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REPORT OF THE WORKSHOP ON AGE READING OF FLOUNDER (WKARFLO)

20–23 MARCH 2007

ÖREGRUND, SWEDEN

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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Contents

Executive summary

Knowledge of the biology and stock structure of the Baltic flounder were reviewed and discussed. Studies suggest that there are two different ecological and genetic types of flounder, a northern type with demersal eggs and a southern type with pelagic eggs.

Participants presented national sampling, processing and age determination methods. Regular sampling of flounder is done in Sub-divisions 23–29 and 32. Most institutes determine ages from whole otoliths, but the sectioning and staining method is preferred by a few countries.

Results from an exchange experiment were presented. Altogether six samples of whole otoliths and four samples of sectioned and stained otoliths had been read by 4–10 persons. The overall agreement for whole otoliths were 62% (CV=20%) while for sectioned and stained otoliths agreement was lower, 53 % (CV=22%).

Age determination by reading whole, burnt or sectioned otoliths was evaluated in a WS experiment. From each sampled fish one otolith was left whole and the other one, either broken and burnt or sectioned. Participants were asked to state the number of winter rings and to mark the rings on a photo of the otoliths. The sectioned otolith technique had the highest percentage agreement (51%) and lowest CV (16%), while the burnt and whole otoliths had a lower level of agreement (40%) and higher CV (20 and 22% respectively). The markings on photos revealed large disagreements among readers where the actual rings were located.

After extensive discussions, a re-reading of two sectioned samples from the exchange program was done. The percentage agreement was significantly higher in the re-reading compared to the original readings in both sets of otoliths (70% compared to 59% and 62% compared to 48%). Results indicate a higher consistency among readers than obtained prior to the workshop.

A first draft of an international manual for age determination of Baltic flounder was discussed during the meeting. It was agreed that the objective of the manual is to provide quality assurance among and within national laboratories.

It was recommended that sectioning and staining of otoliths should be used for the age determination of Baltic flounder. The second-best method is the broken and burnt method.

Training of age determination by sectioned otoliths will be achieved by an intercessional exchange program using sectioned otolith samples from Germany and Sweden.

Considering the fresh insights into the age determination of flounder a 2nd workshop is recommended to take place in 2008. The terms of reference should include:

- a) evaluation of the 2007 exchange experiment
- b) experiment on broken and burnt otoliths
- c) experiment on influence of length information on age determination
- d) updating of an international manual
- e) measures to update national reference collections
- f) protocol for updating historical data

1 Terms of Reference

2006/2/ACFM35 A Workshop on Age Reading of Flounder [WKARFLO] (Co-chairs Johan Modin, Sweden and Ann-Britt Florin, Sweden) will be established and take place in Öregrund, Sweden 20–23 March 2007 to:

- a) Review the sample processing techniques of the different age reading laboratories and try to standardise the processing techniques of calcified structures;
- b) Agreement on age determination criteria (e.g. date of birth 1st of January, one annual growth zone consists of one opaque and one translucent zone, interpretation of the first ring);
- c) Discuss disagreements in age reading results from the sets of the calcified structures read during the exchange and at the workshop and try to agree on the age reading method;
- d) Determine at the end of the workshop the precision in age reading and the relative bias (if possible the absolute bias);
- e) Prepare a manual for age reading (date of birth, interpretation of rings and edges, period of opaque and translucent ring formation);
- f) Make recommendations on how to improve the age reading quality and how the age reading techniques can be validated.

WKARFLO will report to ACFM, RMC and PGCCDBS by end of March 2007

2 Agenda and participation

Altogether 19 national representatives from nine countries participated in the meeting. The list of participants is presented in Annex 1. The adopted agenda is presented in Annex 2.

3 Biology and stock units of Baltic flounder

The European flounder (*Platichthys flesus* L.) is distributed in the shelf areas of the North East Atlantic including the Baltic Sea. Despite its marine origin it tolerates very low salinity and is common in most of the Baltic, including the Gulf of Finland, the Gulf of Riga and the Bothnian Sea (review in Florin 2005).

Flounder spawns in spring. The onset of spawning is influenced by rising sea temperatures and consequently the spawning period differs between different areas in the Baltic. In the Kattegat spawning starts in February-April, while in the Gotland basin spawning occurs in April-May, and in the Åland Sea spawning takes place in May-June (Molander, 1964; Curry-Lindahl, 1985).

In the Baltic Sea eggs hatch in five to six days at 10°C and in ten days at 5°C. On hatching the larvae are about three mm long and live a pelagic life until 7–10 mm long (Bagge, 1981, Sager and Berner, 1988). The settling of larvae occurs in late summer in shallow coastal areas. The settling of larvae coincides with metamorphosis. Like all flatfishes, pelagic larvae of flounder are symmetric, but after the metamorphosis the larvae turns into a flattened, asymmetric shape adapted to its demersal lifestyle. The time of the metamorphosis is dependent on environmental conditions, typically temperature. The size at metamorphosis in the Baltic is believed to be below ten mm (Bagge, 1981, Grauman, 1981, Sager and Berner, 1988). Laboratory experiments with North Sea flounder has shown that metamorphosis occurs between 60 and 80 days and at a size of 8–10mm (Hutchinson and Hawkins, 2004). Results indicate that the size at metamorphosis is less variable than the time from hatching to metamorphosis.

At the end of their first year the flounder off Bornholm and to the east thereof is about 4–5 cm long, but somewhat larger towards the western areas (Bagge, 1981, Antoszek and Krzykawski, 2005). The mean lengths and weights show no apparent change from September to March (mean length for these months are 45.5 mm, mean weight 1.15 g), and increase again in late spring (Weatherley, 1989, Gårdmark *et al*., 2007). The greatest growth rate in length is achieved during the second year. Thereafter growth rates gradually decline with age (Zemskaya, 1960, Antoszek and Krzykawski, 2005). The growth of females is faster than that of males. Sexual maturity is reached at an age of 2–3 years and at a size of 15–20 cm (Molander, 1955, ICES 2005, Muus *et al*., 1999). Males mature before females.

During winter the flounder moves to deeper water but the juveniles return to the shallow water in the early summer next year. Juvenile flounders are generalists, they feed on a wide spectrum of prey, and main items are copepods and amphipods. There appears to be no food overlaps with juvenile turbot, which often co-occur with juvenile flounder in coastal nursery grounds (Ustups *et al*., 2007). Adult flounders are principal consumer of the Baltic Sea molluscs.

Most flounders are rightsided, i.e. they have both eyes on the right side of the head, however about a third of the flounders are left-sided. The proportion of leftsided flounders differs in different part of the distribution area but the reason for this is unknown. The asymmetric body shape is mirrored in the sagittae otoliths: the upper otoliths are asymmetrical with the nucleus closer to the rostrum and the lower otolith is symmetrical with a centrally positioned nucleus.

There are two ecological types of flounder in the Baltic: one southern and one northern. In the southern Baltic, the flounder migrates between coastal feeding areas and spawning in the deep basins and have large, pelagic eggs adapted to floating despite the low salinity (Bagge, 1981, Nissling *et al*., 2002). Salinity determines buoyancy of eggs and the pelagic eggs require a minimum salinity of 10% in order to float. Furthermore the success of the spawning also depends on the oxygen content. Oxygen contents below 1 g/m^3 are critical for egg survival (Bagge, 1981). Oxygen content determines distribution of spawning flounder and lowest bound of survival of eggs (Grauman, 1981). Hence the two most important factors which influence flounder recruitment are salinity and oxygen content during spawning.

The other ecological type occurs in the northern Baltic, where flounders are more stationary and spawn in shallow bank or coastal areas. The eggs of these flounders are smaller, more thick shelled and demersal. The minimal required salinity is lower, only 6–7 psu (Solemdal, 1967).

A recent study has shown that there are genetic differences between flounders from the northern and southern part of the Baltic, corresponding to the outlined ecological types (Florin & Höglund, unpublished).

An attempt to divide flounders into meaningful assessment stock units, based on differences in genetic, ecology and migration patterns, and using the ICES Sub-division system was done at the WKAFAB meeting 2006 (Gårdmark *et al*., 2007). This resulted in the following suggested stocks: one in Sub-division 24 and 25, one in Sub-division 27 and one in Sub-division 29–32. The flounder stock composition in Sub-division 26 and 28 is more complicated since both harbour both types of flounders. Sub-division 26 is believed to host two stocks: one in the southwestern, and one in the northeastern part. In Sub-division 28 there are probably three stocks: one on each side of the Gotland deep, and one in the deeper area (Gårdmark *et al*,. 2007).

4 Review of national sampling, processing and age determination (ToR a)

Routine age determination of flounder varies between national laboratories. Table 4.1 presents an overview of the amount of age determinations and participant reader experience by countries.

Table 4.1 Overview of the amount of age determinations and participant reader experience by countries

Country	Sampling of flounder by Baltic Sea Sub-divisions	Annual number of otholiths sampled and aged	Preparation method used	Number of readers in 2006	Readers experience in years for flounder (other flatfishes)
Denmark	24, 25	No routine samples	No age determinations	None	
England	(Sub-area IV)	No routine samples	Breaking and burning	2	0(41); 0(15)
Estonia	28, 29, 32	1300	Whole otoliths	2	2;
Latvia	26,28	1500	Whole otoliths	2	1(12); 1(3)
Lithuania	26		Whole otoliths		
Germany	24	3500-4500	Whole otoliths	2	20(20); 0(0)
Polen	24,25, 26	About 1300	Whole otoliths Sectioned otoliths	2	32(32) retired; 1(2)
Russia	26	500-800	Breaking and burning Whole otoliths	2	0.5; ?
Sweden	23, 25, 27	1000	Sectioned & stained	2	2(20); 1(1)

4.1 Denmark

4.1.1 Sampling of flounder

Flounder are sampled during the Baltic International Trawl Survey (BITS) in ICES Sub-divisions 24 and 25 in quarter 1 and 4. Only length frequencies and total weights are recorded. There are no specific Danish research surveys for flounder in the Baltic Sea. Otolith samples were occasionally collected at the BITS in the 1980s.

Flounder caught in the commercial fisheries are sampled in the Danish discard project. Only length frequencies and total weights are estimated. No otoliths are collected.

Danish age readers have no experience in age determination of flounder.

4.2 England

4.2.1 Sampling of flounder for age determination

At the present time flounder are only sampled on one research cruise each year. This takes place in the southern North Sea and Eastern English Channel during July and August. Samples have been taken for a number of years but routine ageing has not been carried out. Ageing has been investigated with some of these samples and the methods of breaking and burning or sectioning and staining have been found to be the most accurate methods for fish from these areas.

4.2.2 Removal of otoliths

At sea the otoliths are removed by cutting the head open using a vertical cut following the lateral line through the head of the fish. If samples were collected on markets then they would probably be taken through the gills so as not to cause too much visible damage to the fish. At sea the membranes covering the otoliths are removed and the otoliths are stored in plastic trays. When taken on markets the otoliths are stored in small paper packets.

4.2.3 Preparation of otoliths

It is considered that whole otoliths are not a particularly accurate method of age determination for flounder, particularly in older fish. As with most other flatfish species, accurate ageing is only obtained by the methods of breaking and burning or sectioning and staining. Both these methods utilise a protein band that is formed every year at the junction between the end of each translucent zone and the beginning of the opaque zone (for simplicity referred to herein as the annual protein band). When burned or stained this protein band shows very clearly as a thin line giving a much more precise interpretation of the age. If samples of flounder were routinely aged then the sectioning and staining method would be the preferred method due to the convenience of checking results and training new staff. Broken and burned otoliths do not always keep very well for future reading. Both methods are summarised below.

Breaking and burning method

The otolith is broken transversally across the nucleus and the broken surface is gently burned in a small flame from a spirit lamp. The annual protein bands are thus burned producing a thin brownish-black line at the end of each translucent zone (Christensen, 1964). The burned section is then mounted in a piece of plasticene and the broken surface viewed under a microscope using reflected light. A clear thin oil or water is used to clear the surface to aid the identification of annual rings. Water is recommended as the result is similar and, if the otolith needs more burning this can be achieved whereas once oil has been brushed onto the surface, further burning is not possible.

Sectioning and staining method

First of all, the otoliths are carefully positioned in rows in specially prepared aluminium moulds using the nucleus to accurately align each otolith. A video camera and television monitor is used during this to increase the accuracy of the mounting process. There are five or six rows in each mould and approximately 10–15 otoliths are positioned in a row, depending on the size of each otolith. Once the mould is completed, it is filled with black polyester resin. When the resin has set the polyester block is removed from the mould and a thin section, approximately 0.5 mm thick, is cut transversally through the nucleus from each row of otoliths using a diamond saw. The resulting sections are stained for 30 minutes using the following solution

100 ml Neutral red solution from SIGMA-ALDRICH
1 gram sodium chloride
0.5 ml glacial acetic acid

The sections are rinsed in tap water and placed in a fumigation cupboard to dry. During the staining process a mild decalcification takes place on the surface of the otolith and the annual protein bands are stained dark pink. The stained sections are then viewed under a microscope using mainly reflected light, but transmitted light is also used for some otoliths or a combination of the two. Water can be used as a clearing agent but sections can usually be aged dry without this.

4.2.4 Interpretation of otolith annuli

The otolith preparation methods described produce a similar result on the surface of the section, either burning or staining the annual protein band that is deposited on the outside of each translucent zone. Splits do not usually burn or stain in quite the same way as the annual protein bands and the sections are aged by counting the thin dark rings produced by the burning or staining process. The otoliths are aged using a magnification of approx. x 20–60 and all fish are aged to a January 1st birthday. During the first few months of each year the last protein band will not be visible on the edge and an allowance is made for this when ageing.

4.3 Estonia

4.3.1 Sampling of flounder for age determination

Fisheries independent data on flounder in the Baltic Sea is collected during the coastal fish gillnet survey by the staff from Estonian Marine Institute. Flounder is also collected from the commercial fisheries (mostly gillnetting) (Table 4.2).

Besides otoliths the following information is recorded: location, date, gear characteristics (mesh size), total length (mm), weight (g) and sex of the fish.

The Estonian Marine Institute has a database that contains almost ten years of flounder data. Flounder otoliths have been collected since 1997 during annual gillnet sampling of coastal fish (mesh sizes 17–60 mm). Altogether eight areas are sampled annually. These areas extend through ICES Sub-divisions 28, 29 and 32.

Table 4.2. The number of flounder samples to be collected by ICES Sub-division, gear type and year quarter (one sample corresponds to 50 individuals):

Gear type	Year quarter	ICES Sub-division			Sampling per gear and year quarter
		28	29	32	
Gillnet	2	0	1	1	2
	3	1	1	2	4
	4	1	1	3	5
	Total	**2**	**3**	**6**	**11**
Trap net	2	1	1	2	4
	3	1	1	2	4
	4	1	1	3	5
	Total	**3**	**3**	**7**	**13**
Seine	3	0	1	0	1
	4	0	1	0	1
	Total	**0**	**2**	**0**	**2**
	Grand total	**5**	**6**	**13**	**26**

In 2003–2005, the total commercial flounder landings (in by-catch of turbot) were 415 tons in average; over 90% landings originated from the coastal gillnet and trapnet fisheries and about 15 t (4%) from trawling. Data from fish surveys indicate increased cpue values for flounder.

Directed fishery is closed from February to June, and the minimum legal size for landings is 21 cm.

In 2005 the flounder landings in SD 28, SD 29 and SD32 were almost equal (35%, 35% and 30% respectively). The landings were the biggest in the III quarter (74%).

4.3.2 Removal of otoliths

For removing the otoliths, a cut is done through the flounders head. Both sagital (sagittae) otoliths are removed by tweezers and stored in paper bags. These bags are stored in room temperature.

4.3.3 Preparation of otoliths

Afterwards in laboratory both whole otoliths are cleaned with water and sunk into water or alcohol. Otoliths are studied using a binocular microscope with reflect light in 16 x magnification over a dark background.

4.3.4 Interpretation of otolith annuli

An annual ring consists of summer and winter ring. Age is determined by reading winter rings.

4.4 Germany

4.4.1 Sampling of flounders for age determination

Otoliths of flounder are sampled on scientific surveys mostly in Sub-divisions 24 and from commercial catches between August and December exclusively in Sub-division 24. The directed flounder fishery decreased drastically since 1990.

The samples are taken length stratified neglecting sex and collecting a maximum of 25 otoliths per length class and sample. Therefore, the age sample size varies according to the length frequency distribution encountered.

The time series starts in 1992 for Sub-division 24.

4.4.2 Removal of otoliths

The cut is made starting from the dorsal edge of operculum in dorsal direction right behind the otolith position. This ideally opens the sacculi of the sagitta otoliths. Both sagittae otoliths are removed with help of a pair of tweezers. The membranes covering the otoliths are removed. The otoliths are cleaned if necessary and put into a hole in a black plastic array with the plain side up.

4.4.3 Preparation of otoliths

The otolith is cleaned and oriented the plain side up. No further preparation is considered necessary before reading.

The reading itself is done in the black plastic array in water. Some readers add a drop of detergent to the water to remove the surface tension. If the readability is bad, sometimes a prolonged soaking in water (up to an hour) can help.

There is no preference for using the symmetric or asymmetric otolith for age determination.

4.4.4 Interpretation of otolith annuli

The “nucleus” is considered to be the area within the first circular round hyaline ring.

The following first hyaline zone around the nucleus is not counted for age because a small flounder of < 10 cm, caught before January 1 and thus supposed to be in its year of birth, already has such a zone.

A hyaline edge is not counted in the second half of the year.

4.4.5 Age readers' experience

A rather constant total of about 1500 otoliths from BITS and national surveys are read annually. The annual otolith numbers from commercial catches vary between 2000–3000. Since 1972 four experts have successively been responsible for age determination of flounder. The overlaps between readers have been at least one year.

4.5 Latvia

4.5.1 Sampling of flounders for age determination

Otoliths are sampled in commercial and scientific surveys in Latvian waters (ICES Sub-division 26 and 28).

Commercial–otoliths come from open sea and coastal fishery. From coastal fishery time series are available from 1995, from open sea- 1960s.

Approximately 1000 juvenile otoliths from juvenile flounder surveys (age groups 0–2) have been collected since 1998.

Twice a year (March and November) flounder otoliths are collected in the international BITS surveys.

4.5.2 Removal of otoliths

For removing the otoliths, a longitudinal (vertical or horizontal) cut is put through the head and cheeks of the fish. Both sagittae otoliths are removed with help of a pair of tweezers. The membranes covering the otoliths are carefully removed. Otoliths are put in special plastic or paper sample bags that are handled with care.

4.5.3 Preparation of otoliths

Besides cleaning the whole otoliths, LATFRA don't prepare otoliths specially.

4.5.4 Interpretation of otolith annuli

Age is analysed from whole otoliths using a binocular microscope (16 x magnifications) with reflected light. Age determination is carried out counting translucent zones.

All otoliths are measured by total length and length of annuli.

4.6 Lithuania

4.6.1 Sampling of flounders for age determination

Flounder otoliths are sampled from surveys (data and samples are available from 2003) and from commercial catches (data are available from 2005). All data comes from Sub-division 26, various depths, various quarters.

4.6.2 Removal of otoliths

The otoliths are removed by a longitudinal cut at 45 degrees through the head and cheeks of the fish. Both sagittae otoliths are removed with the help of a pair of tweezers. Even if otoliths are broken during extraction, all available pieces are collected. Otoliths are cleaned and dried with absorbing paper. Otoliths are stored dry in plastic tubes. Label information on catch location, sample date and individual weight, length, sex and are glued to each tube.

4.6.3 Preparation of otoliths

Each otolith is placed in small bowl with a dark background, filled with water, convex side downwards. Otoliths are studied using a binocular microscope with reflected light and magnification is 16 x.

4.6.4 Interpretation of otolith annuli

Annual ring is visualised like darker and lighter bands (winter and summer rings). Age is determined by reading winter rings (darker bands).

4.7 Poland

4.7.1 Sampling of flounders for age determination

Data are collected in Sub-divisions 26, 25 and 24 during:

- research trawl surveys (BITS) taken in 1st and 4th quarter of the year. Surveys provide independent data for fish stocks assessment and are internationally planned and co-ordinated within the ICES Baltic International Fish Survey Working Group.
- commercial catches during the whole year.

4.7.2 Removal of otoliths

The otoliths are removed by a longitudinal cut through the head and cheeks of the fish. Both sagittae otoliths are removed with the help of a pair of tweezers. The membranes covering the otoliths are carefully removed and the otoliths are put in paper bags.

4.7.3 Preparation of otoliths

Otoliths are prepared either by

- old method (more common). Whole otoliths (covered with water) are read using a binocular microscope (20x magnification) with reflected light.
- new method (less common). The otholits are placed in silicon mould and covered with EpoFix&hardener mixture. After 24 hours the otoliths are cut with high-speed saw (Struers Accutom 50) with a diamond blade. The result is a section approximately 0,6 mm thick. Then the section is viewed with a microscope using transmitted light at a magnification of 20–50x.

4.7.4 Interpretation of otolith annuli

Age determination is made by counting annuli (one opaque and one translucent zone) from the nucleus to the dorsal edge.

4.8 Russia

4.8.1 Sampling of flounders for age determination

In the Baltic Sea flounder otoliths are sampled from international surveys and on commercial vessels (sub-division 26). In other regions flounders are sampled on several commercial vessels. The samples were collected for all seasons in 1998–2001 in different areas of the Barents Sea (West Coastal Area, Rybachiya Bank, Kaninskaya Bank, Kildinskaya Bank, Central Areas of the sea).

4.8.2 Removal of otoliths

The otoliths are removed by cutting the head using usual methods for such sampling. Both sagittae otoliths are removed with help of a pair of tweezers, the membranes covering the otoliths are gently removed. After extraction otoliths are stored in paper bags with all information (gear, time and region of catch, number of fish, lenght, weight, sex, etc.). Sometimes storing in sticky tape is acceptable.

4.8.3 Preparation of otoliths

For most flatfish species ageing has been investigated by methods of breaking and burning of otoliths. First of all, the otolith is broken transversally across the nucleus. Secondly, the broken surface is gently burned in a small flame from a spirit lamp. After that, the burned section is then mounted in a piece of plasticene and the broken surface viewed under a microscope using reflected light. A clear thin oil or water is used to clear the surface to aid the identification of annual rings. For some of the flatfish species, the burning is not required, because the broken surface is quite clear enough for age determination**.** In some Institutes whole otoliths (cleansed by alcohol) are used.

Interpretation of otolith annuli

In the breaking and burning method age is determined by reading winter rings (dark annual protein band). The central round shape band is not counted. In whole otoliths age determination is made by counting opaque zones (consideration with time of sampling).

4.9 Sweden

4.9.1 Sampling of flounders for age determination

Following the EU Data Collection Regulation samples are taken from commercial landings and data collected on age, length, weight, sex and maturity stage. In 2006 a total of 800 individuals was sampled during the third quarter of the year from the gillnet fishery in Sub-divisions 23, 27 and 28. An additional 200 individuals were sampled during the fourth quarter from the demersal trawl fishery in Sub-division 25.

Fishery independent data have been collected in BITS trawl survey in March and November in Sub-divisions 25–28 and in coastal monitory fishing in the autumn (August-October) using standardized coastal survey nets or fykenets in Sub-divisions 23, 27 and 28. Some monitoring series dates back to the 1960s but age determination has only been done during later years.

No regular juvenile surveys are done.

4.9.2 Removal of otoliths

To remove the otoliths, a transversal cut is put through the head and cheeks of the fish. Both sagittae otoliths are removed with help of a pair of tweezers. The membranes covering the otoliths are carefully removed and the otoliths is rinsed in water and dried with absorbing paper. Otoliths are put in paper sample bags that are handled with care, and stored well-ventilated, not too tightly packed together. As the symmetric otoliths are usually easiest to interpret, they are further prepared for sectioning. In samples where the symmetric otolith is crystalline or is missing, the asymmetric otolith is used instead.

4.9.3 Preparation of otoliths

Specially designed aluminium moulds are prepared with a mixture of black polyester resin. Liquid polyester resin is mixed with a hardener and the bottom of the aluminium moulds are covered with the mixture and left to harden for about 12 hours on a vertical surface. The otoliths are glued to the bottom layer with a new mixture of resin and hardener in straight rows on to the solidified resin. The convex side of the otoliths are placed downward and the centre of the nucleus is placed in straight lines. To facilitate the mounting of otoliths on a straight line a surveillance camera connected to a monitor is used. Five rows of approximately ten otoliths fitted in each row. After mounting the otoliths are left to harden for about 12 hours on a vertical surface. Next day the moulds are filled up to the top with polyester resin so that all samples are covered. The moulds are then left to harden for 12 hours.

After hardening, the solid polyester blocks are loosened from the moulds and thereafter each block is set up in a sawing machine (Struers Accutom 50). Thin cross sections, 0.3 mm, are cut through the centre of the otoliths with a diamond cut-off wheel. The cross-sections are etched with hydrochloric acid (1 %) for 40 seconds, rinsed with water and thereafter stained with a solution of neutral red (20 ml, 3.3 g /L), acetic acid (0.1 ml, 100 %) and sodium chloride (0.2 g) for six minutes. The etched and stained sections are placed on a microscope slide (76x51mm). On top of the sections a cover slip is placed and attached with tape.

4.9.4 Interpretation of otolith annulli

Age is analysed from the cross-sections of the otoliths using a binocular microscope (20-63x magnification) with transmitted light through the sectioned otolith slices. Annual rings in the cross-sections can be visualised as narrow distinctly darker coloured bands. Less obvious rings are checked by also using reflected light.

5 Criteria for age determination (ToR b)

5.1 Definition of annuli and date of birth

Normally developed sagittae otoliths of flounder deposit annular growth increments, so called annuli (Fig. 5.1). This pattern is disrupted in crystalline or otherwise abnormal otoliths.

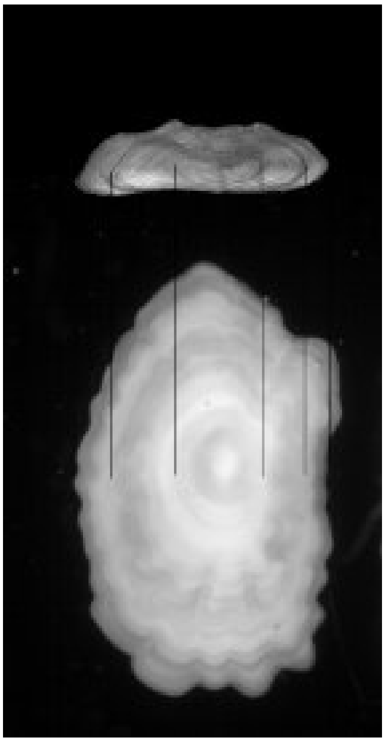

Figure 5.1. Opaque and translucent zones in a 3 year old flounder caught in June. Vertical black lines indicate end of the translucent zones (the start of the new annuli) in a whole (below) and later sectioned (above) otolith.

One annuli consists of one opaque and one translucent zone. The opaque zone represents the seasonal period of fast growth (summer ring) and the translucent zone represents the period of slower growth (winter ring). In Baltic flounder a complete annuli is visible in summer or late summer, when the opaque zone of the new year has started to form outside the translucent zone of the previous year. This transition will be visible earlier in otoliths from flounders that have been caught in the southern part of the Baltic compared to flounders that have been caught in the northern part of the Baltic.

Date of birth is set to the 1st of January. This implies that a translucent zone at the edge of the otolith during the first part of the year shall be interpreted as a part of the previous year annuli, and shall be counted to obtain the correct age. During the later part of the year a translucent zone should not be included as a complete annuli. The season when the translucent zone will be completely visible (followed by an opaque zone) will vary between areas in the Baltic Sea (see section 7).

5.2 Interpretation of the first ring

The diameter of the first annuli (first ring) varies between otoliths. One explanation is that settlement dates vary between individual fishes. Early settlers will experience a longer growth season than late settlers. Monitoring of 0–group flounder in shores around Gotland (Sub-division 28) indicate that newly settled flounder are abundant in mid-June but also occur in mid-September (Anders Nissling, personal communication). Hence, the first summer growth will be long for some individuals and short for others. Presumably the length of the summer growth will be reflected in the diameter of the first opaque zone in the otoliths. The WK participants agreed that the size of the first annuli could vary and that this should be considered in the protocol for age determination of Baltic flounder.

Another problem is the infrequent visibility of a very small innermost ring. This ring has sometimes been labelled as the "metamorphose" ring. During metamorphosis both sagittae otoliths will develop accessory (secondary) growth central on the edge of the larval otolith. Continued growth will be manifested as a starlike appearance in the centre of the otolith. An example is provided in figure 5.2. The question was raised if this pattern can be manifested in otoliths of adult fishes and consequently not be interpreted as a first annuli.

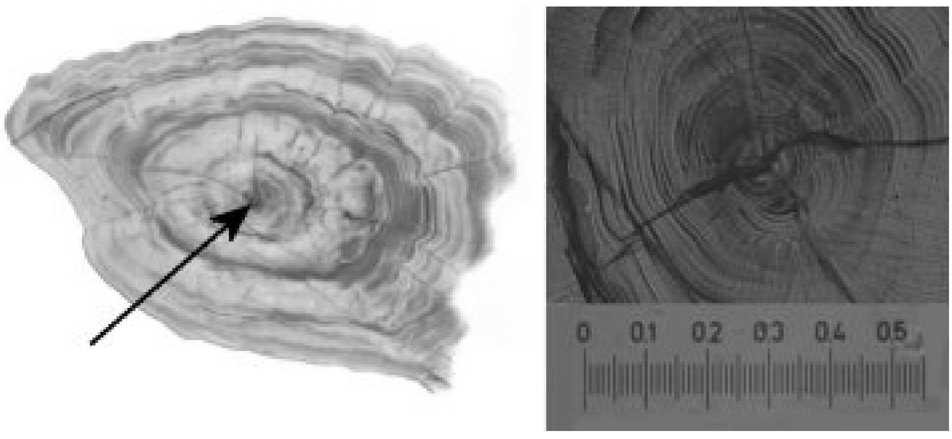

Figure 5.2. A grinded and polished otolith from a 243 mm long flounder caught outside Gotland (Sub-division 28). Arrow indicates position of metamorphosis mark in the left image. Right image shows the typical pattern of the metamorphose circular mark. The scale indicates mm.

A sample from eight 0–group individuals caught on the same date in August 2006 in the Stockholm archipelago (Sub-division 27) was used to establish a linear regression between otolith length (OL) and fish total length (FL). The fishes varied in size from 22 to 86 mm. The calculated regression was OL= 0.0268FL + 0.076 (r2=0.9983). Backcalulation for a 10 mm fish indicates that otolith size should be 0.34 mm, which is more than three times the observed sizes of the "metamorphose" ring in grinded and polished otoliths from adult fishes (Johan Modin, personal communication). Individual measurements are illustrated in figure 5.3 (visible "metamorphose" ring in left image and no "metamorphose" ring in right image). The calculations are based on a very small sample and results are thus not conclusive. The exercise needs to be repeated based on a statistically sufficient number of individuals and using proper back-calculation methodology.

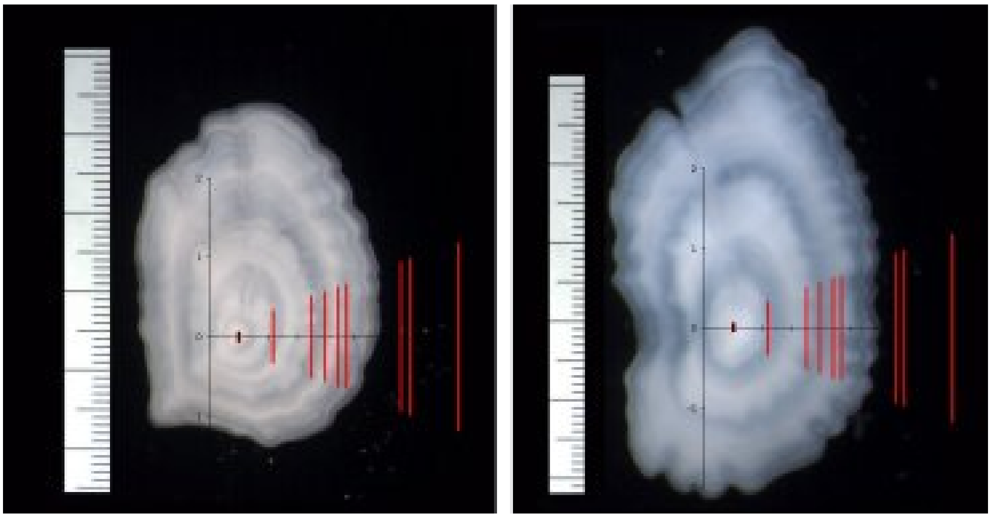

Figure 5.3. Whole otoliths from two adult flounders caught in Subdivision 28. The left otolith displays a visible "metamorphose" ring, while the right otolith shows no visible "metamorphose" ring. Superimposed are observed otolith lengths of eight juvenile flounders. The innermost bar indicates observed otolith size (0.1 mm) of newly metamorphosed larvae of 10 mm in total length. This size is smaller than the back-calculated otolith size from the eight juvenile flounders. The X-axis gives otolith size in mm and the Y-axis gives the juvenile fish size in cm. Only asymmetrical otoliths have been used.

Ocular inspection of sectioned otoliths reveals a less distinct ring pattern of the "metamorphose" ring than subsequent annuli. The patterns were discussed and it was agreed that as a provisional rule, visible so called "metamorphose" rings should not be included as a valid annuli. In doubtful cases the radius of the first visible winter ring and the radius of the otolith should be measured to back-calculate the length of the fish at the formation of the ring in question and to compare this length with the observed length distribution of flounder during the first winter.

6 Consistency among and within age determination experts (ToR c and d)

6.1 Results from the exchange experiment

The exchange of otolith samples was started in spring 2006. In total six otolith samples were prepared by the participating laboratories. Four samples were sent to the Institute of Coastal Research in Öregrund, Sweden, where one of the otoliths was sectioned and stained, while the other otolith was left whole. Two samples consisted only of whole otoliths. All the samples and the number of whole and sectioned otoliths are listed below in the table below.

Table 6.1. Sample origin and the provided number of whole and sectioned otoliths

COUNTRY	WHOLE OTOLITHS	SECTIONED OTOLITHS
Estonia	50	50
Germany	25	25
Latvia	50	50
Sweden	50	50
Latvia 2nd sample	50	
Lithuania	50	

The samples were distributed around the Baltic Sea from one laboratory to another and the results were sent to the coordinator. Two otolith samples were sent also to CEFAS in Lowestoff, where the age was determined by two experienced flatfish age readers. The results of the age determination were analysed using the spreadsheet of Eltink *et al.* (2000). Since in the age determination from the whole otoliths all the readers were regarded as inexperienced and for the age determination from sectioned otoliths only one reader could be regarded as experienced, the results were compared with the modal age of all the readers. The main results of the age determination in the exchange, CV (%) and agreement (%) among age readers, are presented in table 6.2. Detailed results by age reader and modal ages are shown in Appendix 5, tables A5.1–A5.32. Age bias plots are shown in Annex 5, figures A5.1–A5.10.

In general the results revealed a low level of agreement and precision (high coefficient of variation) in relation to modal age. In total for all the samples the average agreement was in the range 45.0–67.9%, average 58.7%. The coefficient of variation was in the range 14.7–28.8%, average 20.4%. The agreement was higher for the age determination from the whole otoliths than from the sectioned otoliths. This could be an effect of reader experience since most of the laboratories use whole otoliths for routine ageing of flounder.

Table 6.2 Agreement and coefficient of variation from samples in the exchange experiment.

SAMPLE ORIGIN	PERCENTAGE AGREEMENT	COEFFICIENT OF VARIATION
Estonian whole otoliths	53.4	28.8
Estonian sectioned otoliths	51.5	28.1
German whole otoliths	67.2	15.3
German sectioned otoliths	58.7	19.7
Latvian whole otoliths	61.0	14.7
Latvian sectioned otoliths	56.7	16.5
Swedish whole otoliths	59.9	22.0
Swedish sectioned otoliths	45.0	22.0
Latvian whole otoliths 2nd sample	67.9	16.7
Lithuanian whole otoliths	65.7	20.4
Average	58.7	20.4
Average whole otoliths	62.5	19.7
Average sectioned otoliths	53.0	21.6

In the samples where age was determined both from sectioned and whole otoliths, the former gave on average higher ages. This could be connected with the “cliff-edge” effect where the annuli of older ages are less visible at the edge of whole otoliths. The result will be a low readability of older age in the whole otoliths. The mean length at age in several samples revealed a rather surprising pattern when flounder length in the older ages did not increase at all (Fig. 6.1). Since it is considered that the females have higher growth rate than males, the results of mean length at age were analysed separately. However, this did not change the previous pattern and in many samples there was no difference between length of females and males. This result could indicate significant errors in the age determination and in the calculated modal age especially for the whole otoliths should be treated with caution.

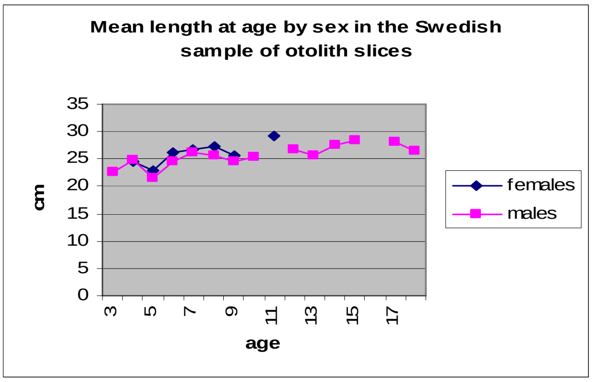

Figure 6.1. Mean length at age by sex as a result of the age determination of flounders in the Swedish samples of sectioned otoliths. Results indicate a low annual increment in length.

6.2 Experiment on different otolith preparing techniques

Otoliths from 50 flounders sampled from commercial gillnet fishery east of Gotland during 14–20 June, 2006 were used in the experiment. For every individual one otolith was kept whole (randomly either the symmetric or the asymmetric), while the other was either broken and burned or sectioned and stained. This resulted in 25 burned, 25 sectioned and 50 whole otoliths.

Apart from the specimens also colour photographs were provided of all sectioned otoliths and also of both whole otoliths from the same individual before the sectioning were done. No photos of the burned otholiths and the corresponding whole otholith were provided. A scale in mm was given on each photo. This means that in total photos of 25 sectioned and 25 whole otholiths were available. Fourteen age readers were asked to give the number of winter rings (translucent rings) seen on the otolith and also to mark the locations of winter rings on the photo. To make it possible to compare how different readers located different winter rings the photos were provided with an arrow along which readers were asked to mark the ring. The position of the arrow was determined by an age reader, not participating in the experiment, trying to make it along the most readable part of the otolith. Furthermore readers were instructed to mark any “metamorphosis” ring seen on the photo but not to include it in the summary of winter rings. Information on sex, length and month of catch were provided. Experience of readers differed from less then one year to more than 30 years. In total six participants could be considered experienced in flounder age reading (more than 1.5 years experience), six could be considered inexperienced (less than 1 year experience) and two participants were highly experienced in flatfish age readings but newcomers to the European flounder from the Baltic Sea.

6.2.1 Results from comparisons of methods (whole, burnt and sectioned otoliths)

The results of the age determination were analysed using the spreadsheet of Eltink *et al.* (2000) and SPSS 14.0 statistical package. There was low agreement between readers and the coefficient of variation (CV) was high for all sets of otoliths (Table 6.3). In fact a threshold of 80% agreement (Eltink *et al.* 2000) was not reached for any of the 100 samples read. However, the sectioned and stained method gave a significantly lower CV and higher percent agreement when compared to whole otoliths (Paired t-test, n=25, $p<0.05$). There was no difference between burned and whole otoliths. Detailed result by age reader and modal ages as well as age bias plots are shown in Appendix 6, tables A6.1–A6.7 and figures A6.1–A6.3.

Table 6.3. Mean and standard deviation (SD) of the coefficient of variation (CV) and percentage agreement of 14 readers on the number of winter rings in differently prepared otoliths. Also the number of samples (N) and the maximum range within a single sample is given.

Method	CV (SD)	% Agreement (SD)	N	Maximum range
Whole	0.22 (0.07)	39 (11)	50	4–16
Broken & Burnt	0.20 (0.07)	40 (10)	25	5–16
Sectioned & Stained	0.16 (0.09)	51 (15)	25	8–16

Since otoliths from each individual fish were prepared as both whole and burnt or as both whole and sectioned, it is possible to compare results of the age determination by method. Thus, the modal number of winter rings for the same fish was compared between the whole or the prepared otolith (sectioned or burnt). Results revealed that the same fish was judged to be much older (average +2 years) when comparing the whole and the sectioned otolith (Paired t-test, n=25, p=0.001, Fig. 6.2). No significant difference was found between whole and burnt otoliths (Fig. 6.2).

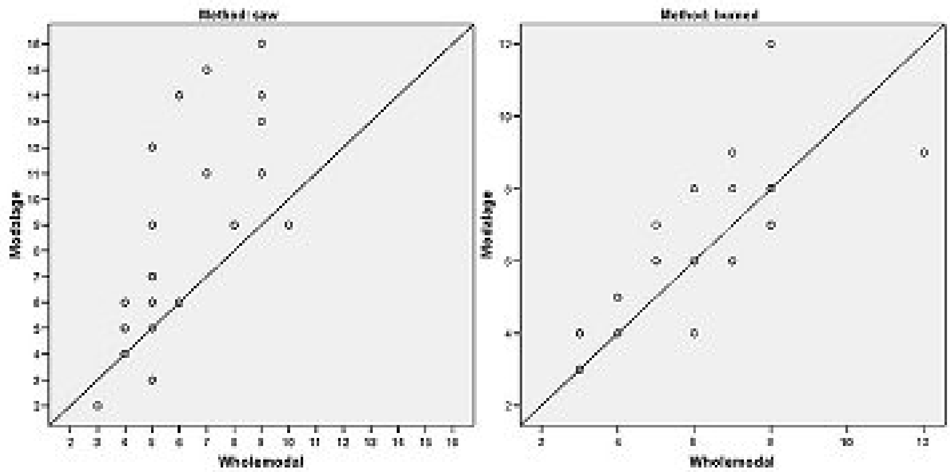

Figure 6.2. Scatterplot of modal number winter rings seen in whole otoliths compared to modal number winter rings of sectioned otoliths from the same individual (left image) and in whole otoliths compared to burnt otoliths (right image). Each mark corresponds to one individual fish.

6.2.2 Results from marking on the images of whole and stained otoliths

When comparing the rings marked by different readers, two things were evident. Firstly, different readers have identified different winter rings on the same otolith. Second, some readers have identified some rings as the metamorphosis ring, which other readers have considered to be the first, or even the second wintering (Fig. 6.3 and 6.4).

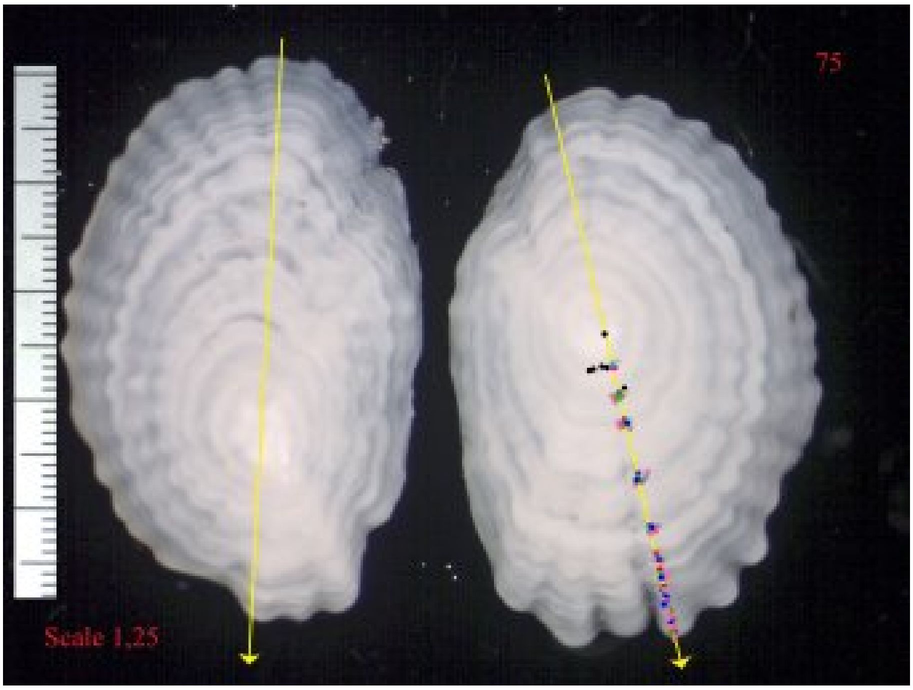

Figure 6.3. Photo of whole otholiths were the interpretation of winter rings by 14 readers have been illustrated. Points are slightly offset to enhance the visibility. Each colour represents the winter rings identified by a specific reader. The black dots are suggested to be metamorphosis rings.

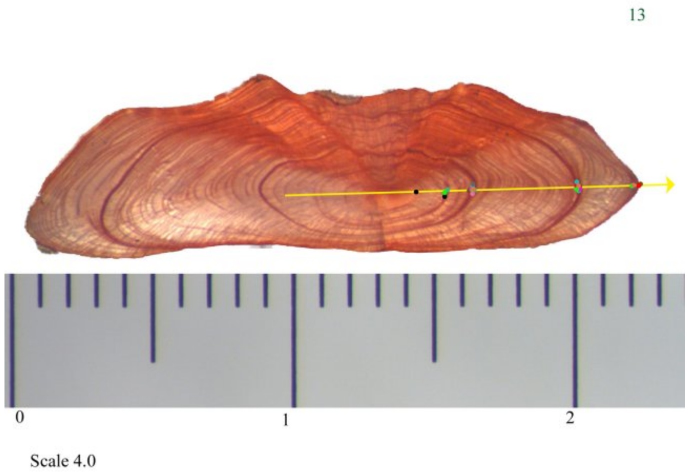

Figure 6.4. Photo of sectioned and stained otoliths where the interpretation of winter rings by 14 readers have been illustrated. Points are slightly offset to enhance the visibility. Each colour represents the winter rings identified by a specific reader. The black dots are suggested to be metamorphosis rings.

6.3 Demonstration of otoliths on the screen and common discussions

After the experiment of winter ring determination from whole, sectioned and burnt otoliths, these structures were viewed on a screen and participants discussed how the winter rings were determined and what could be the correct way of doing it. This also helped to achieve common interpretation in the age determination. The common discussion of the whole otoliths revealed significant discrepancies in the determination of winter rings. This is caused by the structure of the flounder otoliths which usually has very wide translucent winter rings and by the transition between hyaline and opaque zones that is very vague. In addition, translucent rings inside opaque zone (checks or false rings) are common. Moreover, even for whole otoliths where the annuli seemed to be distinct and clearly separable, the determined age differed from the age determined from the sectioned otolith of the same fish. For older whole otoliths the age determination was hampered by „cliff-edge" effect, thus always causing a lower defined age than from sectioned otoliths. It was clear from viewing the sectioned otolith that the „cliff-edge" effect is visible already in flounders at age six. These causes were the background for a general conclusion that whole otoliths should not be used for age determination.

It was concluded that the most convenient method for age determination of flounder is from sectioned and stained otoliths. The discussion revealed good agreement in interpretation of winter rings and determination of flounder age. The main problems were caused with sectioned otoliths which had poor staining. In a few cases there were different interpretations of the first winter ring but this problem could be solved by the measurement of winter rings and back-calculation of the flounder length.

6.4 Re-reading experiment

To evaluate if the discussions and demonstrations during the workshop have made any improvement on age reading, two sets of otoliths that were part of the exchange prior to the meeting were reread at the end of the workshop. The first set was a German sample of 25 sectioned and stained otoliths from Sub-division 24, half of the otoliths were from January and half from August. The second set was a Swedish sample of 20 sectioned and stained otoliths from Sub-division 27 in September. Readers were provided with information on sex, length, month and place of catch of the samples. To compare if any improvement had been made, only the six readers that had participated in both the exchange and the rereading were included in the analysis.

The results of the age determination were analysed using the spreadsheet of Eltink *et al.* (2000) and SPSS 14.0 statistical package. Results show that the percentage agreement was significantly higher in the rereading compared to the original readings in both the German (paired t-test, n=25, p=0.026) and the Swedish (paired t-test, n=20, p= 0.006) sets of otoliths (Table 6.4). The coefficient of variation was also lower in the rereading compared to the original values (Table 6.4), although only significantly so in the Swedish sample (paired t-test, n=20, p<0.001). This is despite that the quality of some of the samples were bad (diffuse stain) during the rereading. Detailed result by age reader and modal ages as well as age bias plots are shown in Appendix 7, tables A7.1–A7.3 and figures A7.1–A7.2.

Table 6.4. Mean and standard deviation (SD) of the coefficient of variation (CV), and percentage agreement in rereading of German and Swedish sectioned otolith from the exchange.

OTOLITH SET	GERMAN	GERMAN	SWEDISH	SWEDISH
	Exchange	Reread	Exchange	Reread
% Agreement (SD)	59 (17)	70 (15)	48 (16)	62 (16)
CV (SD)	0.20 (0.09)	0.16 (0.09)	0.20 (0.08)	0.11 (0.05)

7 Progress towards a manual for age determination of flounder (ToR e)

The objective of an international manual is to provide a protocol for age determination of flounder in the Baltic Sea. The manual and regular inter-calibrations will serve as a means to provide quality assurance. The manual will be updated regularly. It was agreed that the contents of the manual shall be organized in the following sections:

1) Growth
2) Formation of the opaque and hyaline rings

3) Established age determination criteria for otoliths
4) Storage of otoliths
5) Methods of otolith preparation
6) Light source and magnification
7) Confidence levels
8) Training protocol for new readers
9) Images of otoliths marked with ages including text explanations

The contents of the manual sections were discussed and specifically Section 5: Methods of otolith preparation was discussed in some detail (Annex 8). The manual will be further developed by intercessional work during 2007.

8 Recommendations (ToR f)

1) The method of sectioning and staining of otoliths should be used for age determination of flounder in the Baltic. It is recognised that the method requires a costly investment for most laboratories that work with age determination.
2) As a transitional stage the broken and burnt method could be used since it gives a similar otolith ring pattern in the appearance as the sectioning and staining method.
3) The broken and burnt method needs to be further evaluated.
4) Back calculation of fish length from the first ring should be done to estimate location of metamorphosis ring in adult fish and crosscheck with studies on young fish.
5) The precision and the agreement of age determination in Baltic Flounder need to be improved so that it can be used in analytical assessments.
6) After establishing the recommended method, a protocol for an update of historical data needs to be developed.
7) A series of images should be produced, showing the growth of the annual zones throughout the year. This should be done for different parts of the Baltic, and evaluated over time.
8) A reference collection of images with high agreement (>80%) from the exchange should be established. This should be done for different parts of the Baltic, and evaluated and updated over time.
9) An exchange program with sectioned and stained otoliths should be started in May 2007, with two samples one German (spring) from Sub-division 24, and one Swedish from Sub-division 27 (autumn). Samples should be distributed over all length groups for both sexes. The program will be coordinated by CEFAS.
10) Considering the fresh insights into the age determination of flounder a workshop is recommended to take place in the beginning of May 2008. The terms of reference should include:
    10.1) evaluation of the exchange
    10.2) experiment on broken & burnt otoliths
    10.3) experiment on influence of length information on age determination
    10.4) updating of manual
    10.5) updating of reference collection
    10.6) protocol for updating historical data

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Annex 2: Agenda

20 March	10.00	Review of Terms of Reference, selection of rapporteurs, agreement on
	10.30	Presentation: Biology of flounder in the Baltic
	11.00	Coffee
	11.30	Presentation: Stock units in the Baltic
	12.30	Lunch
	13.30	Experiment: age determination by method (whole/broken/sectioned otoliths) using image analysis.
	15.00	Coffee
	15.30	Experiment: cont.
	18.00	End of day
21 March	9.30	Experiment cont.
	10.30	Coffee
	11.00	Presentation: Fishlengths at age derived from length-frequency samples
	11.30	Presentation: Age determination by sectioned and stained otoliths
	12.00	Presentation: Inferences from daily growth zones in otoliths
	12.30	Lunch
	13.30	Discussion on interpretation from live images.
	15.00	Coffee
	15.30	Discussion cont.
	17.00	Agreement/disagreement on otolith structure and annuli.
	18.00	End of day
22 March	9.30	Presentation: Results from the exchange program
	10.00	Results and discussion on the age reading experiment
	10.30	Coffe
	11.00	Presentations: National protocols for age determination
	12.30	Lunch
	13.30	Discussion on manual outline and contents
	14.00	Re-reading exchange otoliths
	15.00	Coffee
	15.30	Re-reading cont.
	18.00	End of day
23 March	9.30	Results from re-reading exchange otoliths
	10.00	Text discussion,
	10.30	Coffee
	11.00	Recommendations including future workshops
	11.30	Summing up
	12.30	End of day

Annex 3: Summary of recommendations

RECOMMENDATION	ACTION
1. Use of sectioned and stained otoliths for age determination of flounder in the Baltic Sea	All national laboratories involved in age determination of flounder.
2. Improve precision and reader agreement of age determination by evaluations and tests of improved methods	All national laboratories involved in age determination of flounder.
3. Sampling of otoliths for studies of otolith seasonal growth	All national laboratories involved in age determination of flounder.
4. Establish protocols for reference collections and for an update of historical data	PGCCDBS, other age reader WK
5. Conduct an exchange experiment with sectioned and stained otoliths	
6. Complete a first draft of an international manual for age determination of flounder in the Baltic Sea	PGCCDBS, other age reader WK
7. Organise work to be reported and evaluated during a 2nd WKARFLO meeting	PGCCDBS, WGBFAS

Annex 4: ToR and justification for new workshop

The 2nd **Workshop on Age Reading of Flounder** [WKARFLO] (Co-Chairs Johan Modin, Sweden and Ann-Britt Florin, Sweden) will take place in Rostock, Germany, 23–25 May, 2008 to:

a) Evaluate the 2007 exchange of sectioned and stained otoliths.

b) Conduct an experiment on the feasibility of broken and burnt otoliths for age determination of flounder.

c) Conduct an experiment on the influence of length information on age determination.

d) Continue to update and prepare an international manual for age determination of flounder in the Baltic Sea.

e) Initiate and update protocols for national reference collections.

f) Establish a protocol for updating historical data.

WKARFLO will report to ACFM, RMC and PGCCDBS by 9 May, 2008.

Supporting Information:

Priority:	To assess the fishery it is necessary to determine the biological characteristics, such as age and length distributions, of the commercial and of research survey catches.
Scientific justification and relation to action plan:	There have been indications (mean weight at age, yearclass estimates) that age determination differs between countries. A previous WS has confirmed substantial differences in age determination by national age readers. A new method has been developed (sectioning and staining of otoliths) and preliminary evaluations during the WKARFLO meeting in 2007 give reasons for improvement. Sectioned otoliths s from national sampling is currently circulated in an exchange program between Baltic laboratories. The exchange program needs to be evaluated and individual age determination experts need to agree on interpretation and quality measures by an international manual in order to assure a consistent age determination process.
Resource requirements:	DCR data collection system.
Participants:	In view of its relevance to the DCR, the Workshop is expected to attract wide interest from ICES Member States that participate in biological sampling of flounder.
Secretariat facilities:	None.
Financial:	To ensure wide attendance of relevant experts, additional funding will be required, preferably through the EU, e.g. by making attendance to the WS eligible under the DCR.
Linkages to advisory committees:	There is a direct link to ACFM through the Baltic Fisheries Working Group.
Linkages to other committees or groups:	This workshop was proposed by PGCCDBS. Outcomes from this Workshop will be of interest to the Living Resources Committee and the Resource Management Committee.
Linkages to other organizations:	There is a direct link with the EU DCR
Secretariat marginal cost share:	

Annex 5: Detailed results from the exchange experiment

The annex contains results (tables and figures) from the exchange experiment on flounder otoliths exchanged during 2006 to 2007.

The presented tables are

A5.1	Mean coefficient of variation per modal age in the exchange experiment.
A5.2	Mean percentage agreement per modal age in exchange experiment.
A5.3	Coefficient of variation by age reader and by modal age in the Estonian sub-sample of whole otoliths.
A5.4	Percentage agreement by age reader and by modal age in the Estonian sub-sample of whole otoliths.
A5.5	Mean length at age by age reader in the Estonian sub-sample of whole otoliths.
A5.6	Coefficient of variation by age reader and by modal age in the Estonian sub-sample of sectioned otolith.
A5.7	Percentage agreement by age reader and by modal age in the Estonian sub-sample of sectioned otoliths.
A5.8	Mean length at age by age reader in the Estonian sub-sample of sectioned otoliths
A5.9	Coefficient of variation by age reader and by modal age in the German sub-sample of whole otoliths.
A5.10	Percentage agreement by age reader and by modal age in the German sub-sample of whole otoliths.
A5.11	Mean length at age by age reader in the German sub-sample of whole otoliths.
A5.12	Coefficient of variation by age reader and by modal age in the German sub-sample sectioned otoliths.
A5.13	Percentage agreement by age reader and by modal age in the German sub-sample sectioned otoliths.
A5.14	Mean length at age by age reader in the German sub-sample of sectioned otoliths.
A5.15	Coefficient of variation by age reader and by modal age in the Latvian sub-sample of whole otoliths.
A5.16	Percentage agreement by age reader and by modal age in the Latvian sub-sample of whole otoliths.
A5.17	Mean length at age by age reader in the Latvian sub-sample of whole otoliths.
A5.18	Coefficient of variation by age reader and by modal age in the Latvian sub-sample of sectioned otoliths.
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A5.20	Mean length at age by age reader in the Latvian sub-sample of sectioned otoliths.
A5.21	Coefficient of variation by age reader and by modal age in the Swedish sub-sample of whole otoliths.
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A5.23	Mean length at age by age reader in the Swedish sub-sample of whole otoliths.
A5.24	Coefficient of variation by age reader and by modal age in the Swedish sub-sample of sectioned otoliths.
A5.25	Percentage agreement by age reader and by modal age in the Swedish sub-sample of sectioned otoliths.
A5.26	Mean length at age by age reader in the Swedish sub-sample of sectioned otoliths.
A5.27	Coefficient of variation by age reader and by modal age in the Latvian sample of whole otoliths.
A5.28	Percentage agreement by age reader and by modal age in the Latvian sample of whole

	otoliths.
A5.29	Mean length at age by age reader in the Latvian sample of whole otoliths.
A5.30	Coefficient of variation by age reader and by modal age in the Lithuanian sample of whole otoliths.
A5.31	Percentage agreement by age reader and by modal age in the Lithuanian sample of whole otoliths.
A5.32	Mean length at age by age reader in the Lithuanian sample of whole otoliths.

Table A5.1. Mean coefficient of variation per modal age in the exchange experiment.

Modal age	Estonian whole	Estonian sectioned	German whole	German sectioned	Latvian whole	Latvian sectioned	Swedish whole	Swedish sectioned	Latvian whole 2	Lithuania whole
2	45.5%	47.2%	0.0%	11.1%						25.6%
3	33.5%	28.2%	20.4%	28.8%	18.9%	9.4%			18.3%	18.4%
4	34.5%	30.2%	13.2%	17.3%	13.7%	12.7%	19.0%	19.3%	21.3%	23.2%
5	34.7%	28.7%	14.1%	18.3%	14.2%	12.9%	28.3%	24.5%	20.8%	22.3%
6	28.8%	10.9%	17.8%	13.5%	9.0%	18.0%	18.9%	14.5%	21.1%	18.3%
7	19.3%	24.2%			14.7%	14.8%	16.9%	16.2%		17.4%
8	8.5%	11.4%			12.7%	25.1%	18.9%	16.4%		
9					15.9%		-	22.3%		
10					19.0%	28.3%	24.5%	27.2%		
11					20.2%	14.8%		20.0%		
12		30.0%						26.7%		
13		-						29.7%		
14		35.8%				23.6%		30.6%		
15								30.5%		
Mean	28.8%	28.1%	15.3%	19.7%	14.7%	16.5%	22.0%	22.0%	19.8%	21.5%

Table A5.2. Mean percentage agreement per modal age in exchange experiment.

Modal age	Estonian whole	Estonian sectioned	German whole	German sectioned	Latvian whole	Latvian sectioned	Swedish whole	Swedish sectioned	Latvian whole 2	Lithuania whole
1										86%
2	58%	50.0%	100.0%	91.7%		50.0%			70%	71%
3	55%	57.5%	68.6%	50.0%	71.4%	87.5%		50.0%	65%	67%
4	55%	45.0%	72.0%	61.1%	62.5%	66.7%	70.8%	58.3%	61%	58%
5	63%	40.0%	60.0%	50.0%	62.5%	62.5%	63.5%	50.0%	54%	54%
6	50%	66.7%	56.0%	61.1%	70.8%	43.3%	56.9%	55.6%	57%	64%
7	50%	51.4%	60.0%		56.8%	52.1%	54.2%	52.2%	40%	57%
8	50%	46.7%			50.0%	41.7%	55.0%	47.2%		
9	-	40.0%			50.0%	33.3%	50.0%	38.9%		
10	-	-			50.0%	50.0%	50.0%	33.3%		
11	50%	-			50.0%	41.7%		50.0%		
12		40.0%			50.0%	33.3%		37.5%		
13		-			-	33.3%		33.3%		
14		40.0%			50.0%	38.9%		33.3%		
15		-				33.3%		33.3%		
Mean	53.4%	51.5%	67.2%	58.7%	61.0%	56.0%	59.8%	43.6%	61.7%	43%

Table A5.3. Coefficient of variation by age reader and by modal age in the Estonian sub-sample of whole otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	All readers
2	26.6%	0.0%	0.0%	33.3%	45.5%
3	29.5%	17.1%	17.1%	24.1%	33.5%
4	15.2%	22.0%	0.0%	10.6%	34.5%
5	9.4%	60.6%	0.0%	0.0%	34.7%
6	26.9%	28.3%	18.2%	8.7%	28.8%
7	8.9%	28.2%	15.4%	5.1%	19.3%
8	6.9%	0.0%	7.5%	7.5%	8.5%
Weighted mean	18.9%	20.5%	11.0%	14.0%	28.8%
RANK	3	4	1	2	

Table A5.4. Percentage agreement by age reader and by modal age in the Estonian sub-sample of whole otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	All readers
2	0%	100%	100%	33%	58%
3	27%	73%	73%	45%	55%
4	0%	40%	100%	80%	55%
5	0%	50%	100%	100%	63%
6	25%	50%	50%	75%	50%
7	13%	63%	38%	88%	50%
8	67%	0%	67%	67%	50%
9	-	-	-	-	-
10	-	-	-	-	-
11	100%	100%	0%	0%	50%
Weighted mean	21.6%	59.5%	67.6%	64.9%	53.4%
RANK	4	3	1	2	

Table A5.5. Mean length at age by age reader in the Estonian sub-sample of whole otoliths.

Age	Reader 1	Reader 2	Reader 3	Reader 4	All readers
2	-	14.6	14.6	12.2	14.4
3	14.6	19.7	18.8	16.2	18.0
4	-	22.3	22.4	19.7	21.1
5	15.8	24.3	23.4	20.6	21.3
6	20.0	25.2	26.0	24.8	23.3
7	21.1	28.5	28.8	28.0	26.4
8	28.2	30.1	32.7	31.7	30.0
9	27.0	-	-	-	27.0
11	27.6	28.0	-	-	27.7
12	-	-	-	-	-
13	-	27.2	-	-	27.2

Table A5.6. Coefficient of variation by age reader and by modal age in the Estonian sub-sample of sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	ALL READERS
2	31%	0%	19%	15%	31%	47.2%
3	19%	15%	11%	14%	9%	28.2%
4	31%	13%	23%	13%	13%	30.2%
5	14%	11%	25%	20%	0%	28.7%
6	14%	9%	10%	0%	9%	10.9%
7	20%	36%	12%	14%	31%	24.2%
8	13%	8%	0%	8%	14%	11.4%
12	0%	0%	11%	11%	0%	30.0%
13	-	-	-	-	-	-
14	0%	26%	0%	11%	0%	35.8%
Weighted mean	18.6%	14.4%	12.5%	12.7%	14.2%	28.1%
RANK	5	4	1	2	3	

Table A5.7. Percentage agreement by age reader and by modal age in the Estonian sub-sample of sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	ALL READERS
2	0.0%	100.0%	83.3%	0.0%	66.7%	50.0%
3	0.0%	81.3%	87.5%	25.0%	93.8%	57.5%
4	0.0%	75.0%	25.0%	50.0%	75.0%	45.0%
5	0.0%	66.7%	0.0%	33.3%	100.0%	40.0%
6	33.3%	66.7%	66.7%	100.0%	66.7%	66.7%
7	0.0%	57.1%	71.4%	57.1%	71.4%	51.4%
8	66.7%	66.7%	0.0%	66.7%	33.3%	46.7%
9	100.0%	100.0%	0.0%	0.0%	0.0%	40.0%
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	100.0%	100.0%	0.0%	0.0%	0.0%	40.0%
13	-	-	-	-	-	-
14	100.0%	0.0%	0.0%	0.0%	100.0%	40.0%
15	-	-	-	-	-	-
Weighted mean	17.0%	74.5%	57.4%	34.0%	74.5%	51.5%
RANK	5	1	3	4	1	

Table A5.8. Mean length at age by age reader in the Estonian sub-sample of sectioned otoliths.

AGE	READER 1	READER 2	READER 3	READER 4	READER 5	ALL READERS
1	-	-	-	-	13.1	13.1
2	-	14.7	14.8	-	16.6	15.2
3	14.7	19.6	18.1	16.5	19.0	18.4
4	15.8	21.4	22.6	18.7	21.2	19.4
5	19.0	24.5	23.5	22.8	24.0	21.8
6	19.3	23.4	26.0	25.4	27.7	23.7
7	22.3	29.0	28.8	28.5	28.4	27.8
8	29.7	29.0	30.1	30.1	28.2	29.5
9	28.3	28.6	-	34.1	-	29.2
10	25.1	-	-	-	-	25.1
11	23.6	-	-	-	25.0	24.5
12	27.1	25.8	-	-	28.0	26.7
13	28.0	27.2	-	-	27.5	27.6
14	27.3	-	-	-	27.2	27.2
15	-	28.0	-	-	-	28.0

Table A5.9. Coefficient of variation by age reader and by modal age in the German sub-sample of whole otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 6	ALL READERS
2	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
3	40.8%	18.0%	14.9%	23.3%	12.0%	20.4%
4	12.4%	26.1%	11.8%	0.0%	11.8%	13.2%
5	16.0%	20.3%	12.4%	14.1%	9.3%	14.1%
6	20.2%	25.1%	16.6%	8.6%	24.8%	17.8%
Weighted mean	21.1%	19.3%	12.3%	11.1%	12.6%	15.3%
RANK	5	4	2	1	3	

Table A5.10. Percentage agreement by age reader and by modal age in the German sub-sample of whole otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 6	AL READERS
2	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
3	71.4%	71.4%	71.4%	42.9%	85.7%	68.6%
4	60.0%	40.0%	80.0%	100.0%	80.0%	72.0%
5	60.0%	60.0%	40.0%	60.0%	80.0%	60.0%
6	20.0%	60.0%	60.0%	60.0%	80.0%	56.0%
7	100.0%	0.0%	0.0%	100.0%	100.0%	60.0%
Weighted mean	60.0%	60.0%	64.0%	68.0%	84.0%	67.2%
RANK	4	4	3	2	1	

Table A5.11. Mean length at age by age reader in the German sub-sample of whole otoliths.

AGE	READER 1	READER 2	READER 3	READER 4	READER 6	ALL READERS
2	19.5	21.8	19.5	19.5	19.5	20.4
3	24.0	25.1	23.5	24.0	24.4	24.3
4	24.7	27.3	26.5	25.1	25.5	25.8
5	27.3	33.0	33.3	27.4	31.3	29.9
6	31.5	33.5	35.0	33.3	34.5	33.7
7	30.3	-	-	33.7	33.0	32.1
8	35.0	-	-	-	-	35.0

Table A5.12. Coefficient of variation by age reader and by modal age in the German sub-sample sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	READER 6	READERS
2	0.0%	0.0%	0.0%	0.0%	47.1%	0.0%	11.1%
3	34.4%	16.8%	15.3%	18.9%	20.6%	14.2%	28.8%
4	15.6%	24.5%	10.6%	9.8%	24.5%	15.8%	17.3%
5	0.0%	26.6%	13.3%	20.0%	17.3%	12.4%	18.3%
6	20.5%	9.1%	10.0%	6.6%	7.0%	10.5%	13.5%
Weighted mean	19.7%	16.7%	11.5%	12.4%	20.0%	12.4%	19.7%
RANK	5	4	1	3	6	2	

Table A5.13. Percentage agreement by age reader and by modal age in the German sub-sample sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	READER 6	ALL READERS
2	100.0%	100.0%	100.0%	100.0%	50.0%	100.0%	91.7%
3	37.5%	75.0%	62.5%	25.0%	25.0%	75.0%	50.0%
4	33.3%	50.0%	83.3%	83.3%	50.0%	66.7%	61.1%
5	100.0%	66.7%	33.3%	33.3%	0.0%	66.7%	50.0%
6	16.7%	66.7%	50.0%	83.3%	83.3%	66.7%	61.1%
Weighted mean	44.0%	68.0%	64.0%	60.0%	44.0%	72.0%	58.7%
RANK	5	2	3	4	5	1	

Table A5.14. Mean length at age by age reader in the German sub-sample of sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	READER 6	ALL READERS
1	-	-	-	-	20.0	-	20.0
2	19.5	21.8	19.5	19.5	23.1	19.5	21.4
3	23.7	25.1	23.5	25.0	27.2	24.7	25.0
4	24.3	27.3	26.5	25.2	27.5	25.0	25.8
5	27.5	33.0	33.3	26.0	37.0	31.3	30.0
6	31.0	33.5	35.0	33.7	33.6	34.5	33.6
7	29.0	-	-	36.0	-	33.0	31.8
8	34.0	-	-	-	-	-	34.0
9	37.0	-	-	-	-	-	37.0

Table A5.15. Coefficient of variation by age reader and by modal age in the Latvian sub-sample of whole otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	All readers
3	23.0%	30.6%	13.2%	15.0%	18.9%
4	21.8%	14.3%	14.3%	11.8%	13.7%
5	6.9%	21.5%	11.9%	7.3%	14.2%
6	0.0%	18.2%	10.0%	7.0%	9.0%
7	13.9%	26.8%	12.5%	7.8%	14.7%
8	7.4%	0.0%	0.0%	0.0%	12.7%
9	8.3%	18.4%	0.0%	8.3%	15.9%
10	23.6%	18.4%	7.4%	28.3%	19.0%
11	6.1%	11.8%	9.4%	0.0%	20.2%
Weighted mean	12.7%	20.0%	10.7%	9.2%	14.7%
RANK	3	4	2	1	

Table A5.16. Percentage agreement by age reader and by modal age in the Latvian sub-sample of whole otoliths.

Modal ge	Reader 1	Reader 2	Reader 3	Reader 4	All readers
3	85.7%	71.4%	85.7%	42.9%	71.4%
4	62.5%	62.5%	62.5%	62.5%	62.5%
5	87.5%	25.0%	50.0%	87.5%	62.5%
6	100.0%	50.0%	50.0%	83.3%	70.8%
7	36.4%	45.5%	72.7%	72.7%	56.8%
8	0.0%	100.0%	0.0%	100.0%	50.0%
9	50.0%	0.0%	100.0%	50.0%	50.0%
10	50.0%	50.0%	50.0%	50.0%	50.0%
11	50.0%	50.0%	0.0%	100.0%	50.0%
12	100.0%	0.0%	0.0%	100.0%	50.0%
13	-	-	-	-	-
14	100.0%	0.0%	0.0%	100.0%	50.0%
Weighted mean	66.0%	48.0%	58.0%	72.0%	61.0%
RANK	2	4	3	1	

Table A5.17. Mean length at age by age reader in the Latvian sub-sample of whole otoliths.

AGE	READER 1	READER 2	READER 3	READER 4	ALL READERS
1	-	10.2	-	-	10.2
2	-	14.2	10.2	-	12.2
3	14.1	15.9	14.7	13.1	14.9
4	16.3	20.5	19.5	16.1	18.3
5	20.6	23.6	22.6	20.6	21.4
6	22.7	24.5	25.7	24.9	24.1
7	24.5	24.7	26.8	26.5	26.0
8	28.1	28.0	34.5	26.7	28.2
9	26.7	27.1	31.2	31.3	29.0
10	29.3	30.8	34.3	31.8	31.6
11	28.5	34.5	-	31.5	31.5
12	33.6	34.3	-	32.6	33.5
13	-	29.5	-	-	29.5
14	35.2	-	-	34.3	34.9
15	-	-	-	36.1	36.1

Table A5.18. Coefficient of variation by age reader and by modal age in the Latvian sub-sample of sectioned otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	READER 5	READER 6	ALL READERS
3	22.0%	12.3%	0.0%	11.3%	12.3%	12.3%	9.4%
4	20.3%	11.7%	12.5%	10.4%	11.6%	15.5%	12.7%
5	0.0%	22.5%	16.3%	0.0%	10.5%	29.6%	12.9%
6	7.2%	27.2%	14.1%	7.2%	26.4%	25.9%	18.0%
7	15.0%	37.4%	14.2%	5.1%	35.0%	16.0%	14.8%
8	0.0%	47.1%	0.0%	0.0%	50.9%	8.3%	25.1%
10	31.5%	0.0%	34.3%	40.0%	5.4%	19.2%	28.3%
11	6.7%	6.7%	17.7%	31.4%	0.0%	0.0%	14.8%
14	8.7%	28.9%	12.5%	14.3%	7.9%	17.8%	23.6%
Weighted mean	13.0%	18.5%	10.8%	9.8%	16.0%	14.8%	16.5%
RANK	3	6	2	1	5	4	

Table A5.19. Percentage agreement by age reader and by modal age in the Latvian sub-sample of sectioned otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All readers
2	0.0%	0.0%	100.0%	0.0%	100.0%	100.0%	50.0%
3	75.0%	87.5%	100.0%	87.5%	87.5%	87.5%	87.5%
4	55.6%	77.8%	77.8%	77.8%	44.4%	66.7%	66.7%
5	100.0%	50.0%	50.0%	100.0%	75.0%	0.0%	62.5%
6	80.0%	40.0%	20.0%	80.0%	20.0%	20.0%	43.3%
7	62.5%	50.0%	50.0%	87.5%	37.5%	25.0%	52.1%
8	0.0%	50.0%	0.0%	100.0%	50.0%	50.0%	41.7%
9	100.0%	0.0%	0.0%	100.0%	0.0%	0.0%	33.3%
10	33.3%	100.0%	33.3%	33.3%	33.3%	66.7%	50.0%
11	50.0%	50.0%	0.0%	50.0%	100.0%	0.0%	41.7%
12	100.0%	0.0%	0.0%	100.0%	0.0%	0.0%	33.3%
13	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%	33.3%
14	66.7%	66.7%	0.0%	0.0%	66.7%	33.3%	38.9%
15	100.0%	100.0%	0.0%	0.0%	0.0%	0.0%	33.3%
Weighted mean	64.0%	60.0%	48.0%	70.0%	50.0%	44.0%	56.0%
RANK	2	3	5	1	4	6	

Table A5.20. Mean length at age by age reader in the Latvian sub-sample of sectioned otoliths.

Age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All readers
1	-	10.2	-	-	-	-	10.2
2	-	14.2	10.2	-	12.3	11.3	11.9
3	13.8	15.9	14.7	13.9	14.8	15.8	15.0
4	18.6	20.5	19.5	18.6	18.8	21.2	19.7
5	21.0	23.6	22.6	21.9	21.2	20.7	21.8
6	20.4	24.6	24.6	23.0	22.5	24.9	23.4
7	24.9	25.0	27.4	25.5	24.0	25.4	25.6
8	26.5	26.7	34.5	28.0	26.1	26.7	27.2
9	26.4	-	31.2	25.2	-	28.7	27.8
10	27.2	29.5	34.3	27.3	23.1	32.6	30.2
11	31.3	27.1	-	30.9	30.5	31.1	30.4
12	31.1	-	-	33.0	-	-	32.2
13	28.5	31.8	-	-	31.8	28.5	30.2
14	32.4	32.5	-	36.1	32.5	30.5	32.4
15	32.6	29.9	-	-	-	-	30.8
16	-	27.6	-	34.3	26.9	-	28.7
17	-	34.3	-	-	27.6	-	29.8
18	34.3	-	-	-	33.5	-	33.7
19	23.1	-	-	-	-	-	23.1

Table A5.21. Coefficient of variation by age reader and by modal age in the Swedish sub-sample of whole otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	ALL READERS
4	17.5%	57.0%	10.6%	0.0%	19.0%
5	11.5%	30.8%	15.7%	7.6%	28.3%
6	7.0%	28.4%	11.9%	0.0%	18.9%
7	6.0%	22.8%	16.9%	19.0%	16.9%
8	0.0%	12.7%	7.2%	12.1%	18.9%
9	-	-	-	-	-
10	0.0%	23.6%	20.2%	25.0%	24.5%
Weighted mean	8.5%	29.6%	13.2%	7.0%	22.0%
RANK	2	4	3	1	

Table A5.22. Percentage agreement by age reader and by modal age in the Swedish sub-sample of whole otoliths.

MODAL AGE	READER 1	READER 2	READER 3	READER 4	ALL READERS
4	50.0%	50.0%	83.3%	100.0%	70.8%
5	68.8%	12.5%	87.5%	86.7%	63.5%
6	76.9%	8.3%	38.5%	100.0%	56.9%
7	83.3%	50.0%	33.3%	50.0%	54.2%
8	100.0%	0.0%	60.0%	60.0%	55.0%
9	100.0%	100.0%	0.0%	0.0%	50.0%
10	100.0%	50.0%	0.0%	50.0%	50.0%
Weighted mean	75.5%	22.9%	59.2%	81.3%	59.8%
RANK	2	4	3	1	

Table A5.23. Mean length at age by age reader in the Swedish sub-sample of whole otoliths.

AGE	READER 1	READER 2	READER 3	READER 4	ALL READERS
3	-	23.7	22.5	-	23.4
4	24.0	24.5	23.9	23.8	24.0
5	25.2	24.5	25.5	25.6	25.4
6	25.9	25.8	26.4	26.2	26.1
7	26.3	26.2	28.3	27.4	26.8
8	28.6	26.8	28.6	28.9	28.1
9	25.7	25.9	-	-	25.8
10	27.3	26.4	-	28.0	26.7
11	-	26.3	-	-	26.3
12	-	27.0	-	-	27.0
13	-	26.9	-	-	26.9
14	-	28.0	-	-	28.0

Table A5.24. Coefficient of variation by age reader and by modal age in the Swedish sub-sample of sectioned otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All readers
4	11.8%	13.3%	0.0%	11.8%	0.0%	16.3%	19.3%
5	0.0%	47.1%	0.0%	12.9%	40.4%	23.6%	24.5%
6	6.6%	31.0%	19.0%	0.0%	16.9%	12.2%	14.5%
7	5.0%	19.2%	13.7%	17.5%	22.0%	15.0%	16.2%
8	12.4%	14.1%	20.0%	7.3%	9.8%	9.6%	16.4%
9	6.7%	10.0%	0.0%	12.5%	6.0%	6.7%	22.3%
10	5.1%	22.2%	9.5%	6.9%	15.9%	20.7%	27.2%
11	0.0%	11.8%	0.0%	15.7%	0.0%	22.3%	20.0%
12	7.4%	8.0%	16.7%	15.3%	0.0%	14.1%	26.7%
13	0.0%	0.0%	10.8%	6.9%	0.0%	11.2%	29.7%
14	17.0%	9.4%	32.6%	0.0%	13.7%	30.7%	30.6%
15	3.9%	10.8%	24.7%	12.4%	7.1%	13.3%	30.5%
Weighted mean	6.3%	15.9%	12.4%	9.4%	10.7%	14.0%	22.0%
RANK	1	6	4	2	3	5	

Table A5.25. Percentage agreement by age reader and by modal age in the Swedish sub-sample of sectioned otoliths.

Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All readers
3	0.0%	100.0%	100.0%	0.0%	100.0%	0.0%	50.0%
4	75.0%	75.0%	100.0%	75.0%	0.0%	25.0%	58.3%
5	100.0%	50.0%	0.0%	50.0%	50.0%	50.0%	50.0%
6	83.3%	33.3%	0.0%	100.0%	66.7%	50.0%	55.6%
7	87.5%	62.5%	12.5%	62.5%	42.9%	42.9%	52.2%
8	50.0%	50.0%	16.7%	50.0%	66.7%	50.0%	47.2%
9	66.7%	33.3%	0.0%	33.3%	33.3%	66.7%	38.9%
10	75.0%	75.0%	0.0%	0.0%	25.0%	25.0%	33.3%
11	100.0%	50.0%	0.0%	0.0%	100.0%	50.0%	50.0%
12	25.0%	75.0%	0.0%	0.0%	100.0%	25.0%	37.5%
13	0.0%	100.0%	0.0%	0.0%	100.0%	0.0%	33.3%
14	50.0%	50.0%	0.0%	0.0%	50.0%	50.0%	33.3%
15	66.7%	66.7%	0.0%	0.0%	33.3%	33.3%	33.3%
Weighted mean	62.0%	58.0%	14.0%	38.0%	53.1%	36.7%	43.6%
RANK	1	2	6	4	3	5	

Table A5.26. Mean length at age by age reader in the Swedish sub-sample of sectioned otoliths.

AGE	READER 1	READER 2	READER 3	READER 4	READER 5	READER 6	ALL READERS
3	-	23.7	22.5	-	24.2	-	23.9
4	24.4	24.5	23.8	24.4	24.2	23.8	24.1
5	22.8	24.0	25.6	22.7	22.9	23.9	24.8
6	25.5	25.6	26.4	25.4	25.9	25.0	25.6
7	26.2	26.4	28.3	25.7	26.1	25.5	26.2
8	26.0	25.9	28.6	26.3	26.2	26.4	26.5
9	24.9	25.4	-	26.6	24.6	26.6	25.8
10	25.6	25.1	-	29.0	25.7	26.4	26.3
11	28.3	27.0	-	-	27.7	26.7	27.6
12	25.9	26.8	-	-	26.7	27.1	26.7
13	26.5	26.5	-	-	25.9	-	26.2
14	27.2	27.4	-	-	28.3	26.7	27.3
15	29.2	27.3	-	-	26.9	29.7	28.1
16	-	26.7	-	-	-	-	26.7
17	-	28.0	-	-	28.3	-	28.2
18	-	28.1	-	-	26.5	-	-

Table A5.27. Coefficient of variation by age reader and by modal age in the Latvian sample of whole otoliths.

Modal age	Reader 1	Reader 3	Reader 4	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	All readers
3	22.1%	10.3%	16.4%	14.5%	18.9%	19.5%	31.1%	18.2%	19.2%	15.9%	18.3%
4	18.9%	24.1%	28.1%	10.6%	6.0%	6.0%	34.8%	31.3%	36.1%	14.8%	21.3%
5	16.0%	9.8%	28.9%	0.0%	8.6%	0.0%	18.9%	12.1%	15.3%	17.4%	20.8%
6	10.2%	0.0%	10.2%	0.0%	0.0%	0.0%	12.5%	12.5%	12.4%	10.8%	21.1%
Weighted mean	18.7%	14.2%	20.9%	10.2%	11.3%	10.7%	28.7%	21.2%	23.7%	14.7%	19.8%
RANK	6	4	7	1	3	2	10	8	9	5	

Table A5.28. Percentage agreement by age reader and by modal age in the Latvian sample of whole otoliths.

Modal age	Reader 1	Reader 3	Reader 4	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	All readers
2	100%	100%	100%	100%	100%	100%	0%	0%	0%	100%	70%
3	59%	91%	77%	82%	68%	68%	32%	36%	59%	77%	65%
4	56%	61%	44%	83%	94%	94%	35%	50%	56%	39%	61%
5	60%	40%	60%	100%	80%	100%	60%	0%	0%	40%	54%
6	67%	100%	67%	100%	100%	100%	0%	0%	0%	33%	57%
7	0%	100%	0%	100%	100%	100%	0%	0%	0%	0%	40%
Weighted mean	58.0%	76.0%	62.0%	86.0%	82.0%	84.0%	32.7%	34.0%	46.0%	56.0%	61.7%
RANK	6	4	5	1	3	2	10	9	8	7	

Table A5.29. Mean length at age by age reader in the Latvian sample of whole otoliths.

AGE	READER 1	READER 3	READER 4	READER 7	READER 8	READER 9	READER 10	READER 11	READER 12	READER 13	ALL READERS
1	-	-	-	-	-	-	14.4	-	-	-	14.4
2	15.4	14.6	14.7	15.7	16.5	16.2	17.2	-	-	16.3	15.9
3	18.4	18.8	18.8	19.0	19.1	19.4	20.3	16.6	18.5	19.4	18.9
4	22.0	23.5	23.5	22.6	22.0	22.1	20.6	20.6	20.4	24.6	22.0
5	27.0	26.3	26.9	28.6	30.6	29.9	22.8	21.9	17.6	28.5	25.7
6	30.1	30.4	29.6	28.4	28.1	28.4	23.0	25.3	25.4	33.2	28.9
7	39.0	32.3	33.1	31.4	31.4	31.4	29.0	28.3	28.9	-	30.7
8	-	-	-	-	-	-	30.4	28.4	30.5	-	29.3
9	-	-	39.0	-	-	-	26.8	26.2	27.3	-	28.9
10	-	-	-	-	-	-	-	-	27.4	-	27.4

Table A5.30. Coefficient of variation by age reader and by modal age in the Lithuanian sample of whole otoliths.

MODAL AGE	READER 4	READER 7	READER 8	READER 9	READER 10	READER 11	READER 13	ALL READERS
2	21.4%	16.6%	0.0%	0.0%	31.8%	31.3%	50.4%	25.6%
3	14.6%	8.4%	11.2%	0.0%	24.0%	22.7%	20.9%	18.4%
4	34.7%	14.6%	0.0%	0.0%	27.2%	18.4%	36.2%	23.2%
5	25.9%	11.9%	0.0%	9.3%	39.1%	8.6%	26.1%	22.3%
6	28.3%	0.0%	0.0%	12.9%	0.0%	9.4%	28.3%	18.3%
7	0.0%	7.9%	7.9%	0.0%	27.0%	0.0%	16.7%	17.4%
Weighted mean	22.0%	11.2%	3.8%	1.4%	26.0%	18.6%	30.0%	21.5%
RANK	5	3	2	1	6	4	7	

Table A5.31. Percentage agreement by age reader and by modal age in the Lithuanian sample of whole otoliths.

MODAL AGE	READER 4	READER 7	READER 8	READER 9	READER 10	READER 11	READER 13	ALL READERS
1	0%	100%	100%	100%	100%	100%	100%	86%
2	50%	88%	100%	100%	63%	75%	25%	71%
3	67%	93%	87%	100%	53%	20%	47%	67%
4	53%	67%	100%	100%	27%	60%	0%	58%
5	20%	60%	100%	80%	40%	80%	0%	54%
6	50%	100%	100%	50%	100%	0%	50%	64%
7	100%	67%	67%	100%	33%	0%	33%	57%
8	0%	100%	100%	100%	0%	0%	0%	43%
Weighted mean	54.0%	80.0%	94.0%	96.0%	46.0%	46.0%	24.0%	62.9%
RANKING	4	3	2	1	5	5	7	

Table A5.32. Mean length at age by age reader in the Lithuanian sample of whole otoliths.

AGE	READER 4	READER 7	READER 8	READER 9	READER 10	READER 11	READER 13	ALL READERS
1	-	8.0	8.0	8.0	10.3	8.0	10.7	9.8
2	10.4	12.4	12.6	12.6	15.4	11.6	14.9	13.5
3	15.5	17.1	16.5	16.8	18.1	14.6	20.1	17.2
4	20.4	20.8	19.7	20.1	23.0	18.0	28.2	20.2
5	25.0	19.1	22.5	25.5	27.5	19.8	32.0	22.2
6	29.8	34.8	34.8	29.5	30.8	23.8	35.3	31.7
7	32.4	31.5	31.5	32.2	32.0	33.3	33.5	32.3
8	37.0	33.3	33.3	33.0	-	40.0	-	34.7
9	40.0	-	-	-	-	32.4	-	33.9

The presented figures are

Fig A5.1	The age bias plots for the Estonian sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.2	The age bias plots for the Estonian sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.3	The age bias plots for the German sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.4	The age bias plots for the German sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.5	The age bias plots for the Latvian sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.6	The age bias plots for the Latvian sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.7	The age bias plots for the Swedish sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.8	The age bias plots for the Swedish sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.9	The age bias plots for the Latvian sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
Fig A5.10	The age bias plots for the Lithuanian sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

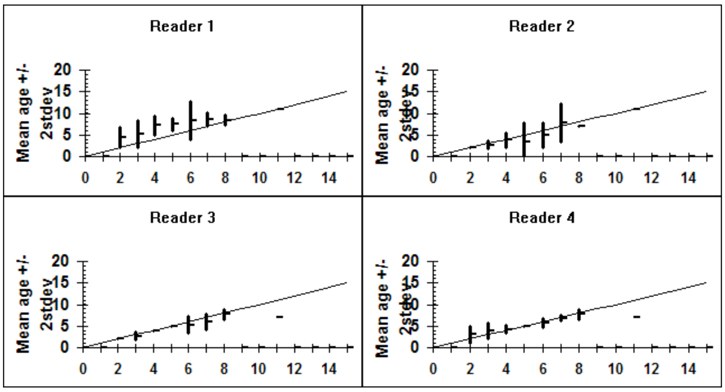


Figure A5.1. The age bias plots for the Estonian sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

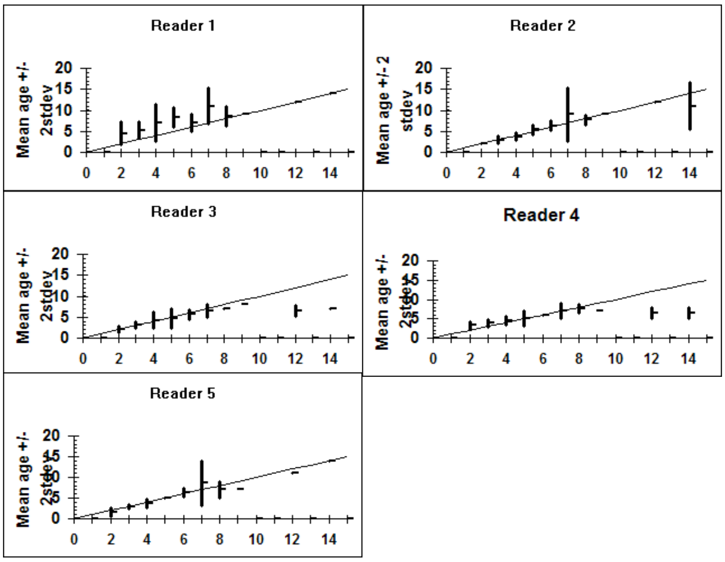


Figure A5.2. The age bias plots for the Estonian sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.

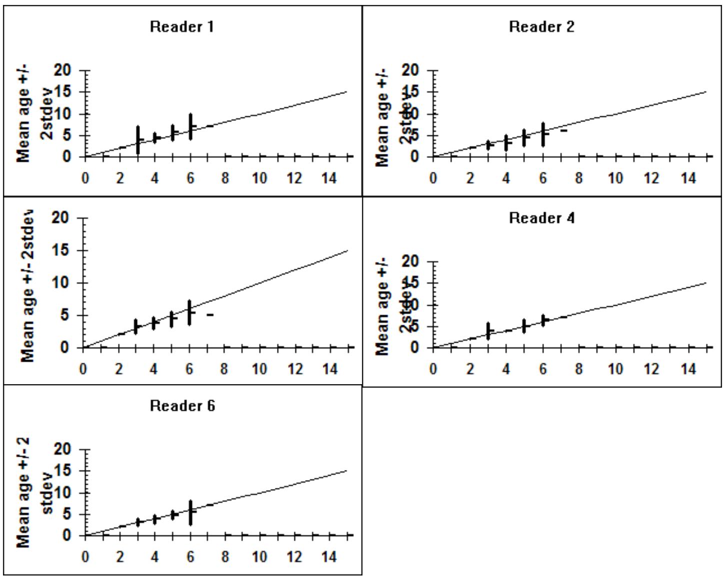


Figure A5.3. The age bias plots for the German sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

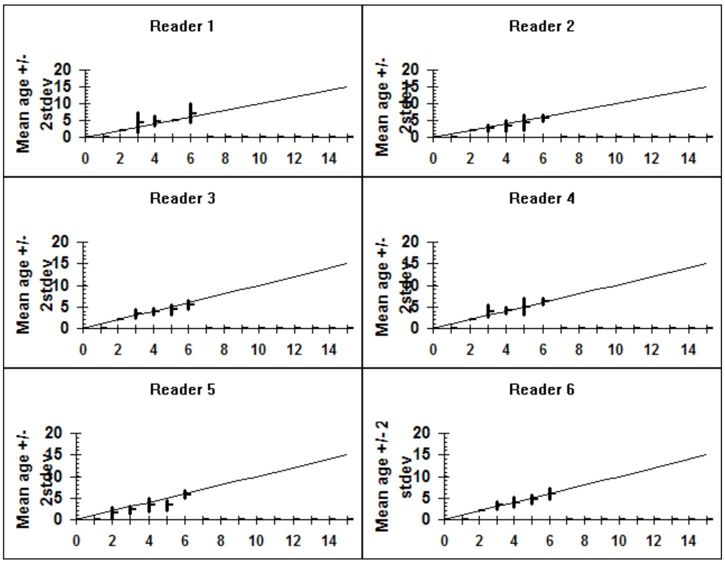


Figure A5.4. The age bias plots for the German sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.

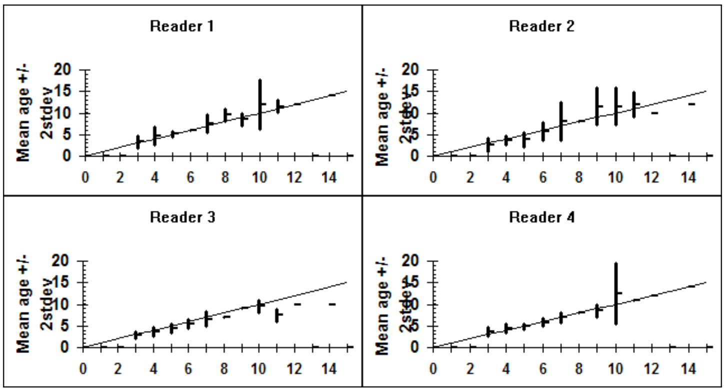


Figure A5.5. The age bias plots for the Latvian sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

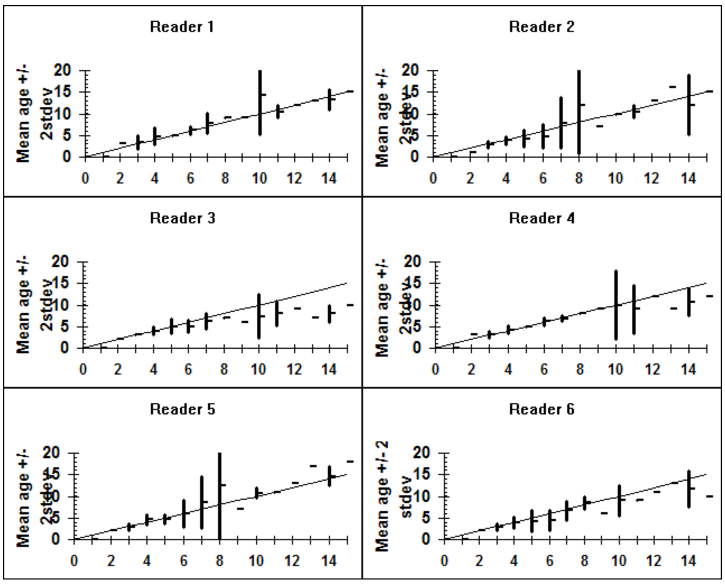


Figure A5.6. The age bias plots for the Latvian sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.

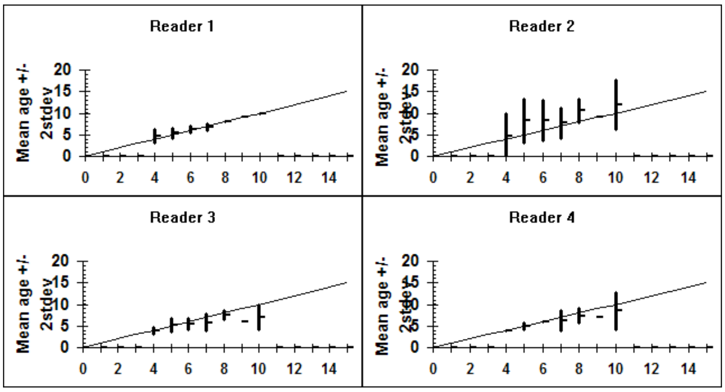


Figure A5.7. The age bias plots for the Swedish sub-sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

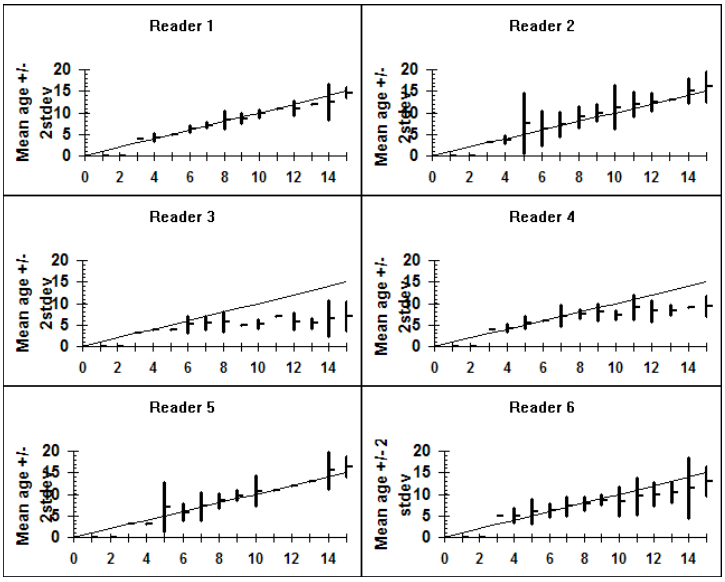


Figure A5.8. The age bias plots for the Swedish sub-sample of sectioned otoliths. The mean age recorded +/- 2stdev of each age reader.

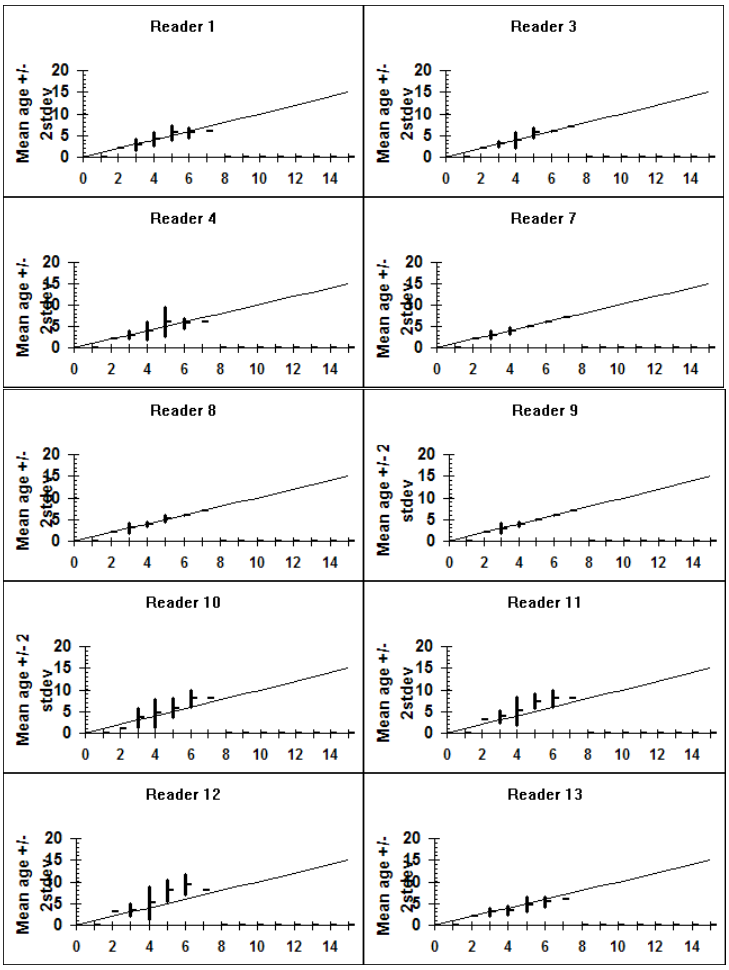


Figure A5.9. The age bias plots for the Latvian sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

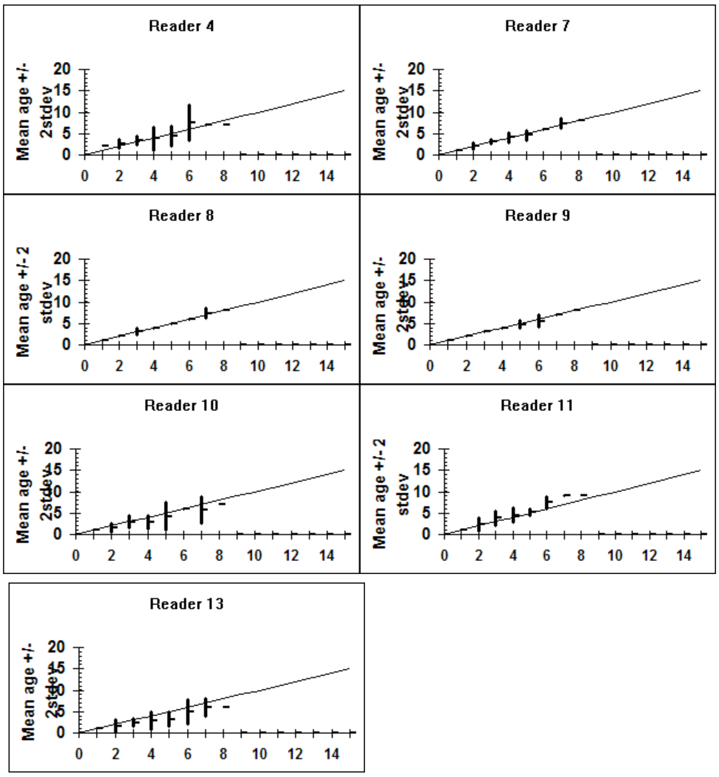


Figure A5.10. The age bias plots for the Lithuanian sample of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

Annex 6: Detailed results from the method experiment during the workshop for different preparation techniques

The annex contains detailed results (tables and figures) from the workshop experiment on age determination by whole, burnt and sectioned flounder otoliths from a Swedish sample taken in Sub-division 28 July, 2006.

The presented tables in the annex are:

A6.1	Coefficient of variance (CV) and percentage agreement by modal age in three sets of otoliths prepared with different techniques: sectioned & stained, broken & burnt and whole otoliths.
A6.2	Coefficient of variance by reader and modal age for age determination of whole otoliths.
A6.3	Percent agreement by reader and modal age for age determination of whole otoliths.
A6.4	Coefficient of variance by reader and modal age for age determination of broken and burnt otoliths.
A6.5	Percent agreement by reader and modal age for age determination of broken and burnt otoliths.
A6.6	Coefficient of variance by reader and modal age for age determination of sectioned and stained otoliths.
A6.7	Percent agreement by reader and modal age for age determination of sectioned and stained otoliths.

The presented figures in the annex are:

A6.1	The age bias plots for the age determination experiment of whole otoliths. The mean age recorded +/- 2stdev of each age reader.
A6.2	The age bias plots for the age determination experiment of broken and burnt otoliths. The mean age recorded +/- 2stdev of each age reader.
A6.3	The age bias plots for the age determination experiment of sectioned and stained otoliths. The mean age recorded +/- 2stdev of each age reader.

Table A6.1. Coefficient of variation (CV) and percentage agreement by modal age in three sets of otoliths prepared with different technique: sectioned & stained, broken & burnt and whole.

	Sectioned		Burnt		Whole	
Modal age	CV	% Agreement	CV	% Agreement	CV	% Agreement
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	50%	-	-	-	-
3	-	50%	-	43%	28%	53%
4	29%	46%	19%	47%	23%	45%
5	-	60%	-	43%	23%	41%
6	15%	55%	18%	42%	23%	41%
7	14%	46%	22%	38%	41%	31%
8	-	-	20%	34%	18%	36%
9	12%	53%	30%	33%	21%	32%
10	-	-	-	-		36%
11	-	-	-	-		-
12	-	43%	-	43%		21%
13	-	-	-	-		-
14	-	57%	-	-		-
15	-	-	-	-		-
Weighted mean	16%	51%	20%	40%	25%	39%

Table A6.2. Coefficient of variation by reader and modal age for age determination by whole otoliths

Modal	Reader number														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0%	15%	29%	15%	18%	18%	46%	36%	23%	0%	48%	15%	43%	13%	28%
4	24%	17%	19%	27%	14%	31%	29%	13%	12%	22%	22%	16%	22%	22%	23%
5	38%	20%	15%	39%	27%	24%	29%	33%	17%	19%	14%	21%	20%	24%	23%
6	11%	32%	15%	16%	27%	29%	22%	23%	8%	17%	7%	11%	27%	27%	23%
7	0%	21%	14%	19%	139%	11%	32%	33%	11%	27%	5%	7%	26%	20%	41%
8	21%	19%	14%	24%	15%	26%	20%	16%	24%	16%	14%	4%	12%	19%	18%
9	28%	32%	5%	8%	34%	17%	19%	15%	16%	17%	22%	7%	6%	12%	21%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-15	20%	21%	15%	23%	35%	23%	26%	23%	15%	17%	16%	12%	21%	20%	25%
RANK	7	9	3	10	14	11	13	12	2	5	4	1	8	6	

Table A6.3. Percent agreement by reader and modal age for age determination of whole otoliths.

MODAL	READER NUMBER														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	100%	75%	25%	75%	75%	75%	25%	25%	25%	100%	0%	75%	50%	25%	53%
4	67%	75%	50%	38%	25%	38%	25%	25%	50%	50%	38%	63%	50%	50%	45%
5	25%	70%	40%	40%	50%	40%	50%	20%	20%	30%	50%	60%	30%	50%	41%
6	57%	25%	63%	50%	25%	0%	50%	38%	50%	0%	75%	63%	38%	38%	41%
7	0%	33%	50%	67%	33%	0%	33%	17%	50%	0%	17%	33%	50%	33%	31%
8	25%	13%	38%	13%	13%	25%	38%	50%	38%	63%	25%	88%	63%	13%	36%
9	0%	50%	75%	25%	25%	0%	0%	25%	25%	25%	25%	50%	75%	50%	32%
10	0%	0%	100%	100%	0%	0%	0%	100%	0%	0%	0%	100%	100%	0%	36%
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	100%	0%	0%	100%	0%	0%	0%	0%	0%	0%	0%	0%	100%	0%	21%
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0-15	40%	46%	48%	44%	32%	24%	34%	30%	36%	34%	36%	62%	50%	36%	39%
RANK	6	4	3	5	12	14	10	13	7	10	7	1	2	7	

Table A6.4. Coefficient of variation by reader and modal age for age determination by broken and burnt otoliths.

MODAL	READER NUMBER														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	25%	12%	31%	37%	16%	16%	29%	18%	20%	22%	17%	12%	12%	25%	19%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	12%	12%	31%	21%	18%	17%	12%	10%	18%	24%	19%	30%	24%	17%	18%
7	29%	7%	12%	12%	42%	15%	23%	23%	8%	23%	6%	0%	27%	14%	22%
8	21%	22%	27%	39%	20%	20%	10%	15%	23%	16%	6%	22%	12%	32%	20%
9	0%	8%	8%	24%	18%	9%	35%	-	37%	14%	0%	11%	31%	0%	30%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0–15	16%	11%	22%	23%	20%	14%	17%	13%	17%	19%	11%	15%	18%	17%	20%
RANK	6	2	13	14	12	4	9	3	7	11	1	5	10	8	

Table A6.5. Percent agreement by reader and modal age for age determination of broken and burned otoliths.

MODAL	READER NUMBER														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	100%	0%	0%	100%	100%	100%	100%	0%	0%	0%	0%	100%	0%	0%	43%
4	80%	60%	40%	0%	40%	40%	60%	60%	80%	40%	40%	60%	40%	20%	47%
5	0%	100%	0%	100%	100%	0%	0%	100%	100%	100%	0%	0%	0%	0%	43%
6	57%	57%	29%	43%	43%	57%	57%	43%	57%	29%	29%	0%	43%	43%	42%
7	0%	75%	50%	50%	25%	50%	50%	50%	50%	50%	25%	0%	25%	25%	38%
8	25%	25%	50%	25%	50%	25%	50%	25%	50%	50%	25%	50%	25%	0%	34%
9	0%	50%	50%	0%	0%	0%	50%	0%	0%	50%	100%	0%	50%	100%	33%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	100%	100%	0%	100%	0%	0%	100%	100%	0%	100%	0%	0%	0%	0%	43%
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0–15	44%	56%	36%	36%	40%	40%	56%	46%	52%	44%	32%	24%	32%	28%	40%
RANK	5	1	9	9	7	7	1	4	3	5	11	14	11	13	

Table A6.6. Coefficient of variation by reader and modal age for age determination of sectioned and stained otoliths.

MODAL	READER NUMBER														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	20%	24%	54%	35%	47%	0%	47%	20%	33%	0%	13%	16%	0%	16%	29%
5	-	12%	20%	33%	25%	12%	27%	0%	10%	0%	0%	29%	0%	0%	-
6	9%	24%	17%	0%	0%	0%	0%	49%	0%	10%	0%	20%	0%	9%	15%
7	9%	15%	8%	9%	27%	0%	9%	9%	7%	13%	7%	9%	8%	8%	14%
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	6%	20%	14%	11%	6%	6%	6%	6%	10%	6%	12%	15%	6%	10%	12%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	7%	0%	0%	0%	7%	0%	6%	6%	7%	0%	7%	7%	13%	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	17%	7%	5%	9%	0%	0%	5%	5%	0%	44%	13%	0%	0%	-
0–15	7%	13%	13%	10%	12%	3%	9%	10%	7%	4%	7%	12%	2%	6%	16%
RANK	5	14	13	9	11	2	8	10	6	3	7	12	1	4	

Table A6.7. Percent agreement by reader and modal age for age determination of sectioned and stained otoliths.

MODAL	READER NUMBER														
age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	all
2	0%	100%	0%	100%	100%	100%	100%	100%	0%	100%	0%	0%	0%	0%	50%
3	0%	0%	0%	0%	100%	100%	100%	100%	0%	100%	0%	0%	100%	100%	50%
4	50%	0%	50%	0%	50%	100%	50%	50%	0%	100%	0%	50%	100%	50%	46%
5	100%	67%	33%	33%	33%	67%	67%	100%	33%	100%	0%	33%	100%	100%	60%
6	67%	33%	67%	100%	0%	100%	100%	67%	0%	67%	0%	0%	100%	67%	55%
7	50%	67%	33%	67%	33%	100%	67%	33%	0%	33%	0%	67%	33%	67%	46%
8	-	100%	100%	0%	0%	100%	0%	0%	0%	0%	0%	100%	0%	0%	-
9	33%	75%	50%	75%	50%	75%	75%	75%	0%	75%	25%	25%	50%	50%	53%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	0%	100%	100%	100%	50%	100%	50%	0%	50%	0%	50%	50%	0%	-
12	0%	100%	0%	100%	100%	0%	100%	0%	0%	100%	0%	0%	100%	0%	43%
13	-	100%	100%	0%	100%	0%	0%	100%	100%	100%	100%	100%	0%	0%	-
14	100%	0%	0%	100%	100%	0%	0%	100%	100%	100%	100%	0%	100%	0%	57%
15	-	0%	0%	50%	0%	0%	100%	50%	50%	100%	0%	0%	100%	100%	-
0–15	47%	48%	44%	60%	48%	68%	72%	64%	16%	76%	12%	32%	68%	52%	51%
RANK	10	8	11	6	8	3	2	5	13	1	14	12	3	7	

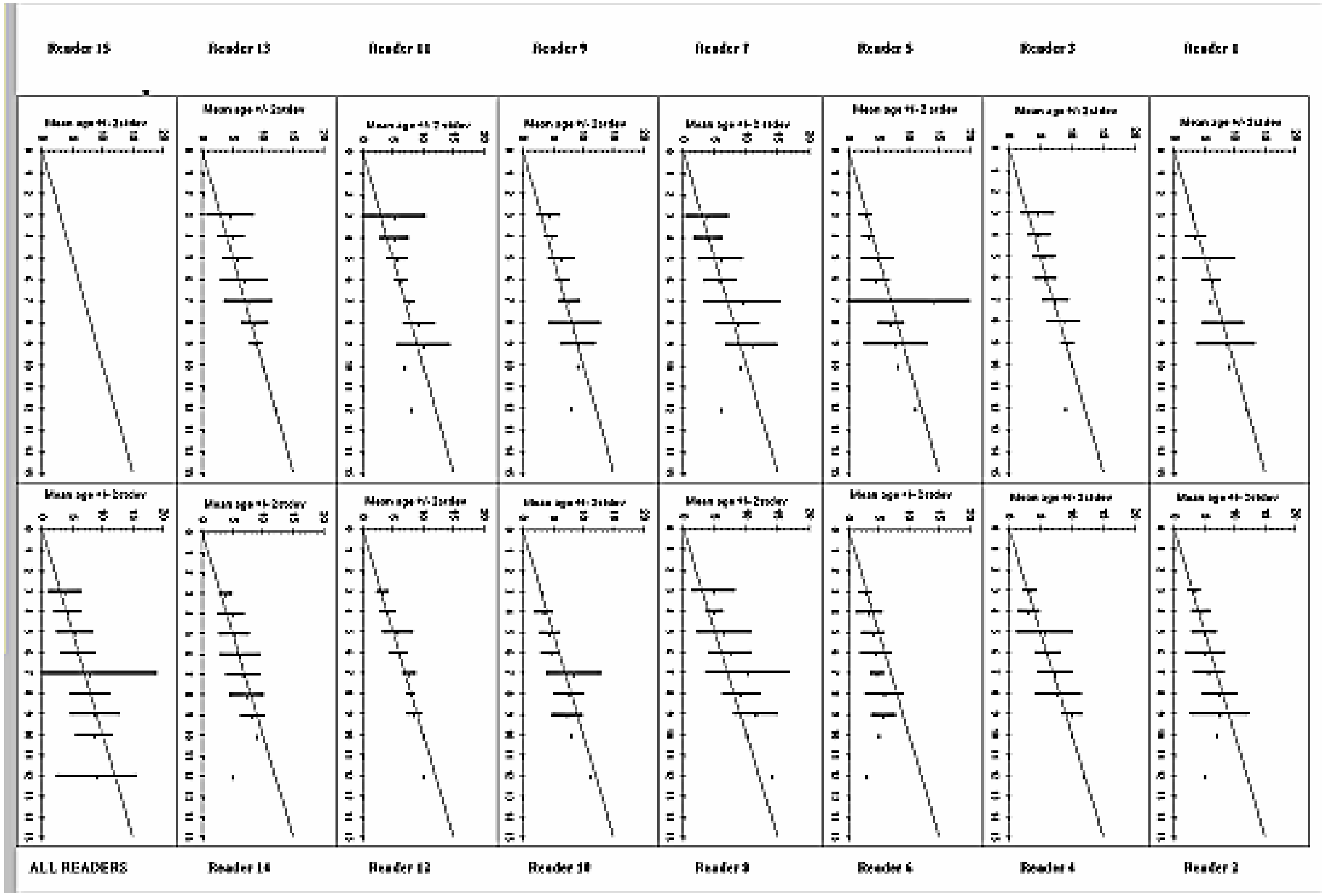


Figure A6.1. The age bias plots for the age determination experiment of whole otoliths. The mean age recorded +/- 2stdev of each age reader.

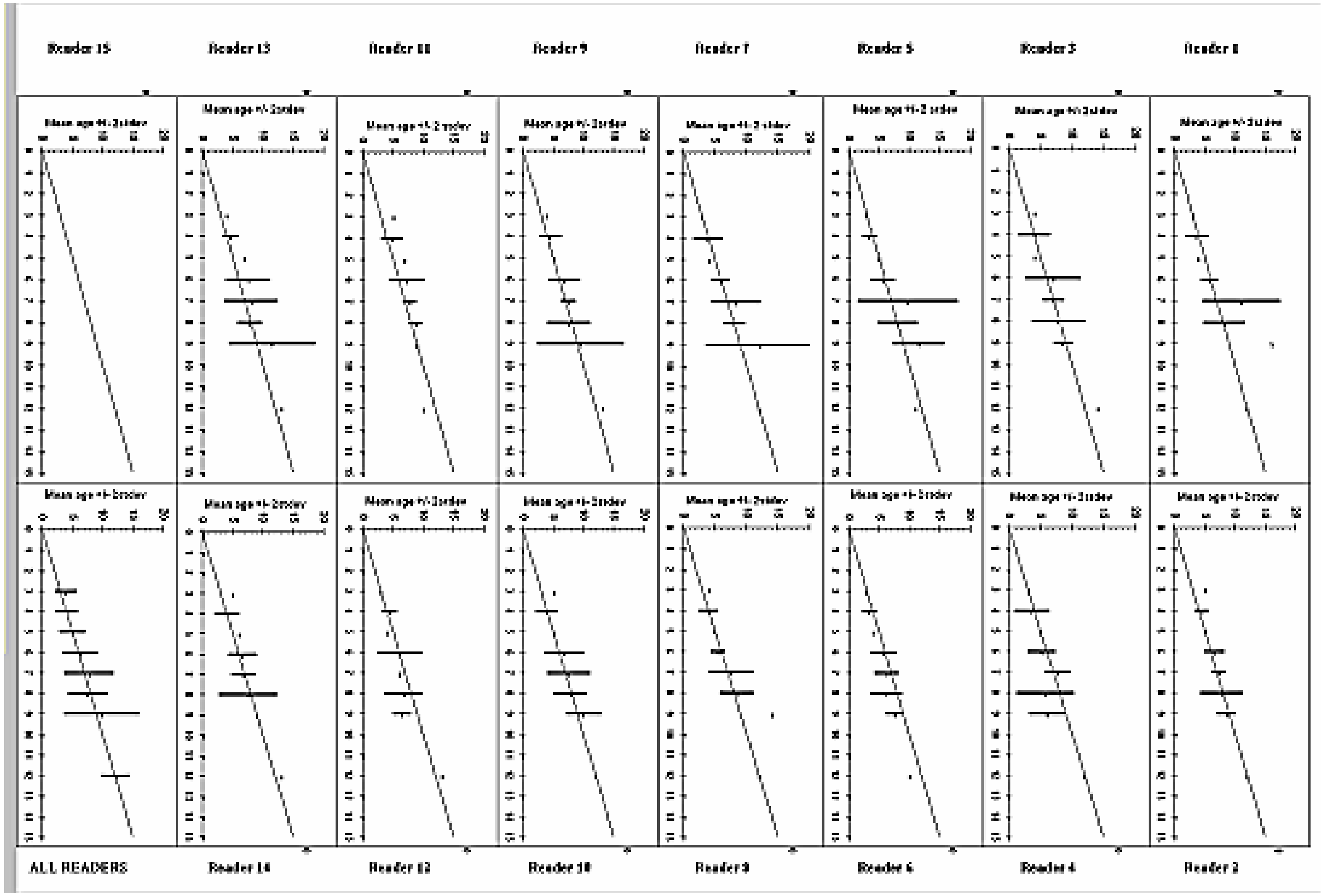


Figure A6.2. The age bias plots for the age determination experiment of broken and burnt otoliths. The mean age recorded +/- 2stdev of each age reader.

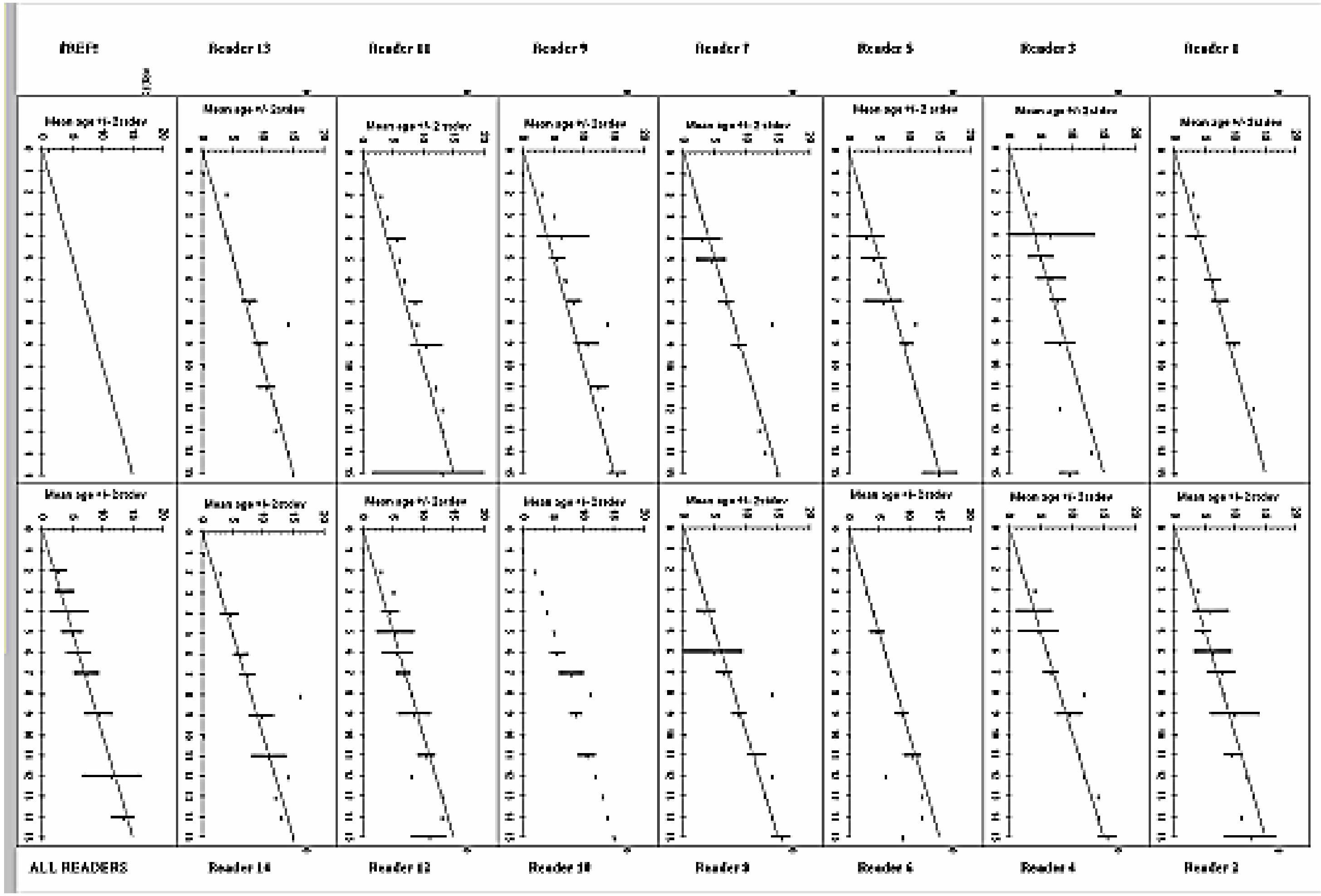


Figure A6.3. The age bias plots for the age determination experiment of sectioned and stained otoliths. The mean age recorded +/- 2stdev of each age reader.

Annex 7: Detailed results from the re-reading experiment of exchange otoliths.

The annex contains detailed results (tables and figures) from the re-reading experiment on age determination by sectioned flounder otoliths. Otoliths were chosen from German sampling in Sub-division 24 during January and August of 2006 and from Swedish sampling in Sub-division 27 in September 2006.

The presented tables in the annex are:

A7.1	Coefficient of variation and percentage agreement in rereading experiment. All 15 readers included.
A7.2	Coefficient of variation by reader and modal age for rereading of German otoliths.
A7.3	Percent agreement by reader and modal age for rereading of German otoliths.
A7.4	Coefficient of variation by reader and modal age for for rereading of Swedish otoliths.
A7.5	Percent agreement by reader and modal age for rereading of Swedish otoliths.

The presented figures in the annex are:

A7.1	The age bias plots for the re-reading experiment of German otoliths. The mean age recorded +/- 2stdev of each age reader.
A7.2	The age bias plots for the re-reading experiment of Swedish otoliths. The mean age recorded +/- 2stdev of each age reader.

Table A7.1. Coefficient of variation (CV) and percentage agreement in rereading experiment. All 15 readers included.

	German slices		Swedish slices	
Modal age	CV	% Agreement	CV	% Agreement
0	-	-	-	-
1	-	-	-	-
2	26%	57%	-	-
3	17%	73%	17%	58%
4	12%	71%	-	47%
5	-	-	-	-
6	8%	81%	-	73%
7	-	-	9%	73%
8	-	-	-	60%
9	-	-	-	73%
10	-	-	14%	36%
11	-	-	-	47%
12	-	-	-	-
13	-	-	8%	60%
14	-	-	-	-
15	-	-	11%	53%
Weighted mean	16%	71%	12%	55%

Table A7.2. Coefficient of variation by reader and modal age for rereading of German otoliths.

MODAL AGE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	21%	14%	24%	20%	20%	25%	0%	25%	21%	0%	25%	0%	0%	21%	16%	26%
3	0%	18%	19%	16%	14%	16%	16%	16%	12%	21%	17%	19%	16%	15%	11%	17%
4	0%	12%	12%	12%	19%	12%	22%	18%	0%	12%	12%	11%	12%	11%	12%	12%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	0%	0%	8%	0%	0%	9%	15%	15%	7%	7%	0%	0%	0%	10%	0%	8%
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0–10	4%	12%	16%	12%	13%	15%	14%	18%	10%	12%	13%	9%	8%	14%	10%	16%
RANK	1	7	14	8	9	13	11	15	5	6	10	3	2	12	4	

Table A7.3. Percent agreement by reader and modal age for rereading of German otoliths.

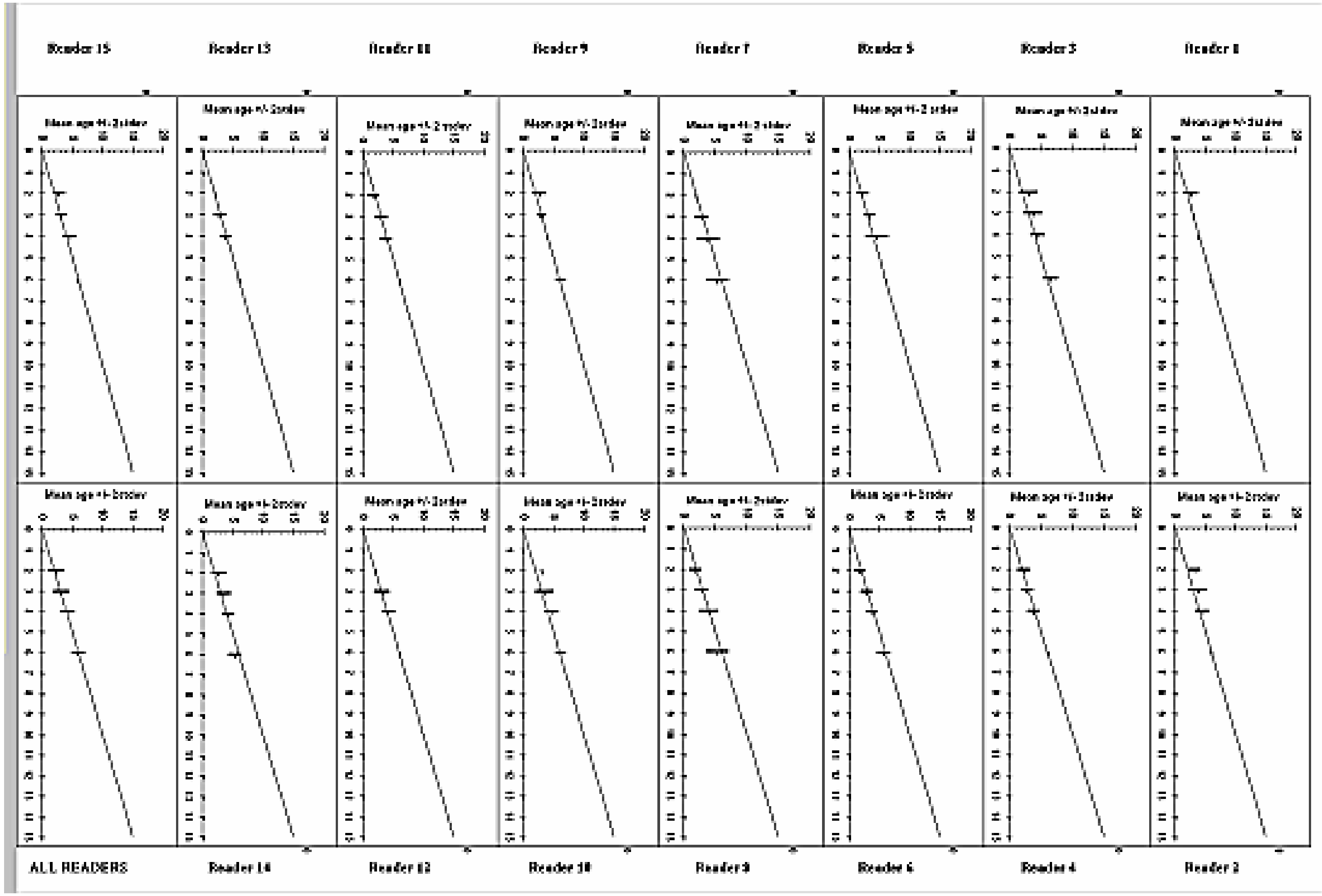
MODAL AGE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	ALL
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	40%	0%	20%	80%	80%	80%	100%	80%	40%	0%	80%	100%	100%	40%	20%	57%
3	100%	33%	44%	78%	78%	78%	78%	78%	89%	67%	78%	67%	78%	56%	89%	73%
4	100%	60%	60%	80%	60%	80%	40%	60%	100%	40%	80%	80%	80%	80%	60%	71%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	100%	100%	50%	100%	100%	67%	50%	50%	83%	83%	100%	100%	100%	33%	100%	81%
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0–10	88%	48%	44%	84%	80%	76%	68%	68%	80%	52%	84%	84%	88%	52%	72%	71%
RANK	1	14	15	3	6	8	10	10	6	12	3	3	1	12	9	

Table A7.4. Coefficient of variation by reader and modal age for rereading of Swedish otoliths.

MODAL AGE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	ALL
3	25%	0%	0%	0%	0%	13%	35%	0%	0%	16%	0%	17%	0%	17%	16%	17%
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	19%	0%	12%	7%	7%	13%	0%	12%	7%	0%	0%	7%	15%	15%	7%	9%
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	16%	15%	10%	15%	17%	6%	18%	16%	13%	12%	5%	6%	13%	13%	19%	14%
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	5%	0%	0%	0%	0%	0%	0%	6%	0%	0%	0%	0%	0%	0%	18%	8%
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	9%	0%	23%	5%	0%	10%	16%	16%	4%	0%	16%	5%	13%	35%	5%	11%
0–15	11%	2%	6%	4%	4%	7%	10%	7%	4%	4%	2%	5%	6%	11%	9%	12%
RANK	15	1	8	5	4	10	13	11	3	6	2	7	9	14	12	

Table A7.5. Percent agreement by reader and modal age for rereading of Swedish otoliths.

MODAL AGE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	ALL
3	33%	100%	0%	100%	100%	0%	33%	100%	0%	33%	100%	67%	100%	67%	33%	58%
4	100%	100%	0%	100%	100%	0%	0%	0%	0%	0%	100%	100%	0%	0%	100%	47%
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	100%	100%	0%	100%	100%	0%	100%	0%	0%	100%	100%	100%	100%	100%	100%	73%
7	75%	100%	50%	75%	75%	25%	100%	50%	75%	100%	100%	75%	75%	50%	75%	73%
8	0%	0%	100%	100%	100%	0%	0%	100%	100%	100%	100%	0%	100%	100%	0%	60%
9	100%	100%	0%	100%	100%	100%	100%	100%	100%	100%	0%	0%	100%	0%	100%	73%
10	0%	33%	33%	33%	33%	67%	33%	0%	33%	67%	33%	67%	33%	0%	67%	36%
11	0%	100%	0%	100%	100%	0%	100%	100%	100%	0%	0%	0%	100%	0%	0%	47%
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	50%	100%	100%	100%	100%	0%	0%	0%	0%	100%	100%	100%	100%	0%	50%	60%
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	50%	100%	0%	50%	100%	50%	50%	50%	0%	100%	50%	50%	50%	50%	50%	53%
0–15	45%	80%	30%	75%	80%	25%	50%	45%	35%	70%	70%	60%	70%	35%	55%	55%
RANK	10	1	14	3	1	15	9	10	12	4	4	7	4	12	8	



Figur A7.1. The age bias plots for the re-reading experiment of German otoliths. The mean age recorded +/- 2stdev of each age reader.

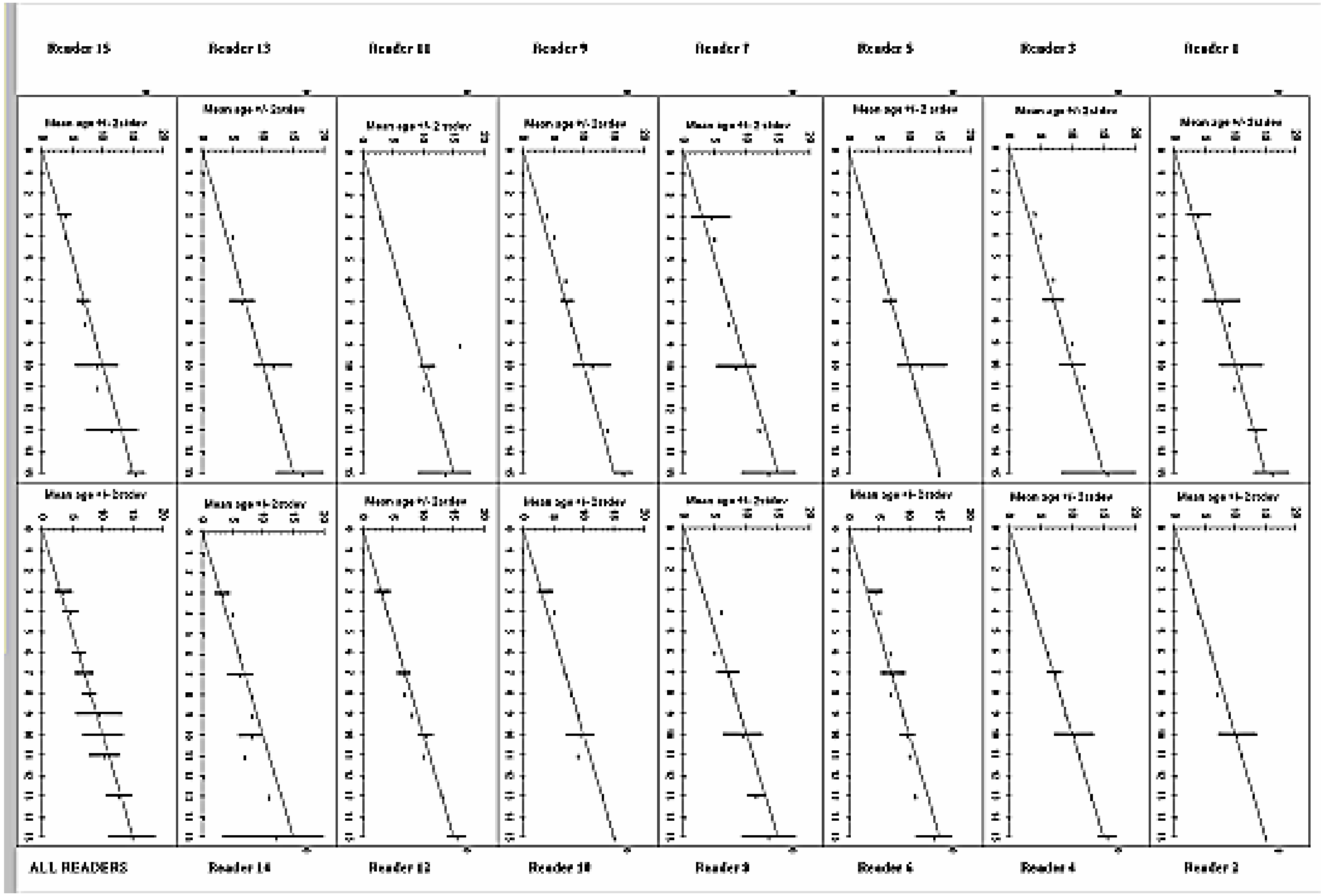


Figure A7.2. The age bias plots for the re-reading experiment of Swedish otoliths. The mean age recorded +/- 2stdev of each age reader.

Annex 8: First manual outline and contents

1. Growth

2. Formation of the opaque and translucent zones

To be copied from the WKARFLO 2007 report

3. Ageing criteria

To be copied from the WKARFLO 2007 report

4. Storage of otoliths

There are a number of storage methods employed by the various countries that collect otoliths from this stock. They range from the traditional paper "envelope" style paper packet to small plastic "self-seal" bags and plastic tubes. Although all of these methods provide for the safe storage of the otolith, it is recommended that paper packets may provide the best alternative. Moisture is easily absorbed by the packet and then evaporated naturally, allowing the otolith to remain dry. This is seen as desirable, as an otolith left in moist conditions will have to be dried out before being read or processed, and prolonged exposure to moisture could alter the appearance of the otolith ring structure. Further, storage in these packets allows them to be stored in small, space saving boxes. The tubes and plastic bags retain moisture and are more awkward to handle.

5. Methods of otolith preparation for flounder age determination

There are several methods of preparation that are commonly used in flatfish species. There relative merits with regard to flounder age determination were discussed at the WKARFLO workshop in Oregrund, Sweden in 2007 and agreed to be as follows:

Whole otoliths

Features:

1) A choice of symmetrical and asymmetrical otoliths to determine the age.

2) It is necessary to follow a ring around the whole of the otolith. A ring should be treated as a true annulus if it is consistent around the whole of the otolith.

3) This method has been most commonly used among institutes reading Baltic flounder otoliths in the past.

Advantages:

1) It is the fastest method for age determination, as almost no preparation is needed.

2) It is also the cheapest method. No additional equipment is required.

3) Most readers are familiar with this method.

4) Storing whole otoliths has an advantage as they can be stored in a variety of ways, using little space.

5) Whole otolith can be used for other methods later if required (sectioned, broken, etc.)

6) If both otoliths are broken (such as during extraction), there is still quite a good chance to accurately determine the age.

Disadvantages:

1) Lowest agreement rates between readers, according to WKARFLO 2007 in Oregrund, Sweden. Because of the difficulties with agreement, this method is not recommended.

2) There is a significant possibility that reading whole otoliths will lead to underaging, particularly of older fish, due to “cliff edge effect” where the otolith lays down growth by thickening of the otolith rather than continuing to grow outwards.

3) If visible, the so-called “metamorphosis ring” can influence age interpretation, because it can be treated as a winter ring when it is really still part of the first summer growth (year zero).

Sectioned and stained otoliths

Features:

1) The stain highlights the boundary at the end of the translucent zone and the beginning of the opaque zone.

2) Knowing how to deal with “cliff edge“ effect could improve age determination, because this method shows all rings.

3) “Lip shape” ring pattern. Only rings of lip shape should be treated as true rings. Round ones should be ignored in the middle.

Advantages:

1) The stain is equally visible in older and younger fish.

2) Agreement at WKARFLO 2007 was seen to be higher using this method than other methods, despite a low level of expertise.

3) Proven track record in providing high levels of agreement in many species of flat fish from a number of areas.

4) Ageing by one reader is easily checked by another reader as several otoliths are in a strip (typically 10–12) and therefore there is less changing of material under the microscope.

Disadvantages:

1) Otoliths are fixed in resin and cannot be moved to aid interpretation of edge, etc.

2) Flounder otoliths from some areas do not stain very satisfactorily, but further experiments with stains and during of staining could improve this considerably.

3) Storage needs to be handled carefully as slices are fragile and prone to breaking.

4) Stain can “leech” out if water or other liquid is applied to the surface as an aid to interpretation (but sections can be re-stained).

5) The cost is much higher than other methods and requires specialist equipment and technical skills in preparation.

6) The time required to process the otoliths is much increased over other methods but ageing time is reduced.

Broken and burnt otoliths

Features:

1) The burn highlights the boundary at the end of the translucent zone and the beginning of the opaque zone.

2) “Lip shape” ring pattern. Only rings of lip shape should be treated as true rings. Round ones should be ignored in the middle.

Advantages:

1) Cost is low.

2) Time requirement for age reading process is lower than in cut and stained method, but higher than in the whole otolith method.

3) A correctly burnt section can show very similar structure to the sectioned and stained slices.

4) Easy to learn.

Disadvantages:

1) Risk of membranes (if they weren't removed thoroughly) being burnt and interpreted as a protein band on the edge.

2) Risk of incorrectly burning (e.g. too long will result in broken pieces, too short will not show up the rings clearly).

3) Re-reading and checking of samples is more difficult due to possible deterioration or loss of burnt pieces.

Conclusion

After reading samples of otoliths prepared in all the above methods, the WKARFLO workshop agreed that the preferred method of reading Baltic flounder otoliths was the sectioned and stained method. It was further agreed that the broken and burnt method offered an interim alternative if the equipment to carry out the sectioning and staining was not available. The whole otolith method was agreed to be the least desirable as it offered low agreement rates and the very high probability to incorrectly assign the age.

6. Light sources and magnification

7. Reading of the otoliths

7.1 Confidence levels

Every otolith should be aged and assigned as either "good", "moderate" or "poor" (denoted by G, M or P next to the age). This is not designed to be a verdict on the otoliths' structure or how well the otolith has been prepared for reading, but an indication of how confident the reader is that they have arrived at the correct age. An otolith may be difficult to read, but if a reader has read it several times in different areas of the otolith and has arrived at the same age every time, they may feel it is a "good" otolith. The definitions of the terms are as follows:

Good (G) No doubt about the age. No other possibility seen.

Moderate (M) Not sure. There could be a possibility that the actual age is +/- 1 year, but on reflection the age given is the best estimate the reader could make.

Poor (P) The otolith really isn't clear and different ages can be obtained from different areas, or the otoliths ring structure is not clear (e.g. many split rings, staining not applied well, etc).

These confidence levels are useful tools for the trainer, as they can see where the trainee is feeling confident about their ages and where they are unsure. It is expected that as the trainee progresses, the use of the "poor" level will decrease while the "good" level will increase. It is hoped that the trainee will have greater agreement with the trainer on otoliths deemed "good" than with those marked "moderate" and the otoliths marked "poor" will show the lowest agreement rate. This distribution of results would demonstrate that the trainee was considering the same kinds of problems that the trainer was.

7.2 Training of new readers

It is important that new readers are trained by readers experienced in the species, and that training takes place over a period of two–three years or more. This is important in order to ensure the effective transfer of skills to the trainee and to maintain the accuracy and precision of the age readings supplied to working groups and for other data requirements.

Baltic flounder have proved difficult to obtain high levels of agreement between age readers and also to show spatial variations in growth, leading to the conclusion that they are not among the easiest of species to learn to read. Because of this, it may take several thousand age readings for the reader to build up a memory library of difficult or unusual otoliths, and a consistent strategy for dealing with problems encountered in determining an age for these fish.

When training to read otoliths, it is crucial that the whole of the year is represented in the samples that are read; covering every month allows the reader to follow the growth of the edge throughout the year and allows for the newly acquired reading skills to be re-enforced regularly. It is also important that reading is spread out, so a reader does not have long periods of not reading followed by short periods of intense reading activity. This is not good practice and lessons that are learnt could soon be forgotten.

The trainer should ensure that the trainee is familiar with:

- The growth and structure of the otoliths and potential difficulties in determining the age (split rings, birthday allocation, slowing of growth rate at maturity, edge growth, etc).
- Light sources and the best way to direct the light onto the otolith to give maximum visibility of the growth.
- The best magnification with which to view the otoliths.
- Allocating a confidence level to the reading of the otolith (good, moderate, poor) and how to use them.

The trainer should initially work through a number of otoliths with the new reader, discussing the characteristics of each otolith and the age that the trainee would assign. This should ideally be done with a “twin eye-piece” microscope, allowing both people to view the otolith at the same time.

The trainee should take this experience and then age a number of fish that are checked, and an agreement level with the trainer established. Any major discrepancies can then be looked at under the microscope and discussed. The trainee should then read samples that are checked regularly and any problems discussed.

For most species, an agreement rate of 95% with the trainer is considered to be the level of attainment required for the reader to be considered “fully trained” and no longer requiring such regular checks. This must be demonstrated over the period of the whole year. However, in more difficult stocks, lower levels of agreement can be set as the target.

Progress in the training can be monitored by the use of a training table such as the text table below:

Reader	L.earner	Species	FLE
Checker	X.pert	Area	26
Year	2006	Month	Jan

	GOOD					MODERATE					POOR				
AGE	-2+	-1	Agreed	+1	+2+	-2+	-1	Agreed	+1	+2+	-2+	-1	Agreed	+1	+2+
0															
1															
2															
3			13					15							
4		1	16	1			2	16							
5		3	8	1			1	9	1				8		
6		4	5	2			3	7				3	7	1	
7		1	3					3	2			5	4		
8		1	1				2	2	1			3	2	1	
9		1	2				1	1				5	5		
10		1		1				1				7	3		
11															
12															
13															
14															
15 +															
Tot.		12	48	5			9	54	4			23	29	2	
%		18%	74%	8%			13%	81%	6%			43%	54%	4%	

Summary :

-2+	
-1	23.7%
Agree	70.4%
+1	5.9%
+2+	

No. checked =	186
No. 99s =	3
Total No. =	189
No. disagreements =	55

No. Males	47
No. Females	142
No. Unsexed	0

Table 1: age reading agreement

This table should be filled in regularly so the trainer can monitor progress. The trainee identifies each otolith as either "good", "moderate" or "poor" when assigning the age depending on their confidence, as previously discussed. The age used in the table is the trainer's age, and the trainee's age is then compared. In the example table, the trainee has given an age of 7 to 2 fish in the "good" section that the trainer has aged at 6 years old (highlighted). Using this table, it can easily be detected if the trainee has any bias in their ages (a tendency to either over or under-age compared to the trainer) and how good their agreement is with the trainer for each confidence level.

The trainer and trainee should then look for patterns in bias and look at otoliths where these problems occur in order to address the discrepancies. In Table 1, although the agreement rate for “good” and “moderate” fish is okay, there is significant bias because the trainee is under-ageing compared to the trainer. This is particularly bad in the case of the “poor” otoliths, with 43% of the fish under-aged compared to just 4% over-aged.

A reader can only be said to be fully trained when their agreement rate with the trainer is consistently high throughout the year and no bias is perceived to be present.

8. Images of otoliths marked with ages including text explanations