Report of the Work Shop on age estimation of sprat.

Institute of Marine Research, Flødevigen, Arendal, Norway, 14-17 December 2004.

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1. Introduction

There are difficulties in following strong and weak cohorts of sprat in the North Sea and Div IIIa (ICES 1998, 2000, 2003). It is assumed that this is mainly caused by problems in age determinations. Exchanges of sprat otoliths and Work Shops have been performed regularly since 1994 in order to solve some of the ageing problems (Torstensen 1994, 1996, 2002). The last exchange performed in 2001-2002 revealed that estimation of sprat ages was inconsistent, with an overall CV of 28%.

The ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS), recommended at its March 2003-meeting (ICES 2003), that an age reading workshop for sprat should be organised by Norway in 2004.

An otolith exchange was organised prior to the workshop to clarify current problems in the age estimations. Samples of otoliths were circulated from May to December and the report of the exchange is presented in Annex 1. Otoliths are here equivalent to the largest of the three pair of otoliths in fish, the sagittae.

The WS took place at the Institute of Marine Research (IMR), Flødevigen, Arendal, 14-17 December 2004. Digital images of otoliths used in the analyses were made and prepared by IFREMER and Øystein Paulsen, IMR-Flødevigen.

1.1. The main aims

a) to analyse the results of the otoliths being exchanged in 2004.
b) to try to include techniques to validate the age reading methods
c) to discuss, if possible, otoliths processing techniques which might help to clarify the ring structures

1.2. List of participations

The meeting was attended by:

Jan Beintema	Netherlands
Michele Casini	Sweden
André Dijkman-Dulkes	Netherlands
Guus Eltink,	Netherlands
Gudrun Gentschow	Germany
Folke Halling	Finland
Knut Hansen (part-time)	Norway
Marianne Johansson	Sweden
Mario Koth	Germany
Birgitta Krischansson	Sweden
Willie McCurdy	UK-Northern Ireland
Else Torstensen (coordinator)	Norway
Jens Ulleweit	Germany

A list with communication details is given in Annex 2.

2. Age estimation

2.1 Examination of the exchange results

Most of the laboratories involved in age determination of sprat in the North Sea and adjacent areas, took part in the otolith exchange (see Annex 1). Unfortunately, not all the readers from the exchange were able to join the workshop. The examination and discussion of the results was thus limited to those present at the workshop.

All but one reader had a general broad experience in reading otoliths and three had a limited experience in working with sprat. Some of the readers are involved in age readings of sprat during survey once a year; others also doing age estimation of commercial samples covering a much broader seasonal and geographical distribution.

A list of otoliths defined as difficult from the exchange (high CV), was prepared and presented to the age readers in the workshop. A sub-selection of 23 otoliths (digital images and the proper otoliths) was presented on a large screen and discussed by using video-microscope display units. By combining the two systems, along with individual tuning of the microscope, the readers had optimal conditions for discussions of inconsistencies. The main purposes of the discussions were to clarify the rationale behind the age attributed by the individual readers, to discuss inconsistencies and to try to reach an agreement.

Results from this otolith exchange showed that there were two main problems that caused disagreements in age reading:

a) interpretation of the first translucent ring and
b) interpretation of narrow opaque zones or fragments of it in the reading area.

The decision by one of the less experienced readers to ignore a first translucent ring as a winter-ring, was based on a visual interpretation of what was a "small" L_1 and on the outline of this first ring; e.g. if the ring followed the outer outline of the otolith or not. The general discussion revealed that it is important to measure what is an acceptable range of L_1 for sprat otoliths collected in a particular area of distribution. As soon as sprat start spawning they produce narrow opaque zones. Interpretations of opaque zones when they appear as more narrow fragments of opaque materials in the outer area of the rostrum, caused inconsistencies in the results of experienced readers. Here, no agreement was reached through the discussion.

2.2 Examination of a 2nd age determination

After the first round of discussing difficult otoliths, a second reading was performed using a sub-sample of the original exchange samples. Three samples (S-2, S-6 and S-7) were selected for the re-reading in order to explore whether the age reading methods of the age readers had improved after the discussions on the difficult otoliths from the exchange.

The reading conditions were not what the readers were familiar with, therefore one sample of otoliths was located to a specific microscope; e.g., all readers read the same samples with the same microscope. The influence, if any, of different, and unfamiliar microscopes, was thus considered to have a low bias effect to the results. After the 2nd reading the results were compared and a new list of difficult otoliths presented. Twenty difficult otoliths where no agreement had been achieved in the second reading were presented and discussed. Full agreement was achieved in only 10% of the otoliths. Interpretations of the otoliths of older sprat, where the opaque zones appear as more narrow fragments of opaque materials with no well defined translucent zones, caused the problems with age estimation and were the main reason for inconsistencies.

2.3 Comparison of the 1st and the 2nd readings

Table 1 and 3 show the sample information and respectively the first and second age readings from samples S-2, S-6 and S-7. Figure 1 shows the age bias plots based on these age readings. The readers considered here are those participating both in the exchange and the workshop for comparison.

Table 2 and 4 show the number of age readings, the coefficient of variation (CV), the percentage agreement and the relative bias by age reader and readers combined for respectively the first and second readings from samples S-2, S-6 and S-7. Figure 2 shows the age bias plots based on these age readings.

The mean CV over all age groups achieved by all readers is lower for the second age readings (CV= 12%) compared to the first readings (21%). Most readers still have a difficulty in determining annual ring of age group 1 (highest CV). The CV is lowest for age group-2 and increases again for age group-3. This indicates that readers are uncertain in determining the first annual ring and again become uncertain at the time the annual growth increment becomes narrow at age 3.

Only six age readers participated both in the first and the second age reading exercise. Four of these readers improved in precision, while two decreased in precision. The same applies to the percentage agreement. The improvements in the reduction of bias can be better observed from the age bias plots. The age bias plots show an improvement from the first to the second age readings (compare Figures 1 and 2).

3. Validation techniques

Preliminary results of validation studies of age estimates of sprat otoliths (sagittae) from the Skagerrak-Kattegat areas, Validation of winter rings, Marginal Increment Analysis and Otolith weight frequency distribution, were presented.

3.1 Validation of winter rings

Lotte W. Clausen, DIFRES, who was not able to participate in the workshop, had prepared a presentation on “Validation of winter rings in sprat”.

Problems with correct age determination may arise from two primary sources; a) individuals may over winter as larvae and a winter-ring may not be discernible; b) more than one translucent zone may be formed in a specific year and thereby adding false winter zones to the total count as suggested for other species like sand eel. Validation of annual ring formation from primary increment formation in otoliths has to either rely on a daily periodicity of the primary increments all year round or an annual cycle in the pattern of the otolith microstructure (Panella 1971).

Production of daily increments in sagittae of larval sprat has been validated from 6 to 29 days under laboratory conditions (Alshuth 1988b). Daily increments have been validated for larval herring under lab, mesocosm and field conditions (Moksness 1992, Johannessen et al. 2000), see however Fox et al. (2004). It has further been found that sprat may over-winter as larvae (Peter Munck. Pers. Comm.) and that primary otolith increments are formed during the winter in both larval sprat (Alshuth 1988a) and larval herring (Moksness and Fossum 1991).

Studies of microstructures in sprat otoliths (sagittae) have demonstrated structural differences between what are defined as true and false translucent (winter) rings (Mosegaard and Baron 1999). When the translucent ring is deposited the width of the daily increments gradually reduces in width (Figure 3 and 4). This pattern can be found in true winter rings in the sagittae of sprat aged 0 – 2 years old (Figure 5). A false winter ring has no gradual reduction in the width of the daily rings in front of it, neither immediately after the translucent zone (Figure 6 and 7). Thus, in otoliths where the age reader is in doubt whether a translucent zone is true or false, the validity of the ring can be examined by reading the otolith microstructure. Figures 8 and 9 illustrate otoliths from two true ages 0 sprats sampled in December with and without a false ring.

3.2 Marginal Increment analysis

Marginal Increment Analysis (MIA) investigates whether or not the translucent rings that are commonly counted to determine fish age are deposited once a year (Campana et al., 2001). For this purpose, the distance between the outermost translucent ring and the otolith edge is usually measured and its development followed throughout several months. In the case a cyclic deposition pattern in time is observed, the annual nature of ring formation is validated. In our study from Skagerrak-Kattegat, we used for this purpose the average distance between the outermost completed translucent ring and the otolith dorsal edge in the area delimited by the antirostrum and pararostrum. MIA was performed for Skagerrak and Kattegat separately using samples collected between February 2003-January 2004. MIA was performed separately for each group of fish presenting the same number of translucent (winter) rings throughout the study period (w-r groups). The increment of 0 w-r group was measured from the nucleus to the edge of the otolith. Age-readers did not have any indication about fish length, sex and sampling date.

The results from MIA pointed out that the otolith translucent zone (winter ring) was laid down once a year during the period analysed (Fig. 10). The increment of the outermost ring increased slowly from February to May. This pattern conforms to the slow growth of sprat during the winter period. The deposition of the new translucent ring was completed during the summer period (June-July). This fact was revealed by the sudden drop in the increment of the outermost ring between May and July. The period July-November represented the period of faster otolith growth. This sinusoidal pattern was common for both Skagerrak and Kattegat and for all w-r groups. Only the 0 w-r group did not follow this pattern, likely due to the long spawning period of sprat in this area (March-July) and, thus, to the different birthday of the specimens analysed. This problem is negligible for older fish with already at least a completed translucent ring. Preliminary analysis of the 0 w-r group indicated that in 2003 the first translucent ring started to be clearly visible not before April-May. The start of the deposition of the translucent ring in older w-r groups was not investigated because of the difficulty in the identification of the translucent ring at the otolith edge in the older fish. Marginal increment analysis showed also that the growth rate of the otolith decreases with increasing age of the fish (Fig. 11). For this purpose the distance between the nucleus and the dorsal edge was measured.

A tentative measurement exercise using the results of marginal increment growth were performed by some of the participants on own otoliths, but due to restricted time, no final results were presented. It is, however, recommended that otoliths taken in the different areas being measured to increase the knowledge of when the different growth zones are laid down.

3.3 Otolith weight

3.3.1 Otolith weight frequency distribution (OWFD)

This method is a variant of the length frequency distribution analysis (LFD) (Campana, 2001). This method assumes that the expected modes of the otolith weight frequency would correspond to the population age-classes. The rationale behind the use of OWFD rather than LFD is that it has been shown that otolith size is more closely related to age than fish size (Fletcher, 1991; Araya *et al.*, 2001). The requirements and assumptions for OWFD and LFD are, however, identical. Both methods are particularly suitable for short living and fast growing species. Random sampling procedure of fish is required to ensure that the samples collected are representative of the population. OWFD was analysed using samples collected by the RV “Argos” during the IBTS (International Bottom Trawl Survey) in Skagerrak and Kattegat in February 2003. OWFD was explored by means of the Bhattacharya method (see Cardinale *et al.*, 2000) using FISAT software.

The results from otolith weight frequency distribution showed that the modes identified by the software correspond to the observed frequency for both males and females sampled in Kattegat in February 2003 (χ^2 test) (Fig. 12). Moreover, the modes correspond to the otolith weight-at-ages determined counting the translucent rings (Kolmogorov-Smirnov test and Anova, $p>0.05$). Separate analyses for females and males were necessary because of the different growth rates (see below). When using fish length frequency distribution, the modes identified by the Bhattacharya method did not correspond to the actual frequency (χ^2 test) (Fig. 13). Therefore, no attempt was made to compare those modes with the otolith weight-at-ages determined by the age-readers.

3.3.2 Otolith growth-area and sex effects

Overall, otolith weight-at-age was higher in Skagerrak than in Kattegat. Moreover, females presented heavier otoliths compared to males in both Skagerrak and Kattegat (Fig. 14). These differences between areas and sexes possibly reflect the different somatic growth. The observed differences between Kattegat and Skagerrak are probably related to different feeding as well as environmental conditions (e.g. higher salinity in Skagerrak) experienced by the fish populations in the two areas. These results put in evidence the necessity of considering area and sex effects in age-validation methods.

The factor sex should be carefully considered before performing any age-validation. In fact, the difference in growth rates between males and females could confuse the outcome of the investigation. Especially in the frequency distribution analysis the separation of the sexes is strongly recommended. Moreover, even very close areas could present populations with different growth rates.

4. Otolith processing technique

Various otoliths processing techniques, which might help to clarify the ring structures, were presented and discussed. The participants at the WS use different preparation methods (otoliths dried for some hours to days prior to mounting, otoliths cleaned with water or 96% alcohol, some using glass cover slip on top of the embedded otoliths, other use it uncovered, some use black plastic plates, other use glass slides, some embed in resin other mount the otoliths using clear nail polish, mounted with the *sulcus acustics* -side down) and different age reading methods in their routine age estimation; reflected light, translucent light and light from the side at a rather low angle. The last implies that the otoliths are examined with fibre optic on a light and a black background. By moving the lights from a rather low angle, the ages can be estimated from the step-like structure (topographic structure) exposed in the area between antirostrum and pararostrum.

At present there is no basis for deciding what otolith processing method is best. **The various laboratories are encouraged to evaluate their otolith processing methods and to examine different techniques.**

5. Preliminary guidelines for age estimation of sprat

From the analyses of the age interpretations, the discussion afterwards and contribution by G. Gentschow, Germany, B. Krischansson and M. Johansson, Sweden, F. Halling, Finland, K. Hansen, Norway and Mc Curdy, UK, preliminary guidelines for age estimation of sprat from the North Sea-Skagerrak-Kattegat area, are prepared and presented in Annex 3.

6. Agreed collection of digital images of sprat otoliths

No sprat otoliths of known age are available. Therefore a collection of agreed sprat otoliths as a useful tool for training of new readers and for calibration and updating of established readers (experienced and inexperienced readers), has been advertised. A reference collection of digitised images of agreed sprat otoliths from the exchange will be prepared, representing otoliths where: a) >90% agreements were attained and b) where the agreement was >10% but <20%.

The reference collections will be prepared on CD and distributed to the laboratories participated in the exchange and in the WS.

7. Conclusions and Recommendations

Various otolith processing techniques, which might help to clarify the ring structures, were discussed. However, ambitions to draft an agreed manual for the process of age determination of sprat ages, including all aspects of the process, were not realized due to time constraint. However, a first version of an agreed guideline for preparation and readings of sprat otoliths, was drafted.

Results from this otolith exchange and age reading workshop showed that there were two main problems that caused disagreements in age readings:

1. interpretation of the first translucent ring and
2. interpretation of narrow opaque zones or fragments of it in the reading area.

Time spent studying the variation in the appearance of sagittae from 0-group sprat can be of great benefit in understanding the depositions of the first winter ring.

Sprat in the North Sea could be spawning until late autumn (Alshuth 1988a) and larvae sprat from the late spawnings would probably not deposit an opaque growth zone before the next year. This might cause a wrong age determination by the age readers, because they are not able to distinguish these fish in age from the fish born in the next year. However, this problem could not be evaluated from this otolith exchange and might only be solved by e.g. counting daily increment rings on otoliths having a large L-1 and showing a clear nucleus (similar to autumn spawning herring).

To increase the knowledge and understandings of the processes of seasonal growth laid down in the otoliths, the WS came up with the following recommendations:

1. Age-validation should be performed in order to confirm the validity of the ageing method used (confirm the periodicity of deposition of the translucent ring) and to investigate the time of deposition of the translucent ring for each age-class.

2. It is recommended to continue the studies of daily increment in order to validate the deposition of the first translucent ring and to determine the time of its formation, and also to determine the spawning time. The prolonged spawning time of sprat (February-July in Division IIIa) likely represents the main problem in the interpretation of the first translucent ring. It is suggested that the studies to be done on otoliths from the various areas.

3.Daily growth increment studies should be carried out on otoliths of spawning fish in order to verify whether the very thin or even fragments of opaque ring structures should be interpreted as summer growth and thereby verify whether the two adjacent translucent rings should be counted as annual zones or not.

4. It is strongly recommended not to consider fish length in age estimation, at least not for the first reading. Otoliths continue growing even when somatic growth stops (for instance due to starvation).

5. Cooperation and coordination between laboratories are recommended in preparation and collection of digital images/sprat otoliths to make an exchange and reference set of otoliths from all the areas and throughout a year

6. Experiences from other area (Baltic) indicate that each lab should check the readability of the otoliths reading the *sulcus acusticus* side, compared with the present method using the other side of the otolith.

7. It is recommended that effort be allocated to improve the agreement between the age-readers from the other laboratories by regular exchange and when problematic otoliths are encountered.

8. It is recommended that laboratories reading sprat otoliths, build up a representative collection of otoliths from all months to be able to follow the seasonal growth of the edge in their particular area to know when a young sprat starts to lay down the first translucent zone, when an older starts and when they start to make the opaque zone.

9. It is recommended that measurements of L_1 in sprat otoliths from the various areas be made to establish the position of the first annual translucent zone (winter ring). The outline, nuance of translucencies and the distance from the nucleus can all vary considerable. It is considered important to know the acceptable range in the current distribution area for including or rejecting the first translucent ring as a first winter ring.

10. It is recommended that the different readers test and compare the results of reading from different otoliths processing techniques.

11. It is strongly recommended that all otolith readers regularly check their precision by re-reading some of the otolith samples each year and that small-scale otolith exchanges should be conducted annually for each stock/species as a quality check. Preferably, exchange of otoliths should be with laboratories that work on the same stock of sprat.

12.These actions should be considered by the coming series of ageing workshops

13. It is recommended to have a next exchange in 2007, followed by a WS if necessary.

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Table 1. Sprat Otoliths Workshop. 1st reading of S-2, S-6 and S-7

RANGE r. 1-9

Sample year	no	Fish no	Fish length	D GG	SE BK	SE MJ	NL AD	D MK	NL JB	MODAL age	Percent agreement	Precision CV
01.feb.04	S-2	5	10,0	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	6	11,5	2	2	2	2	3	2	**2**	83 %	19 %
01.feb.04	S-2	7	8,5	1	1	1	1	1	1	**1**	100 %	0 %
01.feb.04	S-2	8	10,0	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	9	9,0	2	1	1	2	1	2	**1**	50 %	37 %
01.feb.04	S-2	10	10,0	1	1	1	2	1	1	**1**	83 %	35 %
01.feb.04	S-2	11	13,0	3	3	3	2	3	3	**3**	83 %	14 %
01.feb.04	S-2	12	13,0	3	3	3	3	3	3	**3**	100 %	0 %
01.feb.04	S-2	13	12,0	2	3	3	2	2	3	**3**	50 %	22 %
01.feb.04	S-2	14	8,5	1	1	1	2	1	2	**1**	67 %	39 %
01.feb.04	S-2	16	11,5	2	1	1	2	1	2	**1**	50 %	37 %
01.feb.04	S-2	18	10,5	2	2	2	-	2	2	**2**	100 %	0 %
01.feb.04	S-2	19	9,5	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	20	7,5	1	1	1	2	1	1	**1**	83 %	35 %
01.feb.04	S-2	21	9,0	2	1	1	2	1	2	**1**	50 %	37 %
01.feb.04	S-2	22	11,0	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	23	7,5	1	1	1	1	1	2	**1**	83 %	35 %
01.feb.04	S-2	25	10,0	2	2	2	2	2	3	**2**	83 %	19 %
19.nov.03	S-6	1	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	2	12,0	3	4	3	4	4	3	**3**	50 %	16 %
19.nov.03	S-6	3	12,0	3	4	4	3	5	2	**4**	33 %	30 %
19.nov.03	S-6	4	13,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	5	12,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	6	12,0	3	3	3	2	3	3	**3**	83 %	14 %
19.nov.03	S-6	7	11,5	4	4	4	2	4	4	**4**	83 %	22 %
19.nov.03	S-6	8	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	9	12,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	10	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	11	11,5	1	1	1	1	1	1	**1**	100 %	0 %
19.nov.03	S-6	12	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	13	12,0	1	1	1	3	1	1	**1**	83 %	61 %
19.nov.03	S-6	14	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	15	12,5	3	4	4	2	4	4	**4**	67 %	24 %
19.nov.03	S-6	16	14,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	17	12,5	2	3	3	2	3	3	**3**	67 %	19 %
19.nov.03	S-6	18	16,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	19	12,5	2	4	4	2	3	3	**4**	33 %	30 %
19.nov.03	S-6	20	11,5	1	1	1	2	1	1	**1**	83 %	35 %
19.nov.03	S-6	21	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	22	12,5	2	2	2	3	2	2	**2**	83 %	19 %
19.nov.03	S-6	23	14,0	3	4	4	2	4	3	**4**	50 %	24 %
19.nov.03	S-6	24	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	25	11,5	3	3	3	2	3	3	**3**	83 %	14 %
12.des.97	S-7	1	12,5	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	2	13,0	3	3	4	-	3	5	**3**	60 %	25 %
12.des.97	S-7	3	13,5	2	3	4		3	3	**3**	60 %	55 %
12.des.97	S-7	4	11,0	1	1	1	-	1	2	**1**	80 %	37 %
12.des.97	S-7	5	12,5	1	1	1	-	2	1	**1**	80 %	37 %
12.des.97	S-7	6	12,0	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	7	11,0	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	8	11,0	1	2	2	-	1	2	**2**	60 %	34 %
12.des.97	S-7	9	12,5	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	10	11,0	1	2	2	-	1	2	**2**	60 %	34 %
12.des.97	S-7	11	9,0	0	0	0	-	0	0	**0**	100 %	0 %
12.des.97	S-7	12	11,5	1	1	1	-	1	2	**1**	80 %	37 %
12.des.97	S-7	13	13,0	2	2	2	-	2	2	**2**	100 %	0 %
12.des.97	S-7	14	11,5	1	1	1	-	1	3	**1**	80 %	64 %
12.des.97	S-7	15	11,0	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	16	11,0	1	1	1	-	0	1	**1**	80 %	56 %
12.des.97	S-7	17	11,5	1	1	1	-	0	1	**1**	80 %	56 %
12.des.97	S-7	18	10,5	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	19	11,0	1	1	1	-	0	1	**1**	80 %	56 %
12.des.97	S-7	20	12,0	1	1	1	-	0	1	**1**	80 %	56 %
12.des.97	S-7	21	9,5	1				1	2	**0**	100 %	122 %
12.des.97	S-7	22	12,5	2	3	3	-	2	2	**3**	40 %	23 %
12.des.97	S-7	23	10,5	1	1	1	-	1	1	**1**	100 %	0 %
12.des.97	S-7	24	12,0	2	2	2	-	1	2	**2**	80 %	25 %
12.des.97	S-7	25	11,5	1	1	1	-	0	1	**1**	80 %	56 %
			Total read	68	68	68	44	68	68		**83,0%**	**20,7%**
			Total NOT read	0	0	0	24	0	0			

Table 2. Sprat Otolith Workshop. Number of age readings, coefficientof variation (CV), the percentage agreement and the relative biasby age reader and readers combined. 1st reading of S-2, S-6 and S-7

NUMBER OF AGE READINGS

	MODAL age	D GG	SE BK	SE MJ	NL AD	D MK	NL JB	TOTAL
	0	2	2	2	1	2	2	**11**
	1	27	27	27	11	27	27	**146**
	2	24	24	24	19	24	24	**139**
	3	10	10	10	8	10	10	**58**
	4	5	5	5	5	5	5	**30**
	5	-	-	-	-	-	-	**-**
	6	-	-	-	-	-	-	**-**
Total	**0-15**	**68**	**67**	**67**	**42**	**68**	**68**	**380**

COEFFICIENT OF VARIATION (CV)

	MODAL age	D GG	SE BK	SE MJ	NL AD	D MK	NL JB	ALL Readers
	0	141 %	0 %	0 %	-	141 %	141 %	**61,2%**
	1	29 %	0 %	0 %	33 %	54 %	42 %	**29,8%**
	2	15 %	0 %	0 %	11 %	21 %	10 %	**6,2%**
	3	20 %	10 %	13 %	53 %	20 %	24 %	**20,3%**
	4	24 %	0 %	0 %	20 %	18 %	26 %	**26,1%**
	5	-	-	-	-	-	-	**-**
	6	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**25,5%**	**1,5%**	**2,0%**	**26,3%**	**37,1%**	**29,6%**	**20,7%**
	RANKING	**3**	**1**	**2**	**4**	**6**	**5**	

PERCENTAGE AGREEMENT

	MODAL age	D GG	SE BK	SE MJ	NL AD	D MK	NL JB	ALL
	0	50 %	100 %	100 %	100 %	50 %	50 %	**73 %**
	1	89 %	100 %	100 %	27 %	78 %	70 %	**83 %**
	2	92 %	100 %	100 %	95 %	83 %	96 %	**94 %**
	3	60 %	90 %	80 %	13 %	70 %	80 %	**67 %**
	4	20 %	100 %	100 %	0 %	60 %	40 %	**53 %**
	5	-	-	-	-	-	-	**-**
	6	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**79,4%**	**100,0%**	**98,5%**	**54,8%**	**76,5%**	**77,9%**	**82,9%**
	RANKING	**3**	**1**	**2**	**6**	**5**	**4**	

RELATIVE BIAS

	MODAL age	D GG	SE BK	SE MJ	NL AD	D MK	NL JB	ALL
	0	0,50	0,00	0,00	0,00	0,50	1,00	**0,36**
	1	0,11	0,00	0,00	0,82	-0,15	0,33	**0,12**
	2	-0,08	0,00	0,00	0,05	-0,08	0,04	**-0,01**
	3	-0,40	0,10	0,20	-0,88	-0,10	0,10	**-0,14**
	4	-1,00	0,00	0,00	-1,80	0,00	-0,80	**-0,60**
	5	-	-	-	-	-	-	**-**
	6	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**-0,10**	**0,01**	**0,03**	**-0,14**	**-0,09**	**0,13**	**-0,02**
	RANKING	**4**	**1**	**2**	**6**	**3**	**5**	

Table 3. Sprat Otoliths Workshop. 2nd reading of S-2, S-6 and S-7

Sample year	no	Fish no	Fish length	SE BK	D GG	NL JB	NL AD	SE MJ	D MK	RANGE r. 1-8 MODAL age	Percent agreement	Precision CV
01.feb.04	S-2	5	10,0	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	6	11,5	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	7	8,5	1	1	1	1	1	1	**1**	100 %	0 %
01.feb.04	S-2	8	10,0	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	9	9,0	2	2	2	2	1	2	**2**	83 %	22 %
01.feb.04	S-2	10	10,0	1	1	1	1	1	1	**1**	100 %	0 %
01.feb.04	S-2	11	13,0	3	3	3	3	3	3	**3**	100 %	0 %
01.feb.04	S-2	12	13,0	3	3	3	3	3	3	**3**	100 %	0 %
01.feb.04	S-2	13	12,0	3	2	3	3	3	3	**3**	83 %	14 %
01.feb.04	S-2	14	8,5	1	1	2	2	1	2	**1**	50 %	37 %
01.feb.04	S-2	16	11,5	2	2	2	2	2	1	**2**	83 %	22 %
01.feb.04	S-2	18	10,5	2	2	2	2	2	2	**2**	100 %	0 %
01.feb.04	S-2	19	9,5	2	2	2	1	2	2	**2**	83 %	22 %
01.feb.04	S-2	20	7,5	1	1	1	1	1	1	**1**	100 %	0 %
01.feb.04	S-2	21	9,0	2	2	2	2	1	1	**2**	67 %	31 %
01.feb.04	S-2	22	11,0	2	2	2	2	2	3	**2**	83 %	19 %
01.feb.04	S-2	23	7,5	1	1	1	1	1	1	**1**	100 %	0 %
01.feb.04	S-2	25	10,0	2	2	2	2	3	3	**2**	67 %	22 %
19.nov.03	S-6	1	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	2	12,0	3	3	2	4	3	3	**3**	67 %	21 %
19.nov.03	S-6	3	12,0	3	3	3	3	4	3	**3**	83 %	13 %
19.nov.03	S-6	4	13,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	5	12,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	6	12,0	3	3	3	3	3	3	**3**	100 %	0 %
19.nov.03	S-6	7	11,5	3	3	4	4	4	4	**4**	67 %	14 %
19.nov.03	S-6	8	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	9	12,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	10	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	11	11,5	1	1	1	1	1	1	**1**	100 %	0 %
19.nov.03	S-6	12	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	13	12,0	1	1	1	1	1	1	**1**	100 %	0 %
19.nov.03	S-6	14	11,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	15	12,5	3	3	3	3	4	4	**3**	67 %	15 %
19.nov.03	S-6	16	14,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	17	12,5	2	2	3	3	3	3	**3**	67 %	19 %
19.nov.03	S-6	18	16,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	19	12,5	2	2	3	3	3	3	**3**	67 %	19 %
19.nov.03	S-6	20	11,5	1	1	1	1	1	1	**1**	100 %	0 %
19.nov.03	S-6	21	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	22	12,5	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	23	14,0	3	3	3	3	4	3	**3**	83 %	13 %
19.nov.03	S-6	24	12,0	2	2	2	2	2	2	**2**	100 %	0 %
19.nov.03	S-6	25	11,5	3	3	4	3	4	3	**3**	67 %	15 %
12.des.97	S-7	1	12,5	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	2	13,0	3	3	3	3	3	4	**3**	83 %	13 %
12.des.97	S-7	3	13,5	2	2	2	3	3	4	**2**	50 %	31 %
12.des.97	S-7	4	11,0	1	1	2	2	1	2	**1**	50 %	37 %
12.des.97	S-7	5	12,5	1	1	2	1	1	2	**1**	67 %	39 %
12.des.97	S-7	6	12,0	1	1	2	2	1	1	**1**	67 %	39 %
12.des.97	S-7	7	11,0	1	1	2	2	1	1	**1**	67 %	39 %
12.des.97	S-7	8	11,0	1	1	2	2	2	2	**2**	67 %	31 %
12.des.97	S-7	9	12,5	1	1	2	2	1	1	**1**	67 %	39 %
12.des.97	S-7	10	11,0	1	1	2	2	2	2	**2**	67 %	31 %
12.des.97	S-7	11	9,0	0	0	0	0	0	0	**0**	100 %	0 %
12.des.97	S-7	12	11,5	1	1	2	2	2	2	**2**	67 %	31 %
12.des.97	S-7	13	13,0	2	2	2	2	2	2	**2**	100 %	0 %
12.des.97	S-7	14	11,5	1	1	2	3	2	3	**1**	33 %	45 %
12.des.97	S-7	15	11,0	1	1	1	2	1	1	**1**	83 %	35 %
12.des.97	S-7	16	11,0	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	17	11,5	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	18	10,5	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	19	11,0	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	20	12,0	1	1	1	1	1	1	**1**	100 %	0 %
12.des.97	S-7	21	9,5	2	1	2	2	2	1	**2**	67 %	31 %
12.des.97	S-7	22	12,5	2	2	2	3	3	3	**2**	50 %	22 %
12.des.97	S-7	23	10,5	1	1	1	2	1	1	**1**	83 %	35 %
12.des.97	S-7	24	12,0	2	2	2	2	2	2	**2**	100 %	0 %
12.des.97	S-7	25	11,5	1	1	1	1	1	1	**1**	100 %	0 %
			Total read	68	68	68	68	68	68		**85,8%**	**12,0%**
			Total NOT read	0	0	0	0	0	0			

Table 4. Sprat Otolith Workshop. Number of age readings, coefficientof variation (CV), the percentage agreement and the relative biasby age reader and readers combined. 2nd reading of S-2, S-6 and S-7.

NUMBER OF AGE READINGS

	MODAL age	SE BK	D GG	NL JB	NL AD	SE MJ	D MK	TOTAL
	0	1	1	1	1	1	1	6
	1	23	23	23	23	23	23	138
	2	31	31	31	31	31	31	186
	3	12	12	12	12	12	12	72
	4	1	1	1	1	1	1	6
	5	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-
Total	0-15	68	68	68	68	68	68	408

COEFFICIENT OF VARIATION (CV)

	MODAL age	SE BK	D GG	NL JB	NL AD	SE MJ	D MK	ALL Readers
	0	-	-	-	-	-	-	-
	1	0 %	0 %	36 %	42 %	20 %	43 %	14,9%
	2	16 %	18 %	0 %	15 %	20 %	28 %	10,2%
	3	14 %	16 %	14 %	9 %	15 %	12 %	12,0%
	4	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-
Weighted mean	0-15	9,6%	11,2%	14,7%	22,9%	18,5%	29,2%	12,0%
	RANKING	1	2	3	5	4	6	

PERCENTAGE AGREEMENT

	MODAL age	SE BK	D GG	NL JB	NL AD	SE MJ	D MK	ALL
	0	100 %	100 %	100 %	100 %	100 %	100 %	100 %
	1	100 %	100 %	70 %	65 %	96 %	83 %	86 %
	2	90 %	87 %	100 %	90 %	84 %	77 %	88 %
	3	83 %	75 %	83 %	92 %	67 %	83 %	81 %
	4	0 %	0 %	100 %	100 %	100 %	100 %	67 %
	5	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-
Weighted mean	0-15	91,2%	88,2%	86,8%	82,4%	85,3%	80,9%	85,8%
	RANKING	1	2	3	5	4	6	

RELATIVE BIAS

	MODAL age	SE BK	D GG	NL JB	NL AD	SE MJ	D MK	ALL
	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00
	1	0,00	0,00	0,30	0,39	0,04	0,22	0,16
	2	-0,10	-0,13	0,00	0,03	0,03	0,06	-0,02
	3	-0,17	-0,25	0,00	0,08	0,33	0,17	0,03
	4	-1,00	-1,00	0,00	0,00	0,00	0,00	-0,33
	5	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-
Weighted mean	0-15	-0,09	-0,12	0,10	0,16	0,09	0,13	0,05
	RANKING	1	4	3	6	2	5	

Figure. 1. Sprat Otoliths Workshops. 1st readings from samples S-2, S-6 and S-7. The age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

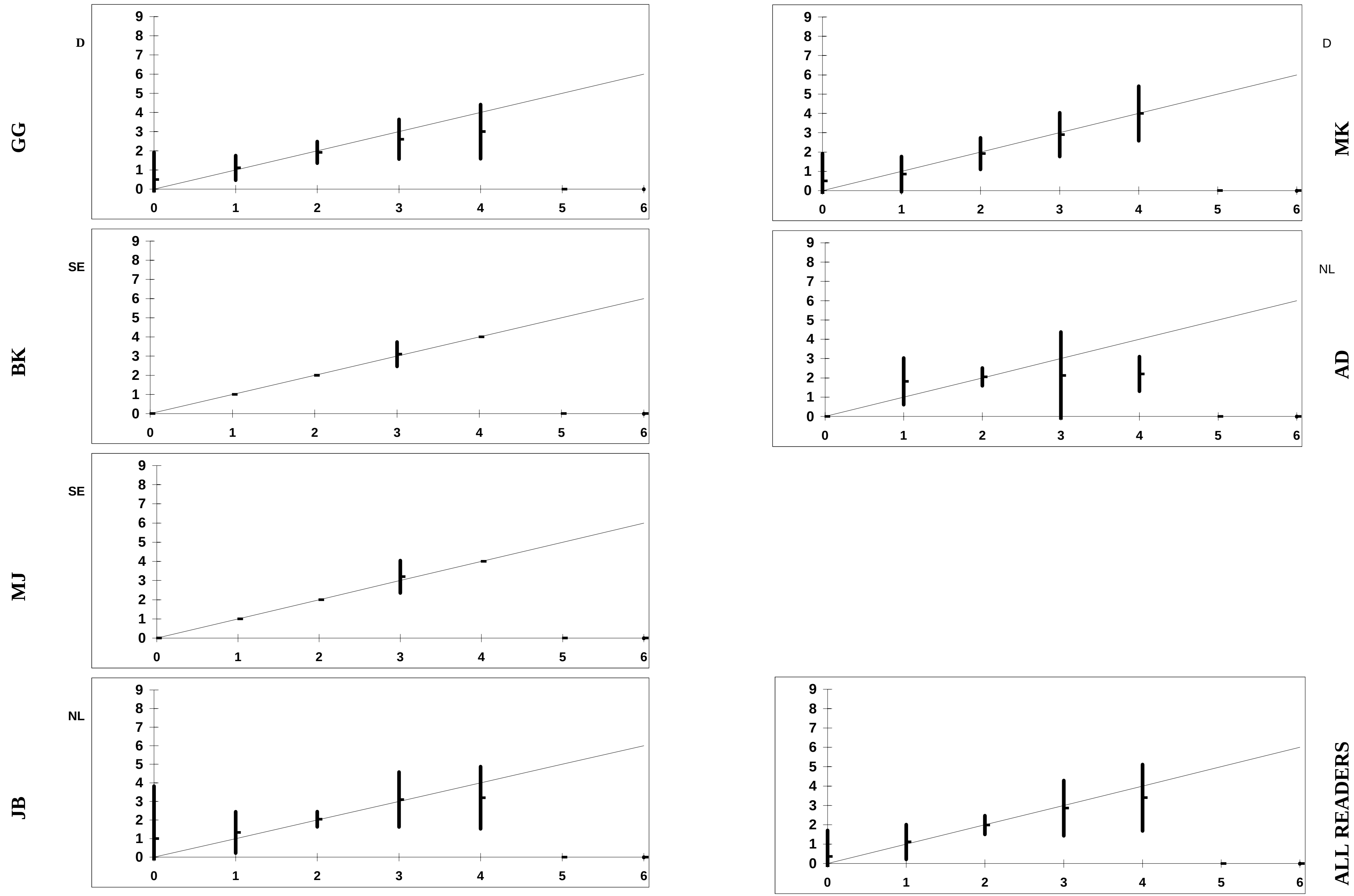
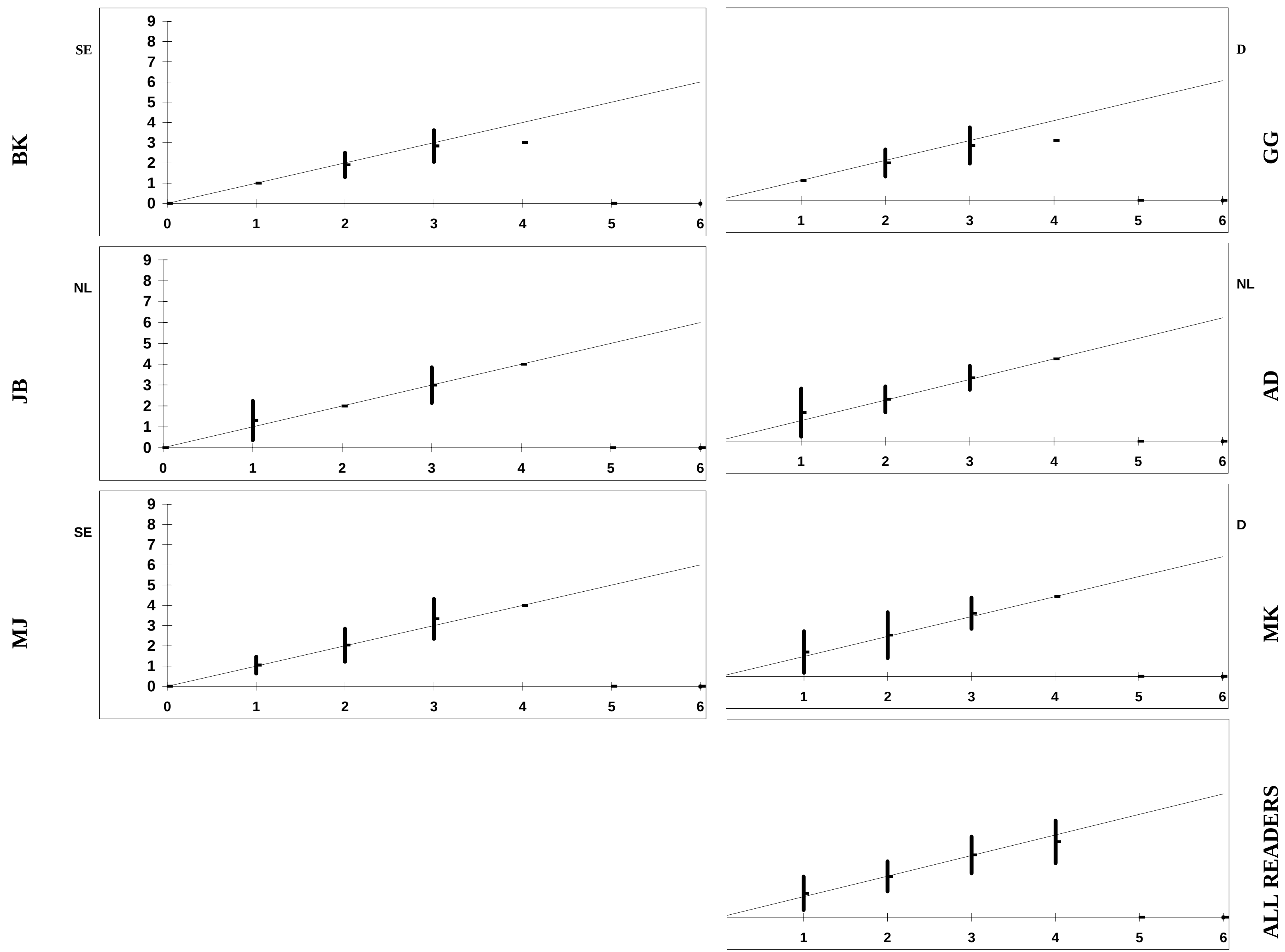


Figure. 2. Sprat Otoliths Workshops. 2nd readings from samples S-2, S-6 and S-7. The age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line).



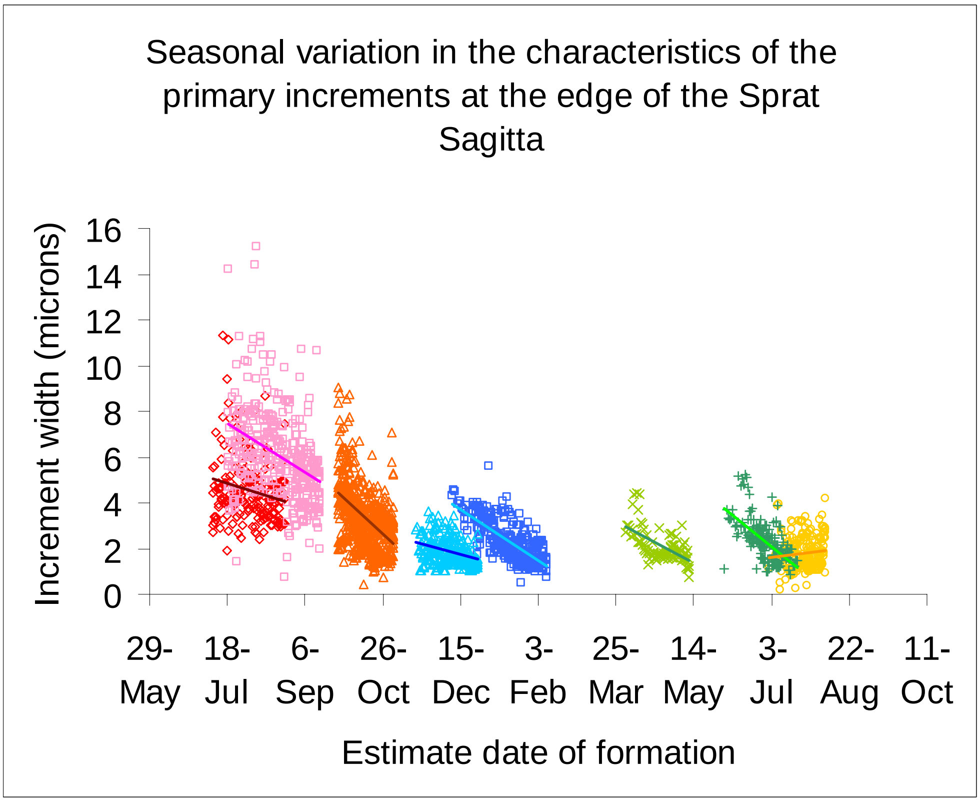


Figure 3. Development of daily increment width on the edge of sprat otoliths from indviduals caught during winter (Mosegaard and Baron 1999).

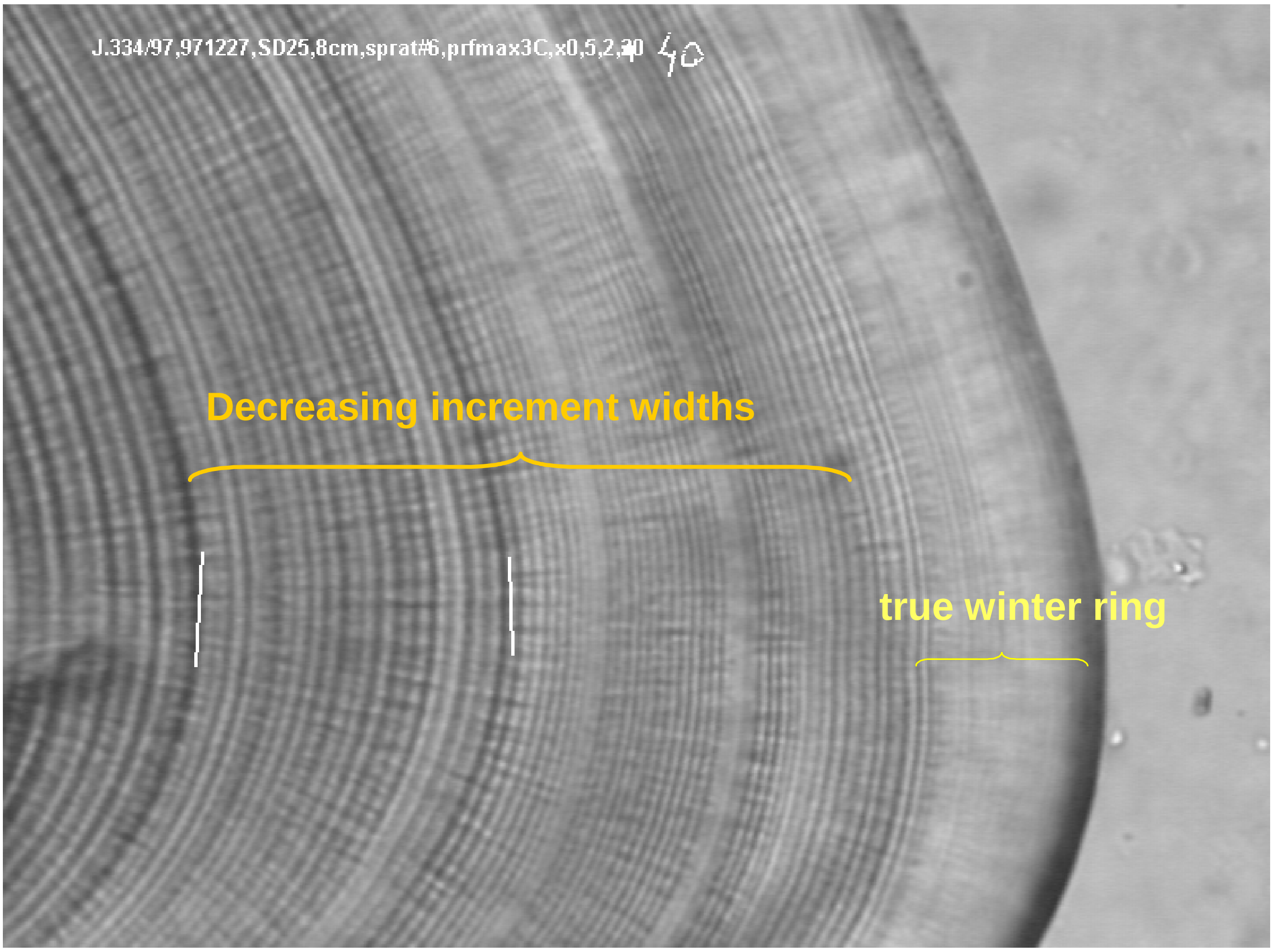


Figure 4. Microstructure development on the edge of sprat otolith from an individual caught in December.

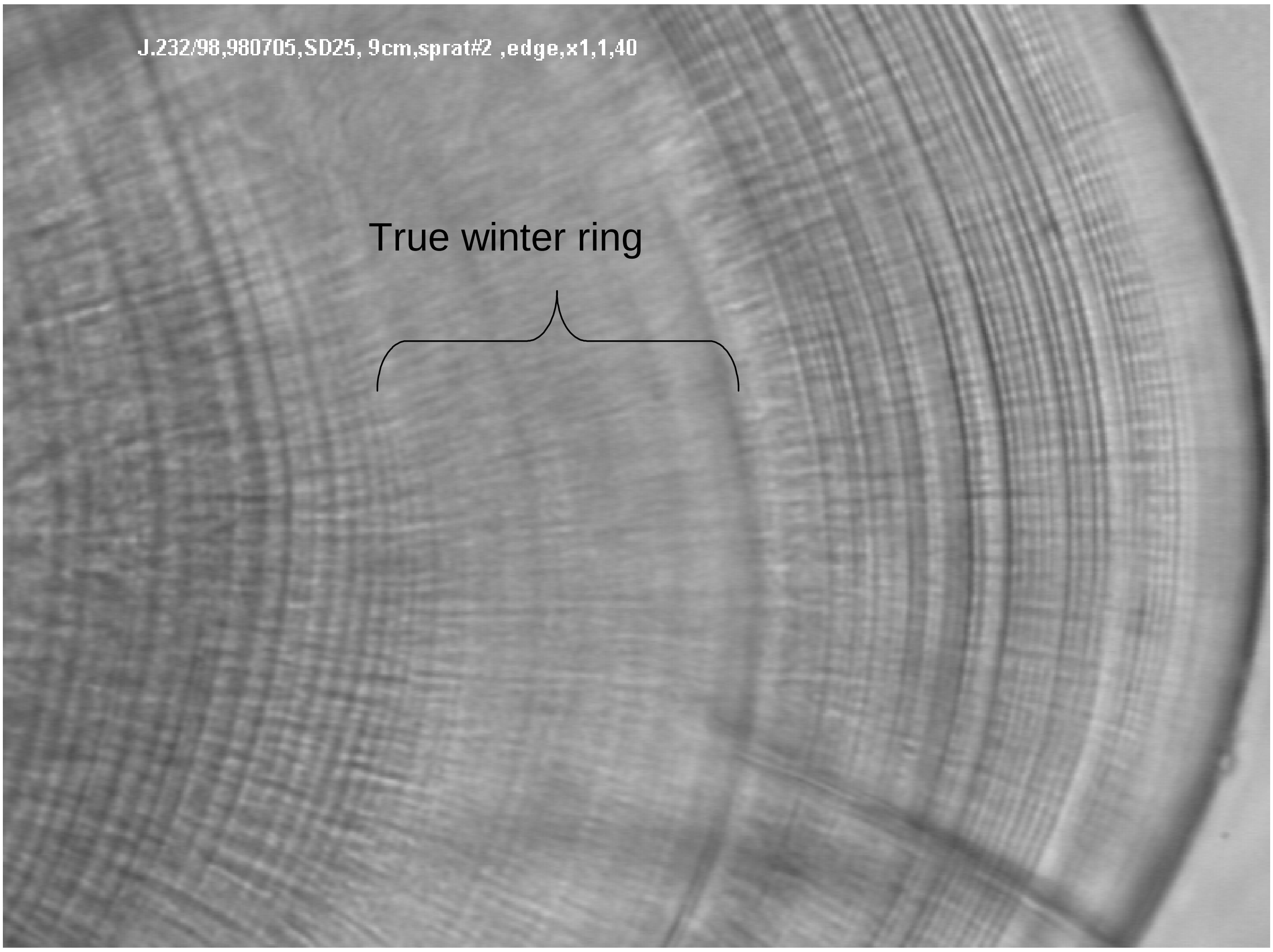


Figure 5. Microstructure pattern surrounding a true winter ring in sprat in an individual caught in July.

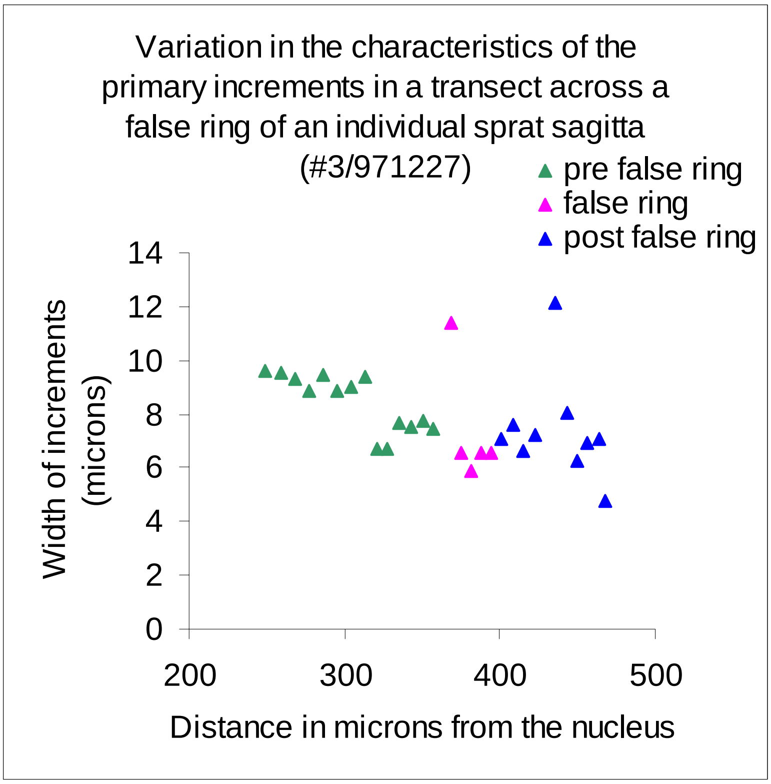


Figure 6. Development in width of daily increments across a false winter ring in a sprat otolith (individual caught in December) (Mosegaard and Baron 1999).

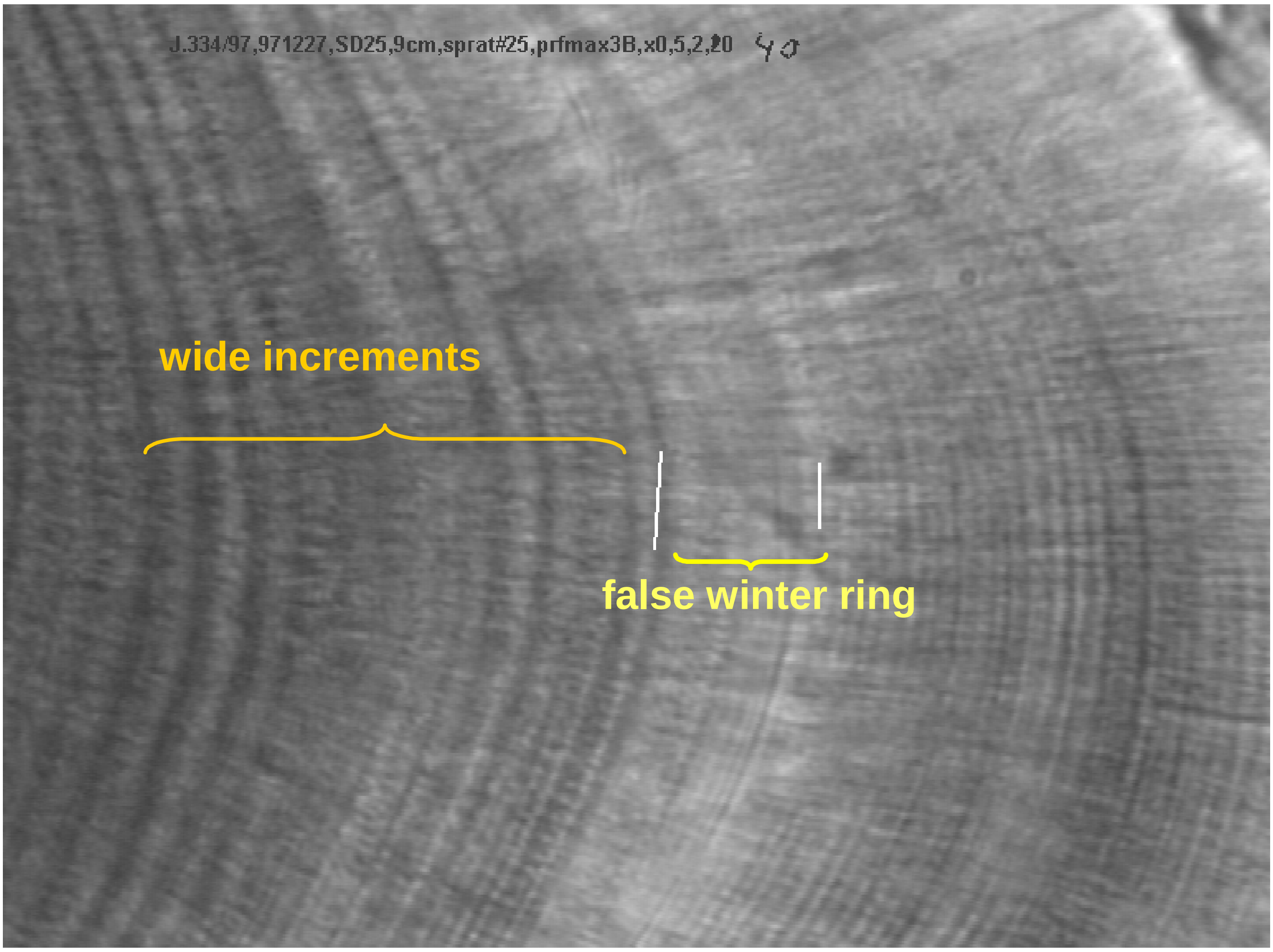


Figure 7. Otolith microstructure across a false winter ring in sprat (individual caught in December).

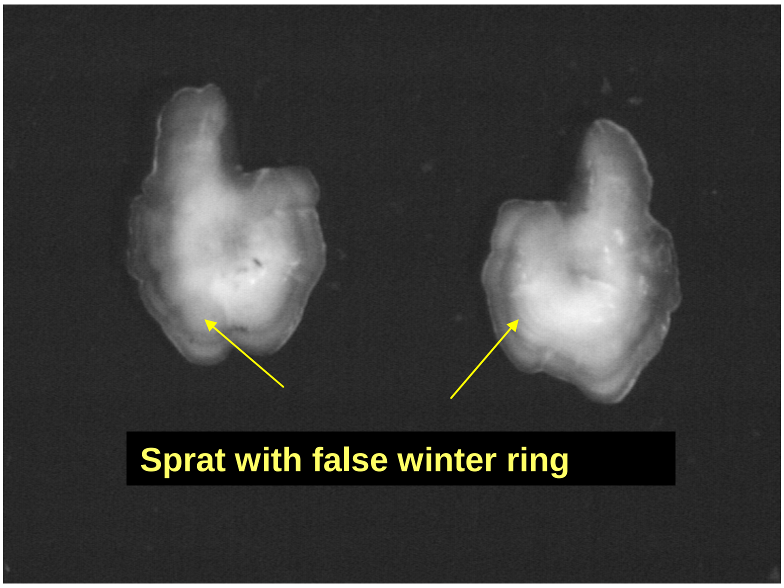


Figure 8. True age 0 sprat with a false winter ring (individual caught in December).

Figure 9. True age 0 sprat without a false winter ring (individual caught in December).

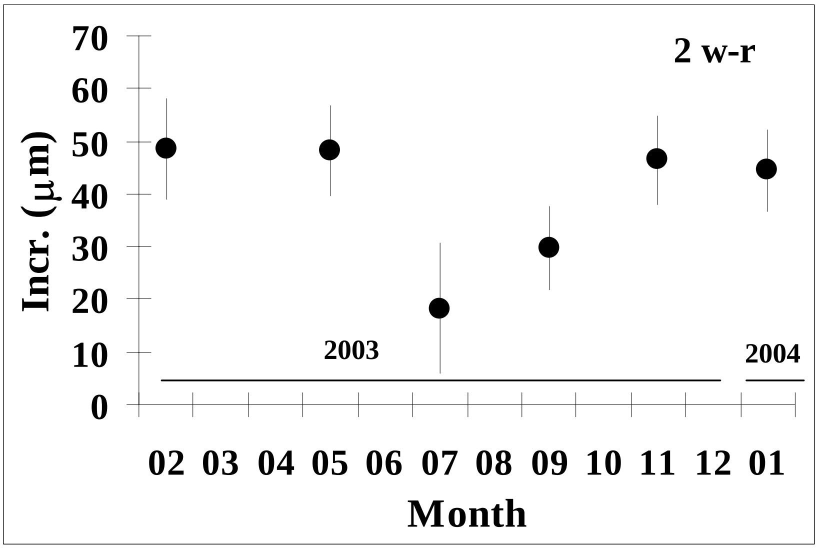


Figure 10. Marginal increment analysis of 2 w-r group in Skagerrak.

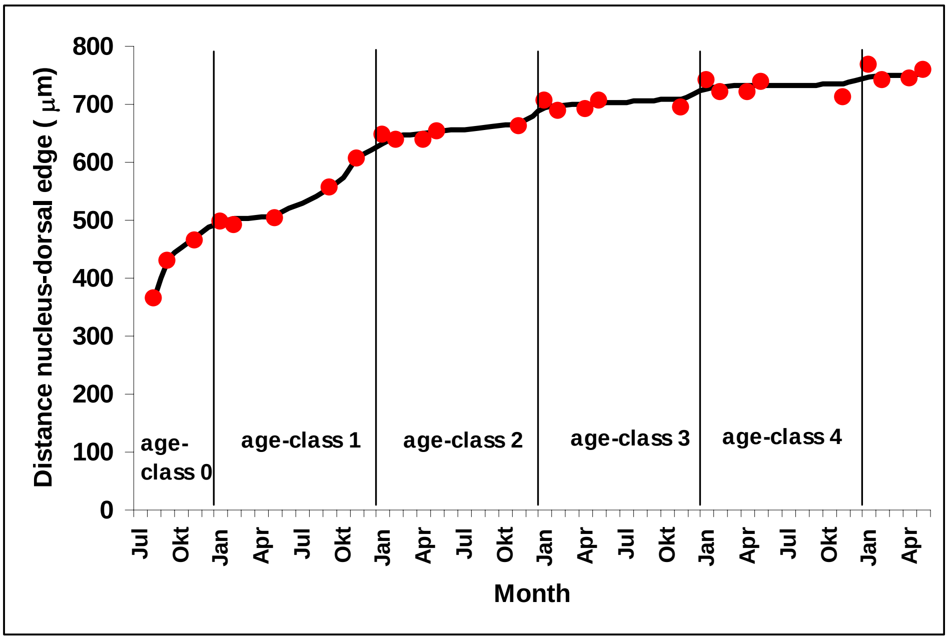


Figure 11. Growth of the otolith in Skagerrak (measured from the nucleus to the dorsal edge).

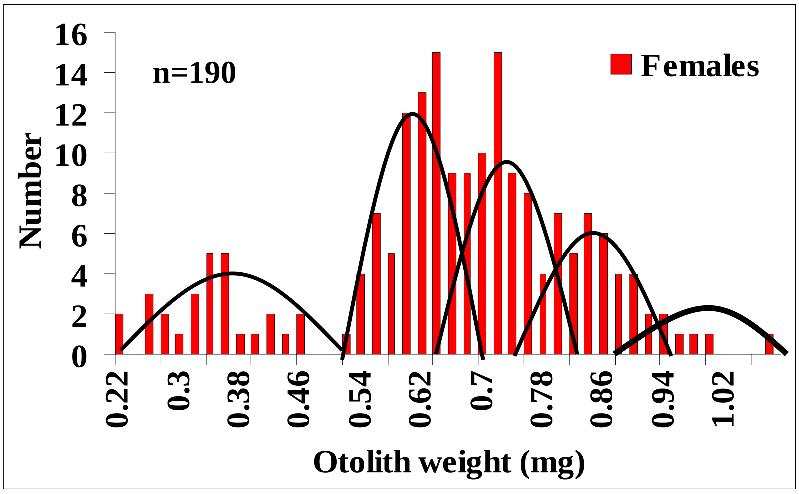


Figure 12. Otolith weight frequency distribution of females collected in Kattegat in February 2003. The modes have been identified by the Bhattacharya method using FISAT software. The modes correspond to the observed frequency (χ^2 test). The modes correspond also to the otolith weight-at-age determined by the age-readers (Kolmogorov-Smirnov and Anova tests)

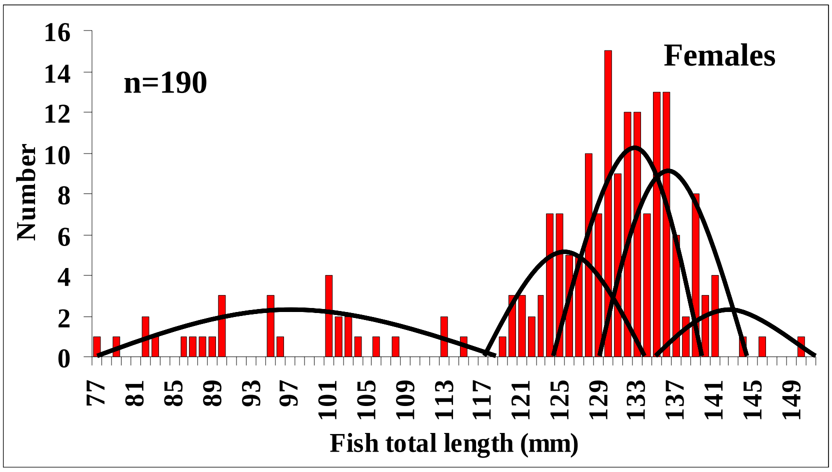
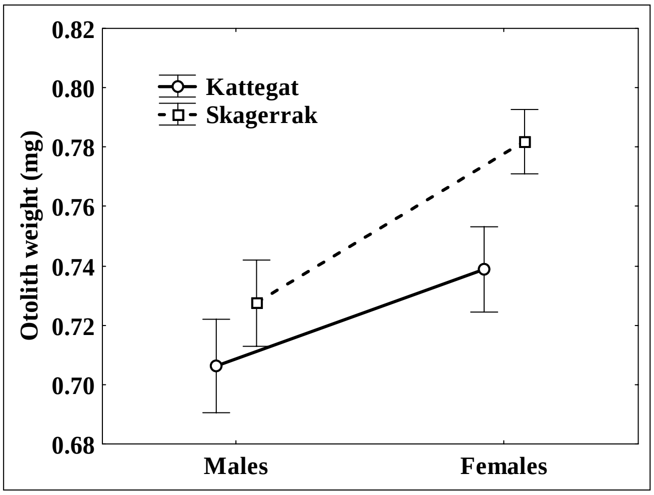


Figure 13. Fish length frequency distribution using the same data set. The modes have been identified by the Bhattacharya method using FISAT software. The modes do not correspond to the observed frequency (χ^2 test).



Factor	df	F	p
Age	**1**	**1441.8**	**< 0.001**
Area	**1**	**20.5**	**< 0.001**
Sex	**1**	**37.5**	**< 0.001**
Area*Sex	**1**	**2.4**	**0.123**

Figure 14. The results of the analysis of covariance between age, sex and area in Div IIIa in February 2003.

ANNEX 1.

Report of Sprat otolith exchange 2004

1. Introduction

The ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PCCDBS) recommended at its March 2003 meeting that an age reading workshop for sprat should be organised by Norway in 2004. Prior to this meeting an otoliths exchange was organised and coordinated by Else Torstensen, IMR/Norway, to detect problems in age reading.

1.1 Participants

Jan Beintema	Netherlands
Stine Bilstrup	Denmark
André Dijkman-Dulkes	Netherlands
Jean-Louis Dufour	France
Mark Etherton	UK(England)
Gudrun Gentschow	German
Knut Hansen	Norway
Marianne Johansson	Sweden
Mario Koth	Germany
Birgitta Krischansson	Sweden
Yves Verin	France
Steve Warners	UK(England)

Communication details are given in Table 1.

2. Material and Methods

Guidelines on how otolith exchanges should be organised and how the analysis of the age readings should be carried out can be found on the internet website of EFAN (European Fish Ageing Network). A spreadsheet for a standardised analysis of the age reading comparisons can also be found on the EFAN website (www.efan.no under "Guidelines"). One can download both the guidelines together with the spreadsheet for the age reading comparisons. For these otolith exchanges it is recommended to make an otolith set for the exchange that consists of an equal number of otoliths from each participating institute in order to enable an analysis on the otolith processing method for each institute.

The set of otoliths was made by otoliths available from France (IBTS-February 2004), Germany (summer herring survey 2003 and IBTS-January 2004) and Norway (commercial catches S-1, S-3, S-6, Survey S-4, S-7). Details are given in the following text table:

Sample	Date	ICES-sq	N fish	Area	Country
1	13.01.1996	37F4	25	North Sea	Norway
2	15.02.2004	38F8	25	North Sea	France
3	30.03.1999	37F0	25	North Sea	Norway
4	14.05.2003	45F8	25	Coastal Skagerrak	Norway
5	11.07.2003	34F2	25	North Sea	Germany
6	19.11.2003	47G0	25	Coastal Skagerrak	Norway
7	12.12.1997	37F2	25	North Sea	Noway
8	22.01.2004	*	176	RF area 6	Germany
9	30.01.2004	*	173	RF area 3	Germany

* "ICES roundfish sampling areas"

The French otoliths were received by IMR-Norway and mounted in the Norwegian way. In S-1-4 and S-6-7, pairs of otoliths were embedded in resin on black plastic plates and in S-5 and S-8-9 on a dual observation glass slide with transparent and black background. For the age determination, sprat birthday is defined as 1 January (ICES 1976).

Most laboratories performing age determination of sprat in the North-Sea-IIIa-area, participated in the exchange.

Age bias plots are a perfect way of showing the age readers both types of age reading errors (affecting precision and accuracy), when calcified structures of known or actual age are available. However, if no age validated calcified structures are available like in this case for the sprat otoliths exchange, then only the age reading errors that affect precision can be estimated. In this case the bias in age reading (accuracy error) can only be shown as relative bias.

3. Results

Table 2 contains the sample information and the input data of the age readings by reader. Three readers aged only part of the total collection. For each otolith the modal age, percentage agreement and CV is calculated based on the age readings. The age readers are ordered from left to right in the order of increasing CV (see Table 4). The modal age was only calculated over the age readings of the first nine readers, which achieved a CV lower than 20%, because the last three age readers had little or no experience in age reading sprat. Otoliths, which appeared to be difficult for age reading, are indicated with a high CV and a low percent agreement. At the workshop the age readers should discuss these difficult calcified structures in order to get an agreement on their interpretation.

Figure 1 shows the age bias plots in which the mean age recorded ± 2stdev is plotted against the modal age. The relative bias, which is an age reading error that affects accuracy, corresponds to the difference between mean age recorded and modal age.

The age readings are in agreement with modal age when the mean age recorded is on the 1:1 equilibrium line (mean age recorded equal to modal age). Readers have a relative bias in age reading when the mean age recorded is lower (underestimation of age) or higher (overestimation of age) than the modal age. The age reading errors affecting accuracy are best described by the relative bias by age group for each age reader, if calcified structures of known age are not available. However, it should be taken into account that relative bias might provide a very serious underestimate of absolute bias, because the comparison is not made to known/actual ages of the otoliths!

The precision errors are indicated by ± 2stdev in Figure 1. The lengths of the error bars indicate the spread in the age readings. A high precision is achieved, if the error bars remain relatively small. However, in Tables 2, 3, 5 and 6 it is preferred to show the precision errors not as standard deviation, but as coefficient of variation (CV = STDEV/mean age estimated), because the standard deviations increase greatly with age, while CV remains far more stable, since it is much less age dependent (see Figure 2). Relatively high CV's for certain age groups indicate specific problems in age reading. The precision errors by age reader are best described by the coefficient of variation (CV) by age group, because the CV's are different by age group.

Table 2 shows the precision (CV) in age reading by modal age, by age reader and all readers combined. The weighted mean CV's by age reader indicate the relative precision in age reading by reader. These can only be used to compare the precision levels in age reading of the age readers for this particular set (possibly age dependent!). The age readers are ranked according the precision they achieved.

Table 2 shows for each reader the weighted mean percentage agreement to the modal age over age groups 0-5. These mean agreements are related to accuracy, but should not be used to express accuracy reached by each reader, because they are very age dependent (see Figure 2). Percentage agreement decreases significantly when modal ages increase. Percentage agreements are, therefore, only representative by age group. The readers are ranked according to their achievement in the weighted mean percent agreement. It should be preferred to express the accuracy reached by reader as relative bias by age group. Relative bias by age group can be assumed to be equal to absolute bias by age group, if from another set with validated age structures it can be proven that there is no absolute bias in age reading! A rough indication of the relative bias by reader is the weighted mean of the relative bias over all age groups by age reader. The age readers are ranked from lowest to highest relative bias. At the end of Table 2 the age readers are ranked based on the average ranking according to CV, agreement and relative bias.

Figure 3 shows the relative bias by modal age as estimated by all age readers combined.

Figure 1 and Table 2 present the age reading method of each individual age reader assuming that modal age represents the best age available for comparison. It shows how the age readers have to correct for the bias in age reading (assuming modal age is correct!) and for what ages they have to try to improve the precision (i.e. reduce the CV). At the workshop Figure 1 and Table 2 are the most important tools to show age readers how they have to improve the age reading method.

Table 3 shows the age compositions and the mean length at age obtained by each reader and all readers combined. The results on mean length at age by reader are presented in Figure 5, which might illustrate age reading problems especially in the younger fish of certain age readers.

The minimal requirement for age reading consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between the readers and the modal age estimates can be tested non-parametrically with a one-sample Wilcoxon signed rank test. The result of this reader against modal age bias test is presented at the bottom of Table 3.

Table 4 shows the precision, percentage agreement and bias achieved by month by modal age. Higher CV's might be observed during the period of opaque deposition on the edge of the calcified structure, because this often causes difficulties in the interpretation.

Table 5 shows the precision, percentage agreement and bias achieved by stratum. The strata are the sub-sets of otliths by age reading laboratory (possibly different preparation techniques). Higher CV's for a certain stratum indicate that these otoliths are more difficult to read for some reason.

Table 6 shows the actual values used for producing the age bias plots of Figure 1.

4. Discussion and conclusions

According to Table 2 only one age reader achieved a precision level CV<10%, eight age readers obtained a CV between 10% and 20% and three readers a CV>20% (the latter being excluded for the calculation of the modal age). <u>This indicates that an improvement in the precision level of age reading is required</u>. The results from sample S-9 indicate that a much better precision appears achievable.

According to Table 2 the CV (precision) achieved by all age readers by age group for the ages 1 to 4 increases respectively from 8.4% to 11.2% to 13.6% and to 14.6%. This indicates that <u>difficulties in the interpretation of the annual rings increases from younger to older the fish</u>. This seems to be due to a misinterpretation of the annual zones after age 1.

According Table 2 and Figure 1 and 3 several age readers <u>underestimated the ages of the older fish</u>. This might also be due to a misinterpretation of the annual zones after age 1. <u>This indicates that a reduction in the age reading bias is required</u>.

The percentage agreement ranged from 62% to 98%. Percentage agreement is neither a measure for precision nor for accuracy, but it is presented because it traditionally is.

From Table 4 <u>it is difficult to evaluate whether a problem in the interpretation of the edge of the otolith exists</u>, because most otoliths are collected in the first half of the year (440) compared to the second half of the year (only 75). If this problem would exist, the precision in age reading would be significantly lower (CV higher) in the second half of the year, when the opaque growth zone appears on the edge of the otolith.

Table 5 shows that the age readings from the different sub-samples of the total otolith set differed considerably in the quality. Based on age readings from most age readers a percentage agreement of 98%, a CV of 1.6% and a bias of 0.00 was achieved for sub-sample S-9 (172 otoliths), while the age readings of sub-sample S-8 achieved a percentage agreement of 89%, a CV of 13.8% and a bias of -0.01. The same institute treated both sub-samples according the same otolith processing technique and both samples were collected in January. The main difference appeared to be that sub-sample S-9 was collected in IBTS “Roundfish area 3” and S-8 in IBTS “Roundfish area 6”. Furthermore, it was discovered that at least one otolith reader had read the otoliths of sub-sample S-8 in the wrong order, because all 176 otoliths were put on one glass plate without otolith number indication. <u>The results of the age readings of sub-sample S-9 indicate that a much better precision and accuracy might be achievable in future than is now achieved for the whole otolith set during this exchange.</u>

Sprat could be spawning until late autumn. It could be that late spawning that juvenile sprat from these eggs would not anymore deposit an opaque growth zone before the next year. This might cause a wrong age determination by the age readers, because they are not able to distinguish these fish in age from the fish born in the next year. However, this problem could not be evaluated from this otolith exchange and might only be solved by e.g. counting daily increment rings on otoliths having a large L1 and showing a clear nucleus (similar to autumn spawning herring). Based on the results of this exchange it appears to be possible to achieve reliable age readings for North Sea / Skagerrak sprat in future, if it can be proven that sprat always produces an opaque growth zone in the year it is born and that by age reading the otoliths of sprat can be assigned to a certain year class.

5. Recommendations

At the age reading workshop a manual on age reading sprat should be written to standardise the age reading method.

At the age reading workshop a manual on the processing technique of sprat otoliths should be written to assure in future a good quality of processed otoliths by all laboratories that provide sprat age readings.

Carry out investigations in counting daily increment rings to prove that sprat always produces an opaque growth zone in the year it is born and to prove that by age reading the otoliths of sprat can be assigned to a certain year class.

Distribute to all age readers that participated in the exchange and workshop a CD with photographs of all otoliths of which the ages were agreed at the workshop. With this digital reference collection all age readers can calibrate their age reading method.

Propose another sprat otolith exchange in 2007 to estimate again the precision and relative bias in age reading. Propose another workshop, if there still appear to be problems in age reading sprat.

5. Reference

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ICES 1976. Report of the Herring Assessment Working group for the area south of 62ºN. ICES CM 1976/H:2

ICES 2003. Report of the Planning Group on Commercial Catch, Discards and Biological Sampling (PCCDBS). ICES CM 2003/ACFM:16

Table 1. Participants in the otolith exchange. Communication list.

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Table 2 The number of age readings, the coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups comined indicate the precision in age reading by reader and for all age readers combined.

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NUMBER OF AGE READINGS

	MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	TOTAL
	0	1	1	1	1	1	1	1	1	1	-	1	1	**11**
	1	191	192	190	190	196	196	192	28	28	9	186	187	**1785**
	2	233	233	229	229	235	234	233	54	54	65	235	234	**2268**
	3	50	51	51	51	51	51	50	32	32	40	50	51	**560**
	4	26	28	28	28	28	28	25	26	26	26	26	28	**323**
	5	2	2	2	2	2	2	2	2	2	2	2	2	**24**
	6	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total	**0-15**	**503**	**507**	**501**	**501**	**513**	**512**	**503**	**143**	**143**	**142**	**500**	**503**	**4971**

COEFFICIENT OF VARIATION (CV)

	MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL Readers
	0	-	-	-	-	-	-	-	-	-	-	-	-	**-**
	1	7 %	12 %	10 %	12 %	20 %	23 %	24 %	24 %	41 %	42 %	22 %	31 %	**8,4%**
	2	6 %	12 %	15 %	15 %	11 %	13 %	16 %	17 %	13 %	22 %	28 %	29 %	**11,2%**
	3	11 %	17 %	13 %	13 %	17 %	15 %	13 %	21 %	21 %	22 %	23 %	16 %	**13,6%**
	4	9 %	10 %	13 %	13 %	18 %	18 %	9 %	17 %	13 %	22 %	15 %	13 %	**14,6%**
	5	0 %	13 %	47 %	47 %	0 %	35 %	13 %	20 %	0 %	0 %	28 %	13 %	**19,4%**
	6	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**6,9%**	**12,4%**	**12,8%**	**13,9%**	**15,7%**	**17,0%**	**18,0%**	**19,3%**	**19,9%**	**22,7%**	**24,5%**	**27,4%**	**10,6%**
	RANKING	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	

PERCENTAGE AGREEMENT

	MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
	0	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	-	100 %	100 %	**100 %**
	1	99 %	98 %	99 %	98 %	95 %	94 %	93 %	93 %	68 %	44 %	95 %	90 %	**95 %**
	2	99 %	95 %	93 %	94 %	94 %	95 %	92 %	89 %	93 %	71 %	76 %	69 %	**89 %**
	3	90 %	78 %	82 %	82 %	80 %	80 %	88 %	69 %	72 %	60 %	56 %	82 %	**78 %**
	4	88 %	86 %	75 %	75 %	71 %	36 %	88 %	19 %	69 %	54 %	77 %	68 %	**67 %**
	5	100 %	50 %	0 %	0 %	100 %	50 %	50 %	0 %	100 %	0 %	0 %	50 %	**42 %**
	6	-	-	-	-	-	-	-	-	-	-	-	-	
Weighted mean	**0-15**	**97,6%**	**94,1%**	**93,0%**	**93,0%**	**92,2%**	**89,6%**	**91,7%**	**71,3%**	**79,0%**	**62,0%**	**80,8%**	**77,9%**	**88,4%**
	RANKING	**1**	**2**	**3**	**3**	**5**	**7**	**6**	**11**	**9**	**12**	**8**	**10**	

RELATIVE BIAS

	MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	-	0,00	0,00	**0,00**
	1	0,01	0,02	0,01	0,02	0,04	0,06	0,07	0,07	0,25	0,67	-0,01	0,11	**0,04**
	2	-0,00	0,03	0,05	0,05	0,04	0,01	0,06	-0,04	0,07	0,31	-0,02	-0,24	**0,01**
	3	-0,06	0,12	0,10	0,10	-0,06	-0,16	0,10	-0,28	0,00	-0,10	-0,16	-0,04	**-0,03**
	4	-0,04	-0,07	-0,04	-0,04	-0,29	-0,71	-0,12	-0,88	-0,31	-0,50	-0,04	-0,32	**-0,28**
	5	0,00	0,50	-0,50	-0,50	0,00	-1,00	0,50	-1,50	0,00	-1,00	0,00	0,50	**-0,25**
	6	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**-0,01**	**0,03**	**0,03**	**0,03**	**0,01**	**-0,03**	**0,06**	**-0,24**	**0,02**	**0,05**	**-0,03**	**-0,09**	**-0,00**
	RANKING	**1**	**4**	**7**	**8**	**2**	**5**	**10**	**12**	**3**	**9**	**6**	**11**	

Overall ranking

	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB
Ranking Coefficient of Variation	1	2	3	4	5	6	7	8	9	10	11	12
Ranking Percentage Agreement	1	2	3	3	5	7	6	11	9	12	8	10
Ranking Relative bias	1	4	7	8	2	5	10	12	3	9	6	11
OVERALL RANKING	**1**	**2**	**4**	**5**	**3**	**6**	**8**	**10**	**7**	**10**	**9**	**12**
Mean Ranking	1,00	2,67	4,33	5,00	4,00	6,00	7,67	10,33	7,00	10,33	8,33	11,00
absolute value of the bias	0,01	0,03	0,03	0,03	0,01	0,03	0,06	0,24	0,02	0,05	0,03	0,09

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Table 3 Upper table: The age compositions estimated by each age reader and all age readers combined.
Midle table: The estimated mean length at age by age reader and by all age readers combined.
Lower table: The reader and MODAL age bias tests: non-parametrically with a one-sample Wilcoxon rank sum test

AGE COMPOSITION

	Age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	TOTAL
	0	1	1	1	1	2	1	1	1	2	-	6	1	**18**
	1	192	192	191	190	189	189	182	31	19	4	210	233	**1822**
	2	235	228	218	220	239	245	228	60	63	63	198	184	**2181**
	3	48	50	57	55	57	64	61	44	35	51	51	61	**634**
	4	24	32	30	31	22	12	28	7	21	23	30	21	**281**
	5	3	3	3	3	4	1	2	-	3	1	4	2	**29**
	6	-	1	1	1	-	-	1	-	-	-	1	1	**6**
	7	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total	**0-15**	**503**	**507**	**501**	**501**	**513**	**512**	**503**	**143**	**143**	**142**	**500**	**503**	**4971**

MEAN LENGTH AT AGE

	Age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
	0	9,0	9,0	9,0	9,0	9,3	9,0	9,0	9,0	9,3	-	11,0	9,0	**9,7**
	1	9,2	9,1	9,2	9,2	9,2	9,2	9,0	11,1	10,8	9,3	9,3	9,7	**9,3**
	2	11,6	11,6	11,6	11,6	11,5	11,5	11,5	11,3	11,1	11,3	11,8	11,6	**11,5**
	3	13,0	12,9	12,4	12,4	13,1	13,7	12,5	14,4	13,5	13,3	12,3	13,3	**13,0**
	4	15,1	14,7	15,2	15,2	15,3	14,3	14,8	13,4	15,0	15,3	14,8	14,8	**14,9**
	5	15,7	13,8	17,0	17,0	15,8	17,5	13,0	-	14,7	17,0	15,4	13,0	**15,4**
	6	-	17,5	17,5	17,5	-	-	17,5	-	-	-	17,5	17,5	**17,5**
	7	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**11,0**	**11,0**	**11,0**	**11,0**	**11,0**	**11,0**	**11,0**	**12,3**	**12,3**	**12,6**	**11,0**	**11,0**	**11,1**

Reader against MODAL age bias test

	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB
MODAL age	–	*	*	*	–	–	* *	* *	–	* *	–	* *

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

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Table 4 Otoliths read, CV's, percentage agreement and RELATIVE bias by month and by MODAL age.

NUMBER OF OTOLITHS

MODAL age	1 Jan	2 Feb	3 Mar	4 Apr	5 May	6 Jun	7 Jul	8 Aug	9 Sep	10 Oct	11 Nov	12 Dec	Nr of otoliths
0	-	-	-	-	-	-	-	-	-	-	-	1	**1**
1	168	5	1	-	-	-	-	-	-	-	3	19	**196**
2	170	11	18	-	-	-	16	-	-	-	16	3	**234**
3	23	2	5	-	5	-	9	-	-	-	4	2	**50**
4	8	-	1	-	19	-	-	-	-	-	2	-	**30**
5	1	-	-	-	-	-	-	-	-	-	-	-	**1**
6	-	-	-	-	1	-	-	-	-	-	-	-	**1**
TOTAL	**370**	**18**	**25**	**0**	**25**	**0**	**25**	**0**	**0**	**0**	**25**	**25**	**513**

COEFFICIENT OF VARIATION (CV)

MODAL age	1 Jan	2 Feb	3 Mar	4 Apr	5 May	6 Jun	7 Jul	8 Aug	9 Sep	10 Oct	11 Nov	12 Dec	Mean CV
0	-	-	-	-	-	-	-	-	-	-	-	0 %	**-**
1	5 %	25 %	31 %	-	-	-	-	-	-	-	25 %	32 %	**8,4%**
2	10 %	17 %	12 %	-	-	-	15 %	-	-	-	10 %	18 %	**11,1%**
3	12 %	5 %	13 %	-	16 %	-	10 %	-	-	-	25 %	27 %	**13,7%**
4	14 %	-	17 %	-	14 %	-	-	-	-	-	28 %	-	**14,8%**
5	20 %	-	-	-	-	-	-	-	-	-	-	-	**-**
6	-	-	-	-	18 %	-	-	-	-	-	-	-	**-**
Mean CV Weighted	**8,1%**	**17,5%**	**13,1%**	**-**	**14,4%**	**-**	**13,6%**	**-**	**-**	**-**	**15,7%**	**28,5%**	**10,5%**

Note: Higher CV's might be expected during months of opaque material deposition and during the juvenile phase, when false rings might occur!

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MODAL age	1 Jan	2 Feb	3 Mar	4 Apr	5 May	6 Jun	7 Jul	8 Aug	9 Sep	10 Oct	11 Nov	12 Dec	Agree-ment
0	-	-	-	-	-	-	-	-	-	-	-	100 %	**100,0%**
1	97 %	85 %	92 %	-	-	-	-	-	-	-	94 %	82 %	**95,1%**
2	90 %	82 %	92 %	-	-	-	88 %	-	-	-	87 %	79 %	**89,4%**
3	84 %	96 %	83 %	-	67 %	-	84 %	-	-	-	48 %	55 %	**78,2%**
4	66 %	-	58 %	-	68 %	-	-	-	-	-	54 %	-	**66,0%**
5	50 %	-	-	-	-	-	-	-	-	-	-	-	**50,0%**
6	-	-	-	-	50 %	-	-	-	-	-	-	-	**50,0%**
Mean CV Weighted	**92,5%**	**84,2%**	**89,2%**	**-**	**66,9%**	**-**	**86,7%**	**-**	**-**	**-**	**79,0%**	**79,9%**	**89,0%**

RELATIVE BIAS

MODAL age	1 Jan	2 Feb	3 Mar	4 Apr	5 May	6 Jun	7 Jul	8 Aug	9 Sep	10 Oct	11 Nov	12 Dec	Mean bias
0	-	-	-	-	-	-	-	-	-	-	-	0,00	**0,00**
1	0,03	0,15	-0,08	-	-	-	-	-	-	-	0,08	0,13	**0,04**
2	-0,02	0,01	-0,05	-	-	-	0,11	-	-	-	0,13	0,06	**0,00**
3	-0,09	-0,04	-0,10	-	0,20	-	-0,11	-	-	-	-0,17	0,09	**-0,06**
4	-0,37	-	-0,25	-	-0,20	-	-	-	-	-	-0,75	-	**-0,28**
5	-0,75	-	-	-	-	-	-	-	-	-	-	-	**-0,75**
6	-	-	-	-	-0,75	-	-	-	-	-	-	-	**-0,75**
Mean Weighted	**-0,01**	**0,04**	**-0,07**	**-**	**-0,14**	**-**	**0,03**	**-**	**-**	**-**	**0,00**	**0,11**	**-0,01**

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Table 5 Otoliths read, CV's, percentage agreement and RELATIVE bias by stratum and MODAL age.

NUMBER OF OTOLITHS

MODAL	SAMPLING STRATA												Nr of
age	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9				otoliths
0	-	-	-	-	-	-	1	-	-	-	-	-	1
1	-	5	1	-	-	3	19	95	73	-	-	-	196
2	5	11	18	-	16	16	4	72	93	-	-	-	235
3	14	2	5	6	9	4	1	6	4	-	-	-	51
4	5	-	1	18	-	2	-	-	2	-	-	-	28
5	1	-	-	1	-	-	-	-	-	-	-	-	2
6	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	25	18	25	25	25	25	25	173	172	0	0	0	513

COEFFICIENT OF VARIATION (CV)

MODAL	SAMPLING STRATA												Mean
age	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	0	0	0	CV
0	-	-	-	-	-	-	0 %	-	-	-	-	-	-
1	-	25 %	31 %	-	-	25 %	32 %	8 %	1 %	-	-	-	8,4%
2	15 %	17 %	12 %	-	15 %	10 %	20 %	21 %	1 %	-	-	-	11,2%
3	11 %	5 %	13 %	17 %	10 %	25 %	27 %	16 %	12 %	-	-	-	13,6%
4	14 %	-	17 %	13 %	-	28 %	-	-	12 %	-	-	-	14,6%
5	20 %	-	-	18 %	-	-	-	-	-	-	-	-	19,4%
6	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean CV	12,9%	17,5%	13,1%	14,4%	13,6%	15,7%	28,5%	13,8%	1,6%	-	-	-	10,6%

Weighted

PERCENTAGE AGREEMENT

MODAL	SAMPLING STRATA												Agree-
age	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	0	0	0	ment
0	-	-	-	-	-	-	100 %	-	-	-	-	-	100,0%
1	-	85 %	92 %	-	-	94 %	82 %	96 %	99 %	-	-	-	95,1%
2	83 %	82 %	92 %	-	88 %	87 %	68 %	80 %	98 %	-	-	-	89,2%
3	85 %	96 %	83 %	62 %	84 %	48 %	64 %	74 %	83 %	-	-	-	77,5%
4	67 %	-	58 %	69 %	-	54 %	-	-	72 %	-	-	-	67,2%
5	50 %	-	-	33 %	-	-	-	-	-	-	-	-	41,7%
6	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean CV	79,2%	84,2%	89,2%	65,9%	86,7%	79,0%	79,5%	88,7%	98,2%	-	-	-	88,9%

Weighted

RELATIVE BIAS

MODAL	SAMPLING STRATA												Mean
age	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	0	0	0	bias
0	-	-	-	-	-	-	0,00	-	-	-	-	-	0,00
1	-	0,15	-0,08	-	-	0,08	0,13	0,04	0,01	-	-	-	0,04
2	0,10	0,01	-0,05	-	0,11	0,13	0,25	-0,07	0,01	-	-	-	0,01
3	0,03	-0,04	-0,10	0,29	-0,11	-0,17	0,36	-0,26	-0,17	-	-	-	-0,03
4	-0,37	-	-0,25	-0,20	-	-0,75	-	-	-0,28	-	-	-	-0,28
5	-0,75	-	-	0,25	-	-	-	-	-	-	-	-	-0,25
6	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	-0,07	0,04	-0,07	-0,07	0,03	0,00	0,15	-0,01	0,00	-	-	-	0,00

Weighted

Table 6 TABLES FOR PLOTTING THE AGE BIAS PLOT FIGURES OF FIGURE 1

SPRAT OTOLITHS EXCHANGE 2004

2STDEV

MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	2STDEV ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	**0,000**
1	0,145	0,249	0,205	0,250	0,424	0,481	0,504	0,525	1,036	1,414	0,441	0,698	**0,456**
2	0,227	0,489	0,599	0,628	0,465	0,507	0,644	0,669	0,529	0,995	1,101	1,007	**0,715**
3	0,627	1,031	0,825	0,825	1,013	0,836	0,833	1,162	1,244	1,265	1,301	0,977	**1,013**
4	0,688	0,756	1,016	1,016	1,317	1,200	0,663	1,032	0,941	1,523	1,197	0,951	**1,174**
5	0,000	1,414	4,243	4,243	0,000	2,828	1,414	1,414	0,000	0,000	2,828	1,414	**2,064**
6	-	-	-	-	-	-	-	-	-	-	-	-	**-**

MEAN AGE

	MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
	0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	-	0,00	0,00	**0,00**
	1	1,01	1,02	1,01	1,02	1,04	1,06	1,07	1,07	1,25	1,67	0,99	1,11	**1,04**
	2	2,00	2,03	2,05	2,05	2,04	2,01	2,06	1,96	2,07	2,31	1,98	1,76	**2,01**
	3	2,94	3,12	3,10	3,10	2,94	2,84	3,10	2,72	3,00	2,90	2,84	2,96	**2,97**
	4	3,96	3,93	3,96	3,96	3,71	3,29	3,88	3,12	3,69	3,50	3,96	3,68	**3,72**
	5	5,00	5,50	4,50	4,50	5,00	4,00	5,50	3,50	5,00	4,00	5,00	5,50	**4,75**
	6	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Weighted mean	**0-15**	**1,82**	**1,87**	**1,87**	**1,88**	**1,84**	**1,80**	**1,89**	**2,17**	**2,44**	**2,68**	**1,81**	**1,76**	**1,89**

MEAN AGE +2STDEV

MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	**0,00**
1	1,150	1,264	1,215	1,266	1,459	1,542	1,572	1,596	2,286	3,081	1,436	1,810	**1,50**
2	2,223	2,514	2,647	2,676	2,503	2,520	2,708	2,632	2,603	3,303	3,080	2,764	**2,72**
3	3,567	4,149	3,923	3,923	3,954	3,680	3,933	3,881	4,244	4,165	4,141	3,937	**3,98**
4	4,650	4,685	4,980	4,980	5,032	4,485	4,543	4,147	4,634	5,023	5,159	4,630	**4,90**
5	5,000	6,914	8,743	8,743	5,000	6,828	6,914	4,914	5,000	4,000	7,828	6,914	**6,81**
6	-	-	-	-	-	-	-	-	-	-	-	-	**-**

MEAN AGE -2STDEV

MODAL age	D GG	DK SB	SE BK	SE MJ	UK SW	UK ME	N KH	F YV	F JD	NL AD	D MK	NL JB	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	**0,000**
1	0,861	0,767	0,806	0,766	0,612	0,581	0,564	0,547	0,214	0,252	0,554	0,415	**0,587**
2	1,768	1,537	1,449	1,420	1,573	1,506	1,421	1,294	1,545	1,313	0,878	0,749	**1,291**
3	2,313	2,086	2,273	2,273	1,928	2,007	2,267	1,557	1,756	1,635	1,539	1,984	**1,958**
4	3,273	3,173	2,949	2,949	2,397	2,086	3,217	2,084	2,751	1,977	2,764	2,727	**2,548**
5	5,000	4,086	0,257	0,257	5,000	1,172	4,086	2,086	5,000	4,000	2,172	4,086	**2,686**
6	-	-	-	-	-	-	-	-	-	-	-	-	**-**

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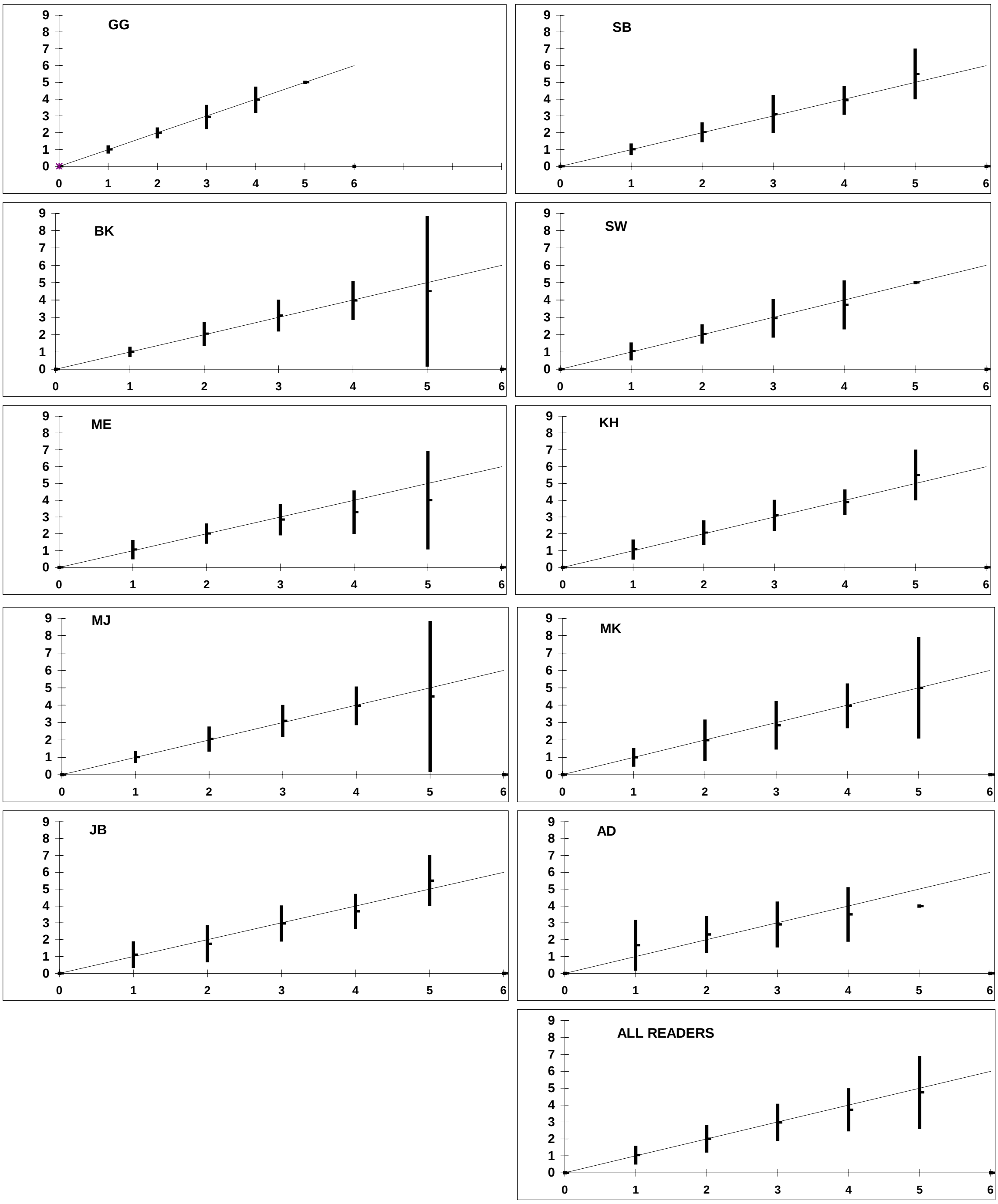
Figure 1

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



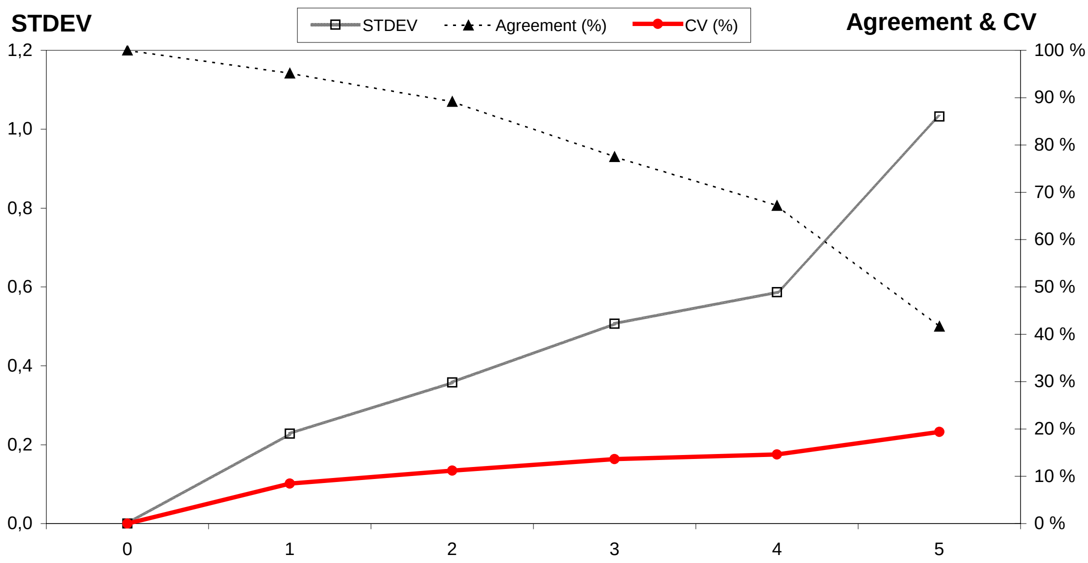


Figure 2 The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age.
CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

SPRAT OTOLITHS EXCHANGE 2004

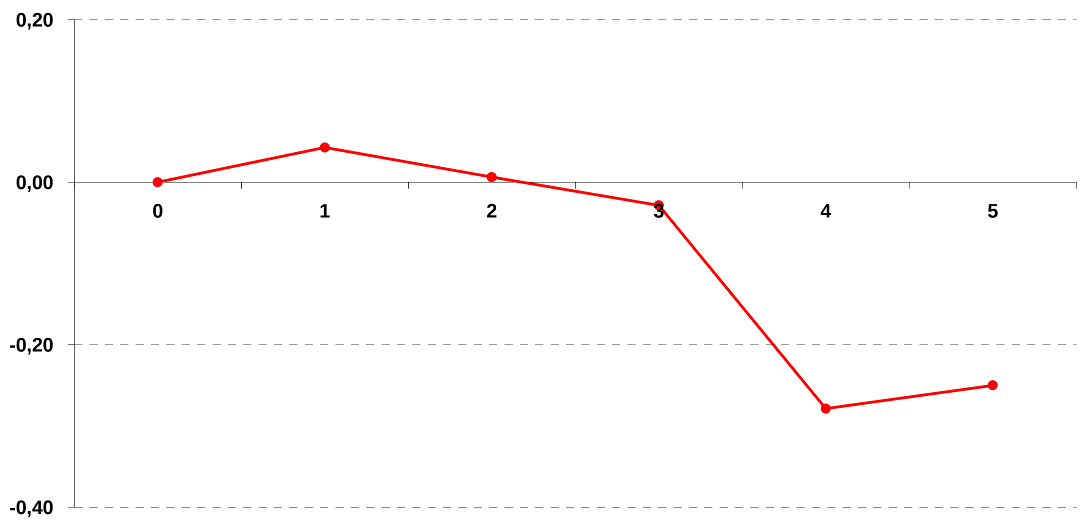


Figure 3 **The RELATIVE bias by MODAL age as estimated by all age readers combined.**

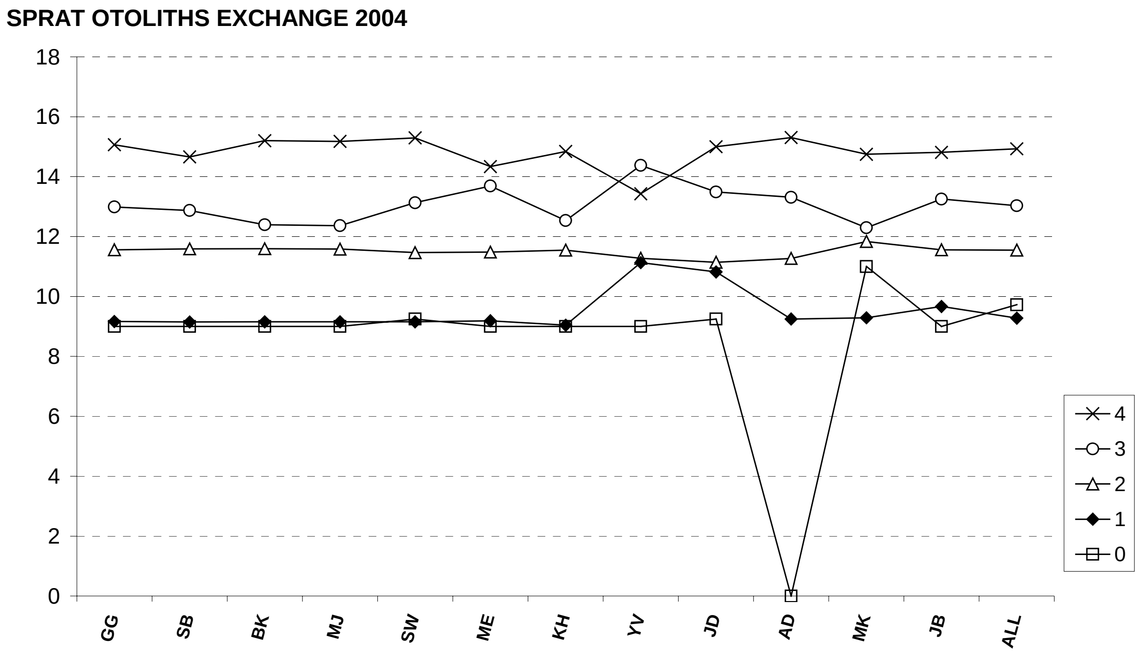


Figure 4 **The mean length at age as estimated by each age reader.**

ANNEX 3.

GUIDELINES FOR AGE DETERMINATION OF SPRAT

Preparation

- The otoliths (*Sagittae*) must be cleaned and dried before mounting
- Mount the otoliths pair-wise with the *sulcus acusticus* –side down.
- Sagittae of sprat are small and often difficult to handle with forceps. A moistened fine paintbrush is a useful tool for handling small otoliths.
- Only otoliths from sprat caught in the same sample, should be mounted on the same plate/slide
- The plates/slides to be labelled with catch dates, sample number and fish numbers. The numbering of fish should not be confusing regarding the arrangement of otoliths (fish numbers) on the plates.
- The otoliths should be mounted randomly according to fish size, to reduce the risk of bias in the age estimations

Observation

Before you start the age interpretation on the ring structures of the otoliths, use a low magnification to get an overview of the whole otolith, its nucleus, the growth zones and the edge structures. Special attention must be given to the edge. The growth on the edge should be recorded according to a code, given for translucent-opaque, or more detailed as narrow-wide, respectively.

Reading

a) Birthday defined as 1 January
b) The accepted best practice advises that the lowest level of illumination and the lowest magnification that permit clear observation of individual translucent and opaque zones should be used.
c) For age determination only translucent zones (winter rings) should be counted. Doubts about a false and real translucent zone, to be checked by the width of the microstructures in the border area, in front of and after the translucent zone.
d) The interpretation of the otolith is done primarily in the rostrum and postrostrum area. However, there should always be another area within the otolith were the counting can be repeated.
e) For sprats being caught during the first half of the year, the translucent zone seen at the edge or where no translucent zone has been laid down, the edge of the otolith is to be regarded as the translucent zone (winter ring). For fish being caught in the second half of the year, a translucent zone at the edge is not counted as a ring.
f) In older sprat (<4 yr) the onset of translucent zones will often be visible on the rostrum tip before being visible elsewhere on the otolith edge.

g) The readings should be made twice, either followed by the second after two- three days or by another reader.
h) The length of the fish should not be used as a criterion for decisions on ages.
i) The readability of the otolith to be noted by a code (easy, doubts, difficult, not readable)