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## Report of the Workshop on Age Reading on Baltic Sprat (WKARBS)

17-20 March 2008

Klaipeda, Lithuania



**ICES**

International Council for  
the Exploration of the Sea

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Conseil International pour  
l'Exploration de la Mer

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## 1 Introduction

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### 1.1 Terms of Reference

According to the Resolutions of ICES 2007 the Workshop on Age Reading of Baltic Sprat (WKARBS) was held in Klaipeda, Lithuania from 17 to 20 of March 2008 to:

- a) Discuss disagreements in age reading results from otolith samples read during the exchanges and determine the possible reasons for that. Check the accomplishment of recommendations made at the previous workshop. Estimate improvement in age reading concerning precision and bias by comparing exchange sets and the sets at the workshop;
- b) Update the manual for age reading (interpretation of rings and edges, period of opaque and translucent ring formation. Make recommendations for further cooperation between Baltic sprat age readers, otolith sample exchange, bilateral cooperation, workshop.

### 1.2 Participants

The meeting was attended by:

Wlodzimierz Grygiel	Poland
Marianne Johansson	Sweden
Olavi Kaljuste	Estonia
Georgs Kornilovs (Chairman)	Latvia
Mario Koth	Germany
Birgitta Krischansson	Sweden
Andrea Müller	Germany
Diana Tarvydiene	Lithuania
Tatiana Vasiljeva	Russia
Alla Vingovatova	Latvia

In the otolith sample exchange and age determination participated also Folke Halling from Finland and Stina Bjørk Stenersen Hansen from Denmark.

### 1.3 Previous sprat age determination workshops

The Baltic sprat age determination was only very occasionally a subject of scientific discussion on an international forum. In the end of 1980s, an informal, ad hoc meeting of sprat age readers from GDR, Poland and Sweden took place in Västervik (Sweden), on board of RV "Argos". In the very beginning of 1990s within the Six State Fisheries Agreement a research sub-group was established including representatives from GDR, Poland and Soviet Union, for a preparation of "Guide for use of Baltic sprat and herring otoliths in fisheries studies" (Aps *et al.* 1992). More wide, and for a first time fully international discussion of Baltic sprat age determination was undertaken during the ICES Workshop in Tallinn (Estonia) in 31.8–4.9.1992 (Anon. 1992). Later in 24–28.11.1997 "Ad hoc Workshop on Baltic Sprat Age Reading" took place and representatives of Estonia, Latvia, Lithuania, Poland and Russia met in Kaliningrad (Russia) for consultations of difficulties with the Baltic sprat age determination and the assessment of the Baltic coastal spring spawning herring

population biomass (Grygiel, 1998). More one consultative meeting concerning Baltic sprat age interpretation, with participants from Poland and Lithuania, took place in Gdynia in 2–16.12.2004, within the POLMARF/WP-3 project (Grygiel 2004).

Results from Baltic sprat ageing intercalibration exercises, available from the past, are as follows:

- during meeting in Tallinn in 1992, based on 588 age determinations, 80% of convergence in age interpretations was obtained, and maximum two age group difference between readers was observed (Anon. 1992),
- during meeting in Kaliningrad in 1997, based on 125 age determinations, the 46-100% range of convergence (depending on a sample origin) in age interpretations was obtained, and maximum three age group difference between readers was observed (Grygiel 1998),
- during meeting in Gdynia in 2004, based on 269 age determinations, 73-76% range of convergence (depending on a sample origin) in age interpretations was obtained, and in the repeated exercises with the same otolith samples 97-98% range of convergence in age determination was obtained (Grygiel 2004).

#### **1.4 Recent cooperation in age determination of Baltic sprat**

In 2004 exchange of sprat otolith samples between the age determination experts of Baltic sprat was initiated by Baltic Sea Regional Project (BSRP). As a result 8 sprat otolith samples were prepared and started their circle around the Baltic Sea. The Finnish and Polish samples were put on plastic plates and covered with Canada balsam. In all other samples loose otoliths were inserted in plastic bags or in plastic plates. It was decided that both sagitta otoliths should be available and that they should not be broken. 11 age readers from 9 countries participated in age determination of these sprat otolith samples. The age determination results were sent to Latvian Fish Resources Agency where they were analysed and when the sample was treated by all the experts the final results of the analysis were distributed between national institutes. The exchange was finished by autumn 2005.

In general the age determination results showed the existence of significant differences in age determination between the readers. Thus according to Wilcoxon signed rank test the disagreement between all individual readings of the readers was stated from 28.9% in the Danish sample till 88.9% in the Estonian sample (on average for all the samples in 65.2% of the cases). The percentage of identically determined age was on average from 36.2% in the Estonian sample till 72.3% in the first part of the Polish sample (on average 58.3% for all the samples).

Therefore it was decided to hold a Workshop in Danish Institute for Fisheries Research, Charlottenlund, Denmark from 24 to 27 January 2006 to:

- 1) Summarize the sprat otolith sample exchange and detect the reasons for different age interpretation;
- 2) Describe national sprat sampling and age determination programmes;
- 3) Organise a comparative age determination of sprat otoliths and evaluate results;
- 4) In the light of the result of the Workshop, identify new research and actions needed to improve the consistency of sprat age reading.

The travel expenses of experts from Estonia, Latvia, Lithuania, Poland and Russia were covered by the Baltic Sea Regional Project.

The meeting was attended by all 11 experts who participated in the exchange.

The age reading results of the otolith samples in the exchange were discussed at the Workshop. It was concluded that the main reason for differences in age determination was the counting of winter rings in the external part of otolith starting with the 3rd winter ring and for older fish the difference in the resulting ages was rather large. At the Workshop the age readers agreed that it would be important that the sprat age is determined similarly in all the national laboratories around the Baltic Sea. In this respect the magnification used could be of a major significance. At first the otoliths should be observed in a smaller magnification (40x) to get the general impression about the age of the sprat. For the determination of ages 1 and 2 this magnification would be sufficient. For older fishes (3+) the counting of winter zones on the edge of the otolith should be performed using higher magnification (100x).

The Workshop also revealed problems with the organisation of the Workshops and age determination of sprat at them. Since most of the readers used qualitative microscopes with high magnification any of the national laboratories had them in sufficient number to hold individual age determination for all the participants. This compelled very ineffective and time-consuming work in queue and definitely influenced the age reading results. One of the ways to accelerate the work is to prepare otolith samples on glass slides and to cover otoliths with nail polish.

The Workshop recommended continuing sprat otolith sample exchange. The samples had to be prepared on glass slides. It was recommended that the age readers do not use binoculars for age determination of sprat, but microscopes with higher magnification. It was also recommended that age readers should follow the development of the sprat stock and the annual feeding conditions and check the consistency of their age readings in respect to the appearance of poor or rich year-classes.

## **2 National protocols for age determination of sprat**

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### **2.1 Denmark**

#### **Sampling and storing**

Every month, sprat landings from trips from the small meshed fishery are sampled for length and age using the protocol defined by the EU directive 1639–2001 which is one sample per 2000 tonnes landed. One sample consists of 100–50 individuals which are length measured and for 100–50 of these the age is determined, depending on where the catch is taken. The sampling of sprat is non-size graded samples.

Both sagitta are taken from each individual, and collected otoliths are kept in trays with one otolith pair per hole from a couple of days to several months until the age determination. After age determination the otoliths are stored in small plastic bags. In the recent years the average number of sprat aged is approx. 2000 a year.

#### **Equipment and preparation of otoliths**

The age estimation is based on the observation of the number of translucent zones in the whole otolith with the sulcus facing down immersed in alcohol in a black tray using a binocular (Zeiss Jena Technival) and reflected light. The magnification varies from 1.6X10 to 2.5X10, the larger most frequently used for older individuals in order

to see the edge of the otolith. Age estimation of sprat caught in Division IIIa and the Baltic Sea is more difficult, since the growth is somewhat slower and the winter rings appear closer together.

## **2.2 Estonia**

### **Sampling and storing**

Sampling type is random sampling. Size of the samples usually is 100 specimens. The samples are collected on monthly basis, according to the fishing activity. The otoliths are stored in the paper otolith booklets. On average 7000 sprats are aged annually.

### **Equipment and age determination**

Microscope Biolam Lomo AY-12 is used for age determination of sprat. The magnification used is 56x and 80x. The otoliths are viewed in transparent light. The otoliths are put into alcohol during the age reading. For the age determination the following otolith structure criteria are used: winter rings are counted, which are clearly presented on the dorsal part of the otolith and on the anterostrum.

## **2.3 Finland**

### **Sampling and storing**

Length stratified sampling is used. The size of the samples is 10–100 specimens. 10 specimens per length group per Subdivision per quarter are collected. The average number of sprat aged is about 1600 per year. The otoliths are stored in pits on transparent plastic discs covered by Canada balsam.

### **Equipment and age determination**

Leitz preparation microscope is used for age determination of sprat. The total magnification is 64x - ocular 16x, objective 4x. The light is fibre optic. Otolith structure criteria used for the age determination: age rings seen from dorsal side with low movable fibre optic from dorsal side.

## **2.4 Germany**

### **Sampling and storing**

The sprat samples are delivered from two different sources: market samples (pelagic trawl) and survey samples (pelagic and bottom trawl).

For sprat it is tried to achieve quarterly 6 market samples per 15 kg in Sub-Division 22 and 24 respectively. The number of fish used for age-reading is fixed by sample to 5 individuals per length-class.



The following fishery independent German surveys are/were conducted every year by RV "Solea":

Survey	Area	Month	Comments
German Bottom Trawl Survey*	Sub-div. 22, 24	Nov/Dec	target species cod
German Bottom Trawl Survey*	Sub-div. 24	Jan/Feb	target species cod
German/Danish Hydro-acoustic Survey (Pelagic Trawl)	Sub-div. 22, 23, 24, partly Sub-div. 21	Sept/Oct	target species herring and sprat
German Hydro-acoustic Survey** (Pelagic Trawl)	Sub-div. 24, 25 partly 26, 27 and 28	May	target species sprat

\* Sampling stopped in 2000, since from 2001 onwards a new bottom trawl was introduced which is catching sprat to a low extent.

\*\* In former GDR times this survey was conducted every year. In 1999 this survey was repeated for the first time after the German reunification. Since 2001 this survey was conducted every year.

During hydro-acoustic survey time at least two hauls are carried out per ICES statistical rectangle. The numbers of fish per length-class used for age-reading are fixed in correspondence to the Baltic hydro-acoustic manual (ICES 2003).

#### Equipment and age determination

Otoliths are not burnt, not broken or embedded, but dried in air only. Microscope Olympus SZ 40/60 with 50–63 times magnification is used. Black ceramic disk in which the otoliths are laid in ordinary tap water.

For age determination hyaline zones (winter rings) are counted. For valid otoliths at least the first two annuli should be traceable throughout the whole otolith. The reading of the otolith is done primarily in the postrostrum; however, the rest of the otolith shall not be neglected.

For individuals being caught during the first half of the year the hyaline edge of the otolith is going to be regarded as annulus, not however in cases when the fish is caught in the second half of the year.

In the centre of the otolith is a very strongly light refracting spot, which is the nucleus. The nucleus is surrounded by a metamorphosis ring, which is not following the outer otolith contour. This marks the metamorphosis from larvae to the young fish. The sprat in the western Baltic Sea spawns between May–June.

The nucleus is surrounded by the central part of the otolith as a very marked hyaline zone. This zone follows the outer contour of the otolith. This zone is followed by an extended broad opaque zone, followed again by the next hyaline zone etc. For otoliths with more than 5–6 annuli these opaque zones become very thin layers on a hyaline background, which however all follow the outer contour of the otolith.

In general sprat otoliths are not easy to read and the annuli cannot be easily recognized. Misleading false rings, caused by hunger, diseases or spawning etc. are not very pronounced and are therefore not having a great effect on the reading process. By contrast problems are encountered when sprat stocks with different growth rate are mixing thus producing also substantial differences in width of the annuli. Mixing of fast growing sprat from the west with slower growing specimens from the east occurs in the Subdivision 24 (Arkona Basin) and Subdivision 25 (Bornholm Basin). The differences in growth rate take place due to the decreasing salinity and length of the feeding season from south-west to north-east and the subsequent decrease of annual growth. The otoliths of sprat from the east are

generally more difficult to read, as a result of the slower growth and the higher age at a given size.

## 2.5 Latvia

### Sampling and storing

From commercial trawl catches 4 sprat samples are taken every month. The samples are random samples consisting of 100 fishes for which length, weight, sex, maturity stage is recorded. From every fish also pair of sagitta otoliths is taken and stored in folded paper booklets. Sprat otoliths are also collected during May and October hydro-acoustic surveys where from every trawl app. 200 fishes are measured and grouped in 0.5 cm length classes. In each length class otoliths are taken from 10 fish for age determination. In each hydro-acoustic survey approximately 2000 sprats are aged.

### Equipment and age determination

Age of sprat is determined using Leica microscope in transmitted light mainly with 100x magnification, but for older fishes even greater. It is considered that magnification less than 100x does not allow to determine correctly age of fishes which are older than 3 years. Age determination of sprat requires distinction and counting of annual growth zones which consist of broader and lighter opaque summer zone and narrower and darker hyaline winter zone. The opaque zone is formed in summer during good feeding conditions while the hyaline zone is formed in late autumn and winter when the feeding intensity of sprat is on a low level. During the formation of annual growth zone in summer inside the opaque zone often narrow hyaline rings are formed. It is especially characteristic for the second annual growth zone which probably is connected with some worsening of feeding conditions. These hyaline rings are regarded as false rings and are not included in counting the age of the fish. Unlike true winter rings they are usually discontinuous. To make certain the distinction between false and true ring it is often necessary to measure the width of the first and second growth zones.

## 2.6 Lithuania

### Sampling and storing

Sampling type is random sampling. Size of the sample is 100 or more specimens. Monthly 10 specimens are taken per every length group, according to the fishing activity. Otoliths are stored in the otolith booklets and plastic bags

### Equipment and age determination

Microscope Biolam Lomo is used. The otoliths are observed with magnification 70 – 140x in transmitted light. The otoliths are put in to 70-90% ethanol during the age reading. In the age determination winter rings are counted, which are clearly presented on the dorsal part of the otolith and on the anterostrum. On average the age is determined from 2000–3500 otoliths per year.

## 2.7 Poland

### Sampling and storing

Sprat samples are collected directly from both, the Polish commercial fishing fleet and the research vessel “Baltica”. Month, ICES Subdivision and type of fishery (for

human consumption and industrial purposes) stratify samples. Sprat sorted out from catch is taken as a random sub-sample (2–3 kg) for total length and mass measuring by 0.5-cm size classes, and 10 specimens from each length classes are randomly selected for detailed biological analyses. During mentioned analyses one pair of otoliths (*sagitta*) are removed from each fish head after prior cutting of the upper bones of the cranium in direction to operculum. Otoliths are stored in black plastic plates with numbered cavities and embedded into the Canadian balsam. The applied Canadian balsam, well known immersion fixative in a microscopic preparation science, performs the task of increasing the sharpness of the otolith morphological structure and preventing otoliths from being damaged during storage or transportation. The use of plastic plates makes it easier to store and displace the research materials, and significantly increases the effectiveness of standard age reading as it eliminates additional manipulations of otoliths, necessary when envelopes are used.

#### **Equipment and age determination**

Otoliths are examined in reflected light using binocular microscope at the magnification of 50 times. Age of sprat from age group 1 and older is usually determined on the dorsal edge of the otoliths (not polished and not breached) where the growth seasonality can be recognized the most clearly (Aps *et al.* 1992). Age is determined from the outer (concave) side of otoliths on which the visibility of the nucleus and hyaline zones is highest. The internal side of the Baltic sprat otoliths is practically not used for standard ageing.

## **2.8 Russia**

#### **Sampling and storing**

Sprat samples are collected on monthly basis according to fishing activity. Samples are random or length stratified containing 150–200 fishes. The otoliths are stored in paper booklets.

#### **Equipment and age determination**

Microscopes Biolam Lomo AY12 and Laboval 4 are used for age determination of sprats. The otoliths are observed with magnification 60–100x in transmitted light. The otoliths are put in ethanol during the age reading. In the age determination winter rings are counted, which are clearly presented on the dorsal part of the otolith and on the anterostrum. On average the age is determined from 5000–7000 otoliths per year.

## **2.9 Sweden**

#### **Sampling and storing**

Random samples of sprat are taken from different fishing boats. We are supposed to take approximately 400 individuals per quarter and Subdivisions 22–24, 25–29 and 30. Each sample consists of 50 individuals. They are measured, weighted and aged. Both *sagitta* are taken from each individual, and the collected otoliths after drying are put on a glass slide and covered with nail polish. After age determination the otoliths are stored in a special box with room for 100 glass slides.

#### **Equipment and age determination**

The age estimation is based on the observation of the number of translucent zones in the whole otolith with the sulcus facing down. We are using a binocular (Leica MZ6)

with reflected light and a magnification of 4.0x10 and a microscope (Zeiss) with transmitted light and a magnification of 100x. We first read the otoliths in the binocular and then we read (not 2 years or younger) in the microscope. Approximately 4000 sprat are aged per year.

### 3 Review of Baltic sprat biology

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The Baltic sprat *Sprattus sprattus balticus* (Schneid.) is the subspecies of European sprat *Sprattus sprattus* (L.) which is adapted to the conditions of low salinity of the Baltic Sea. At present it is regarded and assessed as one stock although previously several stock units were distinguished and separate assessments were performed. Sprat is met almost in all the Baltic Sea except utmost northern and eastern parts of the gulfs.

The sprat stock has peculiar age structure with dominance of one or two age groups. This is connected with substantial differences between year-class strength that could be seen from the assessments of the sprat stock. A general tendency in the estimation of year class strength can be observed. The abundance of poor year-classes is gradually increasing with every next assessment while for the rich year classes it is gradually decreasing. This tendency could be connected with wrong age determination. Supposedly that the estimates of poor year-classes at age 2 could be the most precise because at that age even poor year classes are sufficiently abundant in the samples and the age determination causes few problems.

1980s could be regarded as period of poor reproduction conditions of sprat when the majority of year-classes were poor and respectively the stock size was on a low level. In 1990s the number of rich year-classes and also stock size considerably increased. The number of age groups in the stock is rather high and 17 years old fishes have been recorded. Older fishes more frequently are met in the northern Baltic while in the southern Baltic the fishes till age 8–9 could be met. In ICES assessment working plus group 8+ is used.

The size (length and weight) of sprat is decreasing in the direction from south to north that obviously is connected with longer feeding season in the southern Baltic. The growth of sprat after age of two years is very slow and it is density dependant. There are also differences of size between males and females. The latter are usually 5–10 mm larger than the males of the same age. The growth of sprat is seasonal and is connected with intensive feeding from late spring till autumn. However in spring mainly sprat of age group one is growing while for other age groups the feeding is more important to subsist the reproduction.

The main food items of sprat are different zooplankton and less nectobenthos species belonging to *Copepoda*, *Cladocera*, *Mysidaceae*, *Amphipoda*, *Chaetognata*. Usually intensive feeding takes place during daytime while at night sprat is mainly digesting the food.

The spawning period of sprat is rather prolonged and it could start as early as in February in the south-western part of the Baltic Sea and lasts till the beginning of August. Sprat is a serial spawner having pelagic eggs and it can spawn several batches per spawning season. The fecundity of the sprat is in the range of 5700–34300 eggs depending from the size and age as well from the condition of the fishes. Most of the fish mature at age 2, but some small part of 1 year old sprat from which the majority are males could also spawn. In the beginning of the spawning period sprat

spawns at bigger depth while in summer the spawning moves to higher warmer water layers.

The migrations of sprat are not very clear and there are different opinions about it but seemingly it has less pronounced migrations than herring. The young sprat till age 1 in the second half of the year stays closer to the coast while older sprat during feeding season migrate more to the offshore areas forming feeding shoals.

## 4 Results of the otolith sample exchange

### 4.1 General description of the samples

The description of the sprat otolith samples is given in the text table below.

Country	No. of otoliths	Subdivision	Date of collection
Denmark	51	25	March 2006
Estonia	50	32	I quarter 2006
Finland	50	29	May 2006
Germany	50	24	May 2005
Lithuania	50	26	November 2005
Poland	50	25	III quarter 2005
Sweden	41	26	October 2005

All otolith samples were on glass slides and covered with nail polish. Most of the samples were random samples but some were composed from several samples of a certain time period to provide even distribution of different age groups. It should be noted that Russian sample was broken in the mail and in the Latvian sample the nail polish lost transparency and the age determination was not possible.

### 4.2 Methods of analysis

The analysis of the age readings exchange results was performed using methods developed by Eltink *et al.* (2000). This analysis is based on a reference age when there are no validated ages available. As reference age the modal age was chosen. Each reader's results were compared with the modal age – the best approach available in the absence of knowledge of the true age. Inter-reader bias was examined using the Wilcoxon signed ranks test (Anon 1994). To ensure the anonymity of the results each reader has been assigned a number from 1 to 11, which are constant in all the tables and figures. Tables are put in Appendix 1 and figures in Appendix 2.

### 4.3 The general results of the exchange

The results of comparison of age determination revealed significant improvement in comparison with the previous exchange carried out in 2004–2005. This could be result of discussions at the Workshop 2006 as well as the implementation of the same standards in relation to microscopes and used magnification. According to Wilcoxon signed rank test the average disagreement between all individual readings of the readers had decreased from 65.2% till 29.9% (Table 15). The agreement between readers on average for all the samples had increased from 58.3% till 72.3%. However the coefficient of variation in 4 samples from 7 stayed high over 20% and on average for all the samples combined was 25.9%. The percentage agreement and coefficient of variation separately for all the samples of the exchange is shown in Tables 1–14 and the age bias plots for all the readers combined for all the samples are shown in Figure 1. The average determined age of the samples and their anomalies by reader is shown

in Tables 16–17 revealing that Readers 2, 7, 10 and 11 are giving on average smaller age while Readers 4 and 5 have the opposite tendency. The difference between extreme average determined ages by readers for all the samples was 0.62 (range 0.27–1.38) years and has significantly decreased comparing with previous exchange when in some samples it was above 2. It should be noted that the age readers participating in this exchange were the same as in 2004–2005 except reader 4 who had started the age determination of sprat only in 2006 and thus could be regarded as very inexperienced reader. In many cases the exclusion of reader 4 substantially increases the average agreement and decreases the coefficient of variation.

The agreement between readers decreased with the age of the sprat. For age groups 0 and 1 the agreement was around 90% and it was gradually decreasing till 50% for age groups 6–8. The age bias plots reveal that the closest age readings in relation to modal age have readers 3, 6 and 8. Readers 1 and 5 are slightly overestimating the age and readers 2, 4, 7, 9, 10 and 11 are underestimating the age in relation to modal age in age groups 4+.

#### **4.4 Discussion of the exchange samples at the Workshop**

The age reading results of the otolith samples in the exchange were discussed at the Workshop. The otoliths from the exchange samples were observed on the screen. There could be distinguished three possible problems in age determination of sprat:

- 1) distinguishment of the first winter zone;
- 2) distinguishment between false and true winter rings that is more often important in the second annual growth zone;
- 3) distinguishment of the winter rings in the otoliths of older fish (3+) which are situated in the external part of the otolith.

The observation of otoliths at the Workshop and following discussion revealed practically no discrepancies in determination of the first winter ring. Also distinguishment of the second winter ring was rather compatible and in most of the cases age readers agreed on the distinguishment of false rings which are usual in the second annual growth zone and precedes the second true winter ring. Definitely the main reason for differences in age determination was the counting of winter rings in the external part of otolith starting with the 3rd winter ring. In general, in cases when the determined age varied considerably between the readers, the agreed age was closer to higher assigned age than lower, thus revealing that there are more problems with underestimation of the age.

However, it was not always possible to agree on definite age. The main reason for that were two opinions founding the age determination of sprat. One opinion considered that otoliths belonging to one sample should have similar otolith structure and this should be especially taken into account in the relation to width of the last annual ring or when otoliths are collected in autumn the width of the opaque zone (summer zone) which is on the edge of otolith. Since the yearly feeding conditions for sprat could be rather different in the Baltic Sea it should result in the formation of wider or narrower summer (opaque) zones and this information could be used as biological marker. Some of the readers noticed such phenomenon e.g. indicating that annual ring of 2003 is rather narrow in comparison with the neighbouring rings. However, this was not visible or not taken into account for all the otoliths.

The opposite opinion considered that the growth of sprat and consequently the resulting annual ring could be very different. As a justification for that served the long spawning period of sprat which could result also in substantial differences in the length of feeding season that subsists growth of sprat. The second opinion thus only requires the counting of visible rings, while the first opinion the interpretation of visible rings ties with available information on growth conditions in a certain year.

It was pointed out that age readers are encouraged to record any peculiarities in otolith structure which have regular character and thus could be helpful in age determination.

During the discussion also The Manual for Age Determination of Baltic Sprat was elaborated that is presented in Chapter 6.

## **5 Comparative age determination of sprat otolith samples at the Workshop**

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### **5.1 Results and discussion**

Sample consisting from 50 sprat otoliths collected in the I quarter of 2007 was prepared in Sweden and sample consisting of 45 sprat otoliths collected in II and III quarters of 2007 was prepared in Germany. Taking into account the experience of the previous workshop that sprat age determination could be prevented by insufficient number of available microscopes two microscopes were brought from Latvia. This made the age determination less time consuming and more convenient for the participants. All otolith samples were on glass slides and covered with nail polish.

The analysis of the age readings exchange results was performed similarly as for exchange samples (Eltink *et al.* 2000). The percentage agreement and coefficient of variation in all samples of the exchange is shown in Tables 18–21 and the age bias plots for all the readers are shown in Figure 2.

The average percentage agreement slightly increased in comparison with the results of the exchange and was 76.1% and coefficient of variation decreased and was 17.1%. Readers 3 and 6 have percentage agreement above 90%, readers 1 and 8 above 80%, readers 4, 7 and 10 around 70 % and readers 2 and 11 around 60%. The coefficient of variation was below 10% for readers 1, 3, 6 and 8 and the rest readers had it above 20%. Similarly like in the exchange samples readers 3, 6 and 8 had the highest ranking both in percentage agreement and coefficient of variation. According to the results of age reading at the workshop reader 1 could be also added to this group. Reader 4 had much improved the age determination and the results were much closer than in the exchange. The discussions on the projected otolith images carried out at the workshop definitely had influenced the readers because underestimation of the age which was frequent in exchange samples had decreased and some readers revealed opposite tendency (readers 2 and 11).

### **5.2 General conclusions**

The otolith sample exchange and the Workshop definitely improved the agreement in determination of Baltic sprat age, although it would be desirable to reach higher percentage agreement between readers (target up to 80%) and to decrease the coefficient of variation in age determination (target below 10–12%). The main reason for not reaching the targets was disagreement in interpretation of sprat otolith growth pattern discussed in Chapter 4.4 and some readers have problems with the transition to work with microscopes and higher magnification. The latter could be

solved by bilateral cooperation and help from in this respect more experienced readers. Besides readers should pay attention to the peculiarities in formation of summer zones on sprat otoliths and if this has regular and distinctive pattern they must try to follow it also in the next years. If this is applicable for Baltic sprat it would certainly bring closer the interpretation of age.

## **6 Manual of age determination of Baltic sprat**

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### **6.1 Equipment**

The age of Baltic sprat is determined under microscopes with capacity of high magnification (at least 100x) in transmitted light. Sometimes with older sprat it is necessary to view the edge of the otolith with higher magnification. Loose otoliths on slides could be covered with alcohol for better focusing. When otolith samples are put on slides and covered with nail polish the use of alcohol is not allowable as it could damage the transparency of nail polish.

### **6.2 Otolith structure**

The otolith of Baltic sprat is comprised of annual rings (annulus) which consist of one opaque (summer) and one hyaline (winter) zone. The formation of opaque zone starts in May–June on otoliths of younger sprat while for older specimens it appears only in late summer or for very old fishes even in late autumn. The hyaline zone is formed during winter in the period of poor feeding. The hyaline ring is best seen when it is located between two opaque zones. In microscope the hyaline zones look dark while the opaque zones look lighter.

### **6.3 Age determination**

1<sup>st</sup> of January is considered as the birthday of sprat when fishes become one year older. The age determination of sprat implies counting of hyaline (winter) rings. However, in the first half of the year when the formation of the new opaque zone has not started yet, the hyaline ring on the edge which is usually not visible nonetheless is added to the total number of winter rings. In general it means that in conditions when hyaline ring on the edge is not visible the reader has to determine whether the last opaque zone belongs to the current year or the previous year. When the last opaque zone belongs to the annulus of the previous year it is considered that there is a hyaline zone on the edge and it is also counted (in the first half of the year).

### **6.4 Characteristic features of opaque and hyaline zones**

The hyaline (winter) rings look dark in microscope. The first hyaline ring looks different from the succeeding hyaline rings. It is rather broad and also its colour (brownish) differs from other winter rings. Usually it is situated after the dark central part followed by lighter ring and then comes the first hyaline ring. In some cases the lighter ring is missing and then the first hyaline ring is situated on the edge of dark central part and is not very discernible. The second and the following hyaline rings are usually dark and narrow lines contouring the otolith. The absence of the conspicuous winter ring indicates that this sprat is 0 year (in July–December) or 1 year (in the first half of the year prior to the formation of the new opaque zone) old. Usually the central part of otoliths (1<sup>st</sup> opaque zone) of such fishes is also rather light in comparison with otoliths of older fishes.

The new opaque zone forms in the period of active sprat feeding and growth. It appears earlier on otoliths of younger fishes and later on otoliths of older sprat. In



southwestern part of the Baltic Sea it happens earlier than in the northeastern part. On 1 year old sprat the 2<sup>nd</sup> opaque zone which follows the 1<sup>st</sup> opaque zone (central part of the otolith) and 1<sup>st</sup> winter ring could appear in the end of May–June. Later the opaque zone appears on otoliths of older fishes. The second opaque zone is rather broad and usually broader than the following opaque zones. The 3<sup>rd</sup> opaque zone usually is 1.5–2 times narrower than the 2<sup>nd</sup> opaque zone, but broader than the following opaque zones. In some cases the width of the 2<sup>nd</sup> and 3<sup>rd</sup> opaque zones is almost equal and if it has a regular character it could be used as a biological marker to distinguish a definite year-class. The width of the 4<sup>th</sup> and following annual zones becomes rather narrow and similar although sometimes also between these zones broader and narrower zones could be seen and used for assigning them to a definite year with bad or good feeding conditions and hence formation of broader or narrower opaque zones. In some cases the 3<sup>rd</sup> opaque zone is as narrow as the following opaque zones.

In general it would be advisable to record the cases of regular otolith opaque zone pattern as it could be helpful in the age determination of sprat, especially of older fishes when it is problematic to count hyaline rings on the edge or to decide whether the opaque zone on the edge belongs to the current or previous year.

## 6.5 False rings

False rings are hyaline rings which form inside of the opaque zone due to bad feeding or environmental conditions. Usually they are lighter than true winter rings and are visible only on some parts of the otoliths while the winter rings should be traceable all around the otolith. The general pattern of proportions between 2<sup>nd</sup>, 3<sup>rd</sup> and the following opaque zones could help the discrimination of false ring from true winter ring, however the knowledge of formation of opaque zones in bad and good growth years could be also taken into account if possible.

## 7 Recommendations

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- 1) The Workshop on Age Reading of Baltic Sprat recommends continuing the exchange of sprat otolith samples. Starting from May 2008 three otolith samples (50 otoliths in the sample) should be read annually by the experts of all national laboratories which participate in the age determination of sprat. For the first exchange the samples will be prepared by Latvia, Estonia and Lithuania. The samples will be prepared on slides and the otoliths covered with nail polish. It is desirable that all age groups till age 8 are equally represented in the sample and are not dominated by 1–2 age groups.
- 2) The sample should be treated in maximum 1.5 months and send to the next expert. The results should be sent to the coordinator of the exchange immediately after the treatment of the sample. The coordinator distributes the results of the age reading and analysis after the sample has been read by all the participants of the exchange.
- 3) The Workshop on Age Reading of Baltic Sprat recommends to hold the next Workshop latest in 2011 (the application for the workshop should be submitted in the beginning of 2010).
- 4) The Workshop on Age Reading of Baltic Sprat decided that the otolith sample exchange is coordinated by Olavi Kaljuste from Estonian Marine Institute.

- 5) Workshop reiterates the recommendations concerning the use of microscopes with high magnification for the age reading of Baltic sprat.

## 8 References

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**Annex 1: Tables**

**Table 1. Percentage of agreement with the modal age by reader in the Danish otolith sample, (%).**

MODAL age	Readers											ALL
	8	9	5	1	10	4	3	6	2	7	11	
0	-	-	-	-	-	-	-	-	-	-	-	-
1	100	100	100	100	100	27	100	100	100	100	100	93
2	33	67	67	100	67	0	100	100	100	100	67	73
3	78	83	28	78	83	11	89	89	72	72	72	69
4	80	60	40	20	20	20	60	60	60	60	60	49
5	0	0	100	100	0	0	100	100	100	100	0	55
6	0	100	100	0	0	0	0	0	0	100	0	27
7	-	-	-	-	-	-	-	-	-	-	-	-
8	100	0	100	0	0	100	100	100	0	0	0	45
Weighted mean	82.4	84.3	66.7	80.4	78.4	19.6	90.2	90.2	82.4	84.3	78.4	76.1
RANKING	5	3	10	7	8	11	1	1	5	3	8	

**Table 2. Coefficient of variation by age reader in the Danish otolith sample, (%).**

MODAL age	Readers											ALL Readers
	8	9	5	1	10	4	3	6	2	7	11	
0	-	-	-	-	-	-	-	-	-	-	-	-
1	0	0	0	0	0	36	0	0	0	0	0	24.4
2	22	25	43	0	25	16	0	0	0	0	25	26.3
3	13	14	18	18	14	24	18	18	14	20	20	19.7
4	12	26	20	24	30	33	34	34	18	18	15	29.9
Weighted mean	7.1	8.8	11.0	8.5	9.2	28.0	9.6	9.6	6.7	8.8	10.0	23.2
RANKING	2	5	10	3	6	11	7	7	1	4	9	

**Table 3. Percentage of agreement with the modal age by reader in the Estonian otolith sample, (%).**

MODAL age	Readers										ALL
	8	1	4	7	2	9	5	3	6		
1	100	100	100	100	100	100	100	100	100	100	100
2	100	100	100	100	100	80	100	100	100		94
3	100	90	80	60	70	80	70	90	90		77
4	100	25	83	50	67	83	92	92	92		76
5	100	17	33	100	83	50	83	33	33		59
6	100	0	100	100	100	100	100	0	0		60
7	67	50	67	50	50	17	100	67	67		60
8	100	100	0	0	0	0	100	100	100		60
9	100	0	100	0	0	0	100	100	100		60
10	100	0	100	100	0	0	0	100	100		60
11	-	-	-	-	-	-	-	-	-		-
12	-	-	-	-	-	-	-	-	-		-
13	100	0	100	0	0	0	100	0	0		30
Weighted mean	96.0	56.0	76.0	66.0	68.0	64.0	88.0	80.0	80.0		73.8
RANKING	1	10	5	7	6	8	2	3	3		

**Table 4. Coefficient of variation by age reader in the Estonian otolith sample, (%).**

MODAL age	Readers									ALL Readers
	8	1	4	7	2	9	5	3	6	
0	-	-	-	-	-	-	-	-	-	-
1	0	0	0	0	0	0	0	0	0	0.0
2	0	0	0	0	0	20	0	0	0	9.0
3	0	10	20	15	21	20	32	10	10	14.2
4	0	14	11	23	19	9	7	7	7	12.3
5	0	16	9	0	8	12	8	9	9	11.7
6	-	-	-	-	-	-	-	-	-	-
7	8	13	8	13	13	29	0	7	7	11.2
8	0	0	35	0	0	0	0	0	0	12.3
Weighted mean	0.9	8.8	10.1	10.1	11.3	13.3	9.1	5.7	5.7	10.8
RANKING	1	4	6	7	8	9	5	2	2	

**Table 5. Percentage of agreement with the modal age by reader in the Finnish otolith sample, (%).**

MODAL age	Readers									ALL Readers
	8	5	1	4	2	7	9	3	6	
1	100	100	100	100	100	100	100	100	100	100
2	-	-	-	-	-	-	-	-	-	-
3	100	0	100	33	0	67	33	100	100	59
4	85	85	35	65	65	69	73	77	77	70
5	67	83	83	67	50	50	33	100	100	70
6	67	67	33	33	67	67	0	100	100	59
7	100	67	67	67	0	0	0	100	100	56
8	50	100	0	50	0	0	50	50	50	39
9	0	100	100	0	0	0	0	100	100	44
10	100	100	100	50	0	50	0	50	0	50
Weighted mean	82.0	80.0	54.0	62.0	52.0	60.0	54.0	84.0	82.0	67.8
RANKING	2	4	7	5	9	6	7	1	2	

**Table 6. Coefficient of variation by age reader in the Finnish otolith sample, (%).**

MODAL age	Readers									ALL Readers
	8	5	1	4	2	7	9	3	6	
1	0	0	0	0	0	0	0	0	0	0.0
2	-	-	-	-	-	-	-	-	-	-
3	0	12	0	40	0	17	16	0	0	26.4
4	10	10	22	17	13	14	13	11	11	12.6
5	19	8	8	13	19	19	12	0	0	11.5
6	9	9	17	17	10	22	12	0	0	13.7
7	0	29	8	18	0	0	12	0	0	19.1
8	16	0	7	8	11	11	20	8	8	15.3
9	-	-	-	-	-	-	-	-	-	-
10	0	0	0	7	18	25	0	7	0	12.7
Weighted mean	8.5	9.0	14.3	15.5	10.8	13.4	11.6	6.4	6.1	12.8
RANKING	3	4	8	9	5	7	6	2	1	

**Table 7. Percentage of agreement with the modal age by reader in the German otolith sample, (%).**

MODAL age	Readers											ALL Readers
	8	7	2	9	5	10	4	1	3	6	11	
1	100	100	100	100	100	100	20	100	100	100	100	93
2	88	100	88	88	100	69	0	100	100	100	94	84
3	100	100	67	67	100	100	0	100	100	100	100	85
4	85	92	100	100	77	23	8	92	100	100	92	79
5	100	33	67	67	100	33	67	100	100	100	67	76
6	50	50	0	0	100	50	50	0	100	50	100	50
7	0	0	100	100	100	100	0	0	100	100	0	55
8	100	0	100	100	100	0	0	100	100	100	0	64
9	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	100	0	0	0	0	0	100	100	100	100	0	45
Weighted mean	88.0	86.0	86.0	86.0	92.0	60.0	14.0	92.0	100.0	98.0	88.0	80.9
RANKING	5	7	7	7	3	10	11	3	1	2	5	

**Table 8. Coefficient of variation by age reader in the German otolith sample, (%).**

MODAL age	Readers											ALL Readers
	8	7	2	9	5	10	4	1	3	6	11	
0	-	-	-	-	-	-	-	-	-	-	-	-
1	0	0	0	0	0	0	35	0	0	0	0	29.1
2	16	0	16	16	0	30	24	0	0	0	12	40.0
3	0	0	31	31	0	0	12	0	0	0	0	18.4
4	10	7	0	0	12	14	17	7	0	0	7	17.2
5	0	25	11	11	0	13	20	0	0	0	12	12.7
6	11	13	24	24	0	13	20	24	0	13	0	13.5
Weighted mean	8.2	3.9	8.6	8.6	3.0	14.4	21.9	2.8	0.0	0.5	6.5	26.2
RANKING	7	5	8	8	4	10	11	3	1	2	6	

**Table 9. Percentage of agreement with the modal age by reader in the Lithuanian otolith sample, (%).**

MODAL age	Readers										ALL Readers
	8	10	9	2	7	5	3	6	1		
0	100	0	0	100	0	0	100	100	100	56	
1	83	100	17	100	100	83	100	100	100	87	
2	100	67	33	92	92	67	93	93	79	78	
3	100	78	56	75	75	78	89	78	78	78	
4	50	50	33	33	100	75	100	100	75	69	
5	100	25	67	33	50	25	75	75	50	56	
6	100	0	75	0	0	50	75	100	50	50	
7	-	-	-	-	-	-	-	-	-	-	
8	80	40	25	0	0	60	60	80	40	44	
9	100	0	0	0	0	0	100	100	0	33	
10	0	0	0	0	0	100	100	100	100	44	
Weighted mean	89.8	56.0	38.3	60.5	62.2	64.0	87.5	89.8	71.4	69.1	
RANKING	1	8	9	7	6	5	3	1	4		

**Table 10. Coefficient of variation by age reader in the Lithuanian otolith sample, (%).**

MODAL age	Readers									ALL Readers	
	8	10	9	2	7	5	3	6	1		
0	-	-	-	-	-	-	-	-	-	-	-
1	35	0	22	0	0	35	0	0	0	31.0	
2	0	42	24	14	14	31	14	14	23	22.8	
3	0	14	20	18	14	14	12	17	21	12.0	
4	30	16	12	17	0	12	0	0	22	14.8	
5	0	20	20	13	16	30	10	10	10	15.9	
6	0	12	8	12	0	9	8	0	14	16.9	
7	-	-	-	-	-	-	-	-	-	-	
8	11	20	14	9	17	12	7	6	10	15.8	
Weighted mean	7.8	20.9	18.4	11.2	9.6	21.2	8.4	8.4	15.2	21.0	
RANKING	1	8	7	5	4	9	3	2	6		

**Table 11. Percentage of agreement with the modal age by reader in the Polish otolith sample, (%).**

MODAL age	Readers											ALL Readers
	8	11	7	2	9	3	6	5	1	4	10	
0	100	100	100	100	67	100	100	0	100	100	100	88
1	-	-	-	-	-	-	-	-	-	-	-	-
2	71	75	83	83	96	88	88	8	21	0	63	61
3	75	63	75	88	13	50	38	25	63	25	75	53
4	50	67	83	67	67	50	50	50	33	17	33	52
5	100	50	50	0	50	100	100	50	0	0	50	50
6	100	40	20	0	0	100	100	100	40	0	60	51
7	-	-	-	-	-	-	-	-	-	-	-	-
8	50	0	0	0	0	100	100	50	50	50	50	41
Weighted mean	74.0	66.0	72.0	68.0	62.0	80.0	78.0	28.0	36.0	14.0	62.0	58.2
RANKING	3	6	4	5	7	1	2	10	9	11	7	

**Table 12. Coefficient of variation by age reader in the Polish otolith sample, (%).**

MODAL age	Readers											ALL Readers
	8	11	7	2	9	3	6	5	1	4	10	
0	0	0	0	0	173	0	0	0	0	0	0	300.1
1	-	-	-	-	-	-	-	-	-	-	-	-
2	31	30	23	23	10	18	18	22	17	23	24	39.2
3	17	21	18	12	17	21	22	12	15	34	29	30.4
4	27	16	10	14	14	18	27	24	19	25	22	23.8
5	0	16	16	0	35	0	0	13	0	20	16	20.1
6	0	10	9	12	28	0	0	0	8	10	45	21.8
7	-	-	-	-	-	-	-	-	-	-	-	-
8	16	24	11	24	40	0	0	22	8	8	16	19.0
Weighted mean	21.7	22.2	17.0	16.9	25.4	14.2	15.4	17.0	14.0	21.6	24.6	48.3
RANKING	8	9	6	4	11	2	3	5	1	7	10	

**Table 13. Percentage of agreement with the modal age by reader in the Swedish otolith sample, (%).**

MODAL age	Readers								ALL Readers
	8	3	6	5	1	4	7	2	
0	100	100	100	100	100	100	100	100	100
1	100	100	100	0	100	50	50	50	69
2	100	100	100	94	88	31	94	94	88
3	100	100	100	86	57	57	86	86	84
4	75	75	75	50	50	25	100	100	69
5	100	100	100	100	50	100	0	0	69
6	100	100	100	50	50	50	50	50	69
7	100	100	100	50	50	0	0	0	50
8	50	50	50	100	0	50	50	0	44
Weighted mean	95.1	95.1	95.1	80.5	70.7	46.3	78.0	75.6	79.6
RANKING	1	1	1	4	7	8	5	6	

**Table 14. Coefficient of variation by age reader in the Swedish otolith sample, (%).**

MODAL age	Readers								ALL Readers
	8	3	6	5	1	4	7	2	
0	0	0	0	0	0	0	0	0	0.0
1	0	0	0	0	0	47	47	71	38.6
2	0	0	0	13	18	27	12	12	20.3
3	0	0	0	12	21	16	13	13	12.1
4	22	12	12	13	13	16	0	0	10.4
5	0	0	0	0	13	0	0	0	11.4
6	0	0	0	11	13	11	13	13	9.3
7	0	0	0	9	9	0	0	0	10.0
8	16	8	8	0	7	20	9	0	12.2
Weighted mean	2.9	1.6	1.6	9.3	14.0	18.7	10.4	11.1	15.0
RANKING	3	1	1	4	7	8	5	6	

**Table 15. Results of Wilcoxon inter-reader bias test in the exchange samples, (%).**

Country of the sample	Results of wilcoxon test		
	disagreement	possible disagreement	agreement
Denmark	34.5	5.5	60.0
Estonia	6.7	24.4	68.9
Finland	16.7	16.7	66.6
Germany	38.2	20.0	41.8
Lithuania	50.0	19.4	30.6
Poland	49.1	7.3	43.6
Sweden	14.3	21.4	64.3
Average	29.9	16.4	53.7

**Table 16. Average determined age in the exchange samples by reader.**

Sample	Readers											Average age
	1	2	3	4	5	6	7	8	9	10	11	
Denmark	2.35	2.41	2.35	3.51	2.76	2.35	2.41	2.47	2.47	2.20	2.42	2.53
Estonia	4.58	4.28	4.50	4.48	4.50	4.52	4.22	4.34	4.34			4.42
Finland	4.52	4.48	4.74	4.82	4.70	4.76	4.38	4.76	4.76			4.66
Germany	3.08	2.88	3.12	4.92	3.04	3.10	2.92	3.20	3.20	2.70	3.02	3.22
Lithuania	3.90	3.05	3.63		3.98	3.65	3.56	3.71	3.71	3.38		3.62
Poland	3.66	2.82	2.92	5.30	4.20	2.88	3.00	3.08	3.08	3.06	3.06	3.37
Sweden	3.02	2.88	3.05	3.51	3.15	3.05	2.88	3.10	3.10			3.08

**Table 17. Anomalies of the average determined age from the mean age determined by all readers in the exchange samples.**

Sample	Readers										
	1	2	3	4	5	6	7	8	9	10	11
Denmark	-0.18	-0.12	-0.18	0.98	0.24	-0.18	-0.12	-0.06	-0.06	-0.33	-0.11
Estonia	0.16	-0.14	0.08	0.06	0.08	0.10	-0.20	-0.08	-0.08		
Finland	-0.14	-0.18	0.08	0.16	0.04	0.10	-0.28	0.10	0.10		
Germany	-0.14	-0.34	-0.10	1.70	-0.18	-0.12	-0.30	-0.02	-0.02	-0.52	-0.20
Lithuania	0.28	-0.57	0.01		0.36	0.03	-0.06	0.09	0.09	-0.24	
Poland	0.29	-0.55	-0.45	1.93	0.83	-0.49	-0.37	-0.29	-0.29	-0.31	-0.31
Sweden	-0.06	-0.20	-0.03	0.43	0.07	-0.03	-0.20	0.02	0.02		

**Table 18. Percentage of agreement with the modal age by reader in the German otolith sample determined at the Workshop, (%).**

MODAL Age	Readers									ALL readers
	3	6	7	11	2	1	4	8	10	
0	100	100	100	100	100	100	100	100	100	100
1	100	100	83	67	83	100	100	100	67	89
2	100	100	71	29	71	57	71	71	71	71
3	91	82	55	45	55	82	64	100	73	72
4	100	78	78	56	67	78	67	89	78	77
5	67	0	33	67	33	33	100	100	33	52
6	100	100	100	0	100	100	100	0	100	78
7	50	100	100	50	100	100	0	50	50	67
8	100	100	50	100	50	50	0	100	0	61
9	-	-	-	-	-	-	-	-	-	-
10	100	100	0	0	0	0	0	100	100	44
Weighted mean	93.3	84.4	68.9	53.3	66.7	75.6	68.9	88.9	68.9	74.3
RANKING	1	3	5	9	8	4	5	2	5	



**Table 19. Coefficient of variation by age reader in the German otolith sample determined at the Workshop, (%).**

MODAL Age	Readers									ALL Readers
	3	6	7	11	2	1	4	8	10	
0	0	0	0	0	0	0	0	0	0	0.0
1	0	0	35	39	35	0	0	0	39	26.0
2	0	0	21	27	21	34	38	21	44	27.6
3	10	13	19	27	22	14	24	0	19	19.8
4	0	13	23	28	16	12	12	16	13	14.2
5	12	0	20	11	20	13	0	0	13	13.8
6	-	-	-	-	-	-	-	-	-	-
7	11	0	0	11	0	0	33	9	11	9.1
8	0	0	9	0	9	9	28	0	16	20.3
Weighted mean	3.7	5.6	19.0	22.9	18.4	12.4	16.8	6.9	21.1	18.1
RANKING	1	2	7	9	6	4	5	3	8	

**Table 20. Percentage of agreement with the modal age by reader in the Swedish otolith sample determined at the Workshop (%).**

MODAL Age	Readers									ALL Readers
	3	6	7	11	2	1	4	8	10	
1	100	100	100	95	90	100	95	100	100	98
2	100	100	80	0	0	100	33	100	50	62
3	100	100	60	20	0	80	40	100	80	64
4	100	92	50	42	42	92	33	75	58	65
5	50	100	0	50	0	50	50	50	0	39
6	67	67	67	33	67	67	100	67	100	70
7	100	100	0	100	100	100	100	0	0	67
Weighted mean	96.0	96.0	73.5	58.0	54.0	92.0	66.0	88.0	76.0	77.7
RANKING	1	1	6	8	9	3	7	4	5	

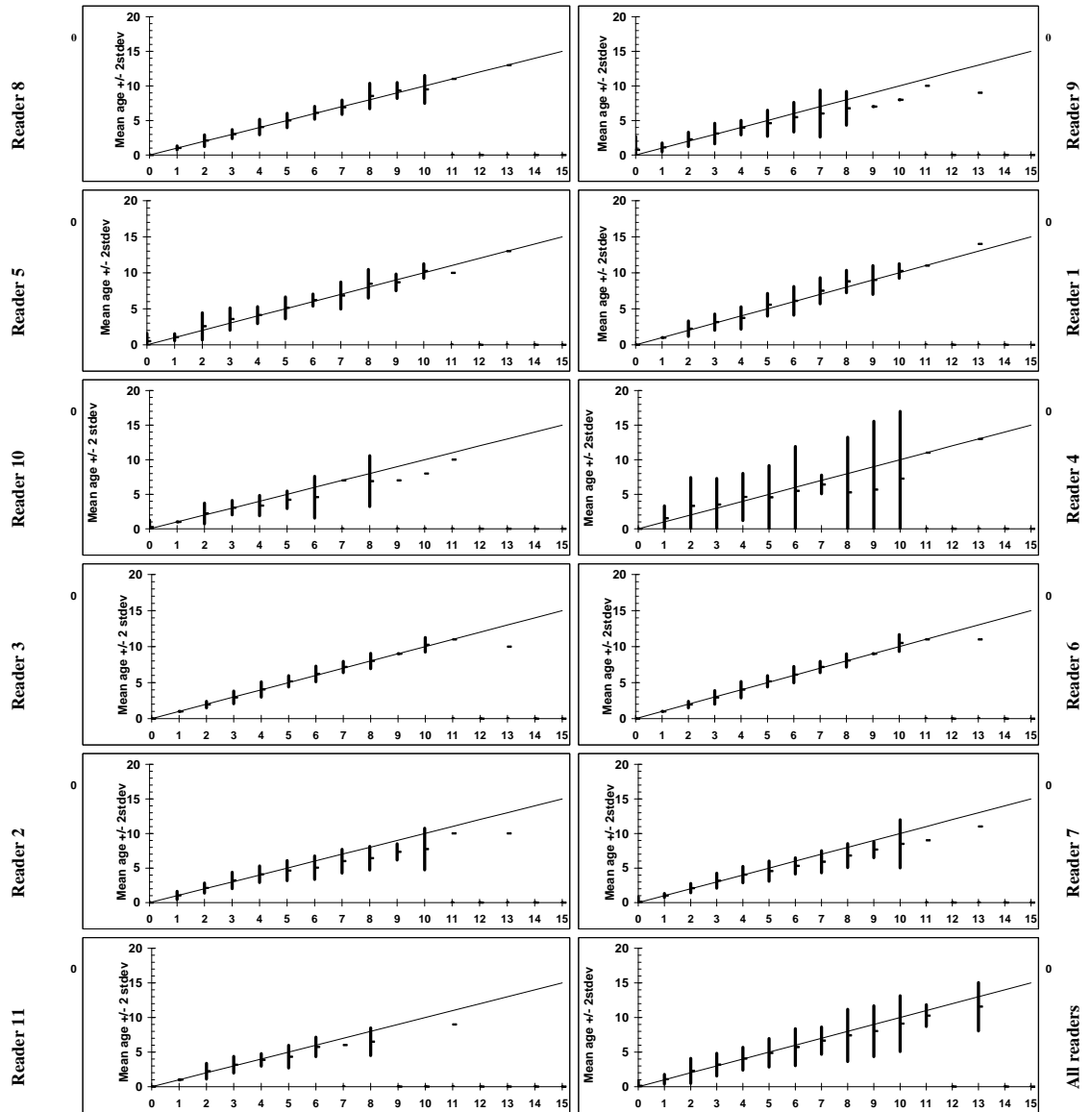
**Table 21. Coefficient of variation by age reader in the Swedish otolith sample determined at the Workshop (%).**

MODAL Age	Readers									ALL Readers
	3	6	7	11	2	1	4	8	10	
1	0	0	0	40	42	0	21	0	0	5.0
2	0	0	52	18	10	0	37	0	55	49.8
3	0	0	16	33	14	14	31	0	14	27.2
4	0	7	25	18	27	7	22	28	17	16.4
5	16	0	11	13	11	13	13	33	11	17.6
6	10	10	10	17	9	10	0	9	0	9.0
Weighted mean	1.2	2.3	14.0	28.0	27.6	4.2	22.0	8.7	12.6	16.2
RANKING	1	2	6	9	8	3	7	4	5	

## Annex 2: Figures

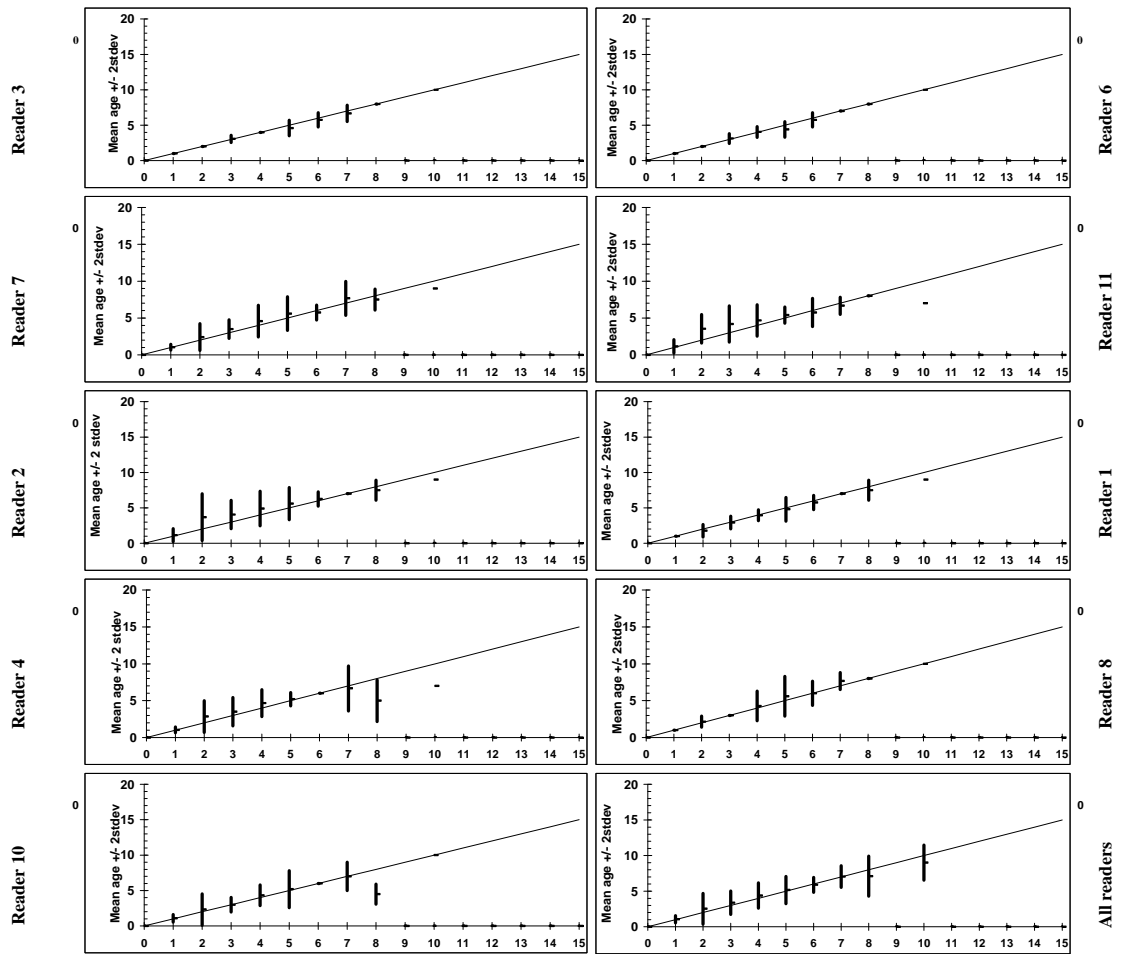
### Baltic Sprat combined samples in exchange 2006

**Figure 1** In the age bias plots below the mean age recorded  $\pm 2$ stdev 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.



**Baltic Baltic Sprat 2nd Swedish sample in exchange 2006**

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



### Annex 3: List of Participants

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