THIRD WORKSHOP ON AGE READING OF EUROPEAN AND AMERICAN EEL (WKAREA3)

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Contents

i Executive summary

The Working Group on Age Reading of European and American Eel (WKAREA) seeks to improve the accuracy and precision of age estimates across the range of the species based on standardized methods and criteria, in order to support stock assessment at local and global levels. The group conducted a collective reading of European eel otoliths extracted from eels sampled in six aquatic systems from the South West Europe area (SUDOE area), which had been poorly represented in previous workshops. In this report the group analyses the results of the intercalibration process, identifies causes of error and their consequences on precision and accuracy, and provides recommendations for future work. The aging performance of advanced and basic readers was poor, suggesting that the growth patterns rather than the reader experience are responsible for the low consistency among readers. The otoliths from the southern part of the eel range presented an overall growth pattern, that is completely different from what has been seen in otoliths from the northern area. The irregular pattern of annuli along with the presence of numerous supernumerary rings complicated the interpretation of the growth pattern in the otoliths used in the exchange. It was concluded that it was impossible to distinguish between annuli and supernumerary rings. In habitats from the south, especially the Mediterranean region it is likely that low river flow during summer, associated with high temperatures, are responsible for the deposition of many supernumerary rings. In view of the uncertainty associated with the age estimation of eels in the southern area, there are concerns in the use of age readings data for stock assessment. To further increase precision and reduce the risk of biased growth estimates in the southern area, it is a priority that mark recapture studies are conducted to ground truth the age and identify patterns of ring formation. A field study to test the effect of summer temperature and river flow on eel growth patterns in the southern area should clarify the patterns of annuli formation.

ii Expert group information

Expert group name	Third Workshop on Age Reading of European and American Eel (WKAREA3)
Expert group cycle	Annual
Year cycle started	2019
Reporting year in cycle	1/1
Chair(s)	Isabel Domingos ,Portugal
	Françoise Daverat, France
	Kélig Mahé, France
Meeting venue(s) and dates	17-18 June, Bordeaux, France, (32 participants)

1 Terms of Reference and reporting

The **Third Workshop on Age Reading of European and American Eel** [WKAREA3], chaired by Françoise Daverat (France), Isabel Domingos (Portugal) and Kélig Mahé (France) met in Bordeaux, France, from 17-18 June 2019 to address the following terms of reference (ToR):

a) Exchange a collection of European eel otolith pictures, including known age eels, with samples prepared using different protocols and representing all the eel sub-populations and their respective environmental types from Portugal, Spain and France (Science Plan codes: 3.3, 4.1, 4.4)

b) Conduct an age intercalibration process with institutions throughout Europe, applying the ageing criteria defined during the Workshop on Age Reading of European and American Eel (WKAREA) to the otolith image library compiled by the workshop.; (Science Plan codes: 3.3, 4.1, 4.4)

c) Develop recommendations on any aspects of the age estimation criteria that could be refined to increase the standardization, precision and accuracy of eel age estimation. (Science Plan codes: 3.3, 4.1, 4.4)

WKAREA3 will report by 1st October 2019, for the attention of WGBIOP and EOSG.

The structure of this report follows the order of the Terms of Reference (ToR) for the meeting, as follows:

Chapter 4 of this report presents the methods and procedures followed during the workshop, describes the sampling area and the samples used in the age calibration exercise, and the statistical analyses used to compare the results obtained on age reading. (**ToR a**).

Chapter 5 presents an overview of samples and readers, the results from the age reading, a discussion and conclusion. (**ToR b**).

Chapter 6 presents recommendations to improve precision and accuracy in future age estimates. (**ToR c**).

The report also includes the agenda of the workshop (Annex 1), the list of participants and their contacts (Annex 2) and a glossary of terms used in the text (Annex 3)

2 Agenda and participant list

The agenda for the workshop is provided in Annex 1 and the list of participants, in Annex 2. Thirty-two attendees (Figure 2.1), representing 12 countries, three from the SUDOE area (France, Spain and Portugal) and nine from elsewhere in Europe (Norway, Sweden, Finland, Ireland, The United Kingdom, Poland, Germany, Greece and Turkey), participated in the workshop. The address list of the workshop participants can also be found in Annex 2.

Figure 2.1 Participants in the WKAREA3 working in groups to identify the main problems in the age reading held prior to the workshop.

3 Introduction

The life cycle of both Atlantic eel species eel is complex. The stocks are panmictic and data on larvae caught at sea indicate that the spawning area is in the southern part of the Sargasso Sea. The newly hatched larvae, leptocephali, are transported by ocean currents to the continental shelf of Europe and North Africa in the case of the European eel (*Anguilla anguilla*), and to the continental shelf of North and Central America, in the case of American eel (*Anguilla rostrata*), where they metamorphose into glass eels and enter continental waters (Benchetrit & McCleave, 2016; Tesch, 2003). Here, they acquire pigmentation, become yellow eels and may remain in coastal waters and estuaries or colonize the river basins, where they spend a variable number of years in their growth phase. At the end of their life in continental waters, they transform into silver eels and start the migration back to the Sargasso Sea to reproduce and eventually die.

The European eel has a widespread distribution that extends across most coastal countries in Europe and North Africa, with its northern limit in the Barents Sea (72°N) and its southern limit in Mauritania (30°N), spreading across the entire Mediterranean basin (ICES, 2017). Besides this widespread distribution, the species inhabits practically all types of aquatic habitats, *i.e.*, lakes, rivers, estuaries, coastal lagoons and coastal/marine waters, where productivity varies substantially, providing diverse and variable environmental conditions during their growth phase. Finally, sex determination occurs late in the life cycle, and there are differences between males and females. Altogether, this wide range of conditions contributes to a large variation in the growth rate.

Age-at-maturity varies according to temperature, habitat type, and density-dependent processes (ICES, 2014). It is therefore expected that silver eels that have grown in estuaries and coastal lagoons are younger than those that have grown in rivers, located at the same latitude. Likewise, growth is expected to be faster in the southern area of distribution of the species due to higher temperatures, which implies that silver eels migrating from latitudes further south are younger than those migrating from northern European rivers.

Age and growth of eels has been extensively investigated but age determination has been considered difficult, as expressed by Bertin (1951) who wrote "*Elle (l'anguille) est le plus déplorable matériel d'étude de la croissance que l'ont puisse imaginer*". This problem is evidenced by the vast literature on age as well as methods used in the preparation of otoliths, since the early 20th century (Daverat *et al.*, 2012). To overcome difficulties in age reading, an effort to standardize age determination criteria and methods for ageing European eel started at the EIFAC Eel Age workshop held in 1987 (Vøllestad *et al.*, 1988). Following this workshop, two other workshops on Age Reading of European and American Eel (WKAREA) have taken place (ICES, 2009a; 2011).

The first WKAREA workshop, held in 2009, had as priority to review the literature on current practices in otolith preparation methods, establish guidelines for age interpretation, and develop a manual for the ageing of Atlantic eels. An exchange of otoliths from eels of known age was also carried out during this workshop, but the results were only used to support the discussions for the report (ICES, 2009a). The effort to standardize the methodologies for otolith preparation and guidelines for age interpretation, carried out during the workshop, resulted in the production of a "Manual for the Ageing of Atlantic Eels (ICES, 2009b).

The second WKAREA workshop, held in 2011, was preceded by an otolith age reading exchange, with eels of known and unknown age to interpret the results of the intercalibration exercise and make recommendations to improve age precision and accuracy (ICES, 2011). The overall agreement ranged from 66.2% to 13.2% and a discussion was conducted to identify the problems in the intercalibration exercise. The manual was updated to include improvements for the different preparation protocols, and a reference collection with otoliths from American and European eel of known age was set up.

Although age reading criteria have greatly improved in recent years, the representativity of eels from the southern part of the species natural spatial range, both in age calibration workshops and in literature has remained very poor or even absent. Most age and growth studies that have been conducted in southern latitudes included only eels from brackish water systems (e.g. Férnandez-Delgado *et al*., 1989; Gordo & Jorge, 1990; Rossi & Villani, 1980), where environmental conditions are more stable than the ones prevailing in freshwater systems, and therefore may account for different growth rates. Furthermore, brackish water systems are highly productive, which may confound the effects of habitat and latitude on eel growth.

The present workshop (WKAREA3) is at the same time, a technical meeting within the frame of the SUDOANG project, and a WKAREA meeting. The main goals were to provide the SUDOANG colleagues involved in age reading with methods and training to estimate the eel age, and to improve eel age estimation by expanding the geographical distribution of eel towards the South of the distribution area, contrary to the two previous WKAREA meetings, which were biased to the central and northern parts of the species range both with samples used (Sweden, Ireland and France) and experts who participated in the meetings.

Growth patterns of eels in Iberian Peninsula, and in the Mediterranean are likely to be much different than eel growth patterns in the northern part. Apart from the temperature patterns across the year, the southern part of Europe is likely to be more affected by strong variations of river flow than the northern part of Europe. Hence, in addition to fill a gap identified in previous workshops on age reading of European and American eels, i.e., the lack of samples from the southern European eel subpopulations, this workshop will bring together researchers from different laboratories, promoting the sharing of different experiences. It is expected that the results contribute to improve knowledge on age of the European eel in its southern range, since the otoliths used in the exchange have been extracted from eels sampled in France, Spain and Portugal.

4 Mthodology (ToR a)

The workshop was proposed as part of the SUDOANG project, funded by Interreg, which aims, among other objectives, to develop standardized methods to collect data on the status of the population in the SUDOE area, in order to support managers in their actions to comply with Regulation EC No. 1100/2007, and accelerate the recovery of the species. Given the time elapsed since the last WKAREA (ICES, 2011) and the interest expressed by some members of the WGEEL in participating, it was decided to extend this workshop to participants outside the project. The preparation of samples was done by members of the SUDOANG. The techniques used in otolith preparation included grinding and polishing along the sagittal or transverse plane. Some images of the whole otolith from eels younger than 5 years were also captured for the exchange.

The participants in the workshop were members of the SUDOANG project, the WGEEL and other ICES groups. All together, these participants represented 22 different institutions from all over Europe. This meeting brought together countries from the northern and southern part of the European eel (*Anguilla anguilla*) range, creating an opportunity for the exchange of samples that had been poorly represented in previous workshops on age reading of European and American eels (WKAREA) (ICES, 2009a; 2011).

The otoliths analysed in the present workshop were obtained from European eels caught in the SUDOE area, within the framework of the project SUDOANG, which established an eel monitoring network consisting of 10 pilot basins. The aquatic systems investigated for eel ageing in this workshop included 5 rivers from the Atlantic (Nivelle, Minho and Mondego rivers) and the Mediterranean (Guadiaro and Ter rivers) coasts, as well as a brackish water coastal lagoon, Bages-Sigean, in the Mediterranean. Except for eels caught in the Bages-Sigean coastal lagoon, all eels were sampled in freshwater habitats (Figure 4.1).

Figure 4.1. Location of the river basins where the eels whose otoliths were extracted for age reading, were captured. The remaining river basins are also part of the eel monitoring network in the SUDOANG project. (https://sudoang.eu/en/project).

The eels used for age reading ranged from 100-728 mm and their distribution per length class are presented in Figure 4.2

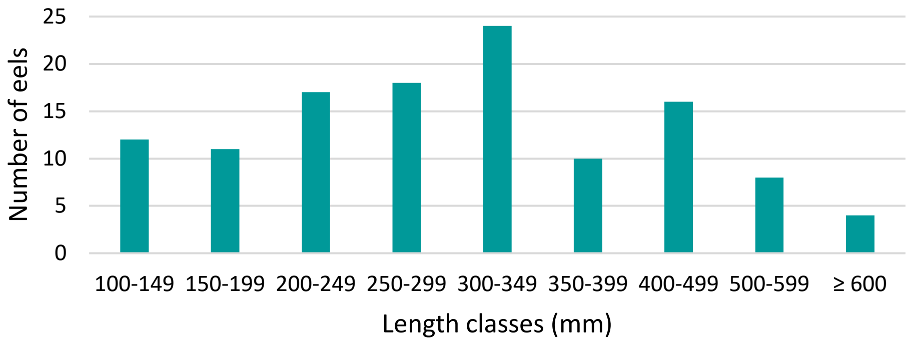

Figure 4.2. Length class frequency distribution of European eel sampled in six river basins, from September 2018 through February 2019 used in the age reading exercise.

The extraction and preparation of otoliths for age reading followed a protocol prepared within the framework of the SUDOANG project - Protocol for otolith preparation and age reading - (available at https://sudoang.eu/en/task-groups/), which was developed according to the methodology defined in the "*Manual for the Ageing of Atlantic Eel*" (ICES, 2009b). The otoliths analysed in the present exchange were embedded in epoxy and prepared in two different ways, which included: 1) grinding and polishing along the sagittal plane, followed by staining; and 2) cutting the otoliths in half along the transverse axis, through the nucleus, and mounting them against a microscope slide. For some eels under 5 years of age, images of the whole otolith were obtained without any preparation (in toto), except for its immersion in 96% ethanol to improve the visualization of growth marks. It is important to mention that, SUDOANG members had not been involved in WKAREA previous meetings. A significant part of SUDOANG members had no experience in preparing eel otoliths using the standardized methods that were implemented within WKAREA1 and WKAREA2 meetings. The methodology of embedding otolith, polishing, and the methodology of picture acquisition was then new to most of the researchers that provided the pictures of samples within the present exchange.

A total of 120 otolith images, uploaded in the ICES web application SmartDots (Event 225), were made available to all participants for age reading. Instructions for participants to start their otolith reading were provided prior to the workshop and readers completed their reading prior to this event. It was not possible to carry out an inter-calibration exercise until the workshop took place due to numerous factors, some related to the lack of information from the available images, which delayed their uploading on the web platform. There was no reference collection of known age available for the samples of the different locations that were exchanged.

In the first day of the workshop the results of the exchange were analysed and discussed based on the on-screen presentation (via Smartdots) of some otoliths considered the most difficult. A review of criteria to estimate age with a special focus on the zero band was also performed during the workshop. Problems on possible double checking and supernumerary rings were discussed in view of the environmental conditions typical of the southern part of the species range (a glossary of terms used within this document is provided in Annex 3). At the end of the discussion, three working groups were set up to identify causes of error in age determination and to point out suggestions to improve precision.

It was agreed to carry out a second reading, after the workshop was over, with a subsample of the otoliths used during the first reading. A random selection was conducted, by river basin, and 59 otoliths were made available to all readers, once again, via the Smartdots platform. Eighteen readers out of the 32 participants in the WKAREA3 workshop did a second reading. In addition, another reader, who could not attend the workshop, participated in the exchange.

The automatic report produced by the Smartdots Application, contains statistical analyses and comparisons of age readings from the Guus Eltink Excel sheet 'Age Reading Comparisons (Eltink, 2000), which are produced in in the form of tables and graphical plots. These analyses include percentage agreement (PA), i.e., the level of accuracy compared to modal age, coefficient of variation (CV), i.e., the reproducibility of age estimation, and bias tests and plots. The formulas are presented below:

- **Percentage Agreement**

The table presents the percentage agreement (PA) per modal age and reader. The PA The is calculated as the ratio between the total number of age readings in agreement with modal age and the total number of age readings for that sample, per reader and modal age.

$$PA = Number\ of\ readers\ agreeing\ with\ modal\ age / Total\ number\ of\ readers * 100\ \%$$

- **Coefficient of Variation**

The table presents the coefficient of variation (CV) per modal age and reader. The CV is calculated as the ratio between the standard deviation (σ) and mean value (μ) per reader and modal age:

$$CV = \sigma / \mu \cdot 100\%$$

These outputs were analysed to help addressing the ToR's from the workshop, and compare improvements following the discussion.

Smartdots also allows the indication of an Age Quality (AQ) score, based on the difficulty presented by the otolith to the reader. Thus, in addition to estimating the age of the otoliths included in the exchange, readers also provided an AQ score for each age assigned. This quality can be classified according to 4 scores:

- **AQ1**: Easy to age with high precision;
- **AQ2**: Difficult to age with acceptable precision;
- **AQ3**: Rings cannot be counted, and the calcified structure is considered unreadable (no age assigned);
- **AQ3_QA**: Rings cannot be counted, and the calcified structure is considered un-readable. Age is assigned for Quality Assurance purposes only.

To determine if there was any significant ($p<0.05$) relationship between the frequency of AQs and river basin or type of preparation, two chi-square (χ^2) tests of independence were performed: one to determine if the proportion of individuals from different AQ scores (ageing difficulties) was independent of the river where eels had been sampled, and the other to determine if the proportion of individuals from different AQ scores independent of the otolith preparation method. In both cases, a post hoc simultaneous test procedure was performed to identify the homogeneous sets of sites or preparation methods for the AQs considered.

5 Analysis of age calibration exercise (ToR b)

5.1 Overview of samples and readers

5.1.1 First reading

A total of 120 sagittal otoliths extracted from European eel with total length ranging from 100 to 728 mm were analysed (Table 5.1). Twenty-one readers completed their readings, including three, classified as advanced readers (Table 5.2). The modal age ranged from 0-14 for the whole set of otoliths (Table 5.1). The distribution of samples per pilot basin is also shown together with their modal age range and length range. Eels smaller than 150 mm are only represented in samples from rivers Ter, Guadiaro, Mondego and Nivelle.

Table 5.1. Overview of samples analysed in the first age reading (Smartdots event 225) for the whole sample (N = 120) and their distribution per pilot basin. Number of samples, modal age range and total length range, are also presented.

Month/Year	ICES area	Pilot basins	Number of samples	Modal age range	Length range
Nov2018	27.7	Bages-SIgean Lagoon	21	2-9	206-728 mm
Oct2018-Feb2019	missing	River Ter	19	0-13	109-598 mm
Sept2018	missing	River Guadiaro	20	0-9	126-518 mm
Oct/Nov2018	27.9.a	River Mondego	20	1-9	140-460 mm
Oct2018	27.9.a	River Minho	21	4-12	182-390 mm
Sep2018	27.8.b	River Nivelle	19	1-14	100-560 mm

Table 5.2. Overview of readers

Reader code	Expertise
R06	Advanced
R08	Advanced
R10	Basic
R12	Basic
R14	Basic
R18	Basic
R20	Basic
R22	Advanced
R24	Basic
R26	Basic
R30	Basic
R32	Basic
R34	Basic
R36	Basic
R40	Basic
R42	Basic
R44	Basic
R46	Basic
R48	Basic
R50	Basic
R58	Basic
R62	Basic

5.1.2 Second reading

A total of 59 sagittal otoliths extracted from European eel with total length ranging from 100 to 728 mm, which were subsampled from the first reading, were analysed (Table 5.3). A total of eighteen readers completed their readings and no advanced readers were included in the exchange (Table 5.4). The modal age ranged from 0-13 for the whole set of otoliths (Table 5.3). The distribution of samples per pilot basin is also shown together with their modal age range and length range.

Table 5.3. Overview of the subsample (N = 59) of otoliths used for the second reading and their distribution per pilot basin. Number of samples, modal age range and total length range, are also presented.

ICES area	Pilot basin	Number of samples	Modal age range	Length range
27.7	Bages-SIgean Lagoon	10	0-10	206-728 mm
Missing	River Ter	9	2-10	109-366 mm
Missing	River Guadiaro	10	2-6	126-316 mm
27.9.a	River Mondego	10	2-6	140-359 mm
27.9.a	River Minho	10	2-13	182-390 mm
27.8.b	River Nivelle	10	1-13	100-560 mm

Table 5.4. Overview of readers

Reader code	Expertise
R06	Basic
R08	Basic
R12	Basic
R14	Basic
R16	Basic
R18	Basic
R20	Basic
R22	Basic
R28	Basic
R30	Basic
R40	Basic
R42	Basic
R44	Basic

R46	Basic
R50	Basic
R56	Basic
R60	Basic
R64	Basic

5.2 Results

5.2.1 First reading-All readers

Measures of Reader Precision

Mean Percentage Agreement (PA) and Coefficient of Variation (CV) for all readers combined, per modal age are presented in Table 5.5. The results indicate that in general, there was a poor level of agreement among readers for all ages. The overall weighted mean percentage agreement (PA) based on modal ages for all readers combined was low (40%), ranging from 21% at modal age 0, to 51% at modal age 3. As for the coefficient of variation (CV), the overall weighted mean was 39%, with a range between 16% at modal age 14, and 62% at modal age 2. The highest CVs occurred at modal ages 1 and 2, indicating that there are difficulties in interpreting the growth pattern at younger ages.

Table 5.5. Summary of the Percentage agreement (PA) and Coefficient of Variation (CV) by modal age, for all readers combined. The total number of age readings (N= 2280) distributed per modal age is also presented. (*) The coefficient of variation is not calculated for modal age 0

Modal age	PA	CV	Number of readings
0	21%	(*)	38
1	47%	62%	111
2	50%	56%	241
3	51%	36%	298
4	47%	37%	305
5	40%	41%	247
6	37%	36%	214
7	30%	35%	246
8	32%	32%	269
9	36%	33%	169
10	26%	25%	62
11	24%	30%	21
12	33%	17%	21
13	26%	46%	19
14	26%	16%	19
Weighted Mean	**40%**	**39%**	

The percentage agreement (PA) for otoliths of the same modal age ranged from 15 to 79% (Figure 5.1). From the 120 otoliths used in the exchange, 13 otoliths had a PA > 60%, with modal age from 1-5. For each modal age, there was a wide range in PA, with the maximum range of 53% at modal ages 2 (26-79%) and 5 (15-68%) showing the high level of discrepancies in the ages assigned by readers to each otolith. The PA decreases strongly at older age groups (modal age 10 and onwards), although a slight decrease is already evident from age 7 onwards. The CV of the whole sample ranged between 9 and 86% (Figure 5.2), which reflects the low agreement and, therefore the discrepancies among readers. The lower variability in the CV observed at older ages (modal age ≥ 10) may result from the small number of otoliths analysed (N = 7).

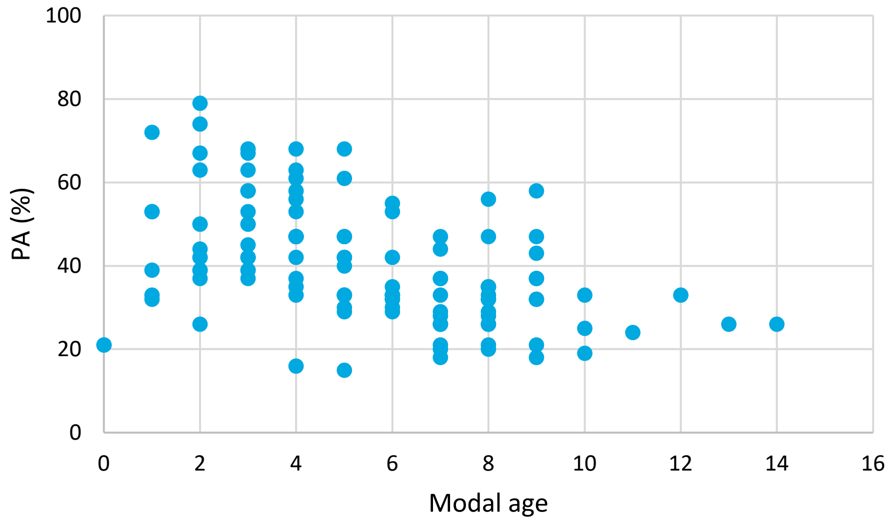

Figure 5.1. Range of the percentage agreement (PA) for otoliths of the same modal age, all readers combined, with modal age ranging from 0 to 14 years.

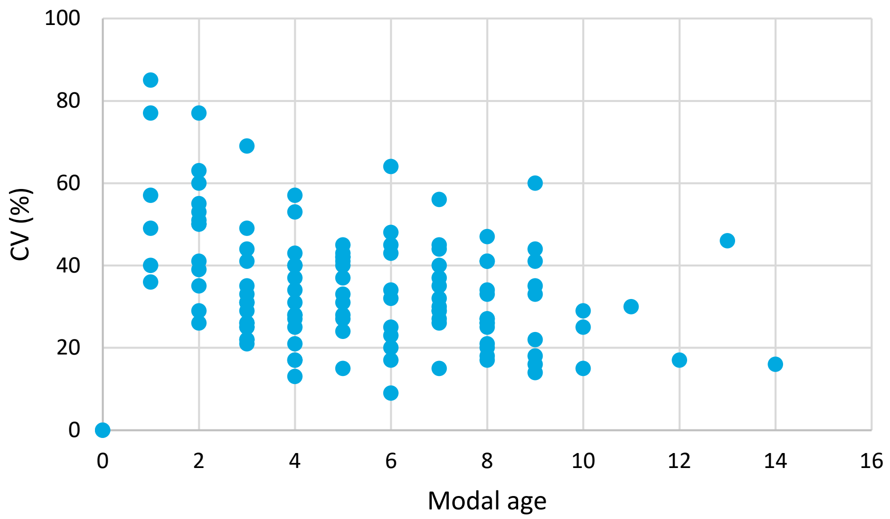

Figure 5.2. Range of the coefficient of variation (CV) for otoliths of the same modal age, all readers combined, with modal age ranging from 0 to 14 years.

The age bias plot for all readers combined (Figure 5.3) shows that in general, there is a good reading agreement with modal age. The lowest variability in the ages assigned by readers occurs at modal ages 1 - 4. An extremely high positive bias and variability among readers is observed at modal age 0, reflecting errors in the identification of the zero band. In contrast, a negative bias is observed at older ages (modal ages 13 and 14), indicating underestimation of age in comparison to modal age.

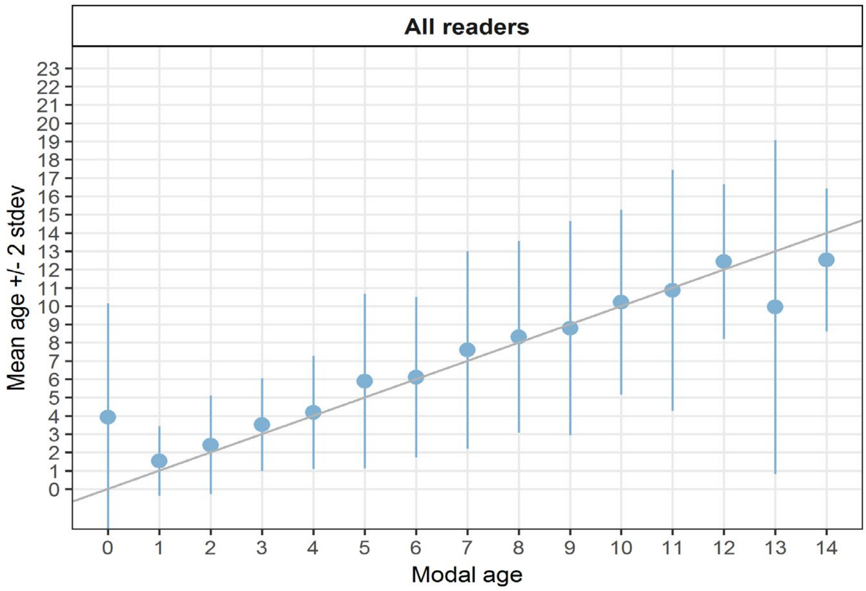

Figure 5.3. Age bias plot of all readers. Mean age recorded +/- 2 stdev of each reader and all readers combined are plotted against modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

Measures of Reader Precision per preparation method

Table 5.6 presents a summary of PA and CV statistics by preparation method. The type and number of otoliths prepared by each method differed between the river basins, with grinding and polishing as the method with the highest number of samples analysed. The best agreement was obtained for otoliths read in toto, with PA ranging from 35% to 68% and a median value of 53%. The PA statistics between readers of otoliths prepared by grinding and polishing (sagittal plane) and otoliths prepared with a transverse section was higher, ranging from 15-79% and 18-55% respectively, both with a median PA value of 33%. The smallest range of CV between readers was obtained for otoliths read in toto (13-53%), and the highest for otoliths prepared by grinding and polishing (15-79%). The median CV was similar for otoliths read in toto (30%) and otoliths prepared by grinding and polishing (29%).

Table 5.6. Summary of otoliths analysed per preparation method and corresponding statistics (PA and CV). Total length range and modal age range are also presented.

Method of preparation	Toto	Transverse section	Grinding & polishing
Number of otoliths	26	34	60
Length range (mm) /**Median**/Mean	109-598 /**290** /305	138-728/ **363** /406	100-560 /**271** /270
Modal age range	1-5	0-13	0-14
PA (%) (Min-Max / **Median**/ Mean)	35-68/ **53** / 53	18-55/ **33** / 35	15-79/ **33** / 37
CV (%) (Min-Max / **Median**/ Mean)	13-53/ **30** / 31	18-77/ **41** / 42	9-85/ **29** / 33
River basins	Mondego,Ter,Bages-Sigean	Mondego,Ter,Bages-Sigean	Nivelle, Minho, Guadiaro

5.2.2 First readings- Advanced readers

Measures of Reader Precision

Compared to the results obtained for all readers combined, the agreement among advanced readers is slightly better (Table 5.7), but still low. The overall weighted mean percentage agreement (PA) was 48%, ranging from 33% at modal age 0, to 67% at modal ages 11, 13, and 14. As for the coefficient of variation (CV), the overall weighted mean was 38%, with a range between 9% at modal age 14, and 66% at modal age 1. The highest CV occurred at modal ages 1 and 2 indicating that there are inconsistencies among advanced reader in interpreting the growth pattern at younger ages.

Table 5.7. Summary of the Percentage agreement (PA) and Coefficient of Variation (CV) by modal age, for advanced readers combined. The total number of age readings (N= 351) distributed per modal age is also presented. (*) The coefficient of variation is not calculated for modal age 0

Modal age	PA	CV	Number of readings
0	33%	-	3
1	44%	66%	18
2	54%	71%	41
3	46%	46%	59
4	56%	46%	41
5	45%	30%	33
6	37%	27%	27
7	43%	28%	28
8	52%	21%	27
9	47%	22%	47
10	50%	17%	6
11	67%	15%	9
12	33%	19%	6
13	67%	14%	3
14	67%	9%	3
Weighted Mean	**48%**	**38%**	

The age bias plot for advanced readers (Figure 5.4) shows there is an overall tendency for readers to overestimate the age at all modal ages except at modal ages 11, 13 and 14, which are slightly underestimated. It should be noted that the level of variability in the ages assigned by readers is high and similar across all modal ages.

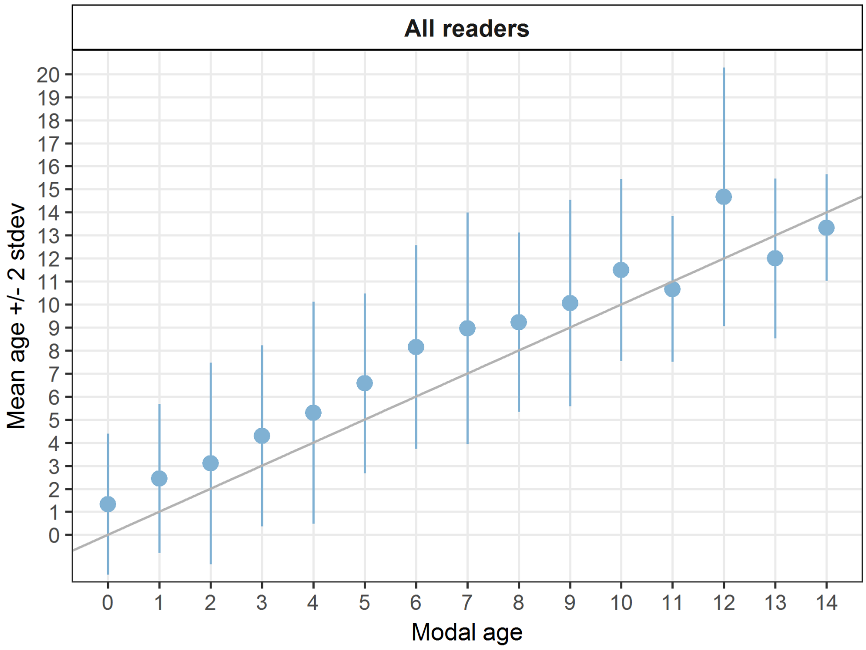

Figure 5.4. Age bias plot for advanced readers. Mean age recorded +/- 2 stdev of each reader and all readers combined are plotted against modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

5.2.3 Workshop exercise – Identification of sources of error

The WKAREA3 participants identified several causes of age determination error that could account for the bad ageing performance. In general, technical issues related to the preparation of otoliths and the acquisition of digital images were pointed out as causes of the poor readability of some otoliths. It should, however, be noted that most otolith images examined in the present workshop were acquired by researchers with little or no experience in the standard methodology approved by WKAREA. The technical aspects discussed include:

- **Poor quality of preparations**
  The preparation of otoliths was done according to the protocols defined in ICES (2009a, b; 2011), but not all criteria were observed due to the lack of experience of some participants in the exchange. The poor quality of the otolith preparations decreased the readability of samples. Specifically, the presence of bubbles in resin, as was the case for some preparations, further complicated reading, by diffracting light or adding artefact into the pictures. The quality of polishing, with scratches remaining on the otolith surface, was also involved. The lack of experience induced over polishing or under polishing, with no more nucleus in the sample, or a nucleus still below the sample surface in some cases.

- **Poor Image quality**
  Digital image capture requires some experience to set the best contrast. Apart from problems with contrast, the quality of images was also considered poor due to the type of light (transmitted or reflected) and focus. In addition, some pictures did not include a calibration scale, which rendered the identification of the zero band particularly difficult. Magnification was also pointed out as a potential source of bias in age determination. Examples of some images with poor quality are presented in Figure 5.5.

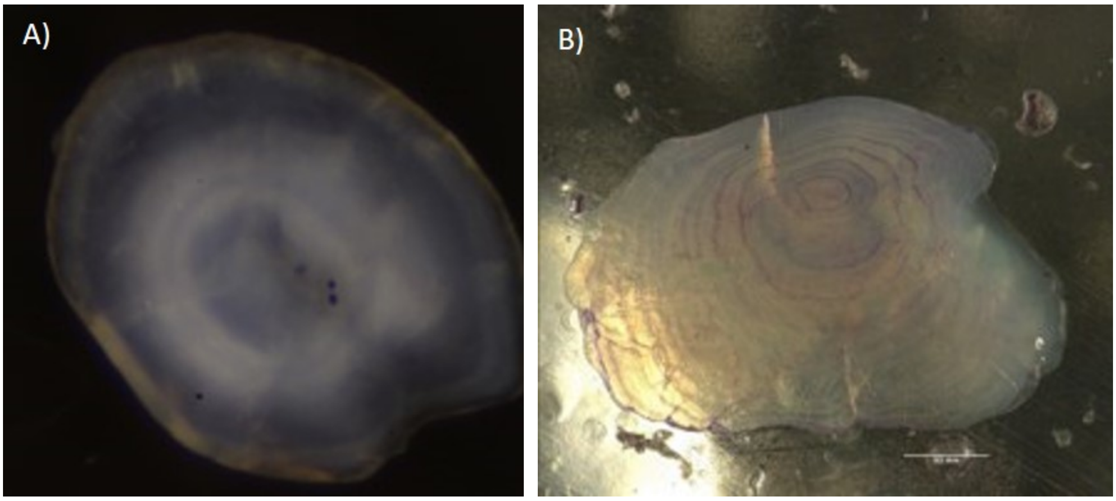

Figure 5.5. Examples of images of European eel otoliths with poor quality. Blurry picture (A) and brightness set too high (B).

Other issues that were identified as greatly affecting the interpretation of age included misidentification of the zero band and difficulties in discriminating between annuli and supernumerary rings, which is one of the main sources of error affecting ageing precision. The main issues addressed included:

- **Misidentification of the zero band**
  There were several errors in the identification of the zero band, which was in some circumstances considered as the first year, contributing to overestimate age. Moreover, in many otoliths there are many check marks between the nucleus edge and the zero band, which further complicated its identification, especially in small eels < 150 mm. An example is presented in Figure 5.6 (A).

- **Difficulty in discriminating between false rings and annuli**
  Although it is easy to identify false checks when they are incomplete or discontinuous, in many situations, there were numerous supernumerary rings, which become particularly highlighted by dying the otoliths. An example is presented in Figure 5.6 (B).

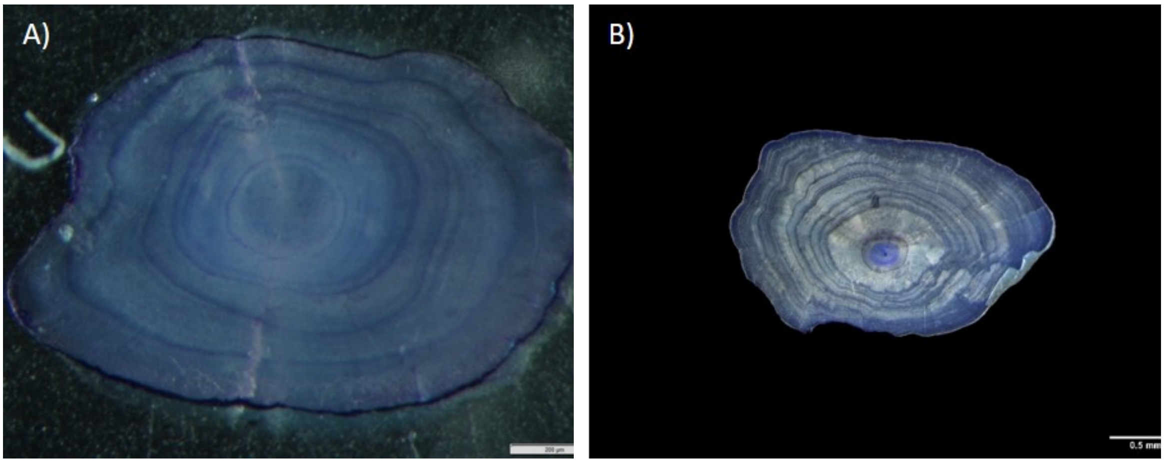

Figure 5.6. Examples of polished, ground and stained otoliths of European eel. Note the presence of many checks after the nucleus (A) and double checks or supernumerary rings (B), that makes identification of the zero band and age reading difficult.

With the feedback from readers at the exchange and the discussion during the WKAREA3 workshop, some important guidelines to follow to improve readability and interpretation of age were recommended:

- **Otolith preparation techniques**: in older eels, when the otolith is not flat, the whole otolith should not be prepared using a sagittal section because there is the risk to lose the rings at the edge. A transverse section is then more appropriate, either embedding in resin, cutting a transverse section followed by polishing or by burning and cracking. The drawback of burning and cracking is that the angle of the section is not controlled, while the diamond saw allows cutting precisely the resin block to obtain a section parallel to the growth axis.
- **Digital image acquisition**: A calibration scale should be included in all images. In addition, it is recommended that the same magnification is used for all images in order to avoid bias due to the different size of the otoliths. Care should be taken because a high magnification may lead to overestimation of the age by highlighting false rings.
- **Additional information**: information on field data and life history details such as location and date of sampling, type of habitat (i.e., brackish or freshwater), eel life stage (i.e., yellow or silver), length and sex should be provided to improve the interpretation of age.
- **Double reading of the otoliths**: two readings should be performed: a first fully "blind" annuli reading, and a second reading, using the additional information listed.
- **Identification of the zero band**: in case the zero band is difficult to identify, readers should follow the recommendation from ICES (2009a, b; 2011) i.e., a diameter of 340-400 µm is used to help defining the location of the zero band.
- **Interpretation of otolith growth patterns**: due to the complexity of growth patterns in eels analysed in this exchange, there is a strong need to validate age determination, i.e., to ground truth the age with mark-recapture. Given the variability observed when considering all samples, it is highly recommended that this validation is done for each river basin.

5.2.4 Second reading- All readers

Measures of Reader Precision

Table 5.8 presents mean Percentage Agreement (PA) and Coefficient of Variation (CV) for all readers combined, per modal age. The results indicate that in general, there was a poor level of agreement among readers for all ages. The overall weighted mean percentage agreement (PA) based on modal ages for all readers combined was low (44%), ranging from 18% at modal age 0, to 65% at modal age 2. As for the coefficient of variation (CV), the overall weighted mean was 33%, with a range between 19% at modal age 11, and 65% at modal age 1. The highest CVs occurred at modal ages 1 and 2, indicating that there are difficulties in interpreting the growth pattern at younger ages.

Table 5.8. Summary of the Percentage agreement (PA), Coefficient of Variation (CV) and relative bias by modal age, for all readers combined. The total number of age readings (N = 1061) per modal age is also presented

Modal age	PA	CV	Relative bias	Number of readings
0	18 %	-	5.82	17
1	39 %	65 %	0.94	18
2	65 %	55 %	0.61	144
3	49 %	31 %	0.32	108
4	49 %	28 %	0.27	180
5	39 %	33 %	0.13	126
6	40 %	33 %	0.41	216
7	40 %	21 %	0.32	72
8	28 %	25 %	-0.33	18
9	28 %	22 %	-0.72	18
10	31 %	31 %	-1.49	72
11	42 %	19 %	-1.19	36
12	-	-	-	0
13	25 %	21 %	-0.75	36
Weighted Mean	**44 %**	**33 %**	**0.21**	

The age bias plot for all readers combined (Figure 5.7) shows that there is an overall tendency for readers to overestimate age up to and including modal age 7, in contrast to the remainder modal ages, which are underestimated. The worst reading agreement was obtained for modal age 0, where age is extremely overestimated, reflecting errors in the identification of the zero band that persisted despite the training held during the workshop. It should also be noted that the range of bias per modal age is high (range from -0.97 to +1.88, with an overall bias of +0.01), showing the variability in the ages assigned by readers, with the lowest variability at modal ages 3 and 4.

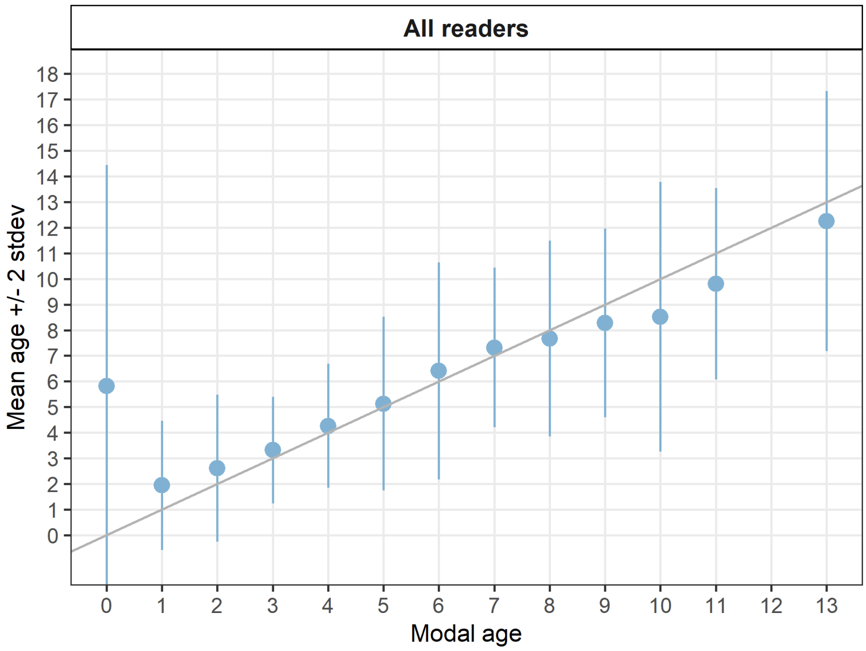

Figure 5.7. Age bias plot for all readers. Mean age recorded +/- 2stdev of each reader and all readers combined are plotted against modal age. The estimated mean age corresponds to modal age, if the estimated mean age is on the 1:1 equilibrium line (solid line).

Measures of Reader Precision per preparation method

Table 5.9 presents a summary of PA and CV statistics by preparation method. The type and number of otoliths prepared by each method differed between the river basins, with grinding and polishing (sagittal plane) as the method with the largest number of samples analysed. The best agreement was obtained for otoliths read in toto, with a median value of 61% for PA, ranging from 39% to 78%. The median value of PA between readers of otoliths prepared by grinding and polishing (sagittal plane) and otoliths prepared with a transverse section, both with 56%, was much lower, despite ranging from 15 to 79% and 18 to 55% respectively. The lowest range of CV between readers was obtained for otoliths read in toto (12-42%), and the highest for otoliths prepared by grinding and polishing (0-65%). The median CV was the same, i.e., 25% for otoliths prepared by grinding and polishing and for otoliths prepared by transverse section.

Table 5.9. Summary of otoliths analysed (N =59) per preparation method and corresponding statistics (PA and CV). Total length range and modal age range are also presented.

Method of preparation	Toto	Transverse section	Grinding & polishing
Number of otoliths	13	16	30
TL range (mm)/ **Median** /Mean	109-594 mm/ **244** /258	138-728 mm/ **348** /401	100-560 mm/ **239** /245
Modal Age	2-5	0-10	1-13
PA (%) (Min-Max / **Median**/ Mean)	39-78 / **61** / 57	18-67 / **36** / 39	17-100 / **36** / 41
CV (%) (Min-Max / **Median**/ Mean)	12-42 / **20** / 22	12-44 / **25** / 28	0-65 / **25** / 29
River basins	Mondego,Ter, Bages-Sigean	Mondego, Ter, Bages-Sigean	Nivelle,Minho, Guadiaro

Quality of age reading

The classification by readers of the difficulty in assigning the age to otoliths from eels sampled in all pilot basins is presented in Figure 5.8 and the classification by otolith preparation method in Figure 5.9. Looking at the scores assigned by all readers to each otolith, it is obvious that a high number of readings was assigned to score AQ3_QA, reflecting the difficulties in assigning age with precision. The difficulties seem to be higher in certain pilot basins such as the Ter, Bages-Sigean and the Guadiaro, where the number of readings classified as AQ3_QA is clearly greater than that obtained for the other river systems (Figure 5.8). As for the type of preparation, it seems clear that the otoliths read in toto were dominated by a score of AQ1, indicating that this was the method that readers considered the easiest.

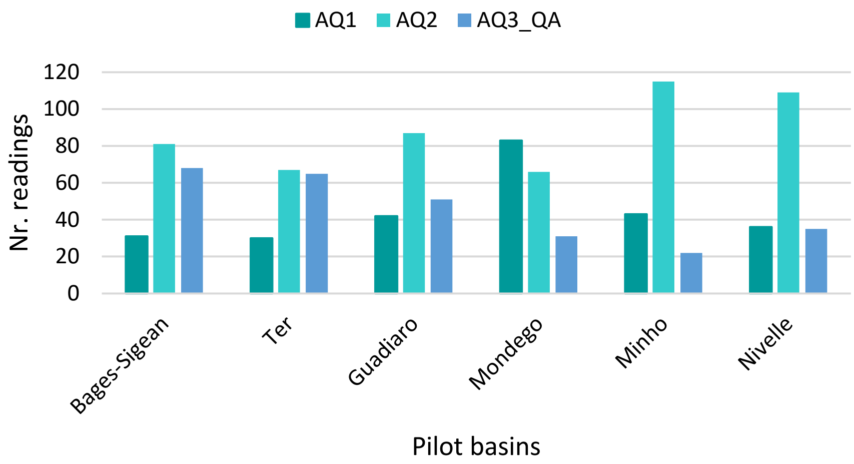

Figure 5.8. AQ scores assigned to each age by all readers for otoliths from each pilot basin. AQ1 - Easy to age with high precision; AQ2 - Difficult to age with acceptable precision; AQ3_QA - Rings cannot be counted, the calcified structure is considered unreadable. Age is assigned for Quality Assurance purposes only.

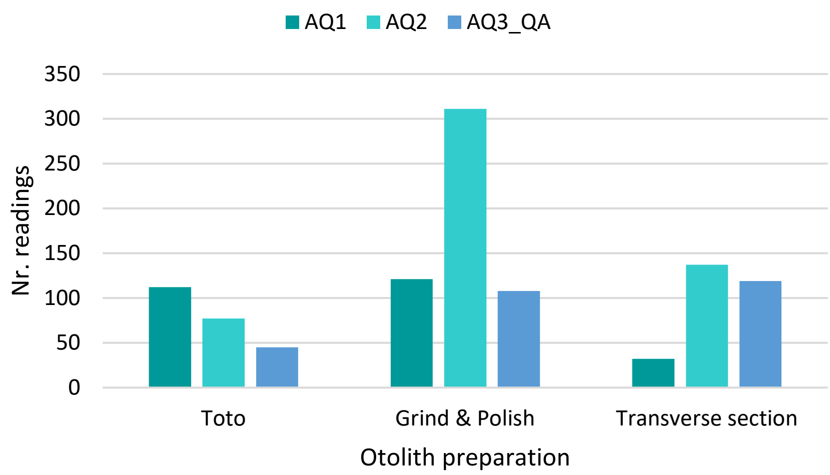

Figure 5.9. AQ scores assigned by all readers distributed type of otolith preparation. AQ1 - Easy to age with high precision; AQ2 - Difficult to age with acceptable precision; AQ3_QA - Rings cannot be counted, the calcified structure is considered unreadable. Age is assigned for Quality Assurance purposes only.

Some examples of the typical otoliths from each river basin are shown in Figure 5.10 and Figure 5.11. In some pilot basins all the otoliths were prepared by grinding, polishing and staining in the sagittal plane (Nivelle, Minho and Guadiaro), In Mondego, Ter and Bages-Sigean, the otoliths were read in toto and in the transverse plane. These images illustrate the sources of error that could have originated such difficulties and hence the bad performance in age reading.

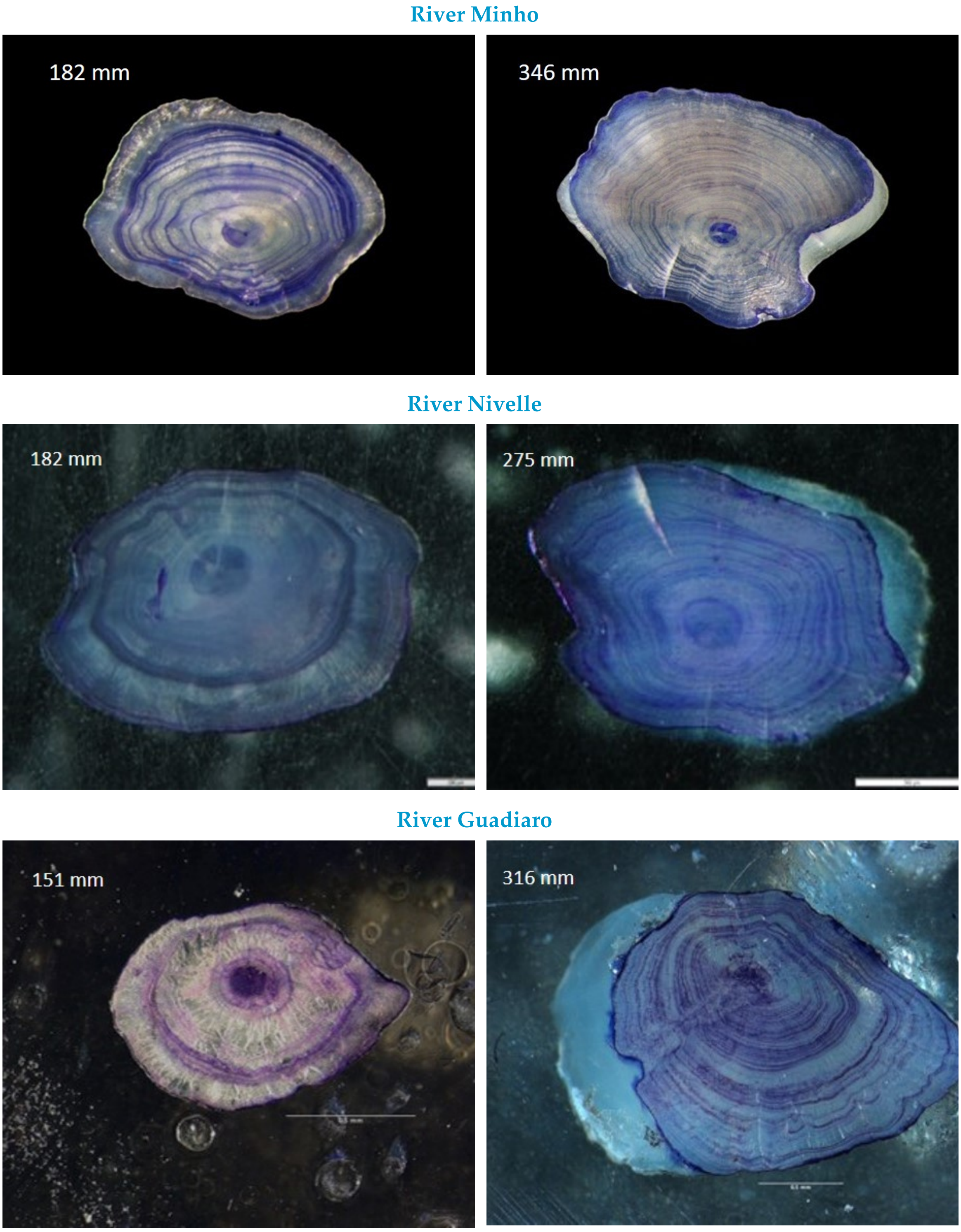

Figure 5.10. Otoliths from rivers Minho, Nivelle and Guadiaro, all prepared by grinding, polishing and staining. Eel total length (mm) is also presented for each otolith

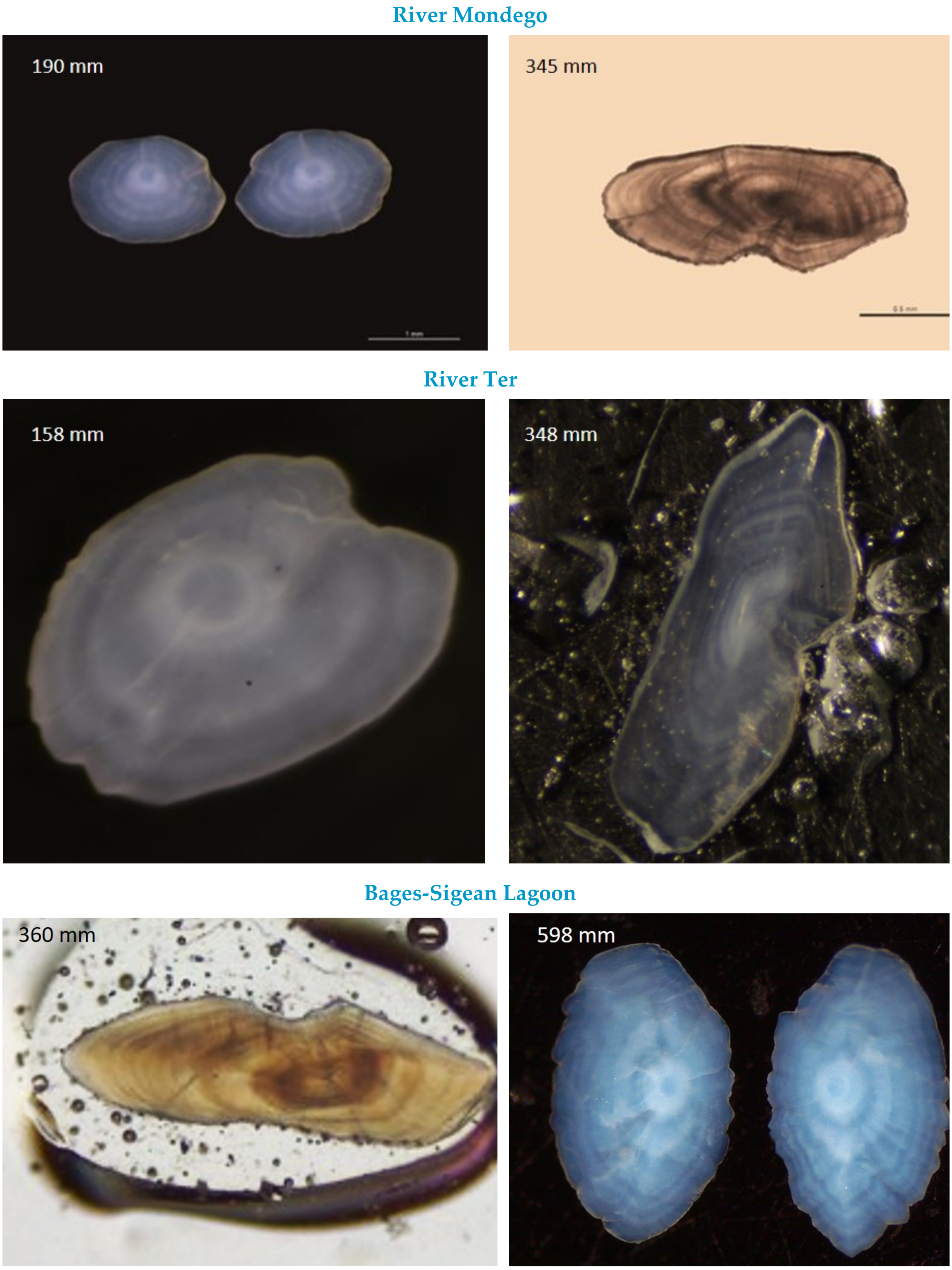

Figure 5.11. Otoliths from River Mondego, RIver Ter and the coastal lagoon Bages-Sigean, prepared by transverse section and without any preparation. Eel total length (mm) is also presented for each otolith.

The results from the Chi-square test showed that difficulties to assign the age are significantly different between river basins ($\chi^2 = 106.74$, df = 10, p-value < $2.2e^{-16}$). The *post-hoc* tests showed that there are differences among river basins, but Mondego is the only one that differs from all the other.

As for the otolith preparation methods there are significant differences in difficulties to assign age between the three methodologies used in the otolith preparation ($\chi^2 = 131.25$, df = 4, p-value < $2.2e^{-16}$) and the *post-hoc* tests showed they are all different ($p < 0.05$).

5.3 Discussion

This was the first WKAREA exchanging otolith images of European eel from the southern range of the species, but the double purpose of the meeting, under the frame of SUDOANG and under the frame of WGEEL, was difficult to achieve. Providing training to SUDOANG members and at the same time expanding the WKAREA knowledge on eels from the southern area was an impossible bargain, which resulted in a poor overall agreement on the estimate of eel age.

The poor results obtained may be due to several reasons, one of them being the lack of experience in the application of the protocols by the scientists who prepared and provided the samples for the age reading exchange. Most of the samples did not meet the quality standards previously agreed upon during the two preceding WKAREA meetings. The readability of the samples was overall poor. The absence of a calibration scale and/or information on eel length made the identification of the zero band particularly difficult in some samples. Another minor issue is that the present set of samples, which were prepared for the exchange of otolith images, included young eels whereas previous WKAREA exchanges were mostly made up of eels older than 5 years.

The second major reason for the poor overall agreement on age estimation is the fact that the participants in the present meeting, both from the SUDOANG project, and the WKAREA, which includes eel experts, had little or no experience in reading eel otoliths from the southern part of their range.

One of the main sources of error affecting the precision of age estimation is the discrimination between false rings or supernumerary rings and annuli, which according to Kullmann *et al.* (2018) is an impossible task, in most circumstances.

Age validation is a necessary step in all growth studies, not only to improve accuracy and precision, but also to provide unbiased data for stock assessment models. A classical reference collection for the species will not serve the purposes of an ageing program because the variation among habitat types seems to overcome the variation among ages. However, stock assessment requires reliable data on growth.

The variability in eel length at a given age can be attributed either to natural causes such as latitudinal variation, habitat types, and local environmental conditions, or to human errors in age reading mainly due to differences in the interpretation of annuli deposition (Domingos *et al.*, 2006). However, the widespread distribution of the European eel, poses major challenges for age determination because growth patterns may differ significantly between the northern part of the distribution area and the southern part.

Temperature is one primary driver of eel growth, due to its strong influence on metabolism and feeding activity. The optimum temperature for European eel growth, under laboratory experiments has been reported to vary between 23 ^{o}C (Sadler, 1979) and 26.5 ^{o}C (Seymour, 1989). The optimum season for growing should be more extended in the Southern part of the distribution area than in the Northern distribution. However, temperature may rise far above optimum in the summer in the Mediterranean region and in the Iberian Peninsula, decreasing the opportunity for growing in summer and maybe inducing a slowing of growth and checks in the otoliths. Interrupted summer growth may occur with a rise in water temperature (Deelder, 1976; Liew, 1974). The presence of false annuli has been widely reported for Atlantic eels, being attributed to handling stress or a rise in water temperature up to 30 ^{o}C, even for short periods (Liew, 1974).

In the Northern area of the species range, a shorter growth season may not result in slower growth if summer productivity of Northern ecosystems is higher than in the ecosystems from the Southern area. The river flow reduction in the summer that is more frequently observed in the Iberian Peninsula and the Mediterranean region has also an effect on river ecosystem productivity and finally on eel growth. The high summer temperatures, typical of southern latitudes, may affect growth similarly to what happens with low temperatures during winter (Domingos et al., 2006), because they promote an increase in the primary production, which in turns contributes to reduce dissolved oxygen and the activity of eels. In a study in the South West of France, it was demonstrated that reduction of river flow had an important effect on eel growth (Yokouchi & Daverat, 2013). So, another plausible explanation for the poor agreement obtained for the eel samples used in the present reading exchange, is that a rise in water temperature in summer along with a decrease in river flow may induce super-annularly checks, especially in the Ter, Guadiana and Mondego, where temperature is higher in summer than in the Minho or the Nivelle. In Bages-Sigean, temperature rises and freshwater input decreases in summer, but the environmental conditions are more stable than the ones from the riverine habitats.

Apart from temperature, there are other major factors that explain eel growth patterns. Habitat is another important one (Daverat *et al*., 2012). Except for samples from the Bages-Sigean Lagoon, samples exchanged in the present age reading came from freshwater systems where growth is known to be slower than in brackish water from the same watercourse (Cairns *et al*., 2009), and the growth patterns are usually more difficult to interpret without any additional information on the habitat.

There was no reference collection of known age available for the samples of the different locations that were exchanged. During the discussions at the present meeting, it was emphasized that a direct measure of growth patterns, providing a direct validation of the age estimation was a priority need. Mark-recapture should provide such validation, and help building a reference collection of otoliths of eels of known age for eels from southern aquatic systems, which will allow to obtain age data that can be used for stock assessment.

5.4 Conclusion

Overall there was a bad ageing performance prior and post workshop. This bad performance was observed for basic and advanced readers, indicating that experience was not the main reason for such results. The poor quality of some preparations has biased the overall agreement between readers. Another bias came from the inexperience of some readers that might have resulted in low precision, but after training the poor results persisted in the second reading. Moreover, the agreement for advanced readers was also low, indicating that the causes have been related to the nature of the otoliths analysed. The irregular pattern of annuli along with the presence of numerous supernumerary rings complicated the interpretation of the growth pattern in the otoliths used in the exchange. It was concluded that it was impossible to distinguish between annuli and supernumerary rings.

The diversity of rivers and readers, as well as the eel length range (100-728 mm) may also have contributed to the discrepancies found between the age assigned by readers. The main conclusion of the workshop was that eel otoliths from the southern area of the range presented a growth pattern completely different from the northern area. To clarify the results obtained, it was agreed that direct validation of length-at-age, with mark recapture studies, is necessary to provide a reference collection of eels for the different habitats occurring in the Iberian Peninsula and the Mediterranean region.

6 Recommendations for future workshops (ToR c)

From the discussions held during the workshop and the results obtained in this otolith exchange, it is recommended that:

- The interpretation of age reading by inexperienced researchers is improved by further training, to meet the standards required.
- Preparation of otoliths is thoroughly trained, and all the guidelines in the protocols set in previous WKAREAs need to be attentively respected.
- Image acquisition is carefully obtained to guarantee the best contrast, the appropriate type of light and a good focus.
- Validation using direct estimates of eel growth in the field is provided, especially for sites where summer growth interruption is expected to occur, and a high decrease of river flow is observed.
- Mark recapture studies are undertaken to ground truth the age.

A field study to test the effect of summer temperature and river flow on eel growth patterns is conducted to determine the pattern of deposition of annuli.

7 References

Benchetrit J. & J.D. McCleave. 2016. Current and historical distribution of the American eel *Anguilla rostrata* in the countries and territories of the Wider Caribbean. *ICES Journal of Marine Science*, 73: 122-134.

Bertin, L. 1951. *Les anguilles*. Payot, Paris, 191pp.

Cairns, D.K., Secor, D.A., Morrisson, W.E., Hallett, J.A. 2009. Salinity-linked growth in anguillid eels and the paradox of temperate-zone catadromy. *Journal of Fish Biology*, 74: 2094–2114.

Daverat F., Beaulaton L., Poole R., Lambert P., Wickström H., Andersson J., Aprahamian M., Hizem B., Elie P., Yalçın-Özdilek S., Gumus A. 2012. One century of eel growth: changes and implications. *Ecology of Freshwater Fish*, 21: 325-336.

Deelder, C.L. 1976. The problem of the supernumerary zones in otoliths of the European eel (*Anguilla anguilla* (Linnaeus, 1758); a suggestion to cope with it. *Aquaculture*, 9: 373-379.

Domingos, I., Costa, J.L., Costa, M.J. 2006. Consequences of unreliable age determination in the management of the European eel, *Anguilla anguilla* (Linnaeus, 1758). Handbook of the ICES Annual Science Conference, 19.-23. September, Maastricht, The Netherlands, CM 2006/J:31. 192 pp.

Eltink, G.W. 2000. Age reading comparisons. (MS Excel workbook version 1.0 October 2000).

Fernández-Delgado, C.; Hernando, J.A.; Herrera, M., Bellido, M. 1989. Age and growth of yellow eels, *Anguilla anguilla*, in the estuary of the Guadalquivir river (south-west Spain). *Journal of Fish Biology*, 34: 561-570.

Gordo, L.S., Jorge, I.M. 1991. Age and growth of the European eel, *Anguilla anguilla* (Linnaeus, 1758) in the Aveiro Lagoon, Portugal. *Scientia Marina*, 55: 389-395.

ICES 2009a. Workshop on Age Reading of European and American Eel (WKAREA), 20-24 April 2011, Bordeaux, France. ICES CM 2009\ACOM: 48. 66 pp.

ICES 2009b. Manual for the Ageing of Atlantic Eel. In Workshop on Age Reading of European and American Eel, (WKAREA) Annex 4, 57 pp.

ICES 2011. Report of the Workshop on Age Reading of European and American Eel (WKAREA2), 22-24 March 2011, Bordeaux, France. ICES CM 2011/ACOM: 43. 31 pp.

ICES. 2014. Report of the Joint EIFAAC/ICES/GFCM Working Group on Eel, 3–7 November 2014, Rome, Italy. ICES CM 2014/ACOM: 18. 203 pp.

ICES. 2017. Report of the Joint EIFAAC/ICES/GFCM Working Group on Eels (WGEEL), 3–10 October 2017, Kavala, Greece. ICES CM 2017/ACOM: 15, 99 pp.

Kullmann, B., Pohlmann, J-D., Freese, M., Keth, A., Wichmann, L., Neukamm, R., Thiel, R. 2018. Age-based stock assessment of the European eel (*Anguilla anguilla*) is heavily biased by stocking of unmarked farmed eels. Fisheries Research, 208: 258–266.

Liew, P.K.L. 1974. Age determination of American eels based on the structure of their otoliths. In: Bagenal, T.B. (ed.). Ageing of Fish. Publ. Unwin Brothers Ld, Surrey, England, 99: 124-136.

Rossi, R. & Villani, P. 1980. A biological analysis of eel catches, *Anguilla anguilla* L., from the lagoons of Lesina and Varano, Italy. *Journal of Fish Biology*, 16: 413-423.

Sadler, K. 1979. Effects of temperature on the growth and survival of the European eel, *Anguilla anguilla*. *Journal of Fish Biology*, 15: 499-507.

Seymour, E.A. 1989. Devising optimum feeding regimes and temperatures for the warmwater culture of eel, *Anguilla anguilla*. *Aquaculture and Fisheries Management*, 20: 311-323.

Tesch, F. W. 2003. *The Eel* (3rd ed.; J.E. Thorpe, Ed.). Oxford, UK. Blackwell Publishing. 408 pp.

Vøllestad, L. A., Lecomte-Finiger, R., & Steinmetz, B. 1988. Age determination of *Anguilla anguilla* (L.) and related species. EIFAC Occasional Paper, 21, 1–28.

Yokouchi, K., & Daverat, F. 2013. Modeling individual growth trajectories of the female European eel in relation to temperature and habitat-use history in the Gironde River, France. *Aquatic Biology*, 19: 185-193.

Annex 1: Agenda

17th June 2019 (Monday)

14.00 - 14.15h Opening session

(I. Domingos FCUL, E. Diaz AZTI)

14.15 - 14.45h Presentation of results of the intercalibration exercise

(F. Daverat - IRSTEA)

14.45 - 17.00h Interpretation of results

(Plenary session)

18th June 2019 (Tuesday)

09.00 - 10.30h Reading consistency and factors related to inconsistency

(F. Daverat - IRSTEA)

10.30 - 13.00h General discussion on training of readers and the reading rules

(Plenary session)

13.00 - 14.30h Lunch

14.30 - 17.00h Agreement on age reading and updates for ICES report

(Plenary session)

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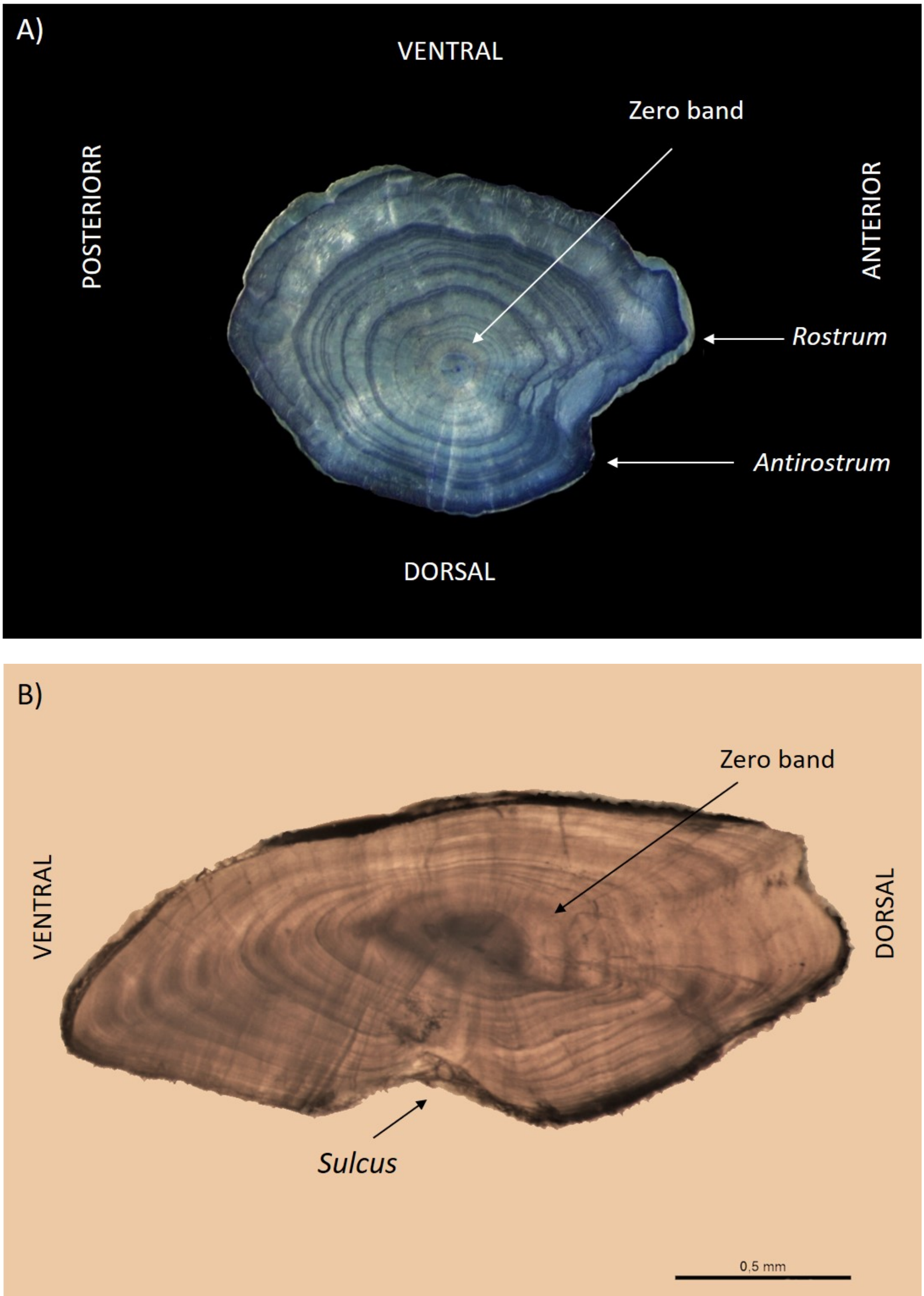
Annex 3: Glossary

OTOLITH TERMS	DEFINITION
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 (~170µm radius from centre)



Sagittal (A) and frontal (B) plane views of European eel otoliths and terminology used. The zero band, referred to in this report, is also indicated. The anterior and posterior regions of the otoliths above are in accordance with the orientation of the eel.