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Report of the Workshop on Age Reading of European and American Eel (WKAREA2)

22–24 March 2011

Bordeaux, France

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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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Contents

Preparation of this document

This publication is the report of the ICES Workshop on Age Reading of European and American Eel, held in CEMAGREF, Bordeaux, France from 22-24 March 2011. The Workshop was chaired by Francoise Daverat.

This report is designed as a stand- alone report covering the Terms of Reference, and should be read in conjunction with the manual of best practice and otolith images that has been drafted as a separate document.

WKAREA eel otolith experts at work (©Guy Verreault)

Executive summary

The workshop commenced with the analysis of the results of the experienced reader inter calibration exercise that had been carried out several months previous the meeting. This intercalibration exercise was based on image exchange for both species. The readings had been performed on a web platform device allowing the positioning of age checks on the pictures and recording the number of checks identified by each reader. A total of 21 readers participated to the exchange. A collection of 117 European eel pictures and 44 American eel otolith pictures were used for the exchange. The overall agreement rate of the readings with the modal age ranged from 66.2% to 13.2%. The results showed that more agreement would have been obtained if the reading rules had been applied more consistently. Some readers discarded some "difficult" otoliths. The absence of metadata such as the location, date of capture and habitat type of the otolith was also identified as a source of misinterpretation of growth patterns. It was recognized for future readings that metadata should be included and that all otoliths would be read, with the addition of a reading confidence parameter. A reference collection composed of 38 A. Anguilla and 19 A. rostrata known age otolith pictures was set up, with one blind file and one fully annotated file. The manual was updated with more precisions included for the different preparation protocols. A protocol for age reading and training age reading and routine age reading was proposed, including the use of the reference collection.

1 Opening of the meeting

The Workshop was opened by the Chair, Francoise Daverat. The Agenda plan for the week was discussed and agreed (see Annex 1). The workshop was attended by 22 participants from 10 countries. Countries represented at the Workshop were Belgium, Canada, France, Germany, Ireland, Italy, Netherlands, Polland, Sweden, UK and Northern Ireland.

2 Terms of reference

The Workshop on Age Reading of European and American Eel [WKAREA-2] (Chair: Françoise Daverat, France) was set up to exchange information by correspondence in 2010 and meet in Bordeaux, France in March 2011:

a) to exchange samples (>100 per species) of European and American eel otolith pictures, including known age eels, with samples prepared using different protocols and representing a range of eel subpopulations, and environment types encountered in both species range;

b) to apply the age estimation criteria defined during the previous meeting in an inter-calibration process involving the exchanged images and a significant number of readers (>20);

c) to analyse readings and interpret the results of the inter-calibration of European and American eel age reading;

d) to make recommendations and feedback on the age estimation criteria to increase age estimation precision and accuracy and improve the inter reader agreement;

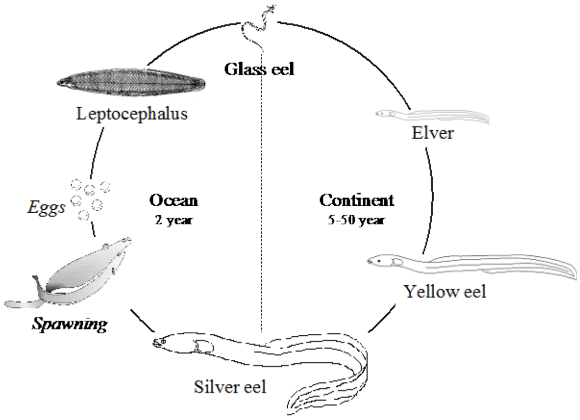
e) to incorporate the findings with the report and manual developed by WKAREA 2009 for formal publication; and

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

3 Glossary

3.1 Eel Terms

Eels are quite unlike other fish and consequently come with a specialised jargon (WGEEL 2008).



The life cycle of the European eel. The names of the major life stages are indicated. Spawning and eggs of Anguilla anguilla and A. rostrata have never been observed in the wild (supplied by Dekker).

Atlantic Eel	The collective term Atlantic eel will be used in this report to cover both *A. Anguilla* and *A. rostrata* where there are no differences. Differences between the species will be indicated by their specific names.
Glass eel	Young, unpigmented eel, recruiting from the sea into continental waters
Elver	Young eel, in its 1st year following recruitment from the ocean. The elver stage is sometimes considered to exclude the glass eel stage, but not by everyone. Thus, it is a confusing term.
Bootlace, fingerling	Intermediate sized eels, approx. 10-25 cm in length. These terms are most often used in relation to stocking. The exact size of the eels may vary considerably. Thus, it is a confusing term.
Yellow eel (Brown eel)	Life stage resident in continental waters. Often defined as a sedentary phase, but migration within and between rivers, and to and from coastal waters occurs. This phase encompasses the elver and bootlace stages.
Silver eel	Migratory phase following the yellow eel phase. Eel characterised by darkened back, silvery belly with a clearly contrasting black lateral line, enlarged eyes. Downstream migration towards the sea, and subsequently westwards. This phase mainly occurs in the second half of calendar years, though some are observed throughout the winter and following spring.
Eel River Basin	"Member States shall identify and define the individual river basins lying within their national territory that constitute natural habitats for the European eel (eel river basins) which may include maritime waters. If appropriate justification is provided, a Member State may designate the whole of its national territory or an existing regional administrative unit as one eel river basin. In defining eel river basins, Member States shall have the maximum possible regard for the administrative arrangements referred to in Article 3 of Directive 2000/60/EC [i.e. River Basin Districts of the Water Framework Directive]."
River Basin District	The area of land and sea, made up of one or more neighbouring river basins together with their associated surface and groundwaters, transitional and coastal waters, which is identified under Article 3(1) of the W F D as the main unit for management of river basins. Term used in relation to the EU W F D.
Stocking	Stocking is the practice of adding fish [eels] to a waterbody from another source, to supplement existing populations or to create a population where none exists.

3.2 Otolith Terms

(updated from Vøllestad, Lecomte-Finiger & Steinmetz, 1988) and see also Figure 3.1

Annual zone: Structural feature of the otolith corresponding to the growth during a complete year of life

Annulus: The theoretical boundary between two successive annual zones

Burning & cracking: The traditional otolith preparation of burning and cracking has been improved by cutting the otolith before burning. Both methods are covered in this manual by the term "Burning and cracking".

Frontal Plane: The flat cut, or cracked, face of a transverse section of an otolith

Growth Check: A boundary between two growth zones, not necessarily annual (also see supernumerary)

Hyaline: See translucent.

Nucleus: The hypothetical or real origin of the otolith; synonymous with focus or core

Opaque zone: A zone that inhibits the passage of light. In transmitted light opaque zones appear dark and in reflected light they appear bright (white)

Radius: A determined measurement from a focus to a specific point

Sagittal Plane: The view of the otolith when lying flat, convex side up. Most grinding takes place on the sagittal plane.

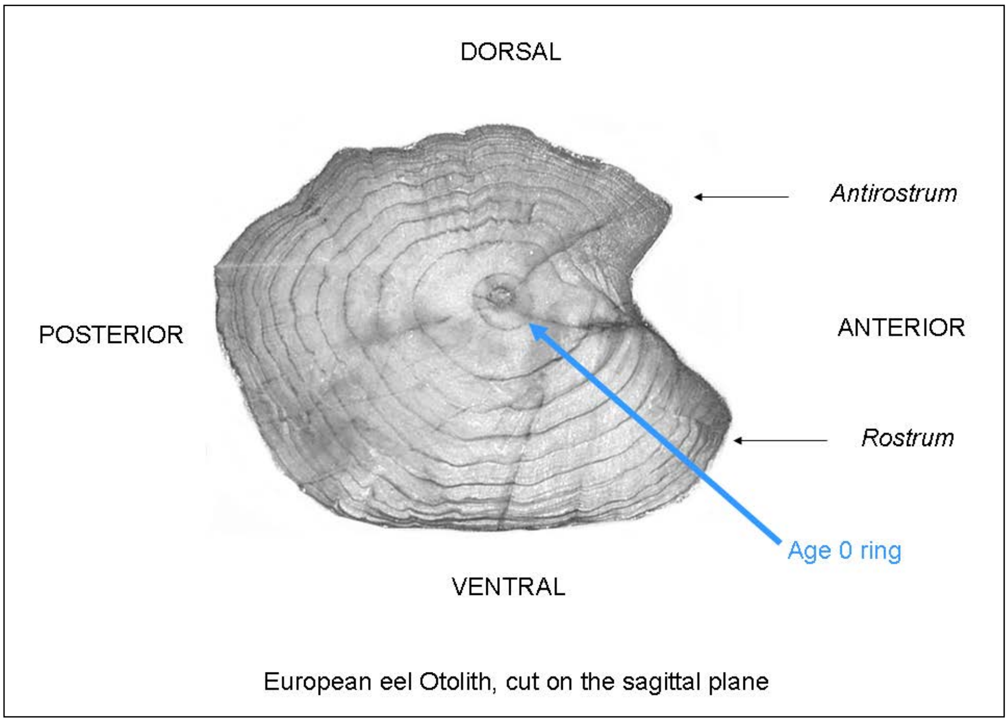
Supernumerary: A growth mark or check not accepted for annual age determination, also referred to as a growth check or false annulus.

Translucent zone: Previously known as the hyaline zone. A zone that allows the passage of light. In transmitted light translucent zones appear bright, in reflected light they appear dark.

Validation: The confirmation of the temporal meaning of a growth increment. Analogous to determining the accuracy of age determination; used in reference to true age.

Verification: Determining the precision (reproducibility) of age determination, used in reference to the precision of estimated age.

Zero band: The first growth check outside the nucleus from where continental age determination commences (~170ìm radius from centre).



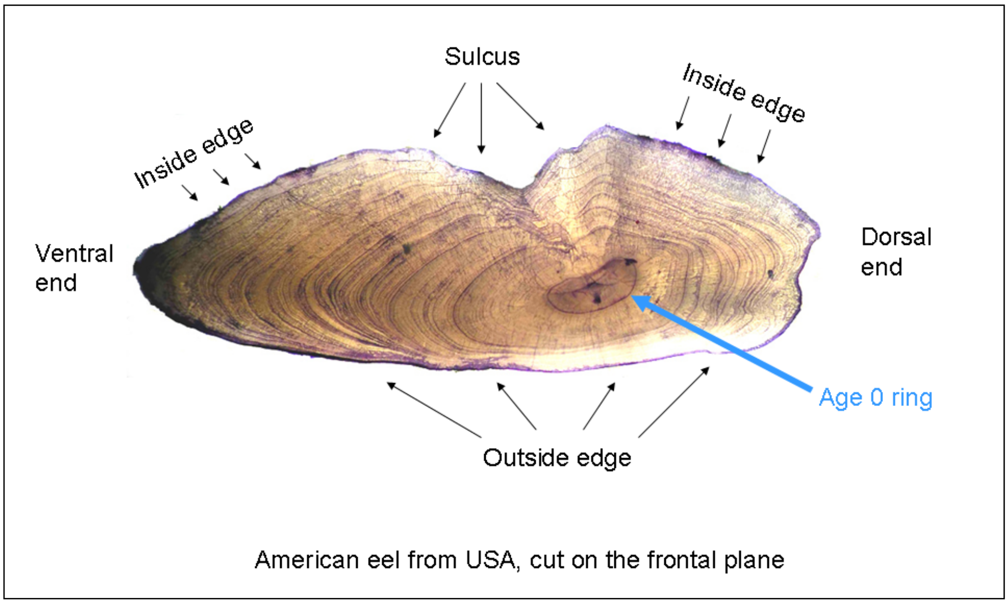


Figure 3.1. Illustrations of sagittal and frontal plane views of otoliths indicating the terminology. The Age 0 ring is equivalent to the zero band referred to in this report. The anterior and posterior regions of the otolith above are in accordance with the orientation of the eel (source: Christine Gazeau, CEMAGREF).

Also useful: http://www.cmima.csic.es/aforo/index.jsp

4 Introduction

An initial session was held to commence the workshop looking at the readings from the exchange. The readings had been performed on a web platform device allowing the positioning of age checks on the pictures and recording the number of checks identified by each reader. A total of 21 readers participated to the exchange. A collection of 117 European eel pictures and 44 American eel otolith pictures were used for the exchange.

The overall agreement rate of the readings with the modal age ranged from 66.2% to 13.2%. An analysis of the readings of the samples with contrasted agreement rates was undertaken by displaying the sample pictures and identifying the sources of consensus and disagreement in the interpretation of annual checks within the participants. A particular attention was given to the eels of known age. The major sources of differences in the interpretation of the age checks were looked at.

This exercise pointed out the need for the knowledge of the a priori growth pattern of an eel in specific habitats

Given the long history of difficulties with eel ageing, it is encouraging to note that the outcomes from the previous and current workshops are very positive and leading to the most robust consensus to date.

5 Analysis of intercalibration exercise

A collection of 161 otolith images was made available to the group: 44 from Anguilla rostrata and 117 from A. anguilla. Images were available from 10 A. anguilla otoliths that had been prepared using the "cracking & burning" method. The remainder (107) of A. anguilla otoliths and all those from A. rostrata were prepared by "polishing & staining".

Twenty-one readers accessed these 161 images and submitted annular marks for between 46 and 160 images, with a total of 2259 readings. The modal age was selected for each otolith based on the readings submitted. Modal ages ranged from 1 to 28 years, with the majority between 1 and 10 years (Table 1).

Modal ages	1 to 5	6 to 10	11 to 20	21 to 28
Number of readings submitted	78	53	25	5

Table 1. Distribution of modal ages amongst the collection of otolith images for the WKAREA 2011 intercalibration exercise

The results of intercalibration exercises can be considered in terms of accuracy (closeness to the true value) or precision (closeness of repeated measures) (Kalish *et al.*, 1995). The % agreement is the most commonly reported measure of both accuracy and precision (Morison *et al.*, 2005), but an age-bias plot in conjunction with the CV is a far superior measure to detect bias and measure precision (Campana et al., 1995). Therefore, the accuracy and precision of the intercalibration exercise were assessed both as the % agreement with the modal age, and the coefficient of variation (SD / Average).

Measures of reader accuracy: Modal v Actual Age

Only two samples of eels with known ages were available for determining the true accuracy of reader's age readings; one from Sweden and one from Canada. Table 2 presents the percentage agreement with the modal age for the total sample (column 1), for the total sample of eels with known ages (column 2) and also the same for the Swedish (column 4) and Canadian (column 6) otoliths on their own. Table 2 also presents the percentage agreement with the actual age for these groups (columns 3, 5 & 7 respectively). Reader rankings changed between groups and the overall % agreement was higher for the modal ages than for the actual ages for the total sample and the Swedish eels, but not for the Canadian eels. This shows that the consensus may not be the most accurate and it is likely that had the metadata been available, the agreement with the actual age would have been better.

Reader	1 Total Modal		2 Known + Modal		3 Known + Actual		4 Swedish Modal		5 Swedish Actual		6 Canadian Modal		7 Canadian Actual	
	Mean %	**Rank**	**Mean %**	**Rank**	**Mean %**	**Rank**	**Mean %**	**Rank**	**Mean %**	**Rank**	**Mean %**	**Rank**	**Mean %**	**Rank**
1	0.66	2	0.74	3	0.40	9	0.62	6	0.29	12	0.57	2	0.57	2
2	0.66	1	1.00	1	1.00	1	1.00	1	1.00	1				
3	0.65	3	0.63	7	0.45	6	0.60	8	0.50	6	0.39	5	0.39	5
4	0.59	7	0.38	17	0.86	2	0.38	18	0.86	2				
5	0.13	21	0.19	19	0.14	16	0.19	19	0.14	16				
6	0.65	4	0.62	8	0.29	14	0.62	6	0.29	12				
7	0.30	20	0.30	18	0.46	5	0.42	17	0.68	4	0.22	7	0.22	7
8	0.55	12	0.67	4	0.10	18	0.67	3	0.10	18				
9	0.46	15	0.60	9	0.40	9	0.60	8	0.40	8				
10	0.52	13	0.52	12	0.38	12	0.52	12	0.38	9				
11	0.50	14	0.51	15	0.62	3	0.50	14	0.28	14	0.90	1	0.90	1
12	0.58	8	0.54	11	0.41	8	0.53	11	0.59	5	0.27	6	0.27	6
13	0.45	16	0.52	12	0.24	15	0.52	12	0.24	15				
14	0.43	17	0.67	4	0.43	7	0.67	3	0.43	7				
15	0.56	11	0.52	12	0.60	4	0.43	15	0.71	3	0.48	3	0.48	3
16	0.36	18	0.57	10	0.33	13	0.57	10	0.33	11				
17	0.36	19												
X	0.57	9	0.43	16	0.05	19	0.43	15	0.05	19				
19	0.59	6												
20	0.57	10	0.67	4	0.14	16	0.67	3	0.14	16				
21	0.60	5	0.81	2	0.40	11	0.76	2	0.38	9	0.41	4	0.41	4
	% Ag	**% cv**	**% Ag**	**% cv**	**% Ag**	**% cv**	**% Ag**	**% cv**	**% Ag**	**% cv**	**% Ag**	**% cv**	**% Ag**	**% cv**
	0.52	0.20	0.57	0.16	0.41	0.16	0.54	0.11	0.37	0.11	0.44	0.21	0.44	0.21

Table 2: Percent age comparisons and rankings for the total otolith sample, the eels of known age and the eels of known age separately for Sweden and Canada.

Plotting the known age against the modal age (Figure 1), shows that readers tended to agree or under-age the otoliths, with the modal age only exceeding the actual age in one case. Care needs to be taken in interpreting modal age compared to variation in individual readings. Examination of individual readings is complex and requires access to the individual otolith images and their interpreted ages but this was not possible in the workshop. However, the experience of a middle ranked reader (Reader X) was used to illustrate some of the reasons for differences between readings and the modal age, and between the modal age and the consensus view or known age.

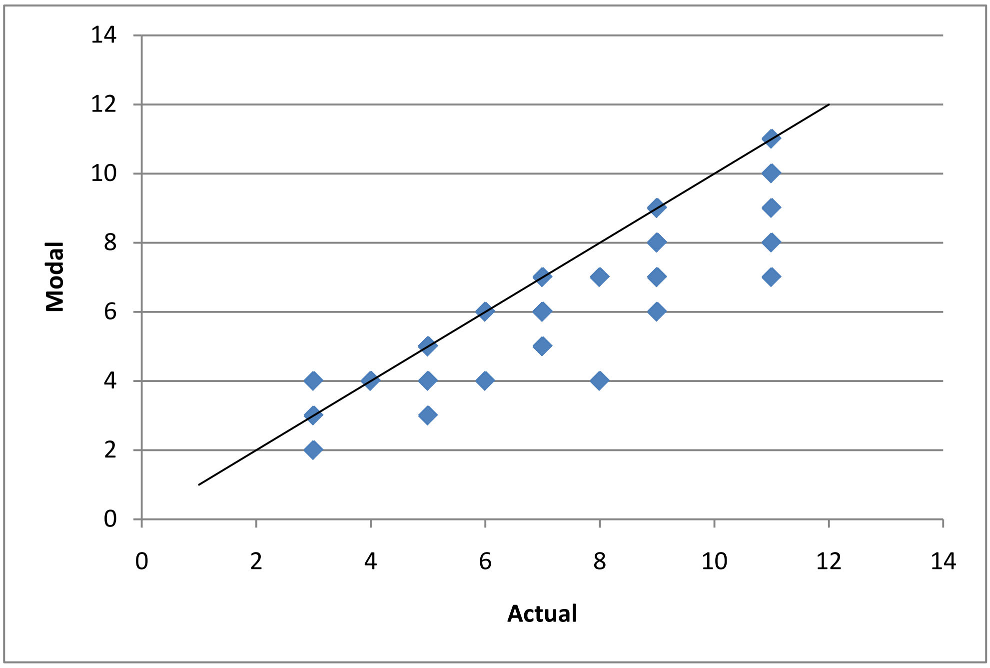


Figure 1: Modal ages plotted against actual known ages for the Swedish and Canadian samples.

Measures of Reader Precision

The % agreement between readers ranged from 100% (2 otoliths, both with modal age 5 years) down to 17%, with most between 26 and 75% (Table 3), and a mean of 52.0%. The precision CV varied from 0.0 to 0.98, with most less than 25% (Table 3), and a mean of 19.9%.

Table 3. Distributions of % agreement and % coefficient of variation (CV) amongst those reading the collection of otolith images for the WKAREA 2011 intercalibration exercise

% agreement band	0 to 25%	26 to 50%	51 to 75%	76 to 100%
Number of readers	9	70	72	10
% coefficient of variation	0 to 25%	26 to 50%	51 to 75%	76 to 100%
Number of readers	122	32	5	2

There was a wide range in % agreement for otoliths of the same age, up to 71%, but this range of % agreement declined with increasing age of otoliths (Figure 2). Similarly, the % coefficient variation ranged as much as 67% across otoliths of the same age, but declined rapidly with increasing modal age (Figure 3). However, the otoliths of older eels were examined by fewer readers (Figure 4) and this introduces a potential bias in the analysis. Those who read the older otoliths were possibly those most familiar with ageing older eels and therefore likely to be relatively accurate and precise. Conversely, those unfamiliar with older otoliths possibly chose to ignore these. As a result, our confidence that the % agreement and % CV statistics are truly representative of intercalibration, declines for older otoliths.

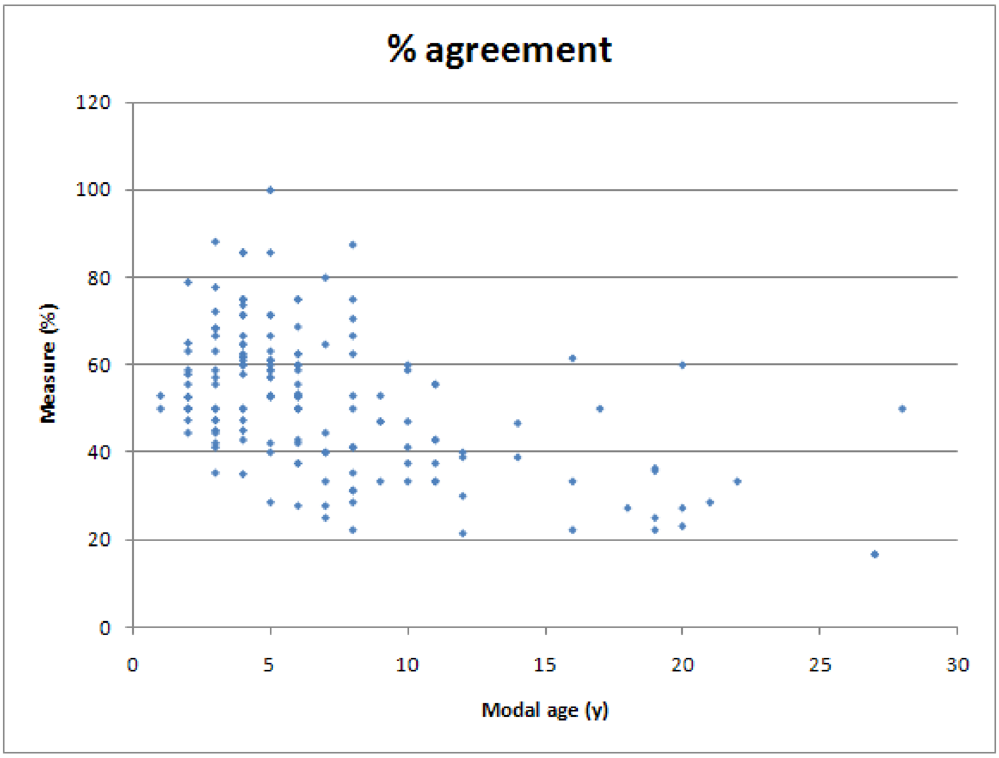


Figure 2: The range of percentage agreement between readers for otoliths of the same terminal age, with ages ranging from 1 to 28 years.

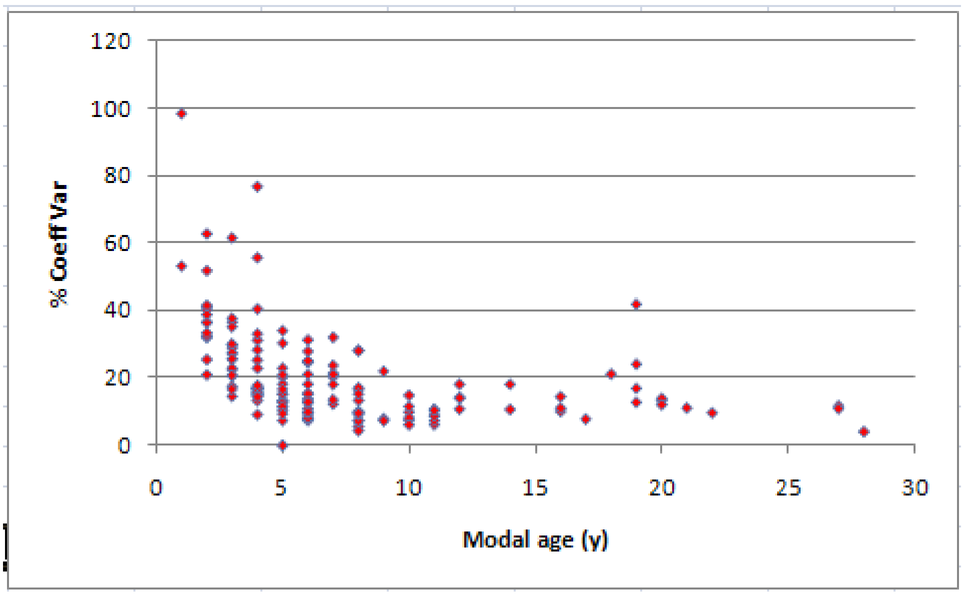


Figure 3: The range of % coefficient of variation between readers for otoliths of the same terminal age, with ages ranging from 1 to 28 years. The data for each terminal age correspond to those for the same terminal age in Figure 2, although the distribution of the data points may differ between the figures.

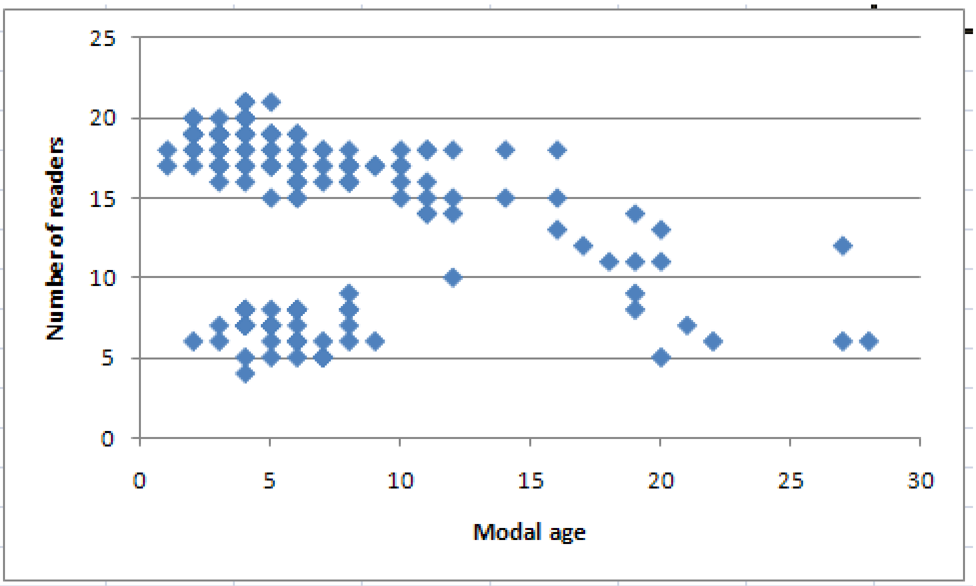


Figure 4: The numbers of readers who submitted ages for otoliths of varying ages, grouped according to the modal ages of these otoliths. The cluster of young otoliths (2 to 9 years) with relatively few readers (4 to 9) in the lower left area of the figure are almost all otoliths from North American eels, suggesting a potential bias according to familiarity (see text for further discussion).

Factors affecting the intercalibration

The workshop identified a number of factors that affected the results or had the potential to introduce biases in the data, based on the choice of statistics used for the analysis, the application of the reading rules and prior experience of readers.

<u>Choice of central statistic</u>

The Modal Age appears to be the most appropriate statistic of central tendency for comparing the accuracy and precision of intercalibration, at least compared to the mean or median, since it is simply the most common (or majority) reading. However, we identified three potential sources of error in the selection of the modal age, so it is not ideal.

In 1 otolith (mrnf_OPG-N21), each of the readers identified a unique age, therefore there was no true mode – a mode was forced by the administrator of the analytical spreadsheet (but an alternative approach could have been to use the mean).

In 15 otoliths, there were two equal modes, and in 3 otoliths there were three equal modes. Usually in intercalibration exercises where there are two or more possible modes, the final selection is based on that of the most experienced reader. Reader experience was not registered in the analysis, however, so readers were listed in alphabetic order and mode assigned to the reader nearest the beginning of the alphabet. Alternative approaches would be 1) to use the rankings assigned as a result of the analysis, but this is perhaps a circular process, so another option is 2) to use the mean of modes.

Five of these otoliths with multiple possible modal ages were from eels of known age. In 2 of these otoliths, the ‘other’ mode was the true age, whereas in the other 3, the known age was not represented by either/any mode.

In 27 otoliths, a secondary peak was within 1 year of the mode. Nine of these were from eels of known age. In 3 of these, the chosen mode was correct. However, in the other 6 otoliths, the secondary mode was correct (4) or closer (2) to the known age. Clearly, the mode selection process could have a significant effect on the results of the intercalibration exercise.

Application of the reading rules

The rules for the intercalibration exercise were clearly defined in the report of WKAREA 2009 but not followed by all those who took part in the exercise for WKAREA 2011. This caused several problems with the analysis. First, some readers ignored images that they found difficult to age, whereas readers should have submitted data for every sample. As a consequence of this, it is difficult to compare the results between readers. Second, some readers already knew the age of some eels because they supplied them and / or had read them previously. These readers / examples should be excluded from a future intercalibration exercise. Third, no metadata (e.g., date of capture, stocking history) were provided with the samples. The experience of a middle ranked reader (Reader X) was used to illustrate the consequence of this lack of metadata and other reasons for differences between readings and the modal age, and between the modal age and the consensus view or known age.

Figure 5 shows the difference between Reader X and the modal age for each eel. The summary statistics for Reader X were:

Total Sample:	161 otoliths
Number Read:	110
Number above modal age:	14 (13%)
Number below modal age:	32 (29%) – of which 27 were one year different

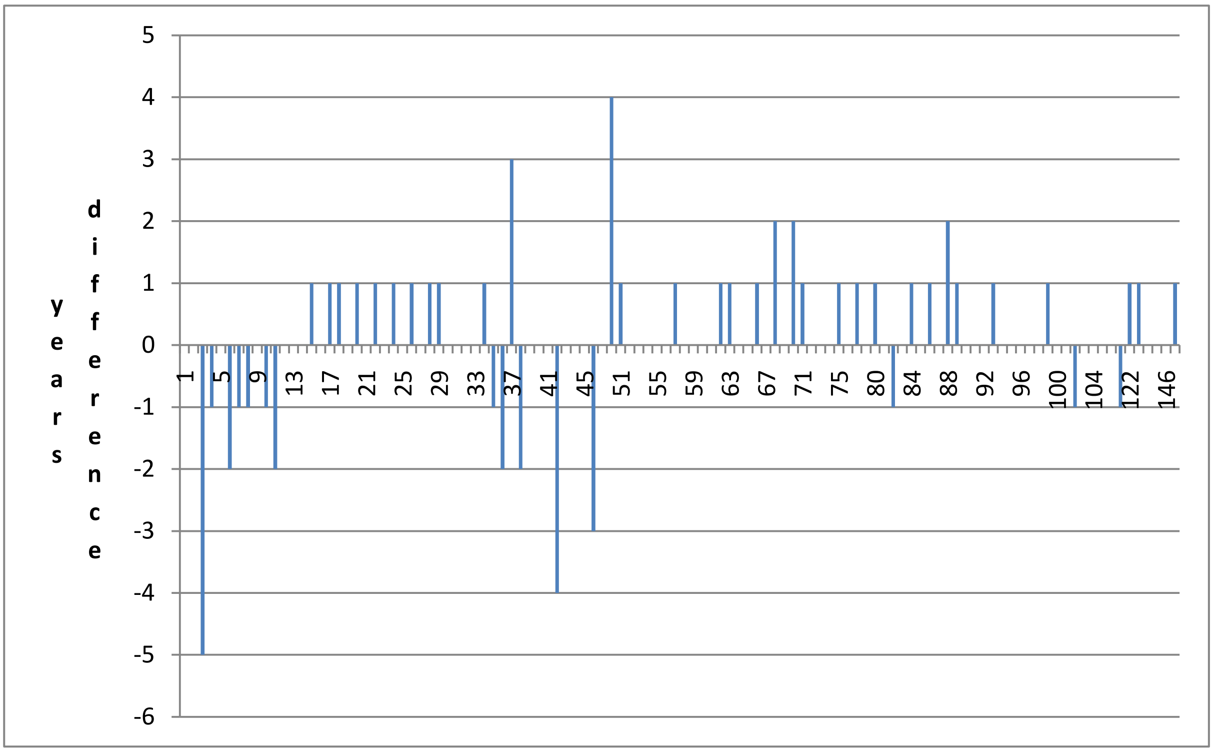


Figure 5: Differences between Reader X and the modal age. The x-axis denotes the eel number and the y-axis gives the difference between the reader and the modal age – positive values indicate higher modal age or Reader X estimating below the mode.

It is clear from Figure 5 that Reader X was generally under-reading by one year with respect to the modal age: 27 less than, and 7 greater than the modal age by one year.

An investigation by the workshop into this phenomenon revealed some differences and explained in many cases why Reader X differed. However, the modal age could also be arrived at by one or more interpretations (see below) and where more than one mode existed, Reader X may have agreed with one of the modes not selected by the software

In the 27 Cases where Reader X under-read the age:

- 7 were as a result of Reader X misidentifying the zero band, partly due to the misleading guide ring in the software (Figure 6).
- 12 were as a result of Reader X not knowing the date of capture and not identifying an annual check on the outer edge of the otolith (Figure 7). However, in 11 of these the modal readers falsely identified an annual band outside the zero band, caused by stress related to quarantine, alizarin marking and stocking and so achieved the correct age by identifying the wrong band (Figure 8).

Therefore, a prior knowledge of the metadata (e.g., date of capture, stocking history) of each eel would have significantly improved the agreement between Reader X and the modal age and also in some cases between the modal and known age.

The presence of growth checks, or false annual bands, also introduces variations in age interpretations (Figure 9). These false annuli were discussed in detail in Chapter 6.3 of the WKAREA 2009 report. An alternative means of validating annual and false annuli, such as otolith micro-chemistry or laser ablation, needs to be investigated in order to cross-validate between methods and determine their accuracy to real age.

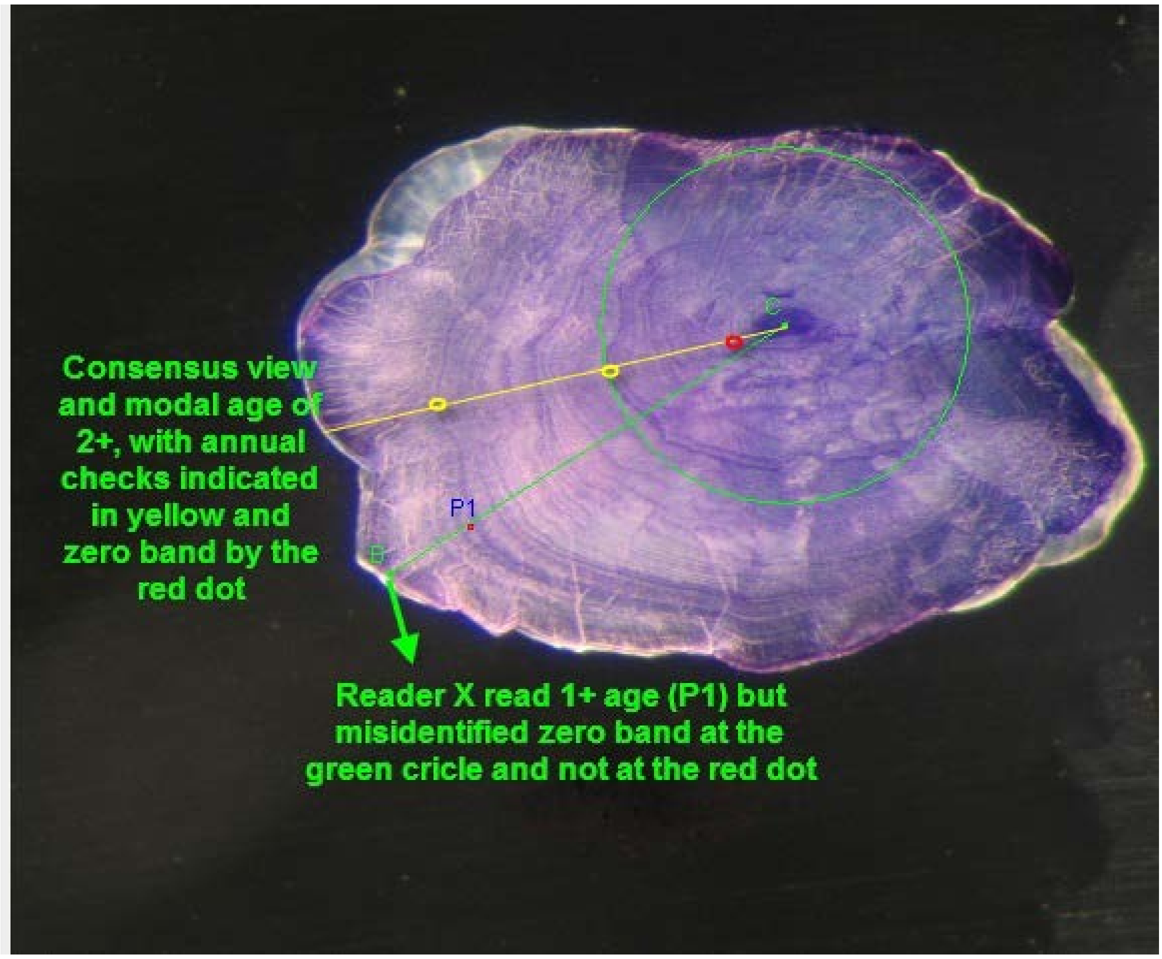


Figure 6. Example of an Italian otolith (lesa Tev .G17) where the guide circle coincided with the first year and not the Zero band, as indicated by the red dot. Reader X aged this one year younger than the mode and the consensus view.

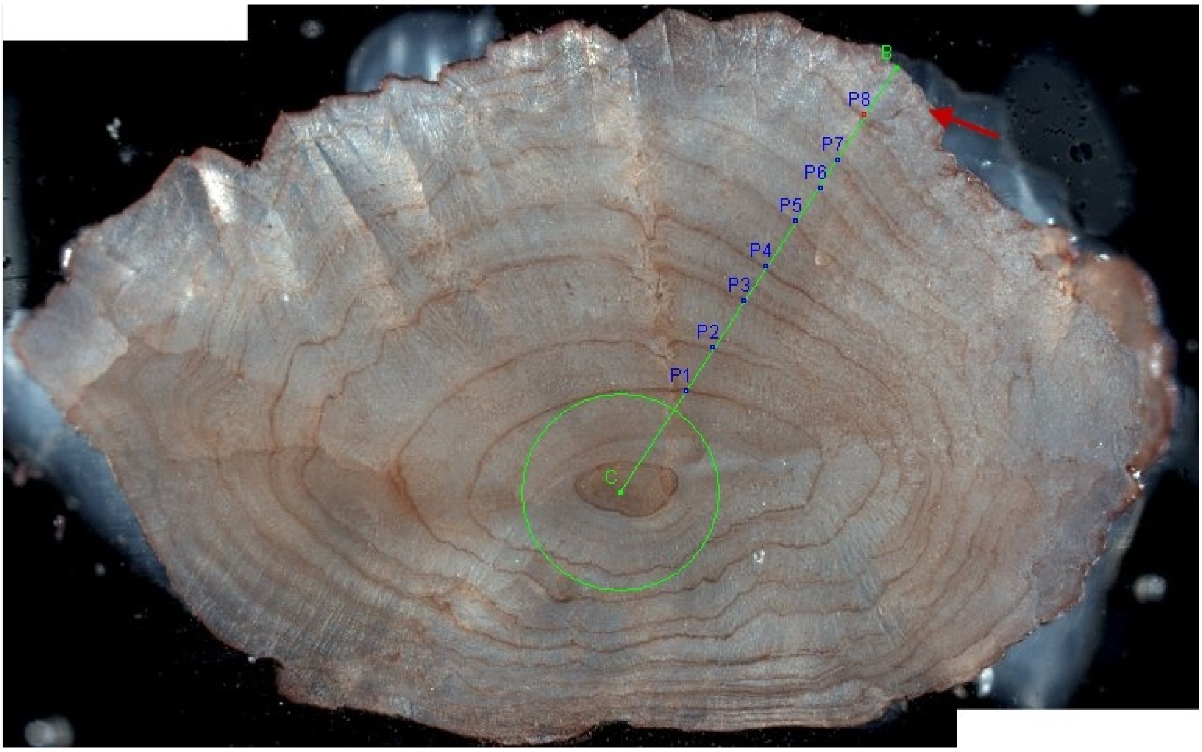


Figure 7: A Swedish yellow eel of known age (sbf 34484) and 9 years of age. The eel was captured on 26th May 2006 at the start of the growing season, but Reader X was not aware of this and failed to identify an annual check on the outer edge indicated by the red arrow.

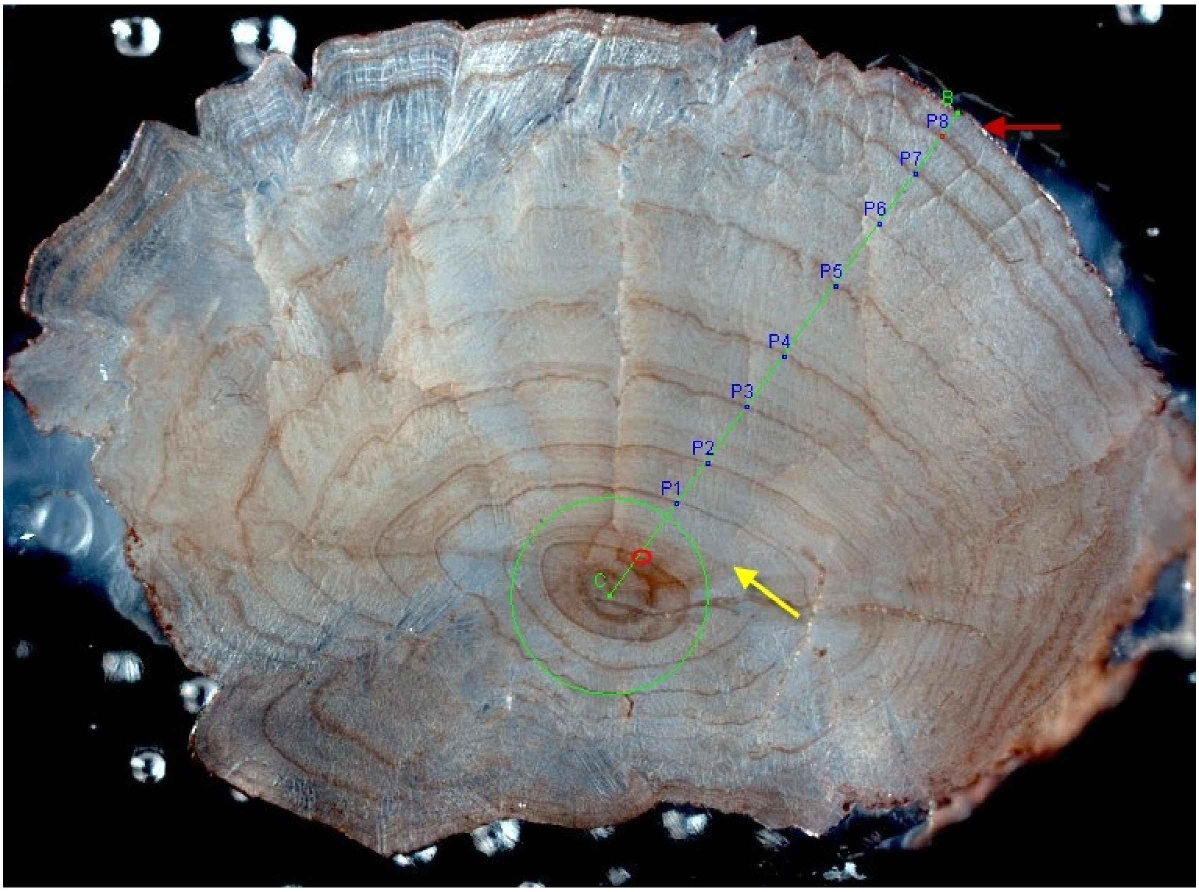


Figure 8: A Swedish yellow eel of known age (sbf 34477) and 9 years of age. The eel was captured on 26th May 2006 at the start of the growing season, but Reader X was not aware of this and failed to identify an annual check on the outer edge indicated by the red arrow. The zero band is indicated by the red circle and the stress check due to alizarin marking and stocking by the yellow arrow. The check indicated by the yellow arrow was erroneously counted as an annual check by some readers.

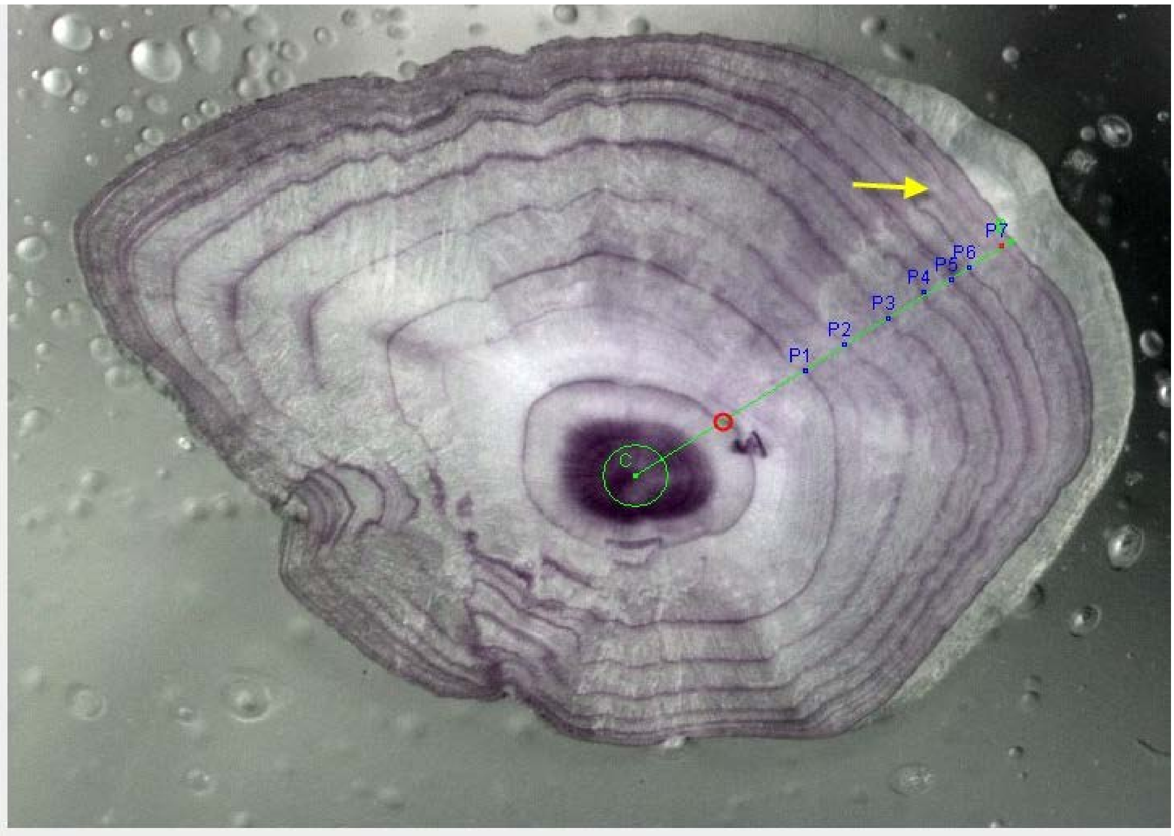


Figure 9: An eel otolith from A. rostrata (Sud-Ouest Y02) showing the zero check (red circle), the consensus age 7+ and the yellow arrow indicating a probable false annulus. Readings were, 6,6,7,7,8,8,9.

Additional analyses for future intercalibration exercises

There are further aspects of the intercalibration data that would be worth exploring during the intercalibration process, but which we have not explored here because of the incompleteness of the present data. For example, analysis should consider how the familiarity of the reader for a species, growth pattern or otolith preparation method might affect their precision across a broad range of samples. We anticipate that familiarity might introduce a bias in the results, but removing this bias will in itself be a significant measure of the success of the intercalibration / reference collection / training guide process. As another example, the analytical software used here also allows for comparison of reader variation per year class of eel. These results can be used to examine in detail whether readers are generally better at identifying younger vs older annuli, or whether biases occur because for example some readers miss marginal annuli or wrongly include stress checks.

We have made no attempt to assess whether the accuracy and precision of this intercalibration exercise are sufficient to support comparisons between studies from different readers across Europe and North America. This is mainly because the data are incomplete due to the various issues discussed above. However, it should be noted that any definition of minimum levels of accuracy and precision must consider 'materiality', i.e. how sensitive to error is the subsequent analysis of data based on ageing by reading otoliths? This sensitivity depends on the age of the otolith and on the intended analysis. For example, reading an age as 5 years instead of 4 would result in 25% error in growth rate, whereas reading 21 instead of 20 years introduces an error of only 5%. In contrast, estimating annual growth rates from back-calculated length-at-age requires correct identification of each annulus.

Conclusions and Recommendations

Our analysis clearly indicates the problems associated with incomplete application of the protocols for reading and for intercalibration. As a consequence, it would be premature to draw conclusions about intercalibration of eel otolith reading across Europe and North America from the present results.

The primary recommendation arising of from our analysis of the intercalibration exercise is that another intercalibration exercise is required, where all the readers faithfully follow the protocols for reading and for completing the intercalibration exercise. The protocols are described elsewhere in this and the 2009 report, but the main lessons learned are that readers should read every otolith, that complete metadata must be available for each otolith, and that readers with prior knowledge of any otoliths should not take part in the intercalibration exercise.

The collection of otolith images for the intercalibration was dominated by images of polished & stained otoliths (151). In contrast, there were only 10 images of otoliths that had been prepared by cracking and burning despite WKAREA 2009 recommending this as the most appropriate method for eel ageing, and particularly for older eels. Similarly, the collection is dominated by younger eels, with only 30 having modal age older than 10 years. Future intercalibration exercises should include a balanced representation of images from the two (or more) preparation methods, and across the entire range of ages, habitats and locations.

6 Reference collection

In the present meeting, based on the analysis of intercalibration exercise, we decided to set up a collection of known age otolith pictures to serve as a reference for age readers training (experienced or inexperienced readers). A total of 38 A. Anguilla and 19 A. rostrata pictures from marked recaptured eels composed this validated reference collection. These validated sets are especially useful to calibrate the age reading method of both experienced and inexperienced readers. Furthermore they can be used as training tools for new readers (Eltink *et al*, 2000).

Every otolith picture of the reference collection was annotated to explain precisely how each check was interpreted. Winter checks and false checks due to stress or marking were pointed out with different colour codes. The reference collection presented in the annex is composed of annotated otolith pictures and associated metadata (length, date and location of capture).

The reference collections is composed of four files of known age eels, two sets of otolith pictures for each species and two sets of annotated otolith pictures where each growth pattern, false checks and zero bands are specifically identified. These four pdf files are available as a stand-alone document.

7 Improvement and update of the manual

7.1 Actual Protocol Improvements Otolith Preparation Methodologies

In order to improve the quality of intercalibration and routine readings some additional recommendations were added to the outcomes of the 2009 workshop.

It was agreed that prior to reading otoliths have to be dried for at least two days to ensure a high quality preparation. Furthermore, vateritic otoliths should be optically identified and excluded from preparation, as they grow chaotically and might lead to incorrect age determinations (see figure 10). Pictures of entire and processed vateritic otoliths are presented (see figure 10) to simplify their identification.

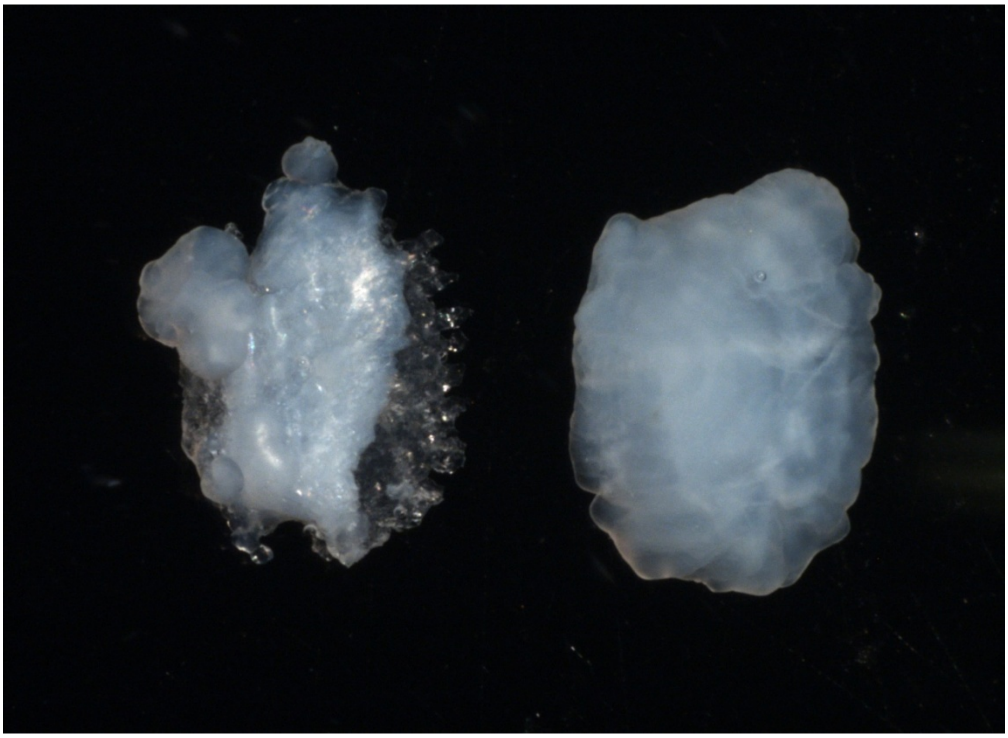


Figure 10: A vateritic (a) and a normal (b) otolith from ***A. anguilla (Elsa Amilhat, Univ. Perpignan)***

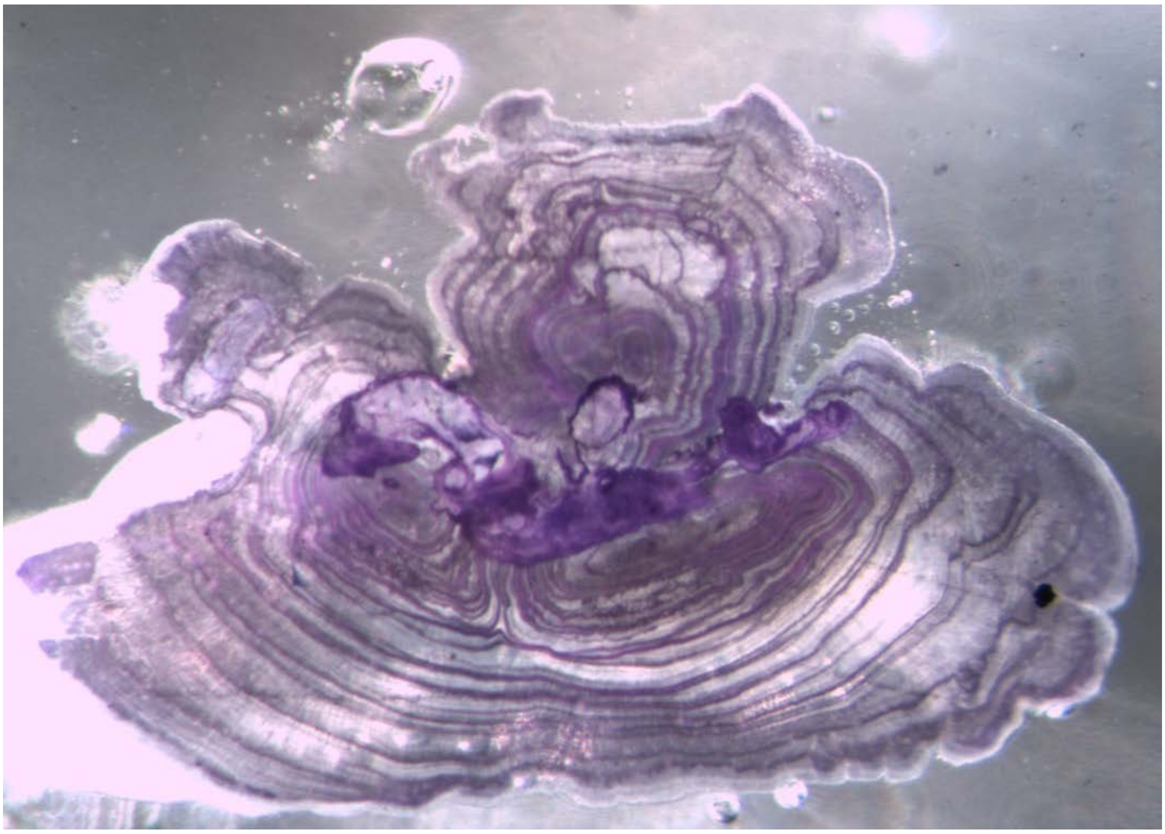


Figure 11: A vateritic otolith prepared by grinding-polishing-staining method from A. rostrata (V. Tremblay, AECOM)

To improve the quality of pictures for intercalibration studies and reading routine a list of recommended image software and their features is provided (Image Pro Plus, Image J, GIMP, NIS Elements, CELL (Olympus), Picasa, MOTIK, COREL Draw suite, Kappa Image Base, Photoshop).

The use of an implemented circular mark in the pictures (Ø 340 μm) to identify the zero band turned out to be helpful and reduces the error of intercalibration readings. There was a consensus that a respective circular mark or at least a proper scale should generally be implemented in otolith pictures. It should however be investigated whether 340 μm are an appropriate diameter to identify the core region of Mediterranean eels, as they tend to grow faster than specimens from further north.

Double bands are difficult to interpret. Regardless of the information stored in these structures the workshop agreed to compare different preparation methods in order to test if double bands are identified similarly. Fiskeriverket and Imares will compare "cut and burn" vs. "grinding-polishing-staining" to shed light on this question. Whether double bands are representing consecutive years or are deposited due to certain environmental events has to be decided individually. The interpretation should include as much life history and habitat information as possible in order to optimize the explanation of double bands.

It was also discussed whether otolith microchemistry could be useful to validate or replace traditional age readings. Despite some potential candidate elements like antimony or lead no procedure is developed yet that improves the accuracy of age determination, making ring counting the method of choice.

a) quality improvement of intercalibration studies

As a result of the recent intercalibration exercise (chapter 1) certain changes are recommended for future intercalibration readings.

The identification of the zero band is considered a problem in the comparability of intercalibration readings (e.g. quarantine marks; see chapter 1.). The quality of intercalibrations would be improved if zero bands are identified and marked by the owner of the picture before uploading. Furthermore, knowledge of the catch date is necessary for the correct interpretation of an outer ring (see chapter 1.). Consequently, the country and location of catch should be indicated as well with respect to different growth seasons. Although the measures suggested above might lead to a lack in objectiveness, there was a consensus that it is a practical approach in order to give every participant a defined area to read, thus enhancing the comparability of readings. The problem of reading the same age by counting different annuli (see chapter 1.) would also be minimized.

Another issue discussed during the workshop was a possible training effect when using pictures that are already known by the reader. In order to eliminate this effect a new set of pictures should be used for each intercalibration reading and participants should not read pictures they contributed themselves.

The number of otoliths read in the intercalibration exercise varied largely between participants. This was partly due to several problems with the online tool (e.g. selection of multiple modes, system failures; see chapter 1.), which are further discussed in chapter X.x. In addition, some participants consciously left out difficult pictures, thus enhancing their average accuracy. In future intercalibrations every participant should make sure to read every otolith and must not leave out any pictures.

To ensure that every participant is given the opportunity for a retrospective analysis of his / her own readings, it was agreed that pictures of the read otoliths – including checkpoints / indication marks – should be stored and accessible for the respective reader. This could be either done by the participant or, even better, provided by the online tool. Further improvements in the online tool are discussed above / below (see chapter x.x). Additionally, it was agreed that handing out the analysis data before an intercalibration meeting would allow for a better preparation of the participants.

b) quality improvement of routine reading

Due to influential differences in the habitat of eel, participants of the workshop agreed that for proper interpretation, it is necessary to keep record of certain data information for each otolith. If available, these datasets should include as many habitat related variables as possible such as capture date, start and duration of the growth period (temperature related), marking or stocking situation, presence of obstacles in the habitat and common growth patterns of the area (slow / fast). Since reading routine indicated problems with the interpretation and validation of the outer bands of the most recent growth period, it was recommended that Håkan Wickström and Francoise Daverat add example pictures of their May / August specimens to illustrate this issue.

To preserve the personal differentiations between annuli and false annuli made during the reading routine, all indication marks / checkpoints should be saved within a screenshot of the otolith for routine reading as well.

Due to differences in quality and elaborateness of prepared otoliths, it was recommended to introduce an affiliated level of confidence within a special column on to the spreadsheet. This level of confidence may help to standardize the readings and classify them for comparable growth rate determination and aging. To determine the level of confidence the readability was thought to be categorized into good, fair and poor. Nevertheless this quality indication needs to take the proportion of discrepancy (interval / estimated age) into account, thus not treating the deviation in the reading of a young eel's otolith the same as in the reading of an old eel. One proposed way to standardise this was to divide the interval of questionable annuli through the expected age of the specimen. The amplitude of the outcome value hence signifies the quality of the reading (the smaller the value – the higher the confidence). Another proposed way to manage this issue was to note the expected age, the oldest and lowest possible age and to include all 3 outcomes into the graphs.

To improve reader training and routine, it was suggested to prepare for the reading of a series of otoliths by studying the reference collection with special emphasis on specimens of known age and those with consensus-aged structures. While reading new sets of otoliths, results should be confirmed by a number of readers. Following the previous protocol this means at least 2 readers and/or 2 readings. If both readers agree on the estimated age of one otolith (consensus), they should compare their checkpoints within the images to see if they have marked & considered autogenic annuli. If there is no consensus between the readers, it is advisable to discuss the disagreements by investigating the images and (while doing this) taking the readability, age interval columns (spreadsheet) and comparable reference pictures into account.

Findings advised that for "reader training and routine" it would be helpful to intermix recently aged samples into the reference collection for the periodic routine / training to keep track of the draft of a single reader.

c) See the existing tool for exchanging otolith for ideas: http:\\webgr.azti.es/:

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Annex 1: List of participants

Name	Address	Phone/Fax	Email
Elsa Almihat	UMR 5110 CNRS – UPVD Université de Perpignan - Centre de Formation et de Recherche sur l' Emvironnement Marin (CEFREM) 58 Avenue Paul Alduy 66860 Perpignan Cedex France	Phone +33 468662186 Fax: +33 468662281	elsa.amilhat@univ-perp.fr
Jean-Christophe Aymes	UMR INRA/UPPA Ecobiop Quartier Ibarron 64310 St Pée-sur-Nivelle France	Phone +33 5 59515984 Fax +33 5 59 545152	jcaymes@st-pee.inra.fr
Pierluigi Carbonara	COISPA Tecnologia & Ricerca Stazione Sperimentale per lo Studio delle Risorse del Mare Via dei Trulli 18/20 70045 Bari Torre a Mare Italy	Phone +39 080 5433596 Fax +39 080 5433586	carbonara@coispa.it
Jennie Dahlberg	Swedish Board of Fisheries Institute of Freshwater Research, Drottningholm Stångholmsvägen 2 178 93 Drottningholm Sweden		jennie.dahlberg@fiskeriverket.se
Francoise Daverat Chair	CEMAGREF Unité de Bordeaux 50 av de Verdun 33612 Cestas France	Phone +33 557 890806	francoise.daverat@bordeaux.cemagref.fr
Nicolas Delorme	CEMAGREF Unité de Bordeaux 50 av de Verdun 33612 Cestas France		nicolas.delorme@cemagref.fr
Marko Freese	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Wuldorfer Weg 204 22926 Ahrensburg Germany	Phone: +49 4102 70860-21 Fax: +49 4102 70860-10	marko.freese@vti.bund.de
Adam Grochowski	Sylwia Stasiak ul. Kollataja 1 81-332 Gdynia Poland	Phone +48 587356206	agrochowski@mir.gdynia.pl
Yvette Heimbrand	Swedish Board of Fisheries Institute of Freshwater Research Stångholmsvägen 2 178 93 Drottingholm Sweden		yvette.heimbrand@fiskeriverket.se
Emil Kuijs	Wageningen UR Centre for Marine Policy P.O. Box 1528 8901 BV Leeuwarden Netherlands		Emil.kuijs@wur.nl
Chiara Leone	Tor Vergata Via Cracovia 1 00133 Rome Italy	+39 06 72595852	leonechiara@hotmail.com
Lasse Marohn	Leibniz-Institute of Marine Sciences IFM-GEOMAR FB3 Marine Ecology Düsternbrooker Weg 20 24105 Kiel Germany	Phone +49 431 600 4554 Fax +49 431 600 4553	lmarohn@ifm-geomar.de
Tomasz Nermer	Sea Fisheries Institute in Gdynia ul. Kollataja 1 81-332 Gdynia Poland	Phone +48-587356206	nermer@mir.gdynia.pl
Anne Odelström	Swedish Board of Fisheries Institute of Coastal Research P.O. Box 109 SE-74222 Ôregrund Sweden	Phone +46 173 46469	anne.odelstrom@fiskeriverket.se

Jan-Dag Pohlmann	Johann Heinrich von Thünen-Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute for Sea Fisheries Wuldorfer Weg 204 22926 Ahrensburg Germany	Phone: +49 4102 70860-21 Fax: +49 4102 70860-10	jan.pohlmann@vti.bund.de
Russell Poole	Marine Institute Furnace Newport Co. Mayo Ireland	Phone + 353 98 42300	russell.poole@marine.ie
Dirk Schaerlaekens	Laboratory of Animal Diversity and Systematics Deberiotstraat 32 bus 02439 3000 Leuven Belgium	Phone +32 16 324296 Fax +32 16 324575	dirk.schaerlaekens@bio.kuleuven.be
Valérie Tremblay	AECOM 2, rue Fusey Trois-Rivières Québec G8T 2T1 Canada	Phone +1 819 373 6820 Fax +1 819373 7573	valerie.tremblay@aecom.com
Guy Verrault	Ministere des Ressources naturelles et de la Faune du Quebec 506 rue Lafontaine Rivière-du-Loup, Qc Québec G5R 3C4 Canada	Phone +1 418 862 8649 ext. 226 Fax +1 418 862 8176	guy.verreault@mrnf.gouv.qc.ca
Alan Walker	Centre for Environment, Fisheries and Aquaculture Science CEFAS) Pakefield Road NR33 0HT Lowestoft Suffolk United Kingdom	Phone +44 (0) 1502 562244 Fax +44 (0) 1502 513865	alan.walker@cefas.co.uk
Håkan Wickström	Swedish Board of Fisheries Institute of Freshwater Research, Stångholmsvägen 2 178 93 Drottningholm Sweden	Phone +466990607 Fax +466990650	hakan.wickstrom@fiskeriverket.se
Kazuki Yokouchi	CEMAGREF Unité de Bordeaux 50 av de Verdun 33612 Cestas France		kazuki.yokouchi@cemagref.fr

Annex 2: Agenda

Tuesday 22 of March 2011 (Cemagref Cestas):

11:00 Welcome of participants

12:00 Lunch

14:00 opening of the workshop, overview of reader inter calibration issues

16:00 Plenary session: interpretation of the readings intercalibration (web platform)

17:30 end of the day

18:07 train to Hotel in Arcachon

19h30 or 20h dinner

Wednesday 23 of March 2011 (Hôtel de la Plage, Arcachon):

9:00 Plenary session: how to increase quality in age reading ?

Age reading protocol = rules for reading, additional data. Necessity of a common data set of pictures for training

11:00 Work on subgroups, reader inter-calibration work

12:30 Lunch

13:30 Work on subgroups (report)

15:30 Plenary

17:30 end of the day

Thursday 24 of March 2011 (Cemagref Cestas):

8h26: train from Arcachon to Cestas 9h15

Collating of the report, circulation and discussion.

13:00 Lunch

End of the meeting

Annex 3: WKAREA terms of reference for the next meeting

a) Find relevant tool for exchanging images and readings with two options improve the tool we have used or join the Azti web platform (including the hosting of the reference collection database)

b) increase the number known age samples in the reference collection

c) repeat the intercalibration exercise applying the protocol (read reference collection first, then read new samples etc…)

d) the intercalibration will be performed on new samples representative of the different methods of preparation and the different habitats and locations

e) Include the « new methods » of preparation that are validated with present agreed methods

Annex 4: Recommendations

We suggest that each Expert Group collate and list their recommendations (if any) in a separate annex to the report. It has not always been clear to whom recommendations are addressed. Most often, we have seen that recommendations are addressed to:

- Another Expert Group under the Advisory or the Science Programme;
- The ICES Data Centre;
- Generally addressed to ICES;
- One or more members of the Expert Group itself.

Recommendation	For follow up by:
1.Set up new validation projects to obtain known age eels from different locations using direct (mark recapture of marked otoliths)	ICES
2. Provide indirect estimation of age with direct estimation of growth rate (mark recapture of fish)	ICES
3. Investigate alternative methods of age estimation such as otolith chemistry (lead radium decay)	ICES
4. Validate new methods of otolith preparation	Other members
5. the validation projects should be funded at international level	ICES
6. DCF should support/manage the reference collection	WGDIM (group on Data and Information management)

After submission of the report, the ICES Secretariat will follow up on the recommendations, which will also include communication of proposed terms of reference to other ICES Expert Group Chairs. The "Action" column is optional, but in some cases, it would be helpful for ICES if you would specify to whom the recommendation is addressed.

Annex 5: Manual for the Ageing of Atlantic Eel (separate document)