ICES WKARDAB REPORT 2010

ICES ADVISORY COMMITTEE

ICES CM 2010/ACOM:44

Report of the Workshop on Age Reading of Dab (WKARDAB)

16–19 November 2010

Hamburg, Germany

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

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

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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Executive summary

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting, in 2008, identified dab (*Limanda limanda*) as a species requiring an exchange and ageing workshop to evaluate and improve the age interpretation based on whole otoliths. The Workshop can build on the results of the otolith exchange organized in 2009 and will be the first ageing workshop for dab.

One otolith set were included in the workshop: whole otoliths from 160 fish of the North Sea recovered during all seasons (January, April, July and November) of 2008 (40 fish per Quarter). Only digital images (Reflected and Transmitted light) are used.

6 countries (Germany, Belgium, Denmark, Netherlands, Irish Republic, France and UK England), including 12 readers, were participated at the exchange.

For dab, one of the main difficulties is the interpretation of the first annual ring, causing uncertainty among the readers during the exchange. Others difficulties were identified as the identification of few rings closed to the edge during the third quarter; Confusion with the hyalin zone and opaque zone as growth rings; Difference of used ligth (transmitted and reflected) and misinterpretation of the occurrence of split rings. During this workshop, the first ring was identified well with an average of 674 μm and a standard deviation of 146 μm.

A manual on the preparation and reading of dab otoliths has been compiled, documented with a reference set of annotated images. These documents can be used as a guideline and can form the template for discussion when refining the interpretation of the growth pattern and for identifying gaps and opportunities concerning the current knowledge of the age estimation of dab.

Mean precision of age estimate for individual fish were Coefficient of Variation (CV) of 12.0% and percent agreement to modal age of 79.3%. There were variations in precision of age estimate between individual fish, with CV ranging from 0 to 49% and percent agreement range from 45 to 100%.

1 Terms of reference

2009/2/ACOM45 The **Workshop on Age Reading of Dab** [WKARDAB] (Chair: Ulrich Damm, Germany) will be established and take place in Hamburg, Germany, 16–19 November 2010, to:

a) Review information on age estimations, otolith exchanges, workshops and validation work done so far.

b) Analysis of the results of exchange programme between ageing labs, using a set of otoliths (images) collection partially from tagging material and from previous WK collection with the purpose of inter-calibration age readers involved in Stock assessment.

c) Report on progress of the compilation of biometrics data of dab otoliths.

d) To revise the age estimation procedures and explore the possibilities to use supplementary information for validating estimated age structures, this include:

i) Otoliths weight distributions

ii) Length distribution in surveys and catches.

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

WKARDAB will report by 3 December 2010 for attention of ACOM.

SUPPORTING INFORMATION:

Priority:	**Essential.**
Scientific justification:	Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of dab stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, an otolith exchange programme and WK should be carried out in order to know the current situation of age estimation of dab which has been subject of concern of ICES WGNEW and make progress towards a solution. For the purpose of inter-calibration between ageing labs an appropriate exchange programme with a set of otoliths will be carried out during the second half of 2009. The aim of the workshop is to identify the current ageing problems between readers from both stocks through a reference collection. To identify the state of art of age estimation after validation studies conducted so far.
Resource requirements :	Before starting the exchange programme, the scientific institutions should make a concerted effort to compile existing information on growth and digital otolith images that can be used as a reference collection.
Participants:	In view of its relevance to the DCR, and ICES WG, the Workshop tries to join international experts on growth, age estimation and scientists involved in assessment in order to progress towards a solution.
Secretariat facilities:	
Financial:	None
Linkages to advisory	ACOM

Priority:	**Essential.**
committee:	
Linkages to other committees or groups:	PGCCDBS, WGBFAS and WGNEW
Linkages to other organizations cost:	There is a direct link with the EU DCR
Secretariat marginal cost share:	

2 Agenda and List of participants

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

3 Review information on age estimations, otolith exchanges, workshops and validation work (ToR a)

Age validation, otolith exchanges and workshops were not identified so far.

4 Analysis of the results of exchange programme between ageing labs (ToR b)

Introduction

In March 2008, the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) agreed about the necessity of organizing an otolith exchange exercise for Dab (*Limanda limanda*) aiming to deal with problems of this species ageing.

The planning group had decided Germany, represented by the Johann Heinrich von Thünen-Institute, to be in charge of the otolith exchange exercise for the Dab, and to organize it in 2009. All the relevant European Institutes were contacted and finally 6 countries (Germany, Belgium, Denmark, Netherlands, Irish Republic, France and UK England), including 12 readers, expressed their interest in participating in the exchange.

Sample collection

The exchange collection consisted of digital images (Reflected and Transmitted light) and whole otoliths from 160 fish of the North Sea recovered during all seasons (January, April, July and November) of 2008 (40 fish per Quarter). The frequency distribution of the total length by sex is given in the Figure 1.

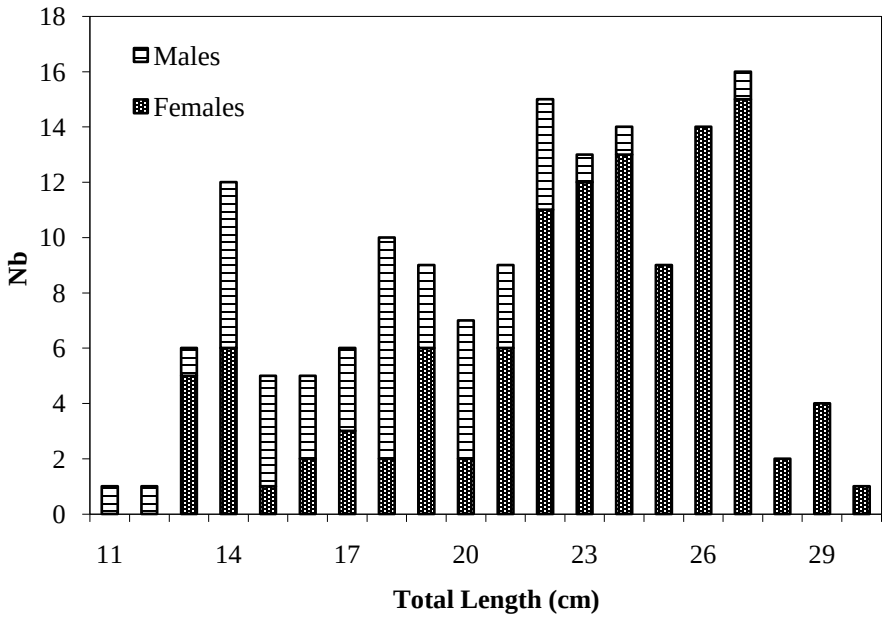


Figure 1: Length frequency distribution of samples analyzed during the exchange program conducted in the 2009.

Results

The spreadsheet (Eltink, 2000) was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink *et al.* (2000). Modal ages were calculated for each otolith red, with percentage agreement, mean age and precision coefficient of variation as a definition (for each otolith):

percentage agreement = 100x(no. of readers agreeing with modal age/total no. of readers).

precision c. v. = 100x(standard deviation of age readings/mean of age readings).

Precision

Precision is defined as the variability in the age readings. The precision's errors in age readings are better described by the coefficient of variation (CV) by age group. This measure of precision is independent of the closeness to the true age (ICES, 2007).

Mean precision of age estimate for individual fish were Coefficient of Variation (CV) of 12.0% and percent agreement to modal age of 79.3%. There were variations in precision of age estimate between individual fish, with CV ranging from 0 to 49% and percent agreement range from 45 to 100%.

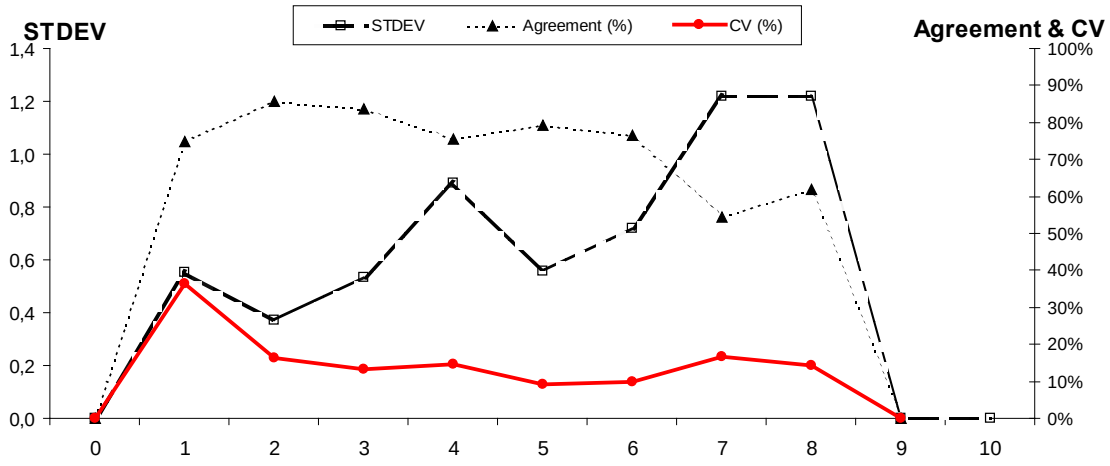


Figure 2: The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age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

On 160 otoliths, 18 were read with 100% agreement and thus a CV of 0%.

Relative bias (Accuracy)

In absence of calcified structures of known age, the age readings can be compared to modal age, which is defined as the age determined for an individual structure whose most of the readers have a preference. Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. The age reading comparisons to modal age provide a low estimate of relative bias compared to absolute bias, when most readers have a similar serious bias in age reading (ICES, 2007).

The minimal requirement for age reading's consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated can be tested non-parametrically with a one-sample Wilcoxon signed rank test. Table 1 shows inter-reader bias test and reader against modal age bias test (-: no sign of bias ($p>0.05$); *: possibility of bias ($0.01<p<0.05$); **: certainty of bias ($p<0.01$)).

Table 1: Inter-reader bias test and reader against MODAL age bias test.

	Denmark Jan Pedersen	France Elleboode Rom	Belgium Ilse Maertens	Belgium rtine Moerm	Germany Helma Koslowsk	Republic Irland Laura Langan	Germany Carmen D	England ark Etherto	Netherlands Peter Groot	Netherlands Marcel de Vries	Germany nny Albrecti	Germany ne Petersen-
Jan Pedersen												
Elleboode Romain	**											
Ilse Maertens	**	–										
Martine Moerman	**	–	**									
Helma Koslowski	**	–	–	–								
Laura Langan	–	**	**	**	**							
Lilly-Carmen Doss	**	**	–	**	–	**						
Mark Etherton	**	–	–	–	–	**	**					
Peter Groot	–	**	**	**	**	–	**	**				
Marcel de Vries	**	*	**	–	*	**	**	*	*			
Conny Albrecht	**	–	–	–	–	**	**	–	**	–		
Christine Petersen-Frey	**	–	*	–	–	**	**	–	**	–	–	
MODAL age	**	–	–	*	–	**	*	–	**	**	–	*

5 To Compile of biometrics data of dab otoliths (ToR c) and To revise the age estimation procedures and explore the possibilities to use supplementary information for validating estimated age structures (ToR d)

These aspects were not approached during the WKARDAB meeting.

6 Identify sources of age determination error in terms of bias and precision

For many images, the annotations of readers were compared (Fig. 3).

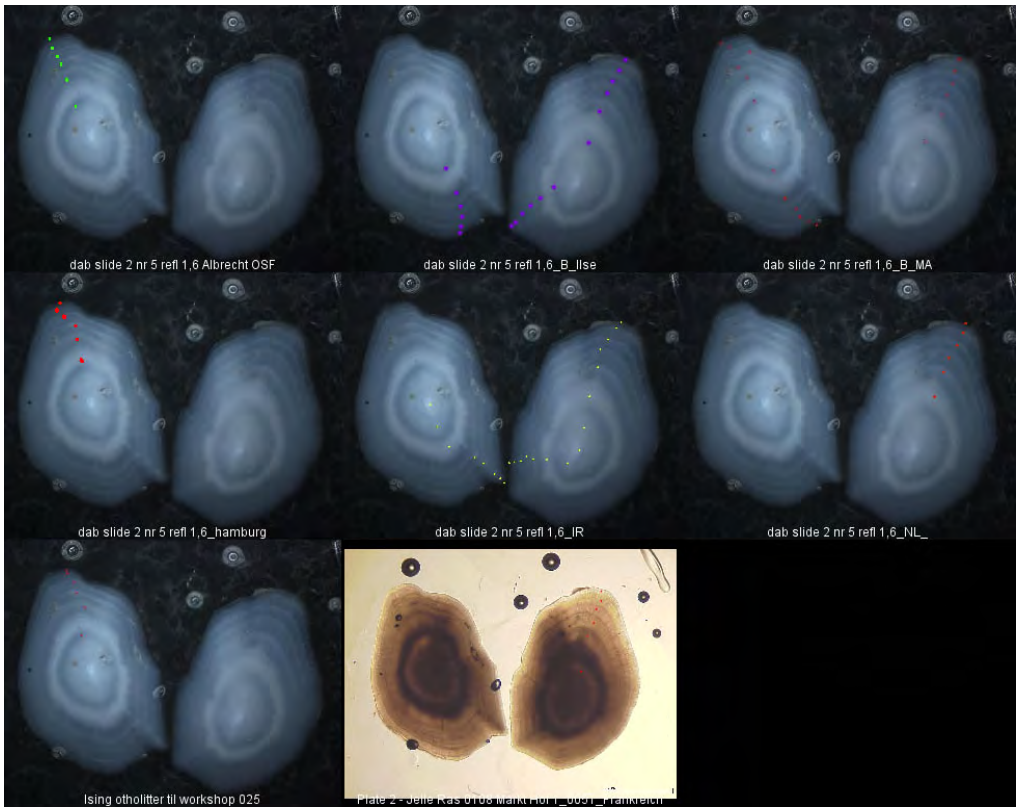


Figure 3: Comparison of the annotations of image "slide_2_Nr_5".

From the discussion during the workshop the following remarks were pointed out:

- Disagreement in the identification of the first annual ring; On some otoliths, there is a first ring with a specific shape which is considered as a false ring (Fig. 4).

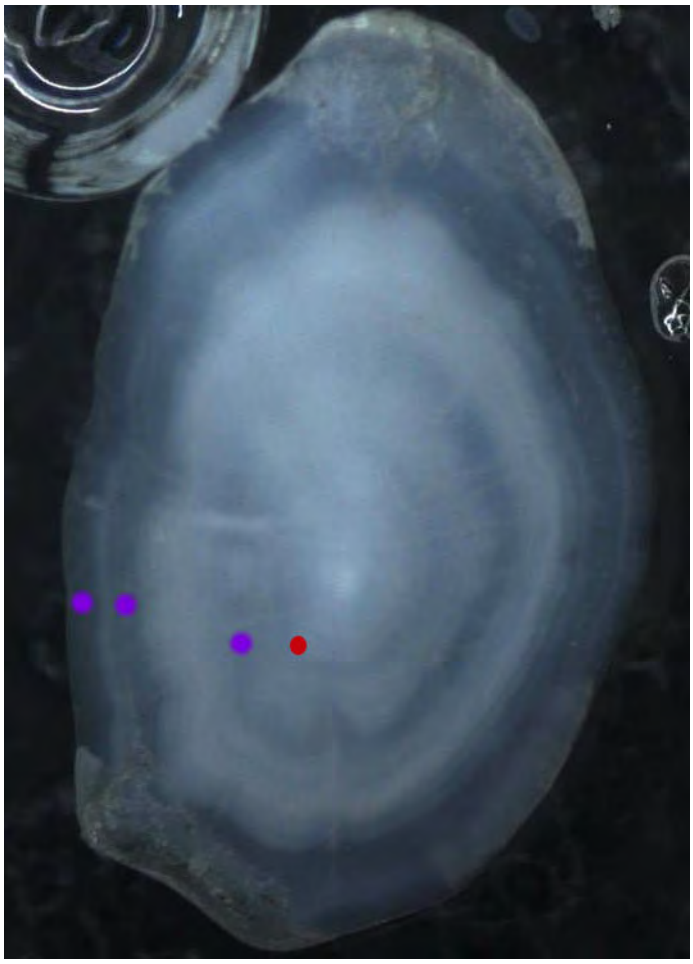


Figure 4: Observation of the growth rings (purple rounds) and the false ring (red round) close to the *nucleus*.

- Disagreement in the identification of few rings closed to the edge during the third quarter;
- Confusion with the hyalin zone (1 reader) and opaque zone as growth rings;
- Difference of used ligth (transmitted and reflected).
- A source of misinterpretation is the occurrence of split rings. Some of the translucent annulus can consist of several thinner translucent bands that can be misinterpreted as true annulus which leads to overestimation of fish age. These bands can be identified as being thinner that true annulus and with less distance between them.

7 Analyse growth increment patterns and provide specific guidelines for the interpretation of growth structures in otoliths

Date of birth

Date of birth is considered to be the 1st of January.

Interpretation of the first *annullus*

Eighteen images with 100% agreement were selected and All the images were analysed using the software TNPC to measure the distances between the *nucleus* and the growth rings (Fig. 5 & 6).

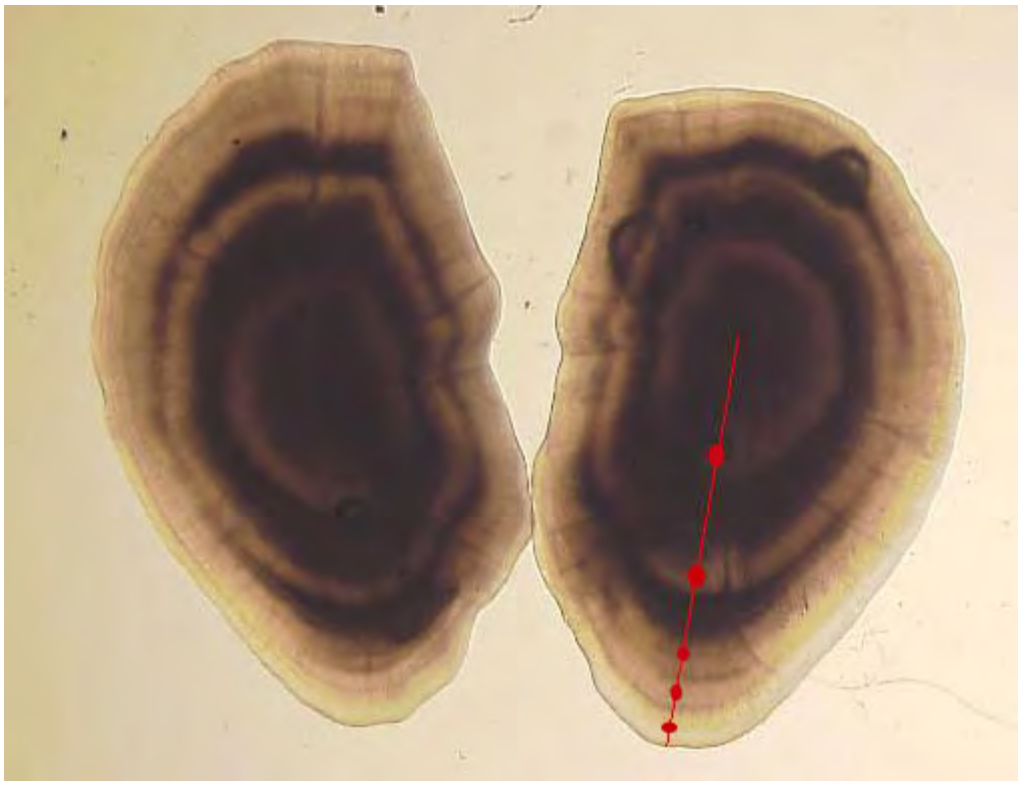


Figure 5: Annotations of the image using TNPC software.

The first ring is identified well with an average of 674 μm and a standard deviation of 146 μm (Fig. 6).

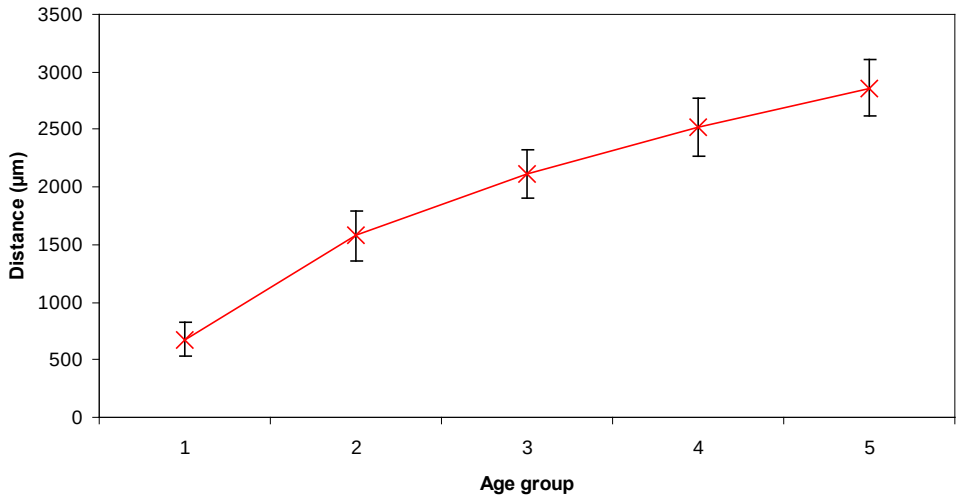
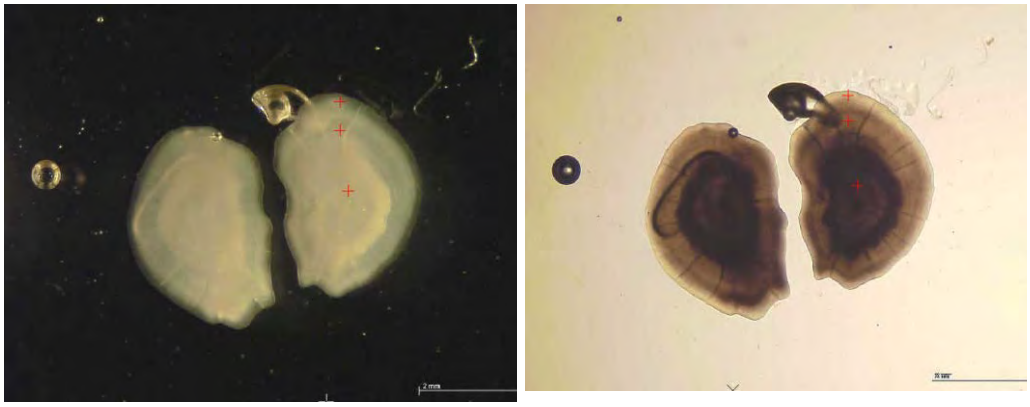


Figure 6: Distance between the *nucleus* and the growth rings.

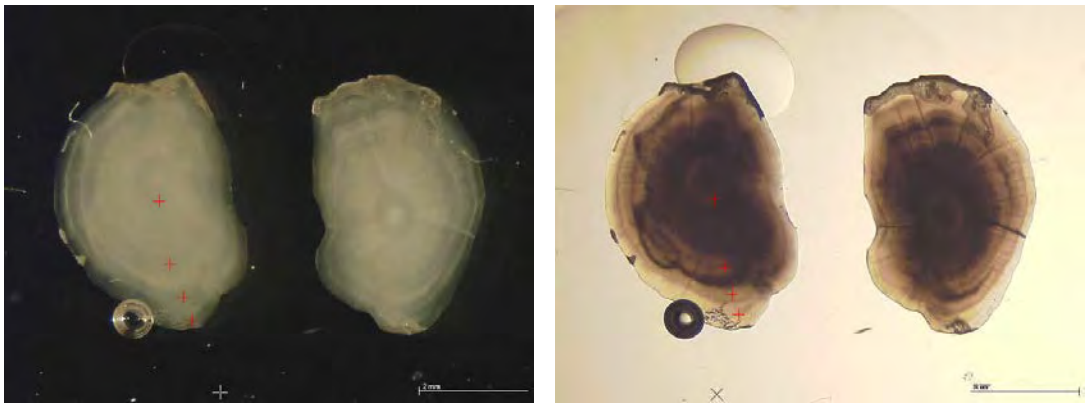
Light

Two types of light settings can be used when viewing otolith in microscope, transmitted or reflected light. For interpreting the structures and assigning a fish age the reader should be able to use both sources of light (Fig. 6).

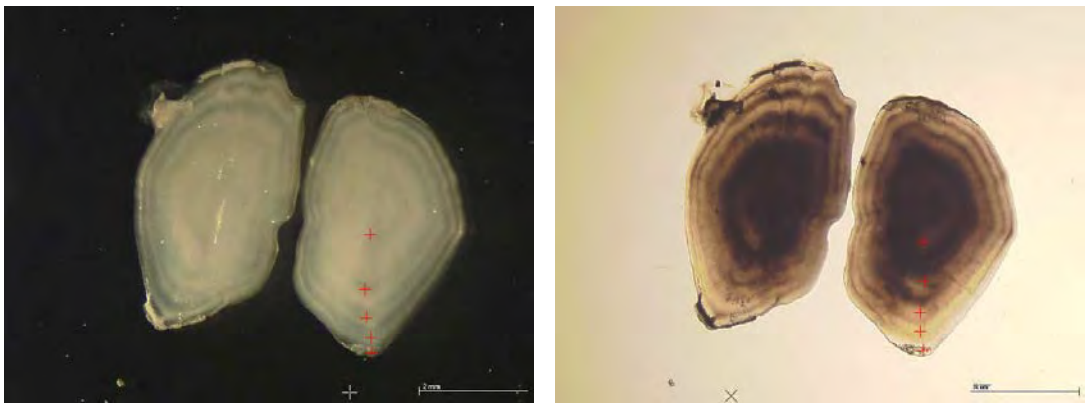
8 Create a reference collection of otoliths



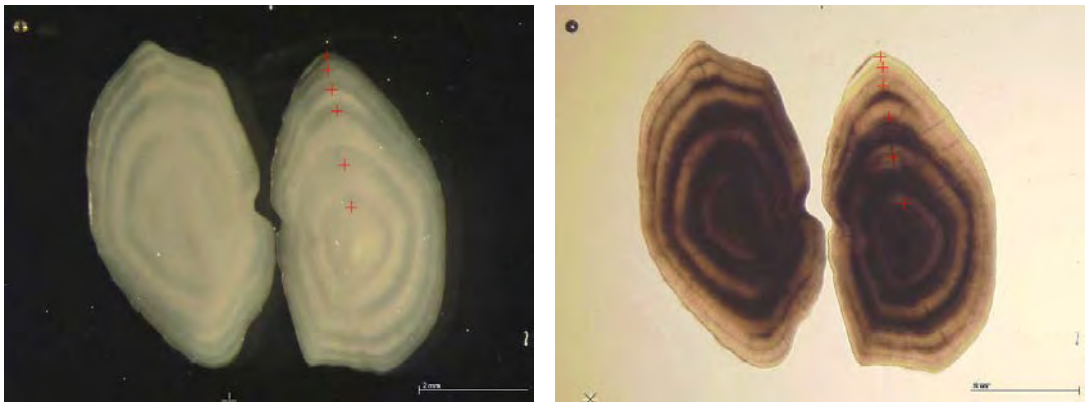
Agreed Age 3 (Relected and transmitted ligths).



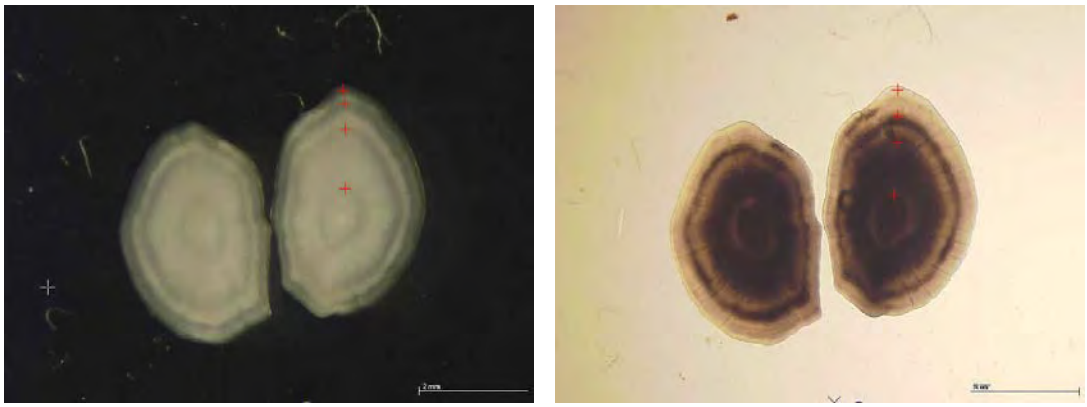
Agreed Age 4 (Relected and transmitted ligths).



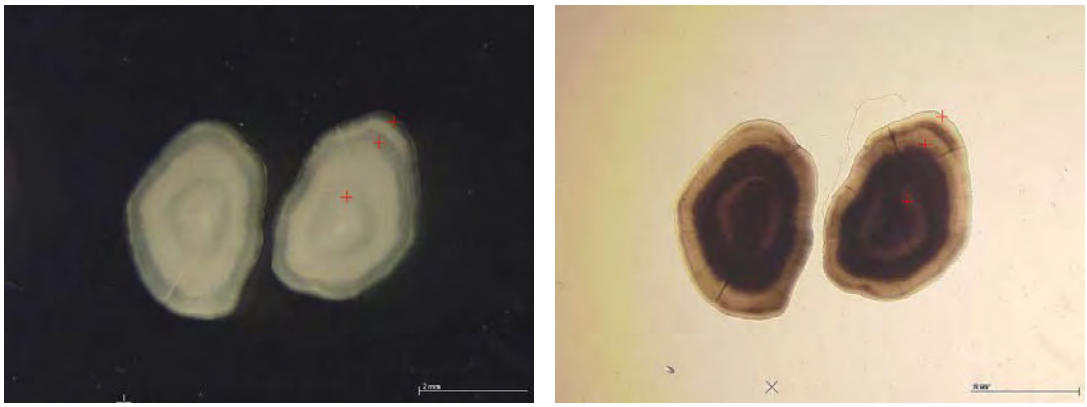
Agreed Age 5 (Relected and transmitted ligths).



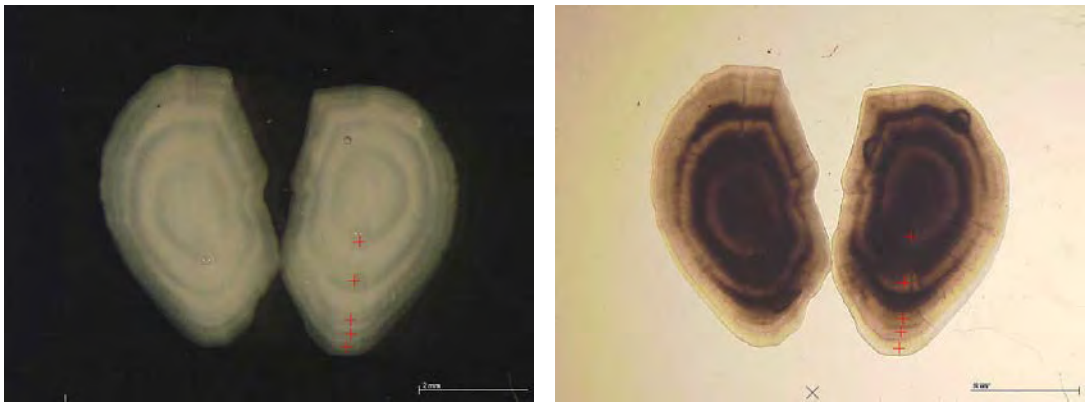
Agreed Age 6 (Relected and transmitted ligths).



Agreed Age 4 (Relected and transmitted ligths).



Agreed Age 3 (Relected and transmitted ligths).



Agreed Age 5 (Relected and transmitted ligths).

9 Bibliography

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M. T. G., Modin, J., 2000. Guidelines and Tools for Age Reading. (PDF document version 1.0 October 2000) Internet : http://www.efan.no

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Annex 1: Agenda

Tuesday 16.

9.00: Opening - Welcome, technical details

All day: Results of the exchange, discussion of examples

Wednesday 17.

Morning: Continued: Results of the exchange, discussion of examples

Afternoon: Reading of a new sample

Thursday 18.

All day: Continued: Reading of a new sample, discussion of selected cases

19.30: Social dinner

Friday 19.

Morning: Discussions: Descriptions of routine procedures

Future work and cooperation

Imaging software and its usefulness, WebGR

Comparison with material from other areas (Irish Sea, Baltic)

Reference collection

Staining, results and suggestions for improvement

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daily timetable:

09:00 Beginning

10:30 Coffee-break

12:30 – 13:30 Lunch-break

15:30 Coffee-break

18:00 End

Annex 2: WKARDAB Workshop List of participants

Name	Address	Phone/Fax	Email
Christine Petersen-Frey	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Palmaille 9 D-22767 Hamburg Germany		christine.petersen-frey@vti.bund.de
Jan Pedersen	DTU Aqua - National Institute of Aquatic Resources Section for Fisheries Advice Charlottenlund Slot Jægersborg Alle 1 DK-2920 Charlottenlund Denmark	Phone +0045 40380261	jpd@aqua.dtu.dk
Kelig Mahe (Rapporteur)	IFREMER Boulogne-sur-Mer Centre P.O. Box 699 62321 Boulogne Cédex France	Phone +33 321 995602 Fax +33 321 995601	Kelig.Mahe@ifremer.fr
Laura Langan	Galway-Mayo Institute of Technology Commercial Fisheries Research Group Marine and Freshwater Research Centre Dublin Road Galway Ireland	Phone +353 91 742525	laural710@msn.com
Lilly-Carmen Doss	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Deichstr. 12 DE-27472 Cuxhaven Germany		lilly-carmen.doss@ifo.bfa-fisch.de
Marcel de Vries	Wageningen IMARES P.O. Box 68 NL-1970 AB IJmuiden Netherlands	Phone +31 317 480900	Marcel.devries@wur.nl

Name	Address	Phone/Fax	Email
Peter Groot	Wageningen IMARES P.O. Box 68 NL-1970 AB IJmuiden Netherlands	Phone +31 317 480900	peter.groot@wur.nl
Romain Elleboode	IFREMER Boulogne-sur-Mer Centre P.O. Box 699 62321 Boulogne Cédex France	Phone +33 321 995687 Fax +33 321 995601	Romain.Elleboode@ifremer.fr
Ulrich Damm (Chair)	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Palmaille 9 D-22767 Hamburg Germany	Phone +49 40 38905 268 Fax +49 40 38905 263	ulrich.damm@vti.bund.de

The list of the participants in the otoliths exchange is presented in the following table :

Name	Address	Phone/Fax	Email
Cornelia Albrecht	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Baltic Sea Fisheries Alter Hafen Süd 2 D-18069 Rostock Germany		cornelia.albrecht@vti.bund.de
Christophe Bonjé	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 1 8400 Oostende Belgium		christophe.bonje@ilvo.vlaanderen.be
Lotte Worsøe Clausen	DTU Aqua - National Institute of Aquatic Resources Section for Fisheries Advice Charlottenlund Slot Jægersborg Alle 1 DK-2920 Charlottenlund Denmark	Phone +45 21362804 Fax +45 33963333	law@aqua.dtu.dk
Ulrich Damm Chair	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Palmaille 9 D-22767 Hamburg Germany	Phone +49 40 38905 268 Fax +49 40 38905 263	ulrich.damm@vti.bund.de

Marcel de Vries	Wageningen IMARES P.O. Box 68 NL-1970 AB IJmuiden Netherlands		Marcel.devries@wur.nl
Lilly-Carmen Doss	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Deichstr. 12 DE-27472 Cuxhaven Germany		lilly-carmen.doss@ifo.bfa-fisch.de
Peter Groot	Wageningen IMARES P.O. Box 68 NL-1970 AB IJmuiden Netherlands	Phone +31 317 780900	peter.groot@wur.nl
Helma Koslowski	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Deichstr. 12 DE-27472 Cuxhaven Germany		helma.koslowski@ifo.bfa-fisch.de
Thomas Lang	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institut für Fischereiökologie Deichstr. 12 DE-27472 Cuxhaven Germany	Phone +49 4721 38034 Fax +49 4721 53583	thomas.lang@vti.bund.de
Laura Langan	Galway-Mayo Institute of Technology Commercial Fisheries Research Group Marine and Freshwater Research Centre Dublin Road Galway Ireland	Phone +353 91 742525	laural710@msn.com
Ilse Maertens	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 1 8400 Oostende Belgium	Phone +32 59 569873	ilse.maertens@ilvo.vlaanderen.be
Kelig Mahe	IFREMER Boulogne-sur-Mer Centre P.O. Box 699 62321 Boulogne Cédex France	Phone +33 321 995602 Fax +33 321 995601	Kelig.Mahe@ifremer.fr
Romain Elleboode	IFREMER Boulogne-sur-Mer Centre P.O. Box 699 62321 Boulogne Cédex France	Phone +33 321 995687 Fax +33 321 995601	Romain Elleboode@ifremer.fr
Martine Moerman	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 1 8400 Oostende Belgium	Phone +32 59 342250	martine.moerman@ilvo.vlaanderen.be

Jan Pedersen	DTU Aqua - National Institute of Aquatic Resources Section for Fisheries Advice Charlottenlund Slot Jægersborg Alle 1 DK-2920 Charlottenlund Denmark		jpd@aqua.dtu.dk
Christine Petersen-Frey	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Palmaille 9 D-22767 Hamburg Germany		christine.petersen-frey@vti.bund.de
Annemie Zenner	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 1 8400 Oostende Belgium	Phone +32 59569823	annemie.zenner@ilvo.vlaanderen.be

Annex 3: WKARDAB terms of reference for the next meeting

The **Workshop on Age Reading of Dab** [WKARDAB] (Chair: ???) will meet in ???, from 2013 to:

a) Review information on age estimations, otolith exchanges, workshops and validation work done so far.

b) Analysis of the results of exchange programme between ageing labs, using a set of otoliths (images) collection

c) To Analyse growth increment patterns

d) To complete the guidelines for the interpretation of growth structures in Dab otoliths

e) To complete the reference collection of Dab otoliths

f) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration')

WKARDAB will report by 2013 to the attention of the ACOM and PGCCDBS.

Supporting Information

Priority:	Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, a WK should be carried out in order to evaluate available information on otolith growth patterns, age determination issues and the current situation of age estimation of Dab.
Scientific justification and relation to action plan:	Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of dab stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, an otolith exchange programme and WK should be carried out in order to know the current situation of age estimation of dab which has been subject of concern of ICES WGNEW and make progress towards a solution. For the purpose of inter-calibration between ageing labs an appropriate exchange programme with a set of otoliths will be carried out during 2012. The aim of the workshop is to continue the work of the first workshop 2010; This aim is to idenify the current ageing problems between readers from both stocks through a reference collection. To identify the state of art of age estimation after validation studies conducted so far.
Resource requirements:	The workshop will be preceded by an exchange.
Participants:	The Group is normally attended by some 15–20 members.
Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM

Linkages to other committees or groups:	PGCCDBS
Linkages to other organizations:	There is a direct link with the EU DCF

Annex 4: Recommendations

Recommendation	For follow up by:
1. Application of the manual agreed by WKARDAB by all ageing laboratories	PGCCDBS
2. Validation study on the first growth ring by daily increment of juveniles	PGCCDBS
3. Validation study of the formation of marginal increment	PGCCDBS
4. Another workshop should be performed in three years to continue promoting standardization of methodologies and practices for age estimation of dab based on the current work done.	PGCCDBS