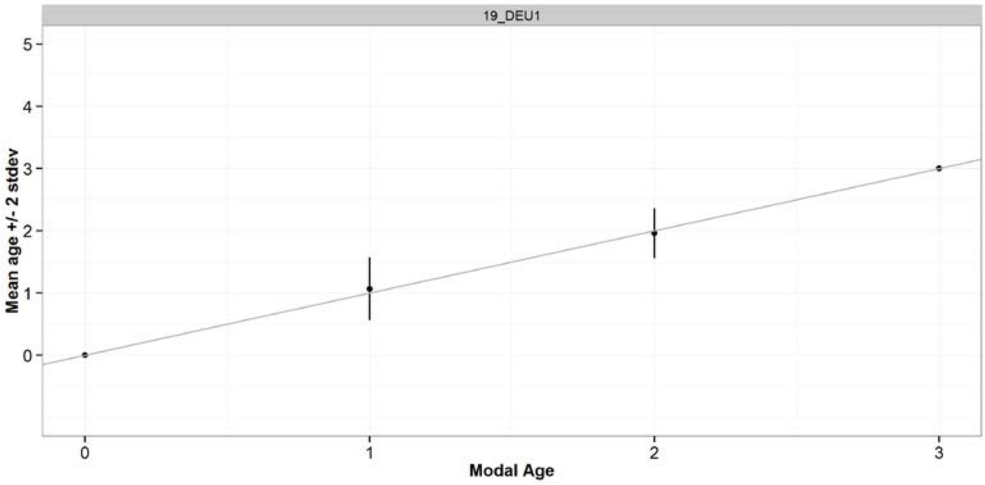
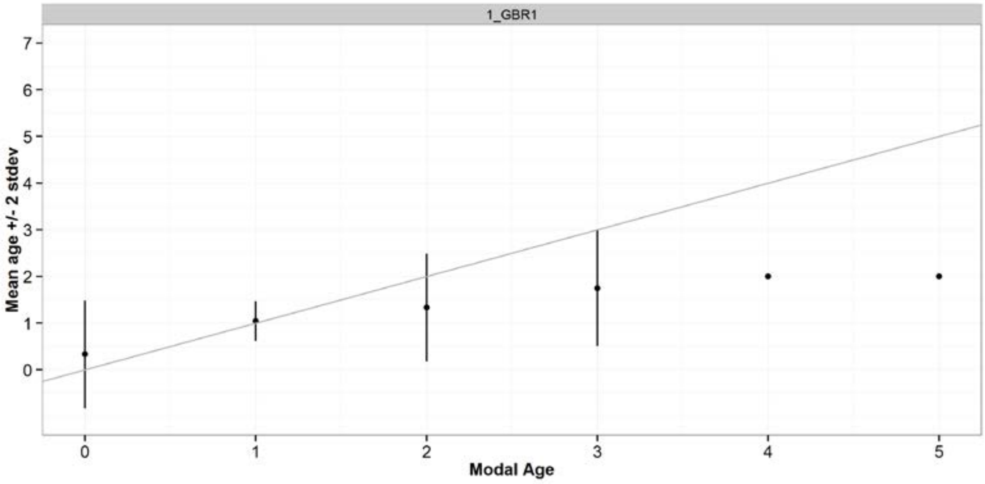


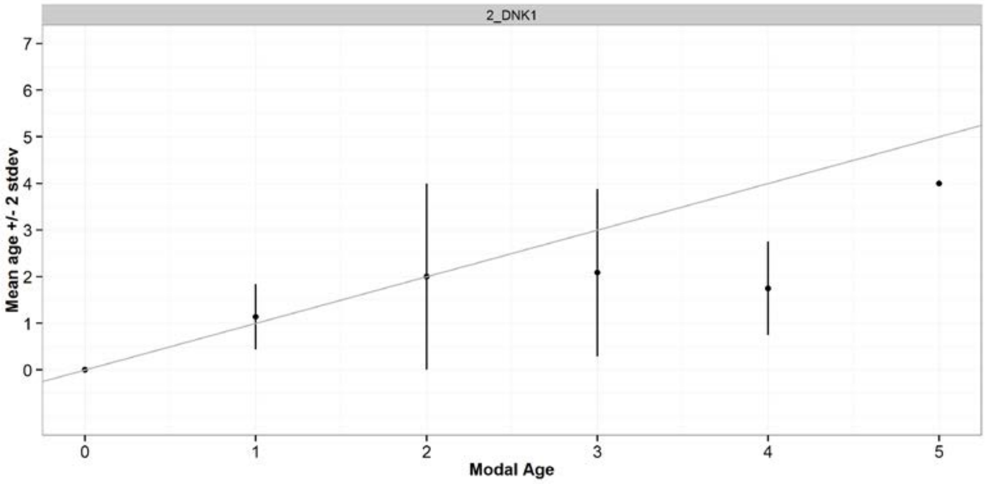
19_DEU1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5

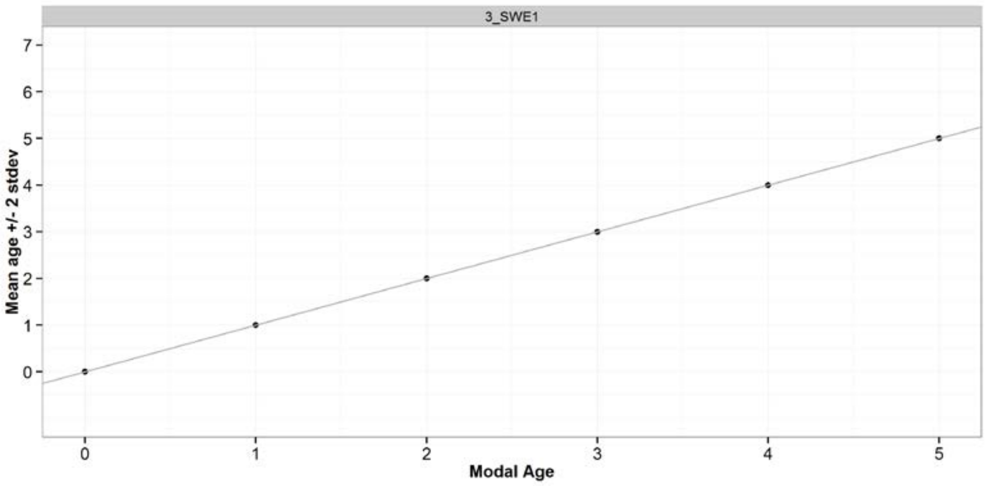
Annex 4: Age bias plots for the workshop exercises

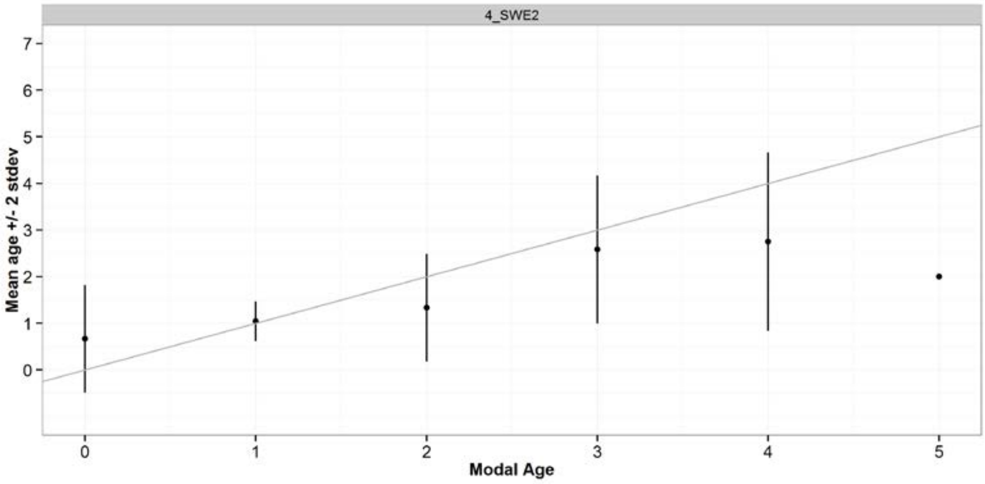
The following graphs are the age bias plots for each reader and for each area/stock. They show the mean age recorded +/- 2stdev of each age reader plotted against the modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line). The reader ID is shown at the top of each graph.

3.a


1_GBR1
Mean age +/- 2 stdev
Modal Age


2_DNK1
Mean age +/- 2 stdev
Modal Age


3_SWE1
Mean age +/- 2 stdev
Modal Age


4_SWE2
Mean age +/- 2 stdev
Modal Age
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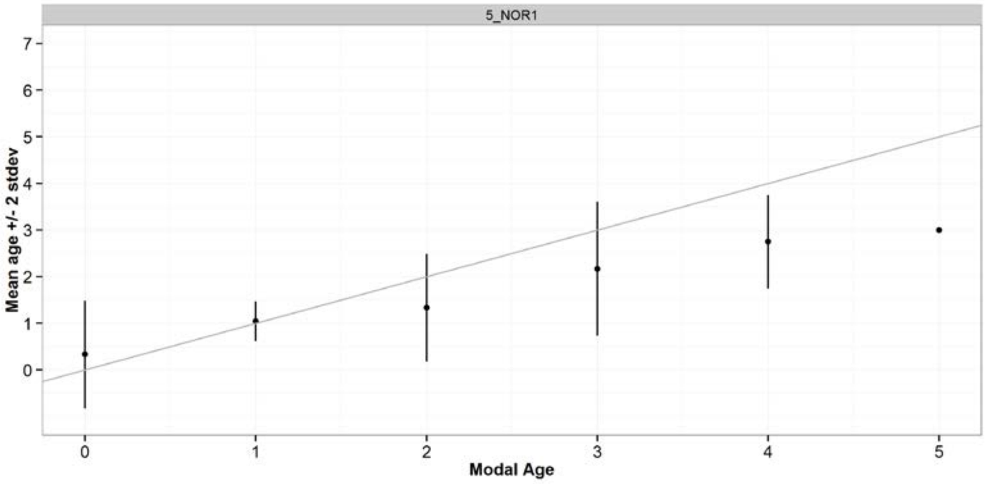
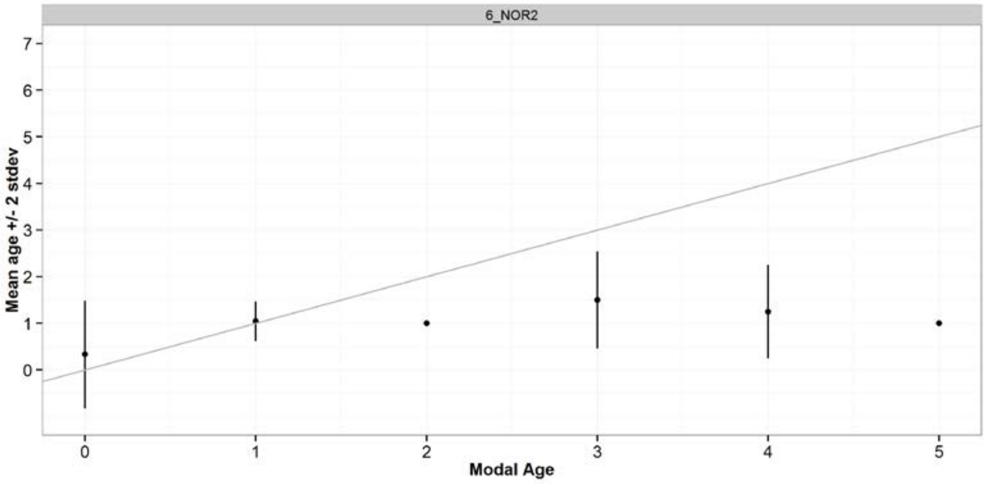
1
2
3
4
5
6
7


5_NOR1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5
6
7


6_NOR2
Mean age +/- 2 stdev
Modal Age
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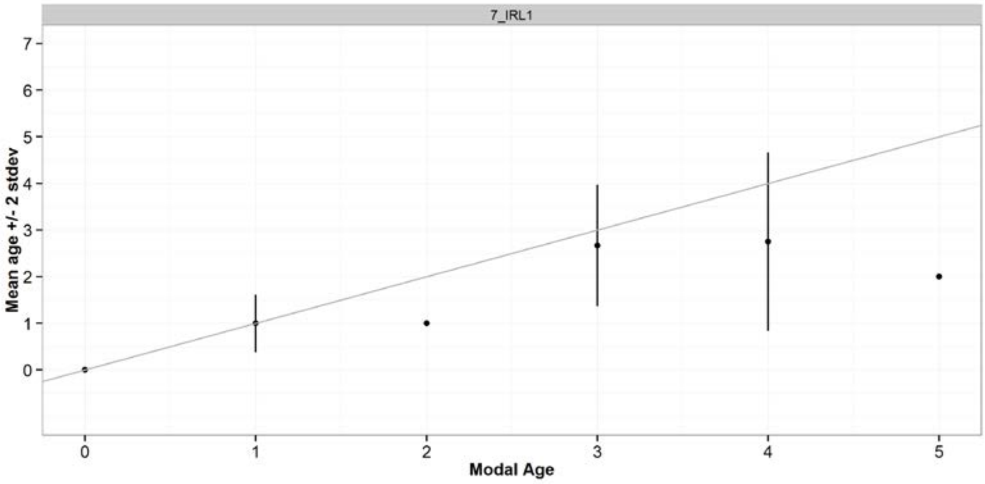
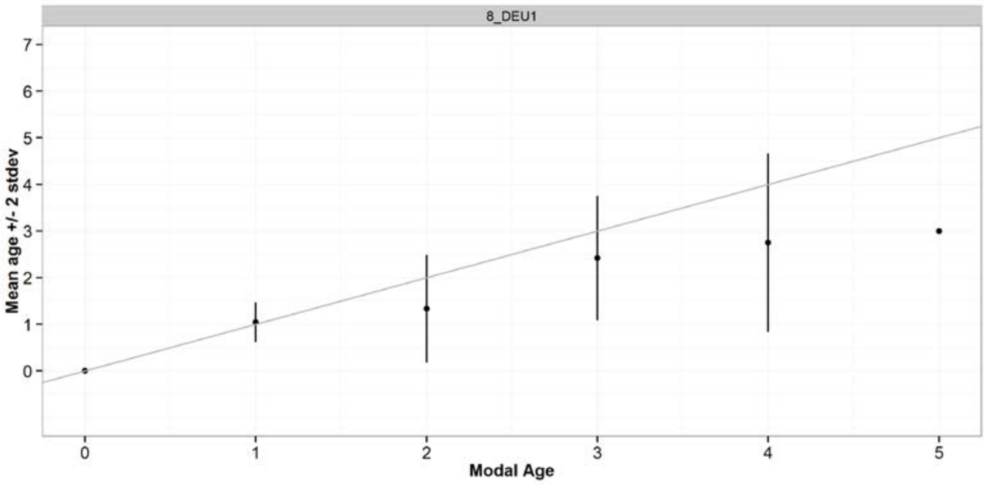
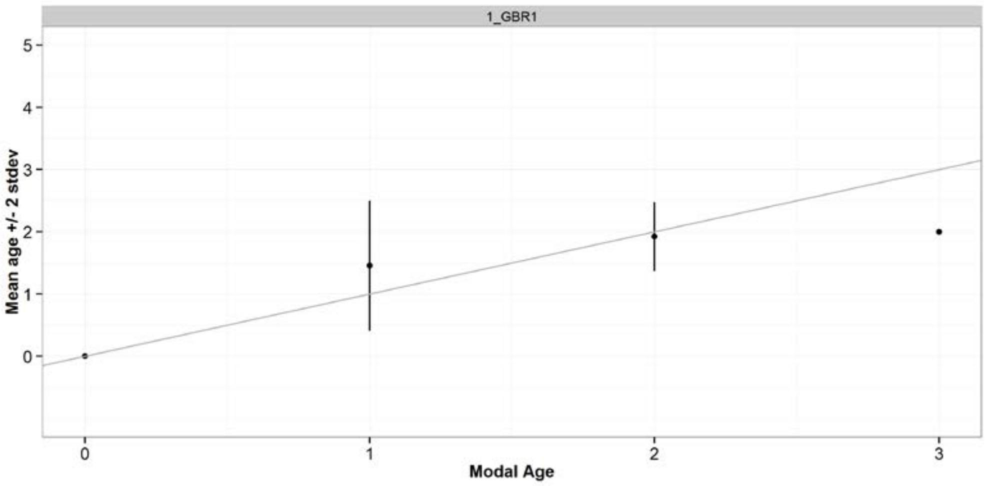
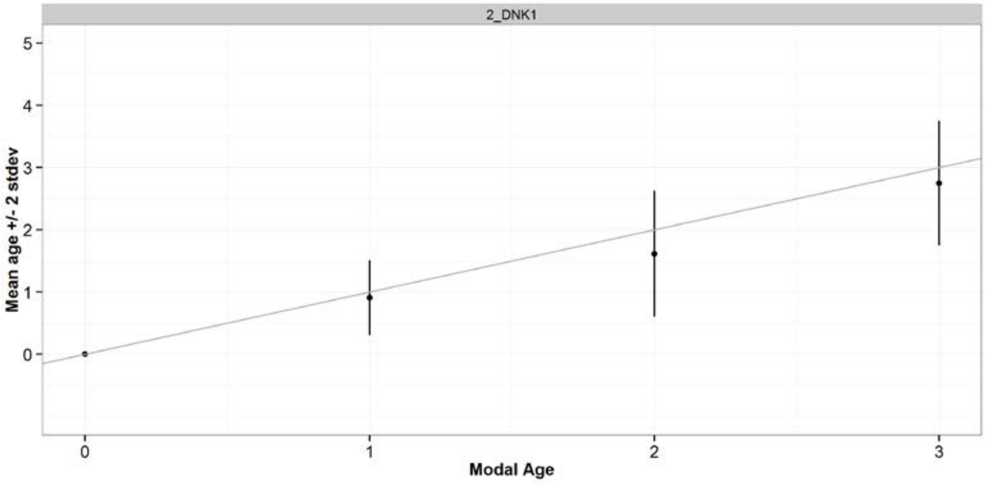
2
3
4
5
6
7


7_IRL1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5
6
7


8_DEU1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5
6
7

North Sea


1_GBR1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5


2_DNK1
Mean age +/- 2 stdev
Modal Age
0
1
2
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4
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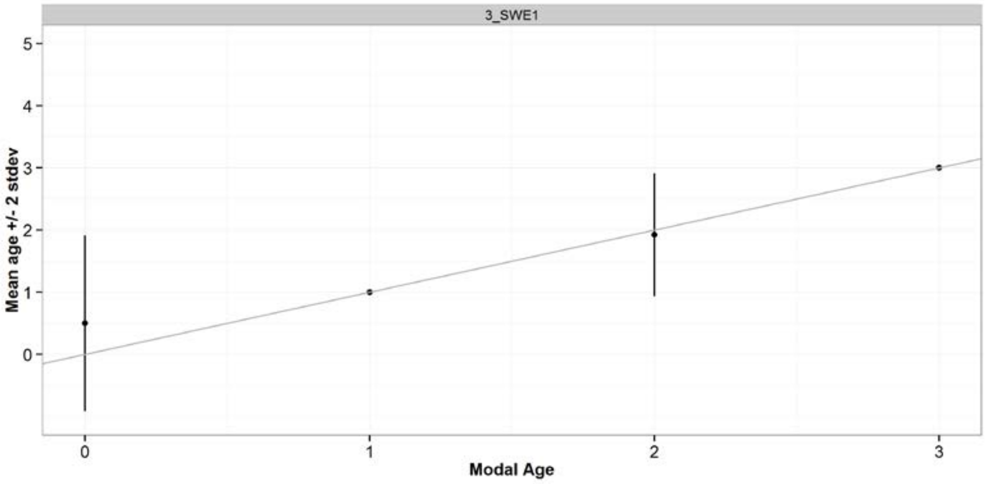
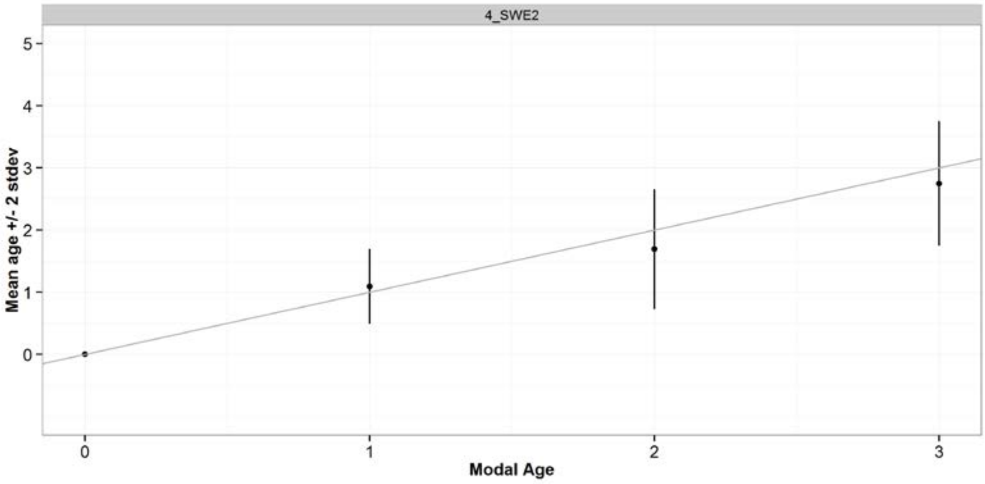

3_SWE1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5


4_SWE2
Mean age +/- 2 stdev
Modal Age
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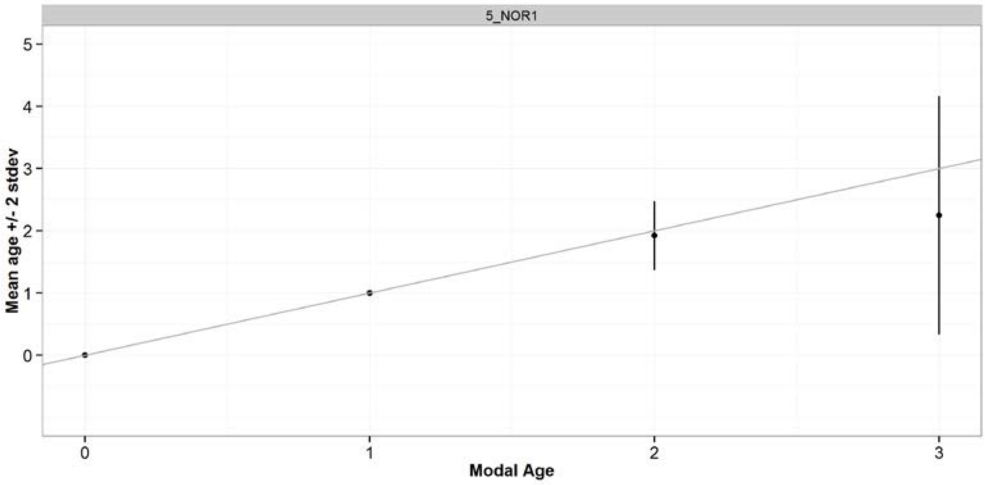
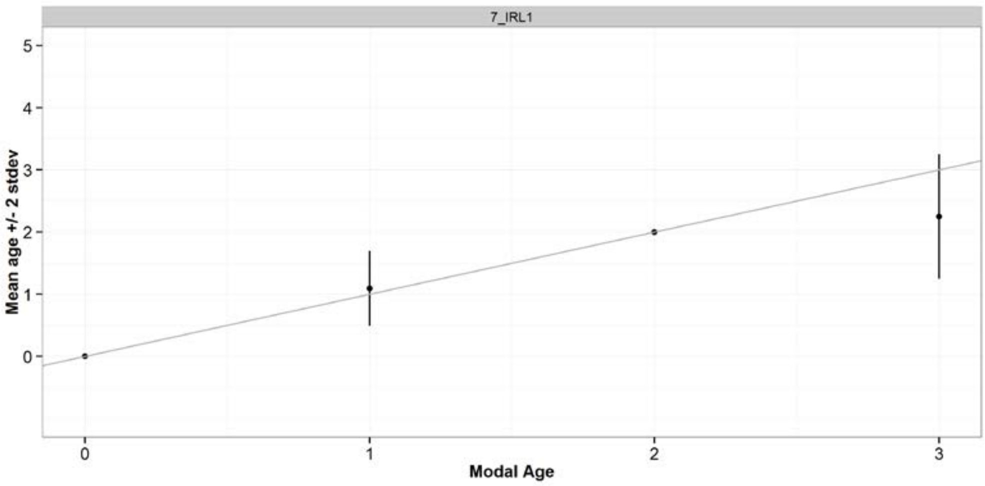
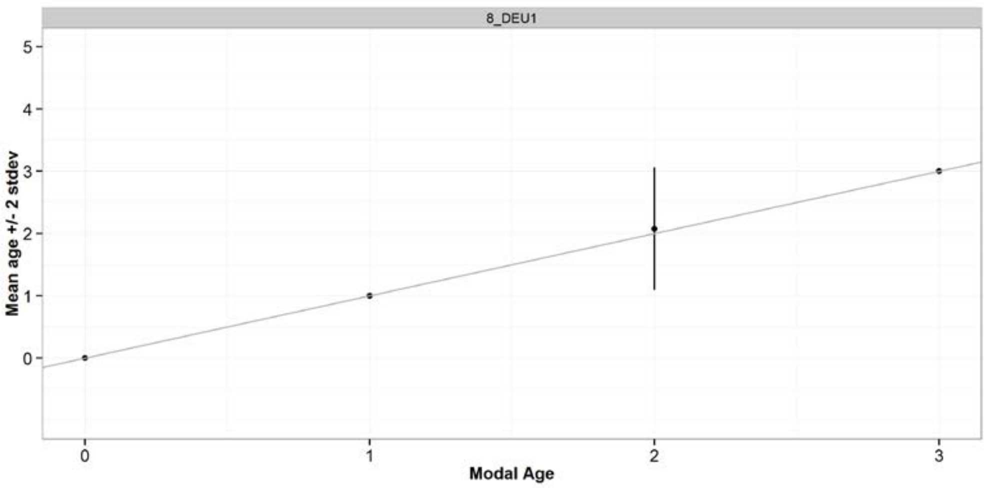
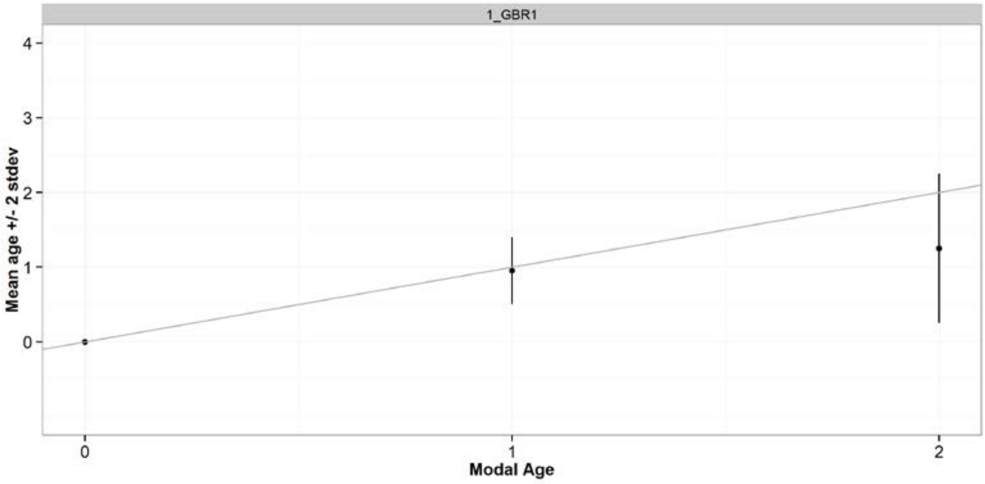
1
2
3
4
5

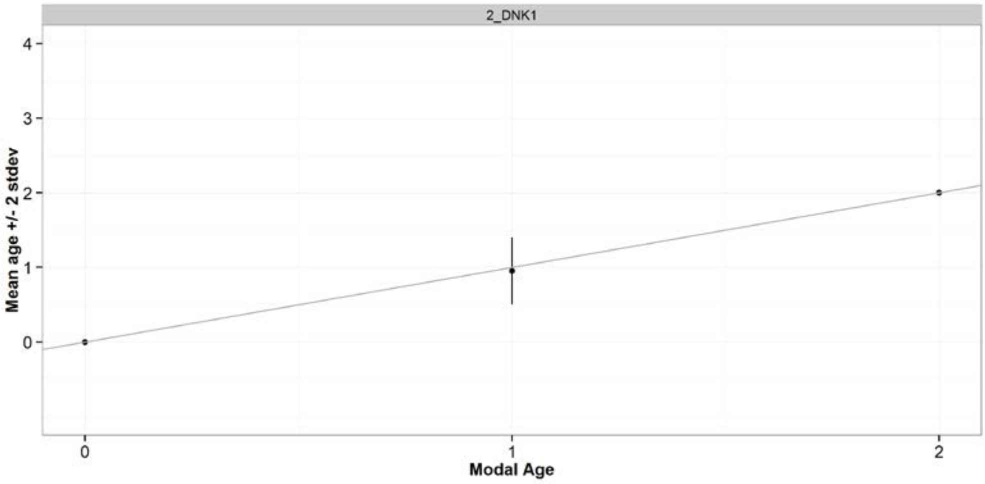

5_NOR1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5

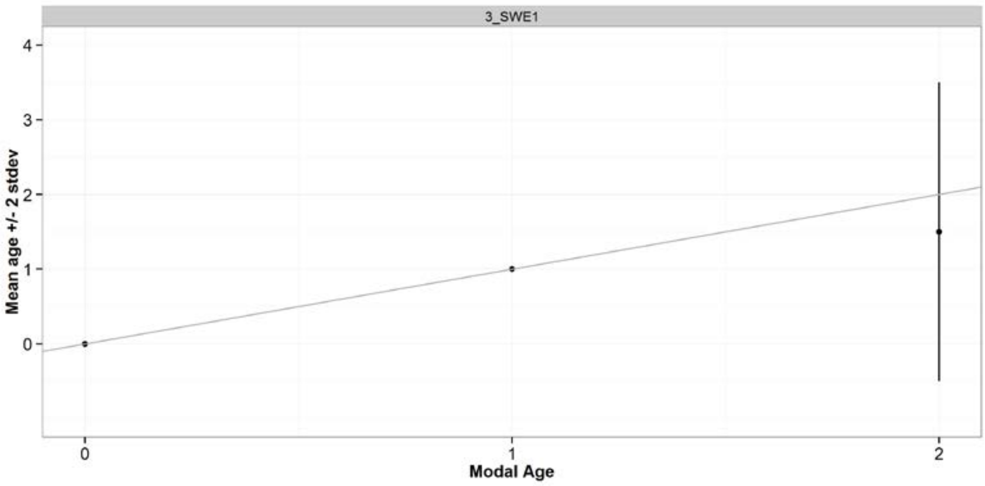

7_IRL1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5

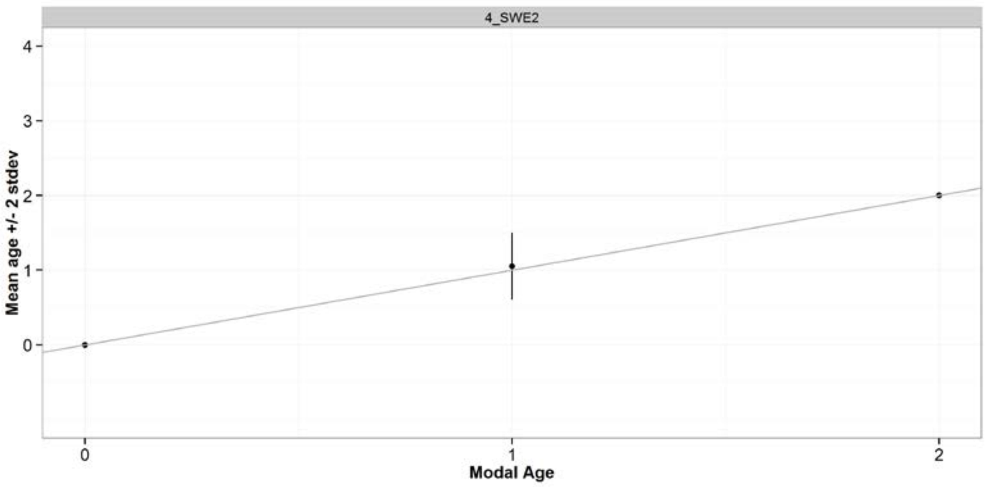

8_DEU1
Mean age +/- 2 stdev
Modal Age
0
1
2
3
4
5

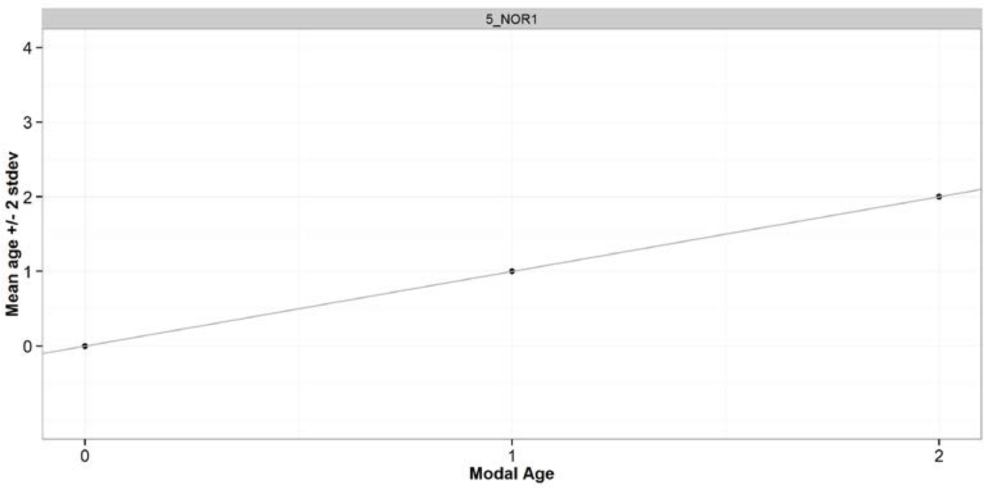
Celtic Seas Ecoregion

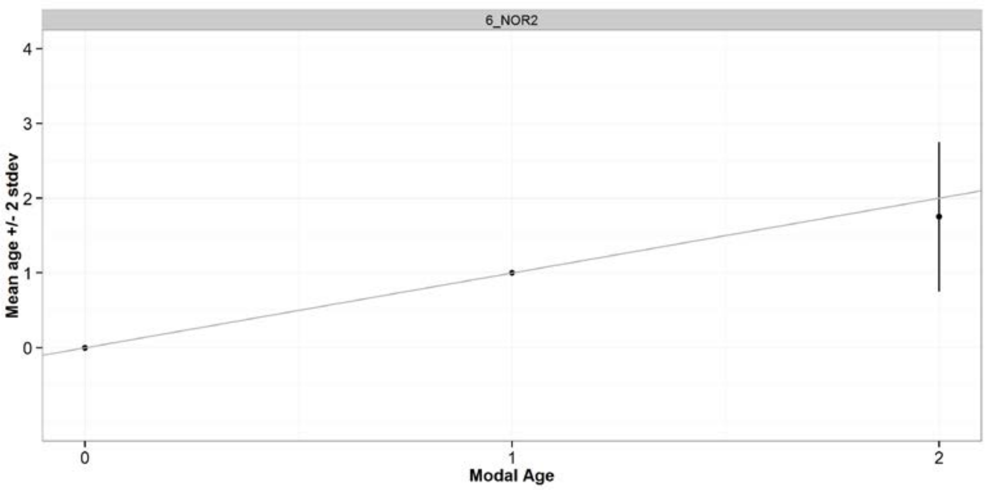


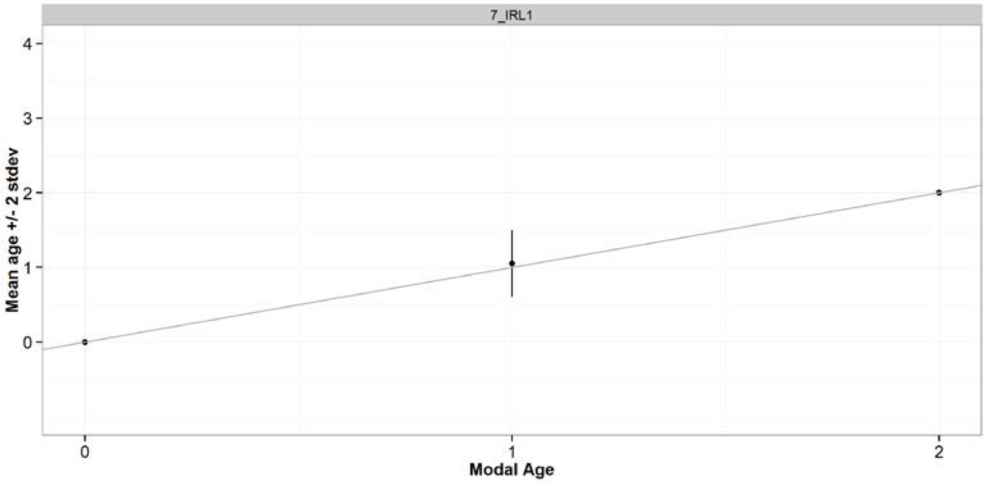


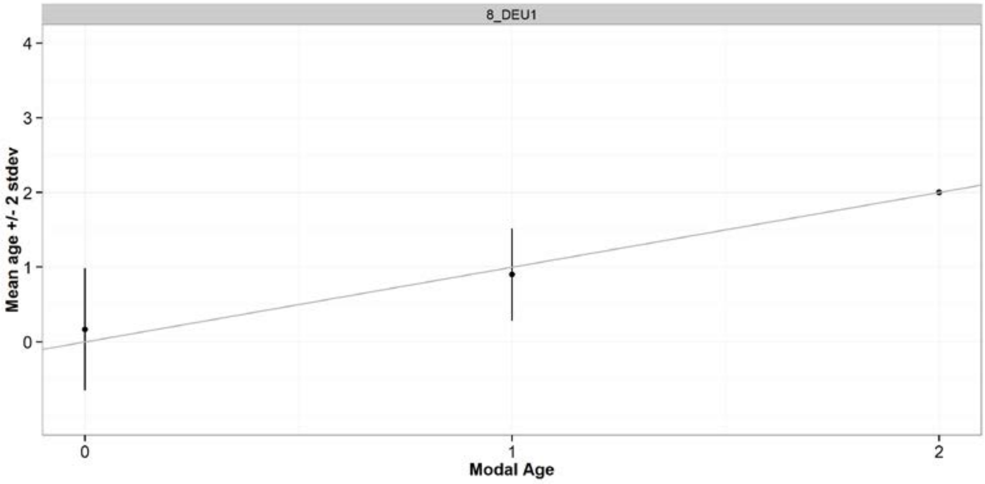



4_SWE2
Mean age +/- 2 stdev
Modal Age


5_NOR1
Mean age +/- 2 stdev
Modal Age


6_NOR2
Mean age +/- 2 stdev
Modal Age


7_IRL1
Mean age +/- 2 stdev
0
1
2
3
4
0
1
2
Modal Age


8_DEU1
Mean age +/- 2 stdev
0
1
2
3
4
0
1
2
Modal Age

Annex 5: Agreed age otolith collection

North Sea

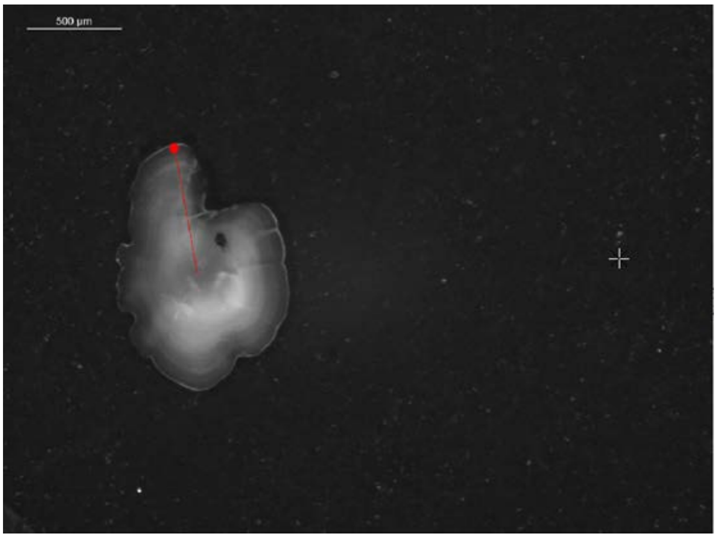


Figure A5.1. 6953228_4B, capture date 14/09/2014. Agreed age 0

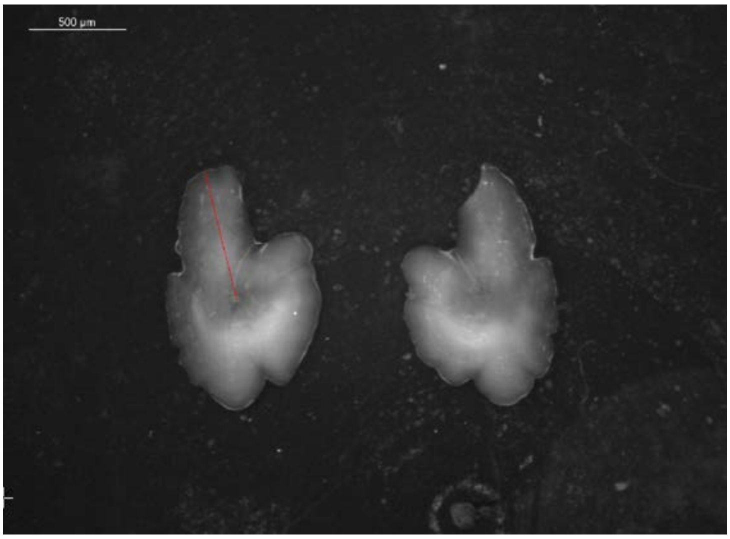


Figure A5.2. 7351932_4C, Capture date 06/08/2016, Agreed age 0

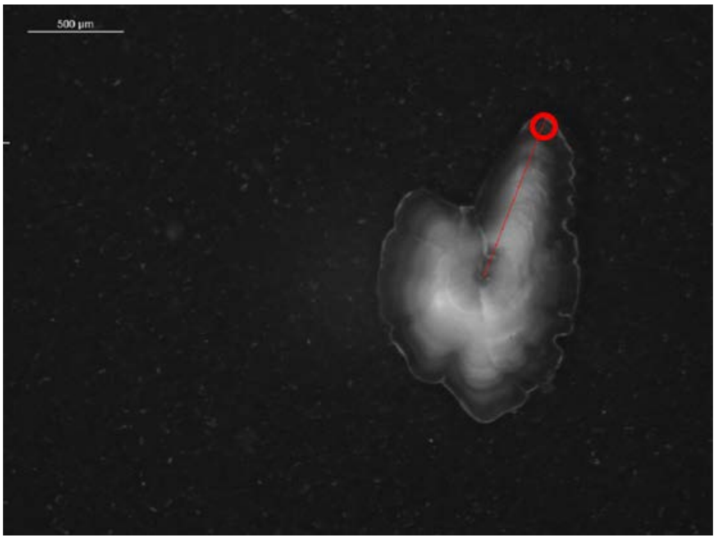


Figure A5.3. 7116403_4B., Capture date 23/06/2015, Agreed age 1

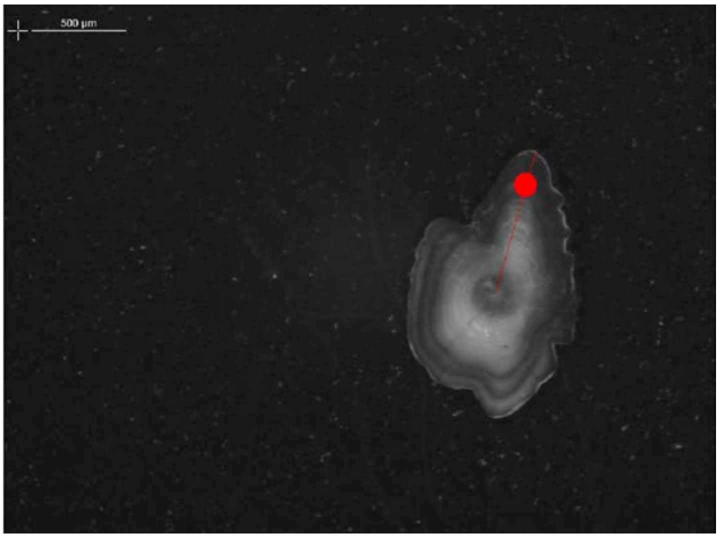


Figure A5.4. 7116379_4B, capture date 23/06/2015. Agreed age 1

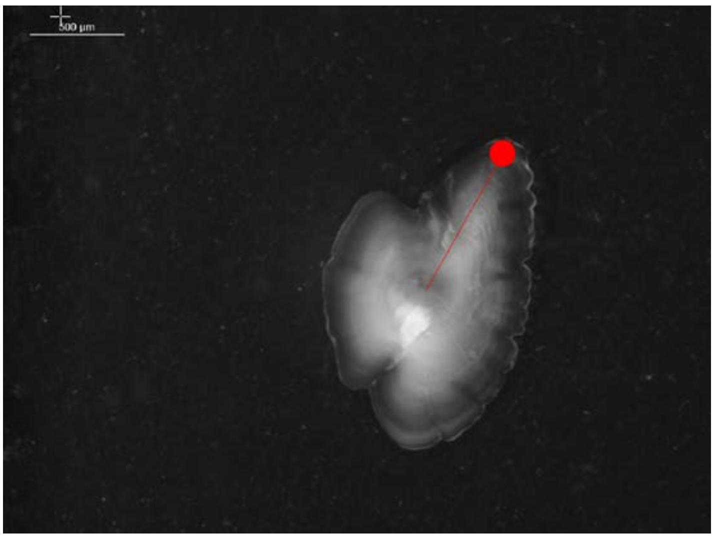


Figure A5.5. 7116356_4B, capture date 23/06/2015. Agreed age 1

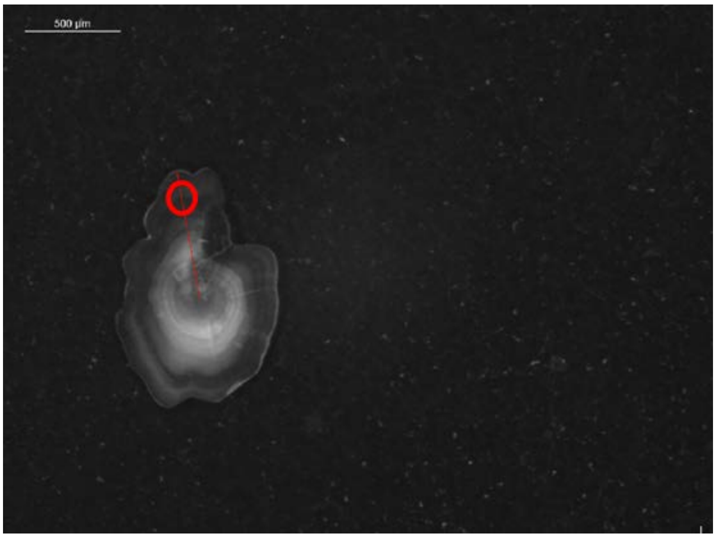


Figure A5.6. 7116359_4B, capture date 23/06/2015. Agreed age 1

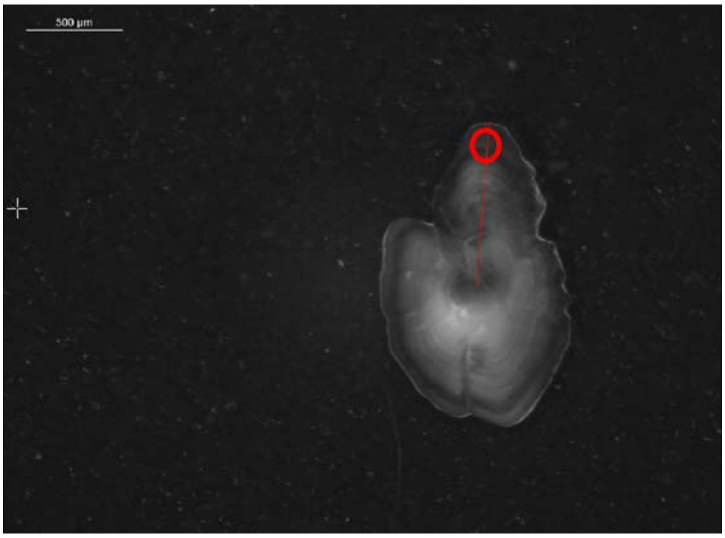


Figure A5.7. 7116383_4B, capture date 23/06/2015. Agreed age 1

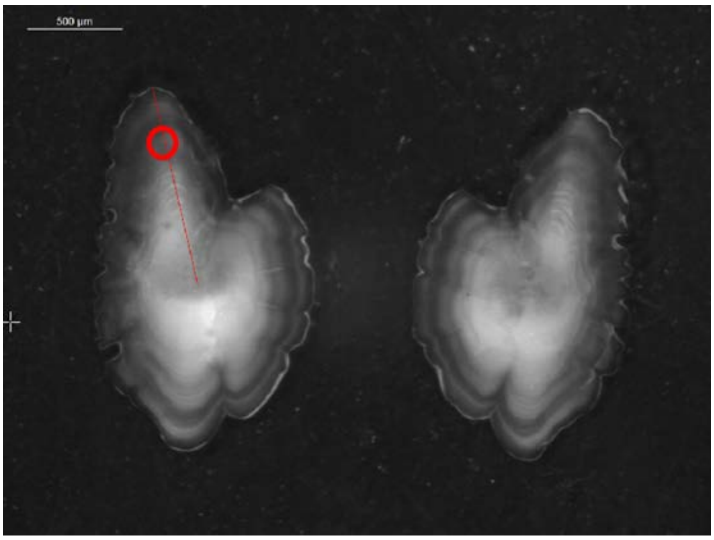


Figure A5.8. 7190397_4b, capture date 24/08/2015, Agreed age 1

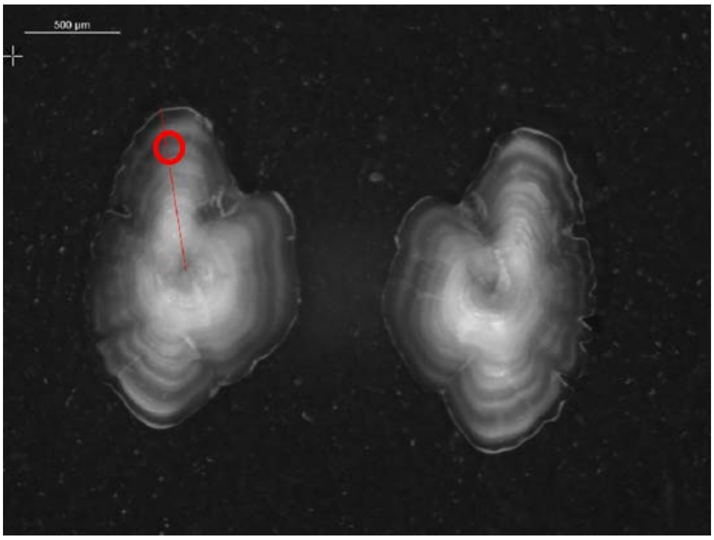


Figure A5.9. 7216460_4C, capture date 09/12/2015, Agreed age 1

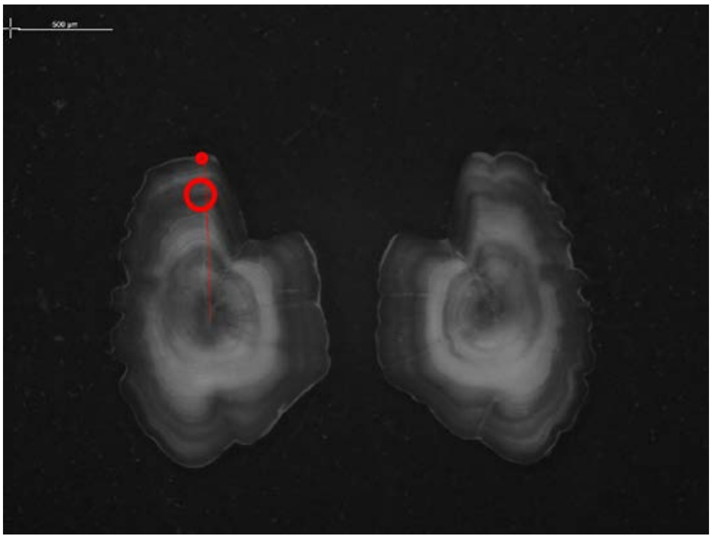


Figure A5.10. 7235987_4B, capture Date 14/02/2016, Agreed age 2

Celtic Seas Ecoregion

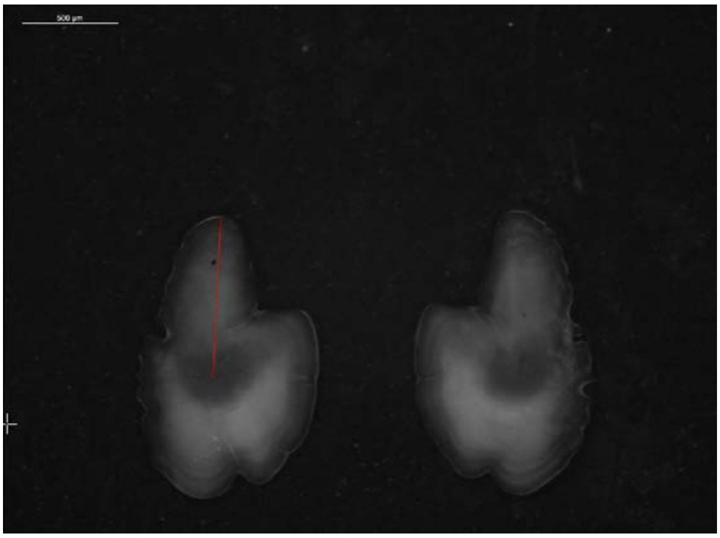


Figure A5.11. IGFS06111320_VIIg, Capture date 05/11/2009, Agreed age 0

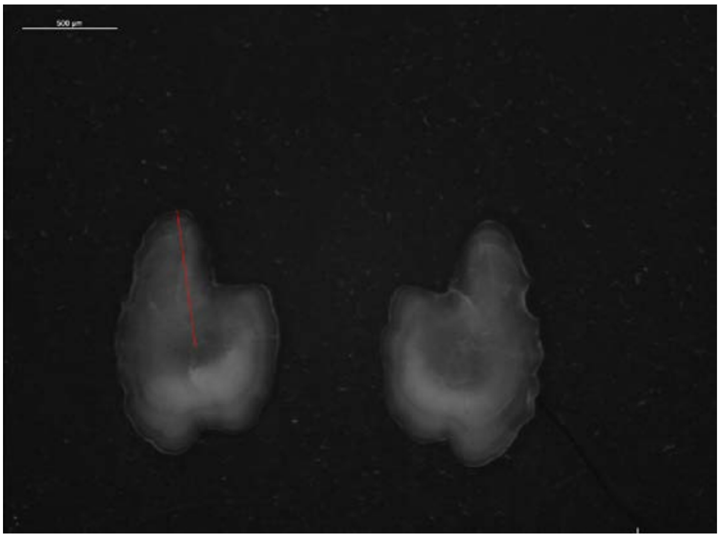


Figure A5.12. IGFS1317840_VIIb, capture date 28/11/2009, Agreed age 0

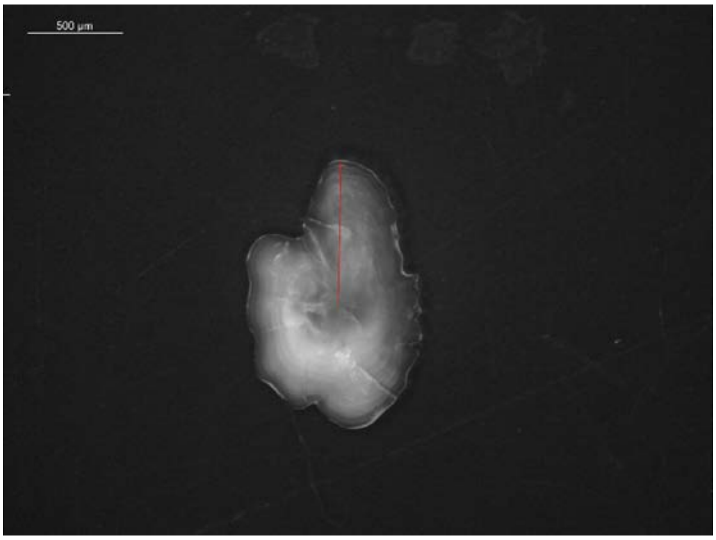


Figure A5.13. IGFS1317832, Capture date 28/11/2009, Agreed age 0

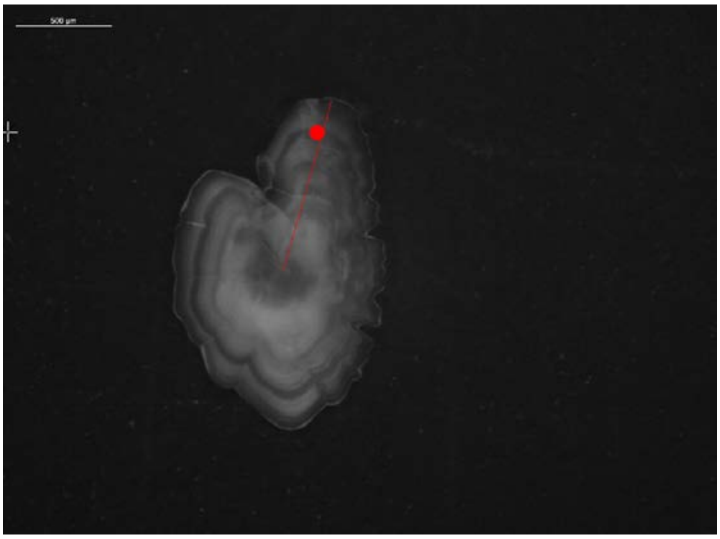


Figure A5.14. 01814S_446_6_VIa, capture date 27/11/2014, Agreed age 1

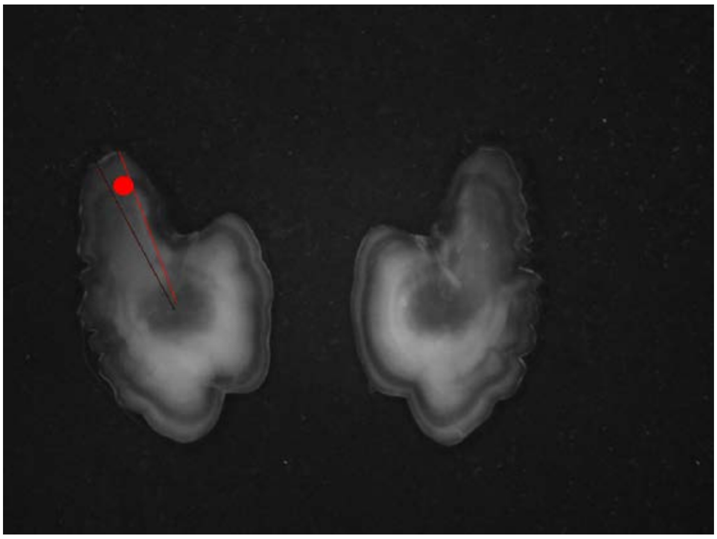


Figure A5.15. NWHAS01071401_VIa, Capture date 01/07/2010, Agreed age 1

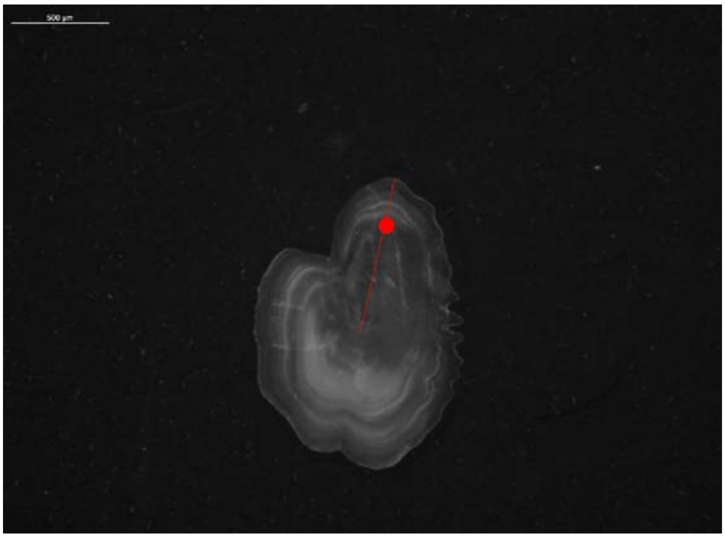


Figure A5.16. ALBA_A14_316_4, capture date 08/11/2010, Agreed age 1

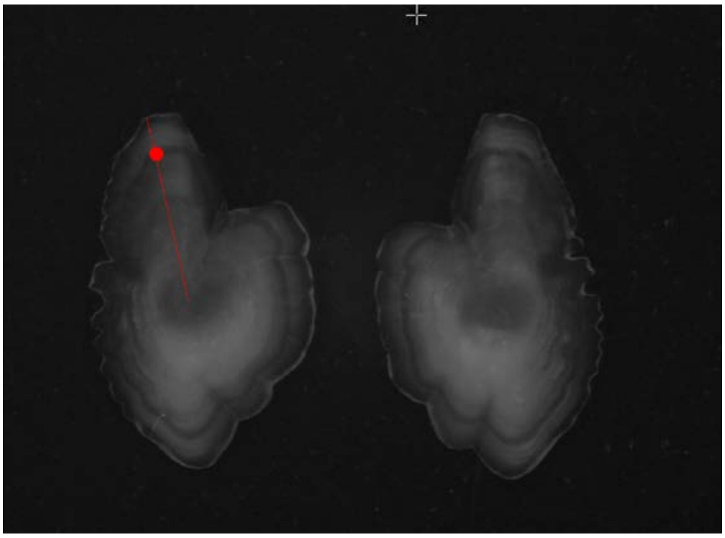


Figure A5.17. OSEP1112, Capture date ??/09/2011, Agreed age 1

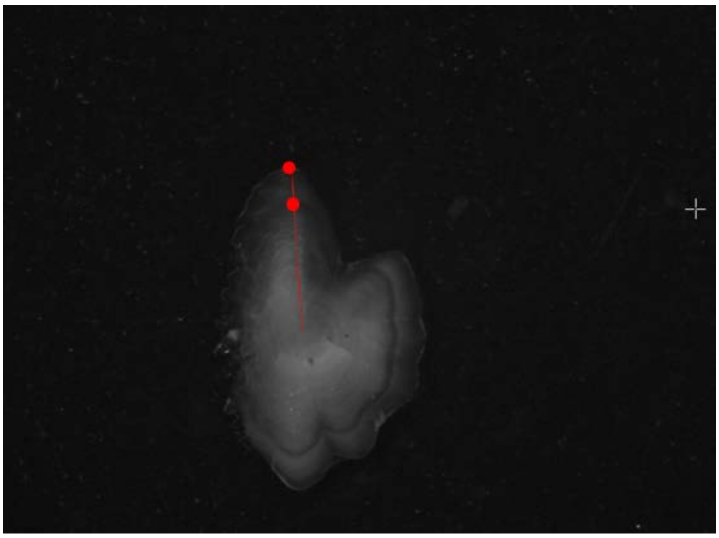


Figure A5.18. 0315S_93_2, Capture date 24/02/2011, Agreed age 2

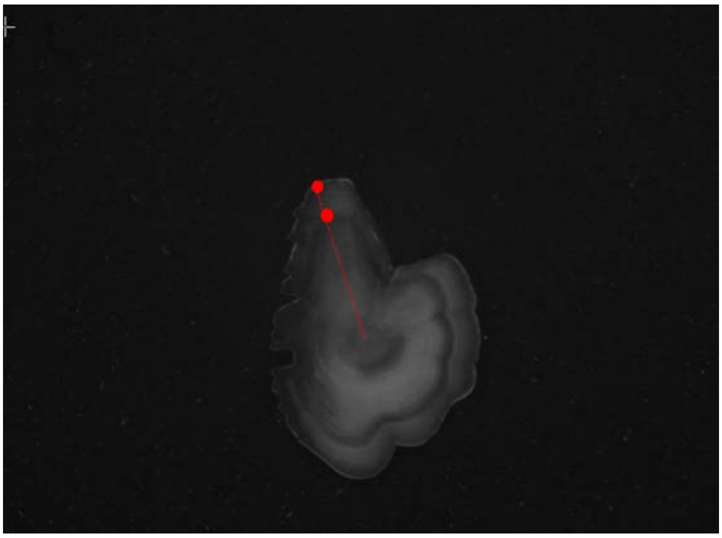


Figure A5.19. 0315S_97_33, Capture date 26/02/2011, Agreed age 2

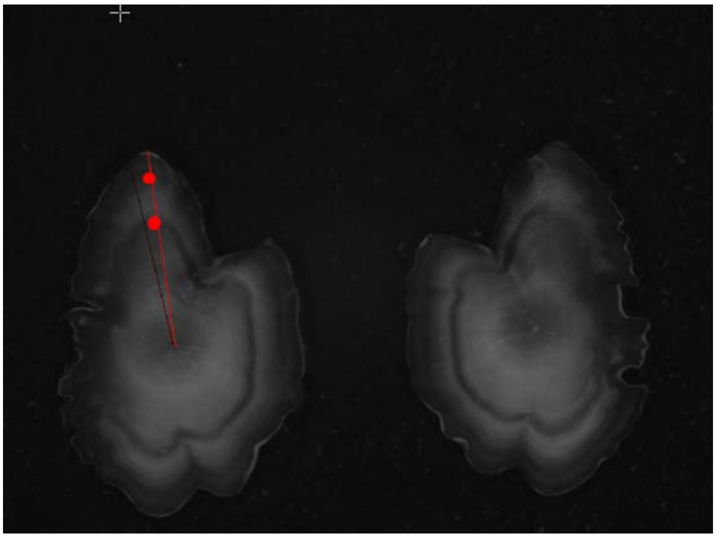


Figure A5.20. OSEP1120, Capture date ??/09/2011, Agreed age 2

Skagerrak and Kattegat

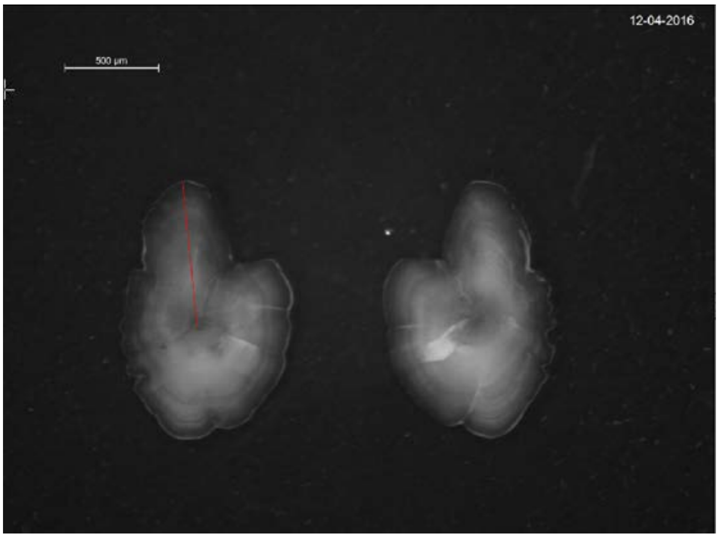


Figure A5.21. 7216510, Capture date 14/12/2015, Agreed age 0

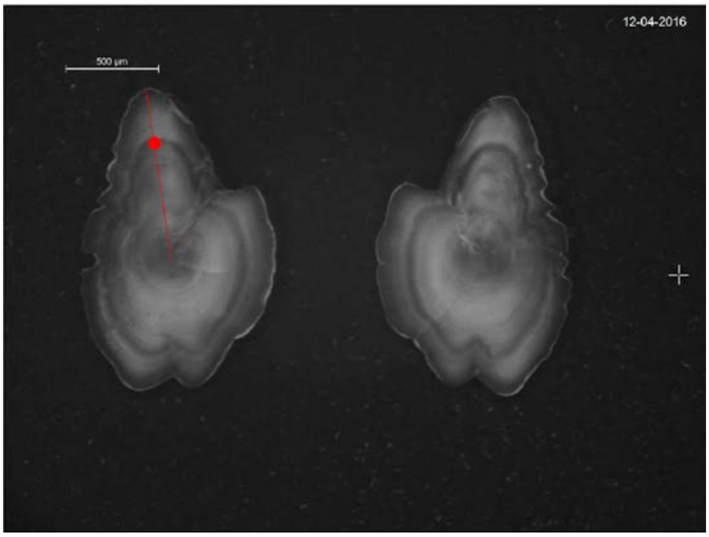


Figure A5.22. 7187388, Capture date 15/10/2015, Agreed age 1

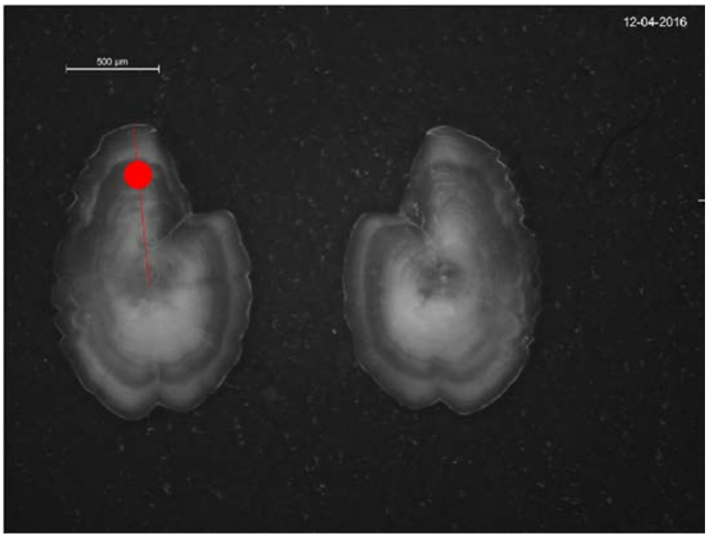


Figure A5.23. 7187393, Capture date 15/10/2015, Agreed age 1

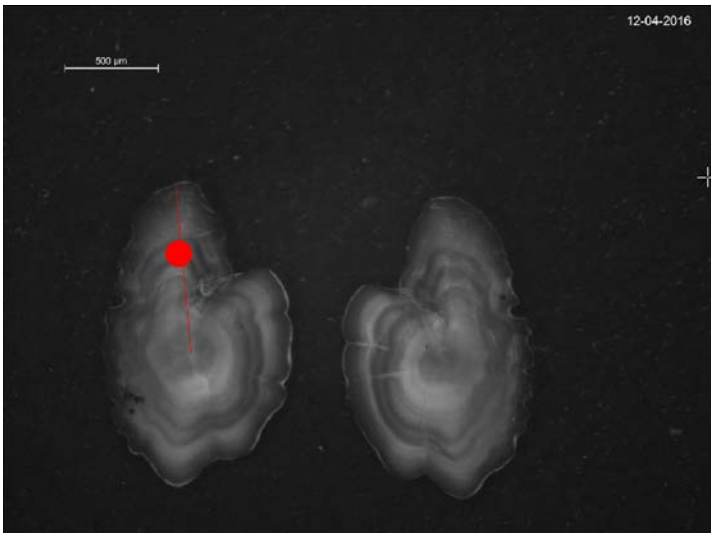


Figure A5.24. 7187165, Capture date 20/10/2015, Agreed age 1

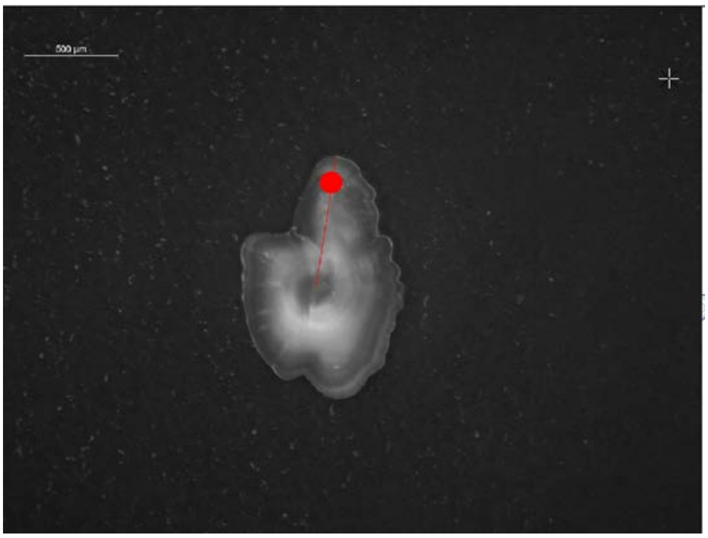


Figure A5.25. 6932325, Capture date 10/06/2014, Agreed age 1

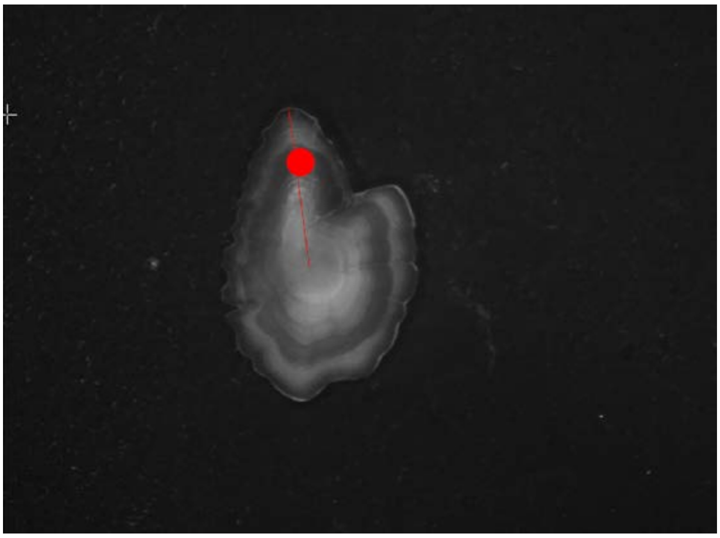


Figure A5.26. 7190495, Capture date 24/09/2015, Agreed age 1

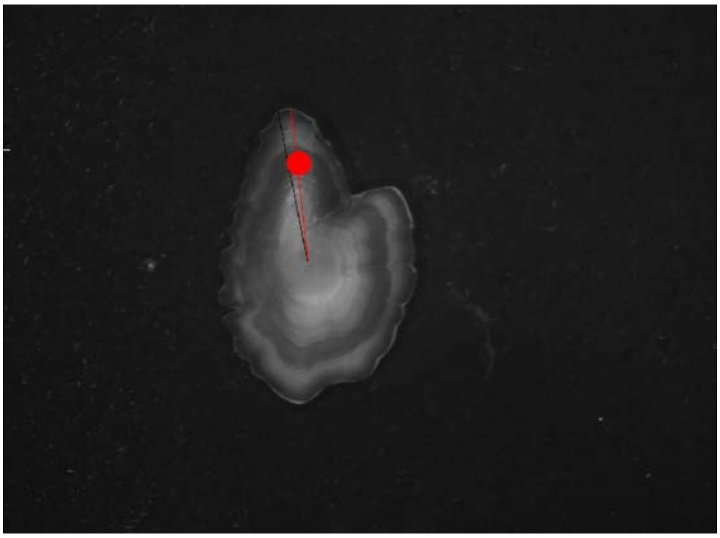


Figure A5.27. 7190488, Capture date 24/09/2015, Agreed age 1

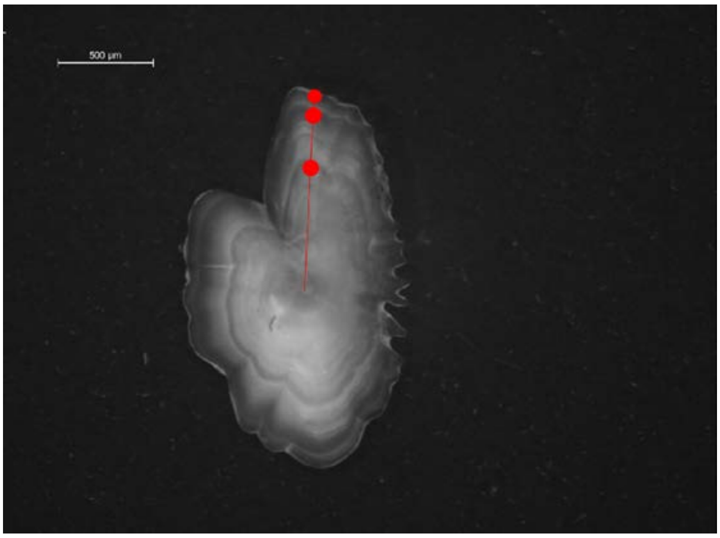


Figure A5.28. 6698258, Capture date 18/01/2013, Agreed age 3

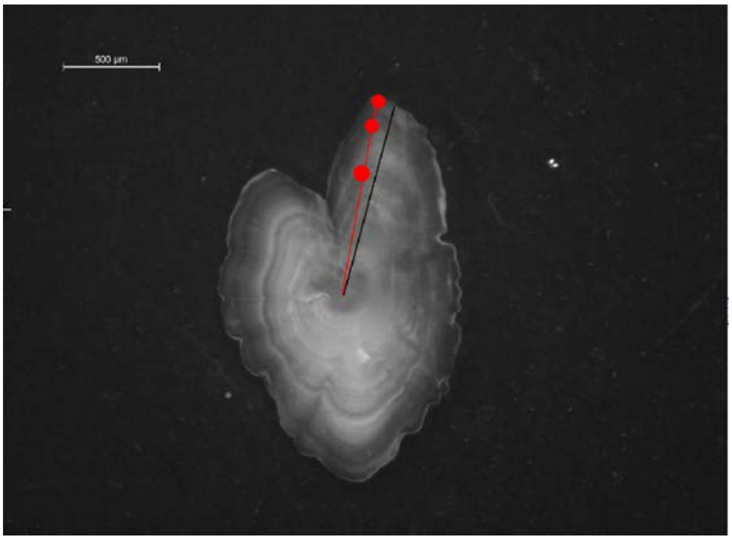


Figure A5.29. 6698260, Capture date 18/01/2013, Agreed age 3

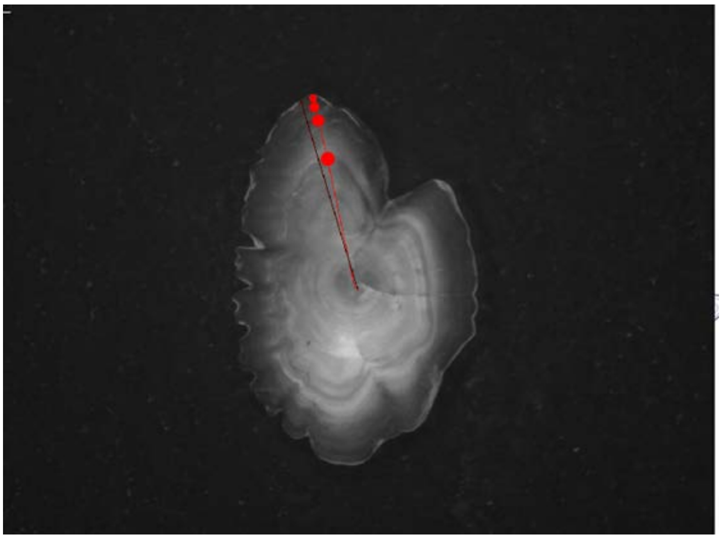


Figure A5.30. 6698268, Capture date 18/01/2013, Agreed age 4

Annex 6: Otolith microstructure examination at IMR Norway

Following WKARSPRAT a microstructure examination study was undertaken at IMR Norway to investigate whether the winter rings identified by the readers at the workshop had the characteristics of a true winter ring as defined by Mosegaard and Baron (1999). The samples were similar in time and position to the samples which were discussed in plenary at the workshop Table A6.1. The ages of the fish were estimated by an experienced age reader who attended the workshop. The microstructure examination of the otoliths was conducted by an experienced herring microstructure reader whose was examining sprat otolith microstructure for the first time.

The following biological parameters were included from each fish: total body length (cm), weight (g) and age (estimated from whole otoliths). The sagittal otoliths were removed from the fish head using a stereoscope and tweezers, the otoliths were carefully rinsed from tissue, before mounted with thermoplastic resin (Buehler Thermoplastic Cement no. 40-8100) on glass slides, for further microstructure analyse (Secor *et al.*, 1991: Mosegaard and Madsen, 1996). Each otolith was photographed with a Nikon SMZ25 with Nikon camera DS-Fi2 (Nikon Corporation, Tokyo) 9.0x magnification (2560 x 1920 pixels) and the otolith length (mm), and otolith width (mm) were recorded using NIS element D software.

The otoliths were then ground and polished on both sides. The otoliths were polished using a series of grinding and polishing films with decreasing grain sizes (Buehler, grit 600–1200) to optimize the visual resolution at a focal plane through the otolith.

The otoliths were examined and through a Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany) with x20 and x40- objective lens, transmitted light. The pictures (2560 x 1920 pixels) were taken with a Nikon DS-Fi2 digital camera and Image Pro Plus 7.0 software (version Media Cybernetics, Bethesda, MD20814) was used to analyse the daily increment width and increment number along the most readable transect.

Table A6.1. Sample information and biological measurements of the six-specimens analysed for otolith microstructure.

Date	Area	ICES Area	Vessel	Series No.	Fish No.	Fish length mm	Fish weight Kg	Age	Readability	Measurement whole Otolith		Number of increments
										Length _mm	Width _mm	
10.01.2016	North Sea	41-05	Cetus	86458	21	8.5	0.003	1	2	1.49	1.01	225
10.01.2016	North Sea	41-05	Cetus	86458	35	6.5	0.001	1	2	1.13	0.82	182
21.08.2016	North Sea	41-35	Hargun	21108	6	13.0	0.017	2	1	1.99	1.27	230
21.08.2016	North Sea	41-35	Hargun	21108	24	10.5	0.01	1	2	1.28	0.93	240
21.08.2016	North Sea	41-35	Hargun	21108	33	11.5	0.013	1	2	1.77	1.21	218
21.08.2016	North Sea	41-35	Hargun	21108	34	11.0	0.013	1	2	1.75	1.1	263

Images of the otolith microstructure have been taken from six fish from the North Sea 2016; two from January and four from August (Table A6.1). These pictures will be available for other experts from the countries participating in the workshop to try to distinguish false rings from the winter rings. When analysing the otolith microstructure of these fish the length of the spawning period should be taken into consideration. It has been reported that spawning may occur from early spring until late autumn in the North Sea (Alshuth, 1988a) and that some fish may overwinter as larvae (Peter Munk, pers. comm.) and lay down daily increments within this period. The structural differences in the increments both before and after the translucent zone should be examined to be able to identify the differences between a false and true winter ring (Mosegaard and Baron, 1999). With this in mind, it can be difficult to trust a count of increments from the centre of the otolith to the first winter ring, as the time for laying down the increments can vary highly between individuals. Increment count of the second zone should be more reliable but we have not been able to establish a minimum or maximum count of increments reasonable for a zone.

When using microstructure to get a better understanding of annual zones, it is important to be aware of the uncertainties that may arise:

1) A bit of the edge of the otolith disappears when polishing the otolith, especially in bigger otoliths.
2) It is difficult to count all the increments. In the first year and especially close to the core the zones are easiest to count due to good growth. As the distance between the increments narrows it becomes more difficult.
3) It is difficult to count along the same axis in all otoliths, as one has to choose the direction which has the clearest increments to count.

Case 1 Fish no 21 (Cetus, Station no. 86458, date 10.01.2016):

This fish is read to be 1 year old, but we are in doubt whether this is 1 or 2 years old (Figure 6.1). Line 1 begins at the centre and ends at the first ring, which we believe is a false winter ring. The second line continues to the second ring, which we believe is the first true winter ring.

In the ground and polished otolith (Figure 6.2) it is possible to count increments along the lines equivalent of Line 1 and Line 2 in the whole otolith. The results show that there are approximately 165 increments along Line 1 (the first 555 microns) and a total of 225 increments from the centre to the edge (696 microns). This gives approximately 60 increments for the last year if Line 1 should count as a year. This is very unlikely, and we are satisfied with this fish being 1 year old.

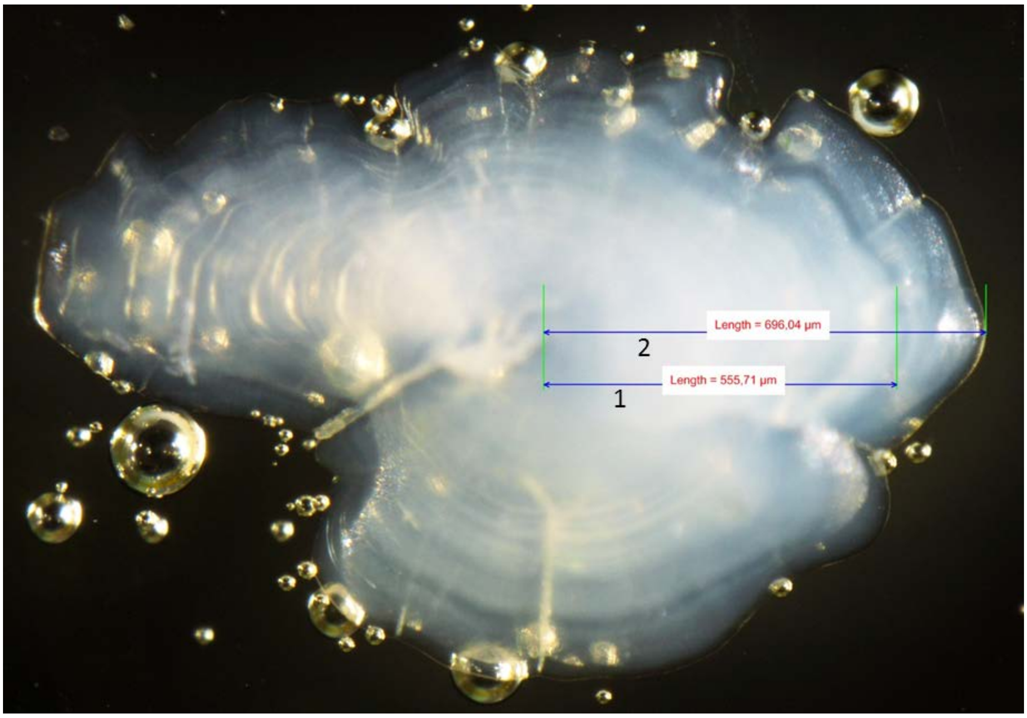


Figure 6.1. Line 1 and 2 refer to the two possible zones in this otolith and the length in microns from the centre.

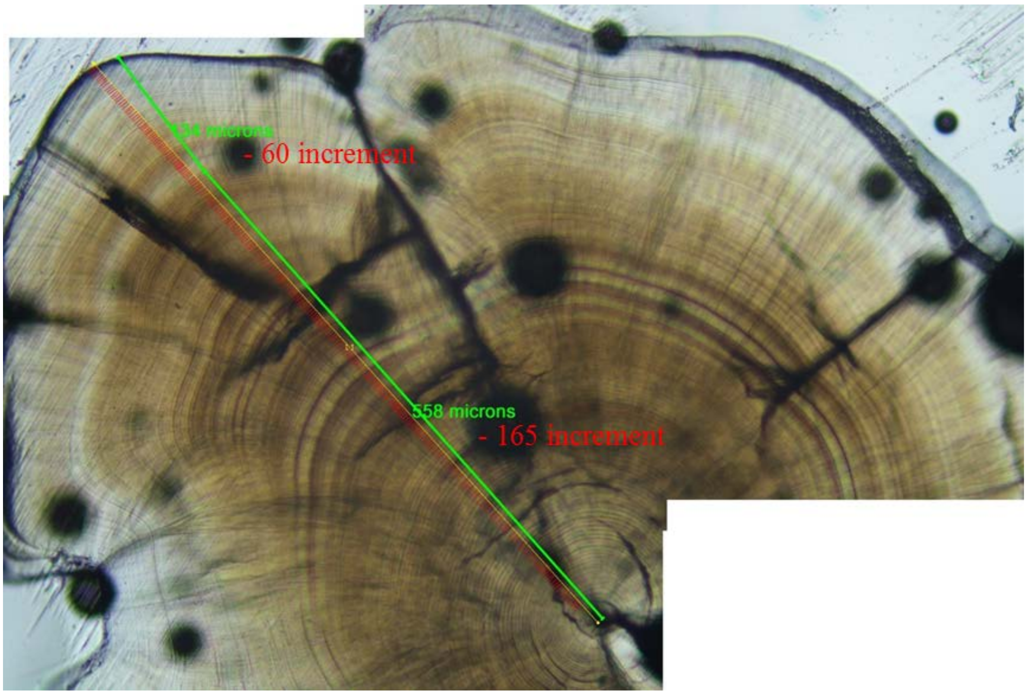


Figure 6.2. The ground and polished otolith with count of daily increments and measurements.

Case 2 Fish no 35 (Cetus, Station no. 86458, date 10.01.2016):

The age of this fish is read to be 1 year old, but there are two bands, which could be taken for a zone (winter ring) (Figure 6.3). The length from the centre to the outermost zone is 540 microns, which on the ground and polished otolith represents 182 increments (Figure 6.4). The very first band has not been considered as a zone, because when reading under a stereo microscope this band is very vague. However, the second band is more likely to being considered a zone. 63 increments were counted in from the second band to the outermost zone, which is unlikely to be a whole year. This otolith is therefore still considered to be from a 1 year old fish.

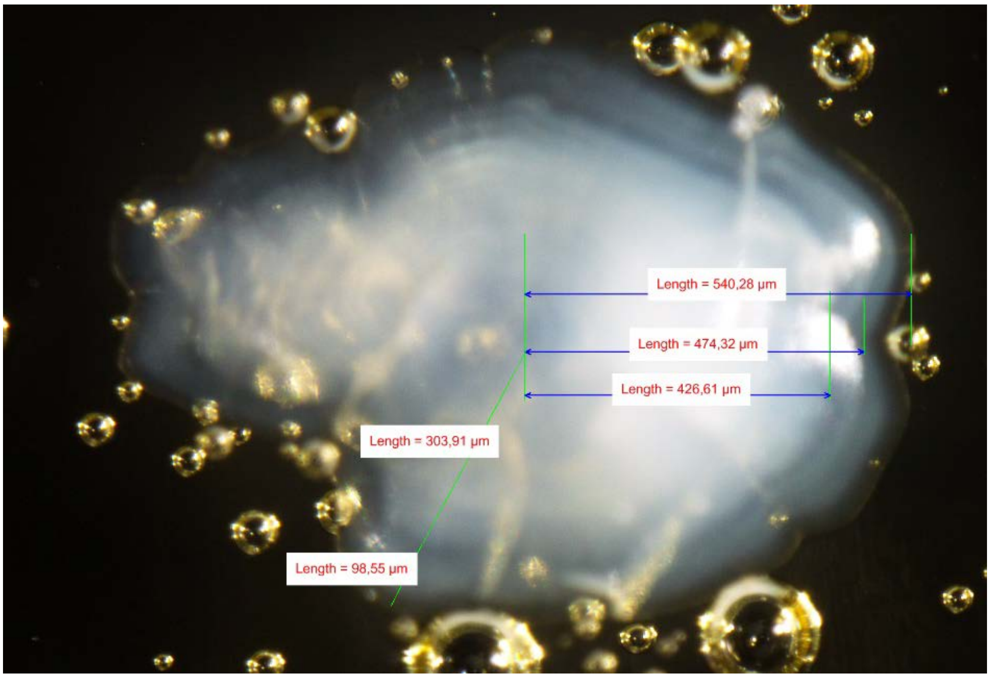


Figure 6.3. The whole otolith with markings of three possible zones. The diagonal green line shows the measurement from the centre to the supposed false year zone (winter ring) (corresponding to the middle blue line) and shows the direction where the increments on the ground and polished otolith (Figure 6.4) are measured.

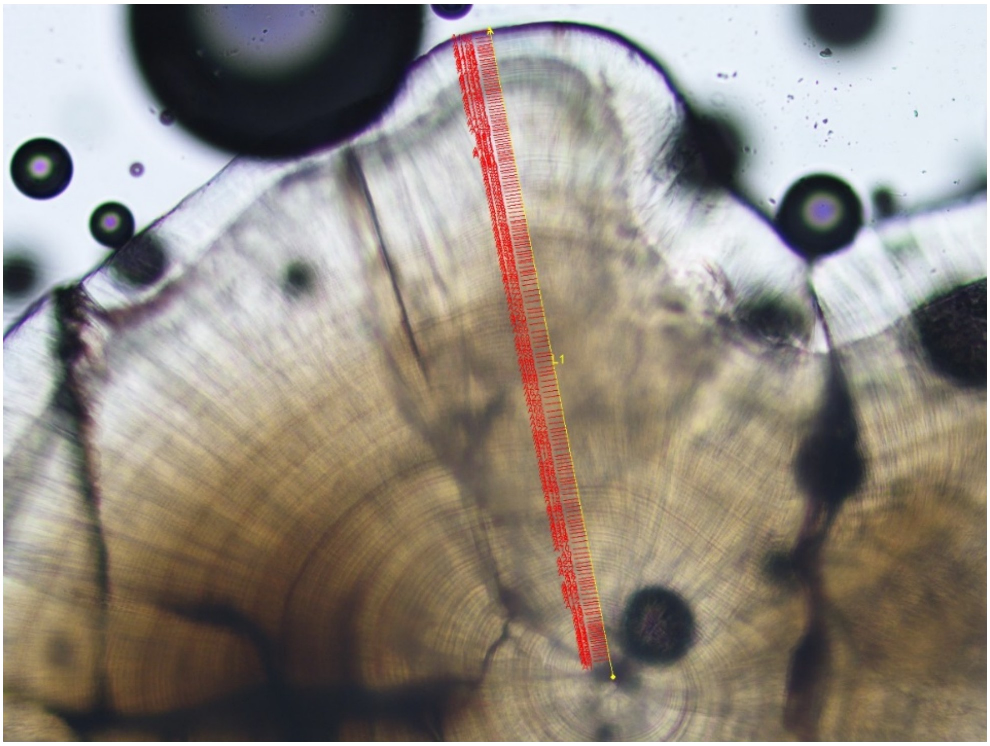


Figure 6.4. The ground and polished otolith with increment count.

Case 3 Fish no 6 (Hargun, Station no. 21108, date 21.08.2016):

This fish is read to be 2 years old. It has two rather clear bands and growth at the edge, as it is captured in August (Figure 6.5). Counting the increments on the ground and polished otolith (Figure 6.6) was difficult and some areas were unclear. The distance from the centre to the first assumed zone (winter ring) was 571 microns (L1), counting 198 increments. The distance from this first zone to the second was 167 microns (between the two green lines in Figure 6.6), but it was not possible to count all of the increments in a section of this transect measuring 123 microns (black lines in Figure 6.6). For the 44 microns, 32 increments were identified. If we assume that all increments are of equal size, there could be a total of approximately 100 increments in the second year. The distance from the second ring to the edge (not visible from the image) is 133 microns, and it was not possible to count the increments here either.

If this fish is two years old, it will have laid down 198 increments during the first year and possibly only 100 during the second year. However, as there are some areas where the increments cannot be counted a definitive count of age cannot be given.

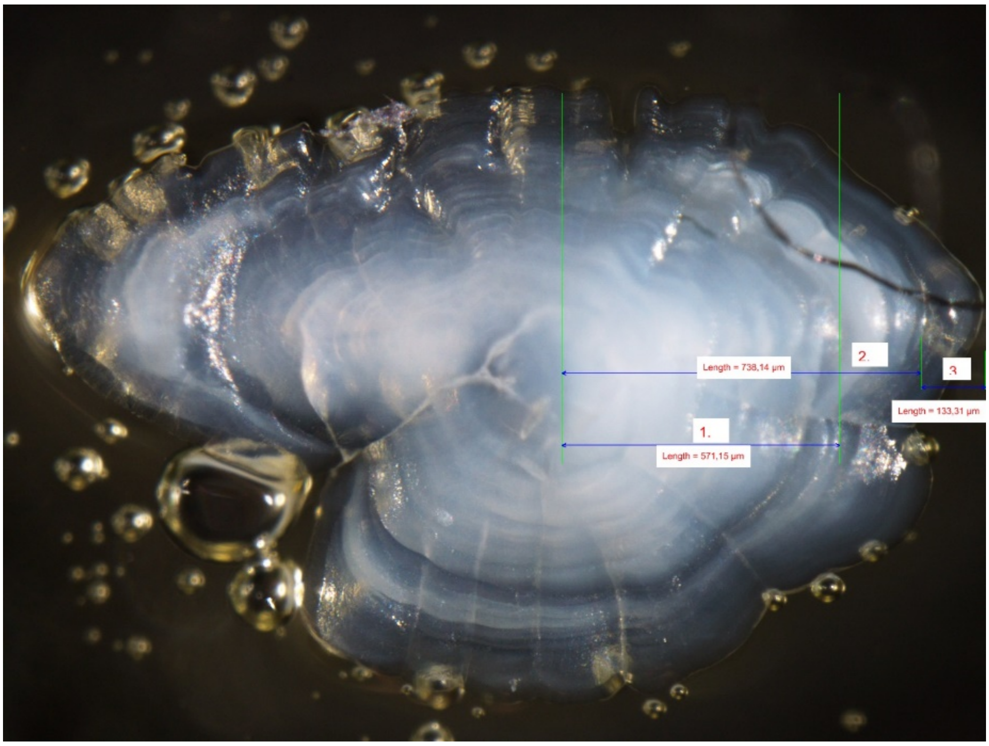


Figure 6.5. Image of the whole otolith showing two possible annual zones.

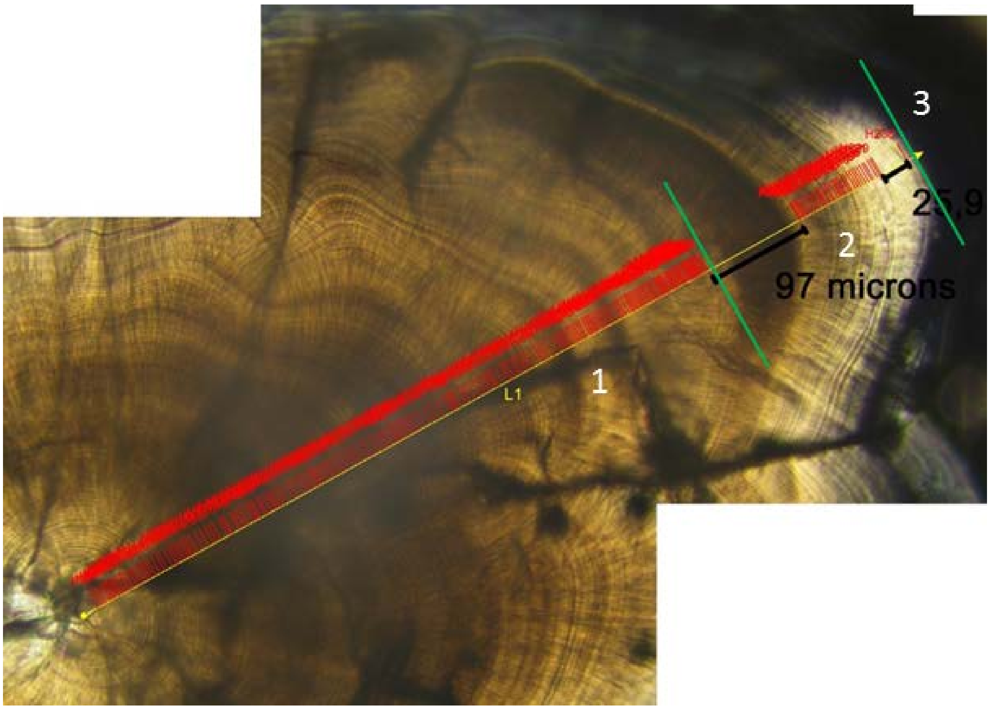


Figure 6.6. Image of the ground and polished otolith, the areas where it was impossible to count increments were along the 97 and 25 micron lines. Green lines represent the possible zones of the whole otolith.

Case 4 Fish no 24 (Hargun, Station no. 21108, date 21.08.2016):

This otolith is read to be 1 year old, but we are in doubt whether this is in fact 1 or 2 years old. Looking at the whole otolith (Figure 6.7) there are two bands, which could be counted as zones (winter rings) and then summer growth to the edge. During the initial readings the innermost band (450 μm from the centre) was counted as a false ring, while the second band was counted as the first winter zone (576 μm from the centre). From the image of the ground and polished otolith we get approximately 93 increments from the centre to the expected false ring, and an additional 31 increments to the expected true ring. There is little doubt that 31 increments is too little for a year, the question is then if the band belongs to the first or the second year's growth. The count of increments to the edge is 116 increments.

If the second band should belong to the first year, we will have a first year increment count of 124 (93+31) and the second year until capture will count 116 increments. If the second band should belong to the second year, we will have a first year increment count of 93 and a second count of 147 (31+116) by August. It is most reasonable to believe that the first scenario is the correct.

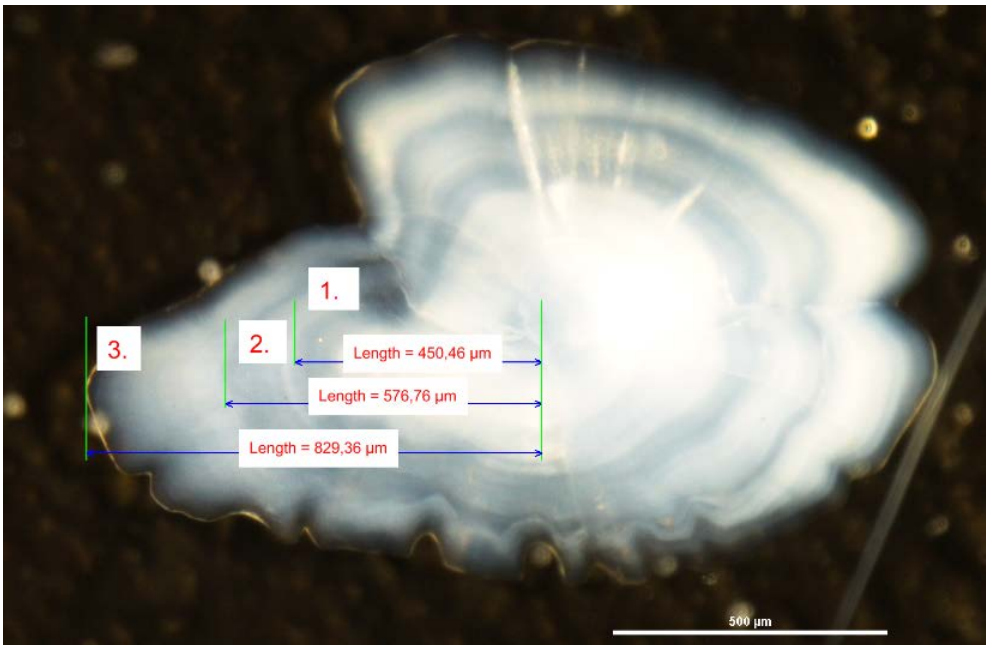


Figure 6.7. Image of the whole otolith, which is read as 1 year old.

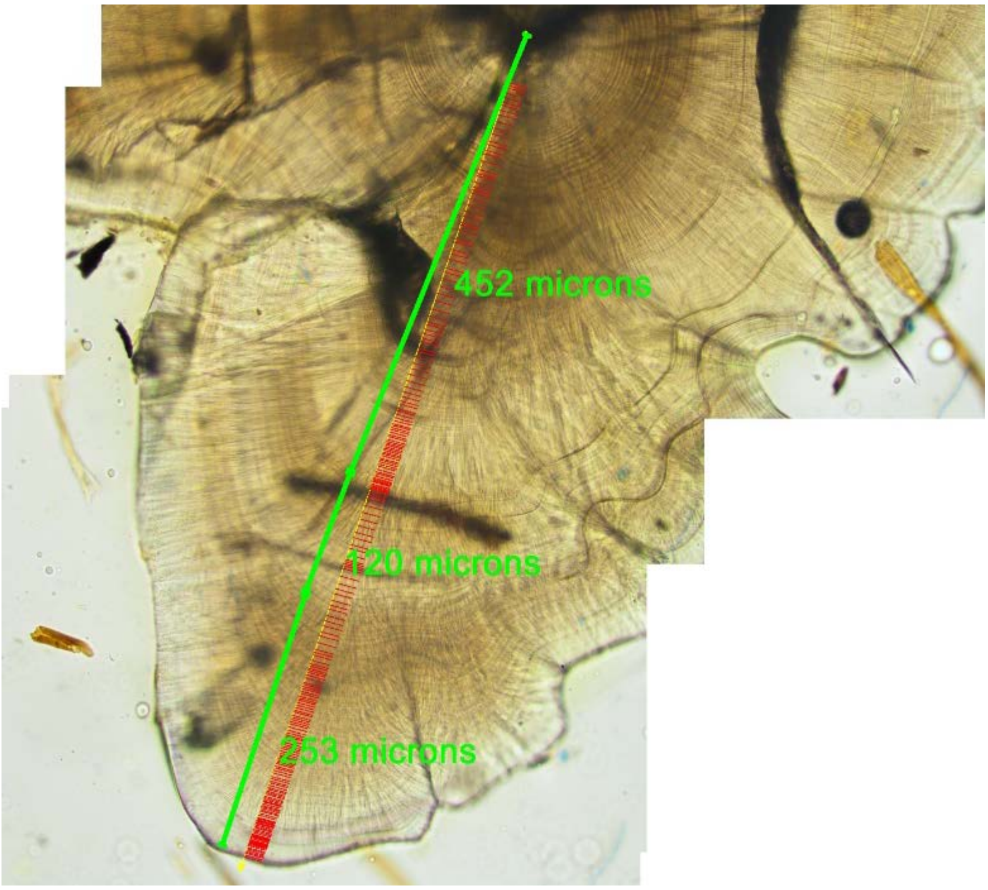


Figure 6.8. Image of the ground and polished otolith with a total of 240 increments.

Case 5 Fish no 33 (Hargun, Station no. 21108, date 21.08.2016):

This otolith is aged to be 1 year old, but we are in doubt whether this could also be 2 years old (Figure 6.9). The first band is expected to be a false zone (634 µm and 147 increments), while the second band is expected to be the first winter zone (777 µm and 211 increments). The increment count (Figure 6.10) of the distance between the expected false zone and the expected first zone is 64 (211-147), which is too small a count to be a zone of its own, so we would expect this fish to be 1 year old at capture.

It is difficult to say from the count of increments if it is most correct to include the second band to the first year or to the last year. The growth from the second band to the edge is 90 µm and 7 increments but the increments in the last zone are rather broad, and it is likely that the outermost increments have been polished away in the preparation process. Nevertheless, it is not possible to use this count in order to conclude whether the second band belongs to the innermost zone or the outermost zone.

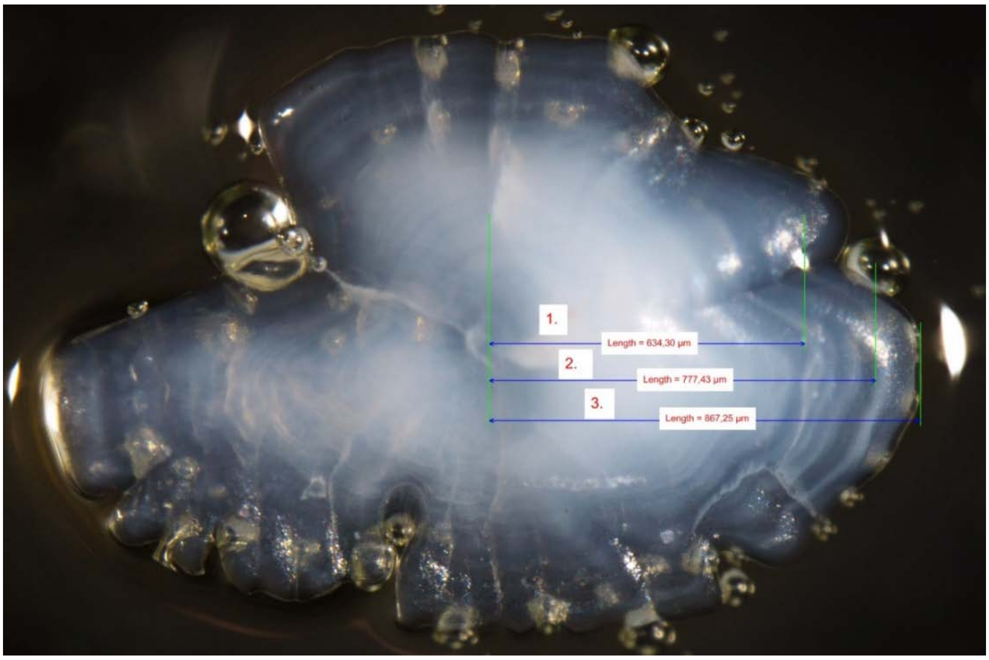


Figure 6.9. Image of a whole otolith, which is read to be 1 year old with an assumed false zone.

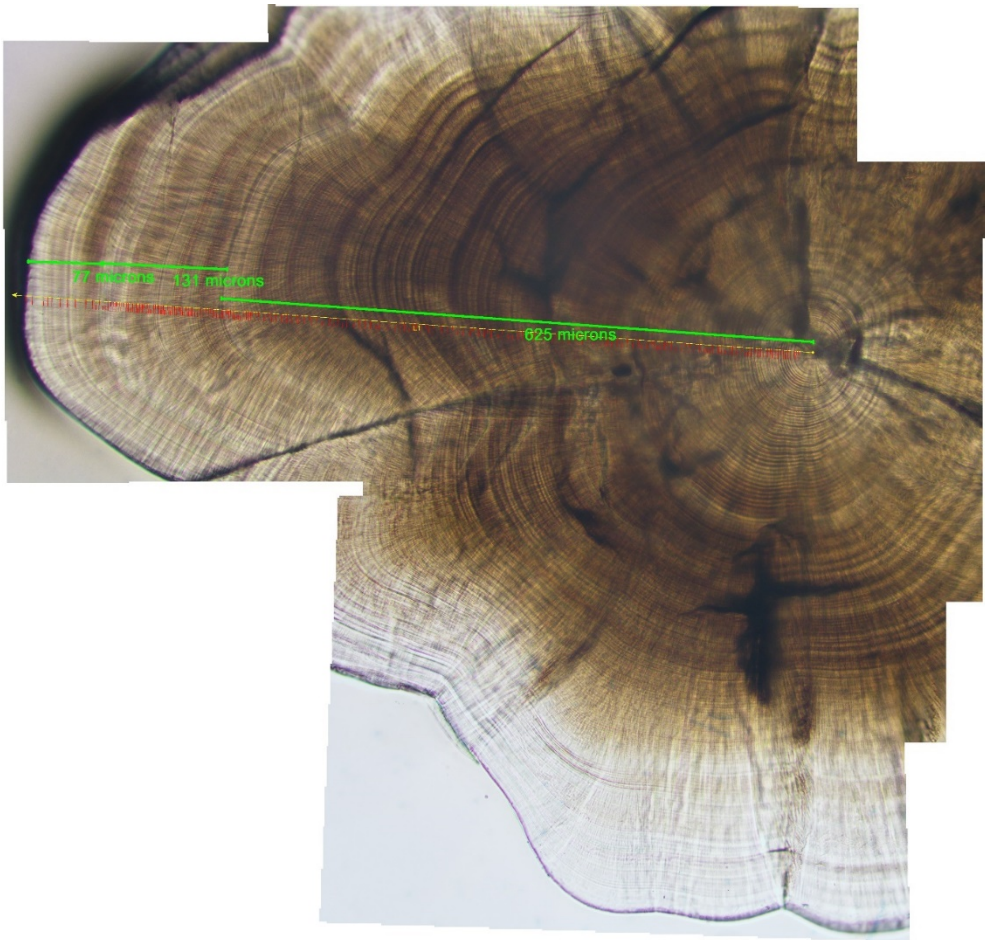


Figure 6.10. Image of the polished and ground otolith.

Case 6 Fish no 34 (Hargun, Station no. 21108, date 21.08.2016):

This otolith is read to be 1 year old, but we are in doubt whether this could also be 2 years old. The first band around 439 microns is expected as a false zone (Figure 6.11), while the second band around 628 microns is expected to be the first winter zone. The increment count to the first band was 134, and the second band contained 55 increments, indicating that it cannot be a zone by itself (Figure 6.12). The distance from the last counted zone to the edge (August 21) was 177 microns and 74 counted increments (Figure 6.13). From this we still believe that the fish is one year old, but it is difficult to say if the second band belongs to the first or the second growth zone.

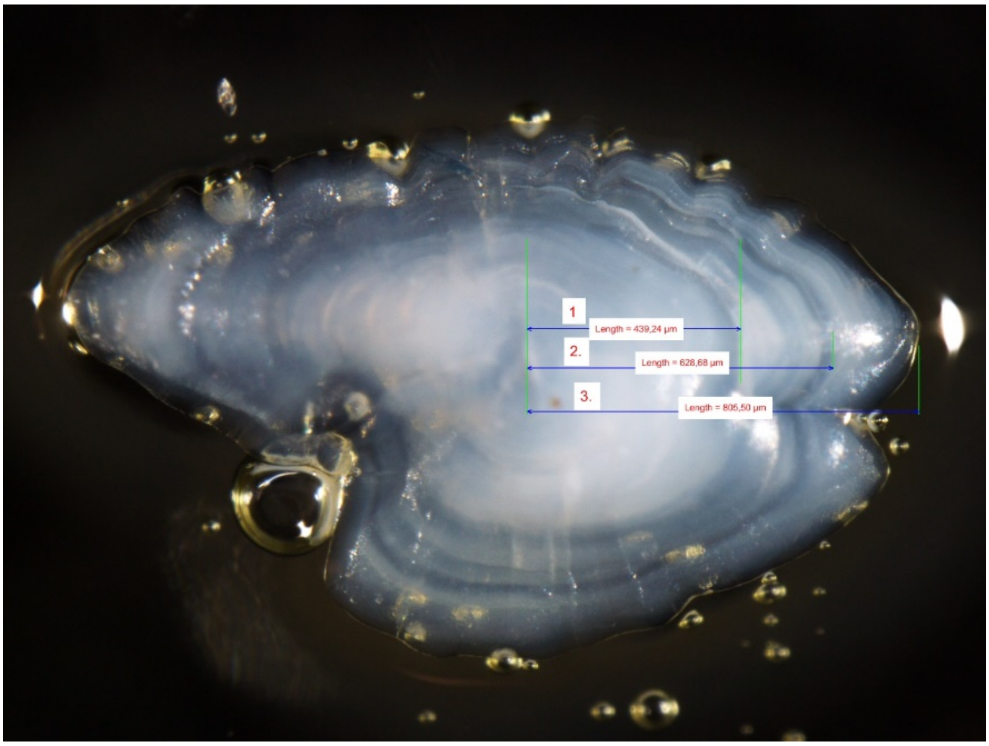


Figure 6.11. Image of the whole otolith showing the two possible zones

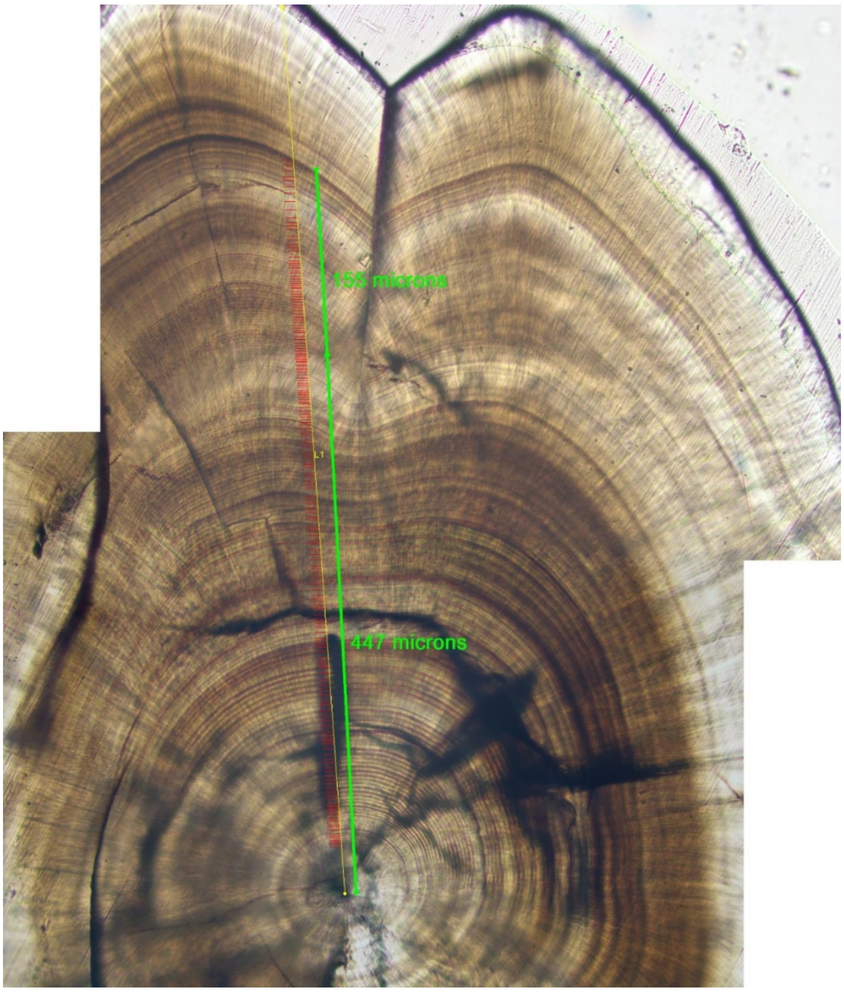


Figure 6.12. Image of the ground and polished otolith, with increments detected from the centre to the end of the second band (expected first true winter zone).

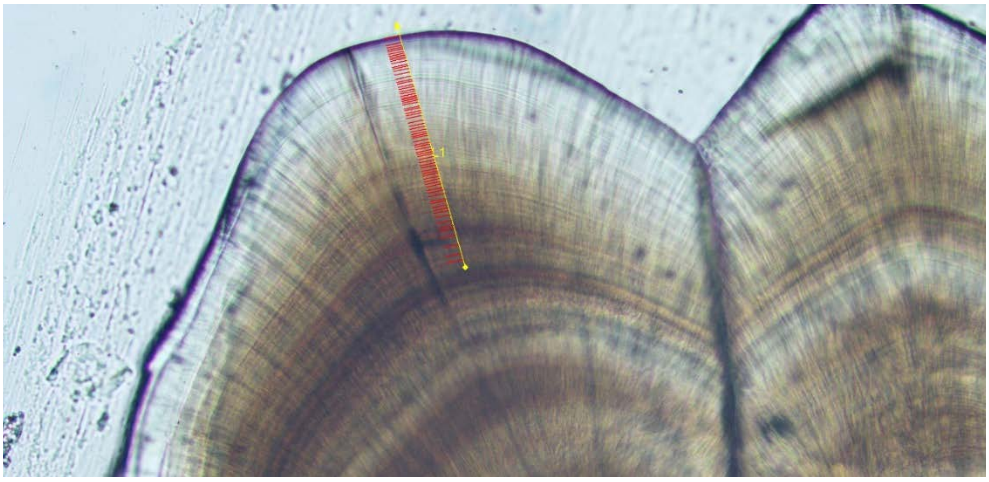


Figure 6.13. Image of the ground and polished otolith, with increments detected from the end of the second band (expected first true winter zone) to the edge.

Conclusion

The use of microstructure analysis has not given us an exact answer to the correct age of these difficult sprat otoliths, but it has given us a hint in the right direction.

In this study, we have seen that the increment width was highly varied between the different summer and winter zones, which makes it difficult to use this method for ageing. In the ageing process of these six otoliths, we have had a few zones which we have dismissed as annual zones due to the count of increments being around 60–70, while we have counted a second annual ring based on an estimated and uncertain count of only 100 increments.

Increment count of the first year growth varied from 116 to 232, but a variation here is more reasonable due to the large variation in spawning time.

One thing that we found to cause most trouble was that the true winter rings are narrow and look similar to what we interpret as a false zone. Therefore, it is difficult to distinguish a real winter zone from a false zone. This will need more work.

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Annex 7: Recommendations

Recommendation	Adressed to
1. Formulation of a readability scale for short lived species which considers the degree of uncertainty by number of years.	WGBIOP
2. For exchange purposes the annotation mark should be placed at the end of the translucent zones.	WGBIOP
3. That a future workshop should include a presentation on the biological drivers within the sprat stocks.	HAWG

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