WORKSHOP ON AGE READING OF EUROPEAN SARDINE (*SARDINA PILCHARDUS*) (NE ATLANTIC AND MEDITERRANEAN) (WKARAS2)

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WORKSHOP ON AGE READING OF EUROPEAN SARDINE (*SARDINA PILCHARDUS*) (NE ATLANTIC AND MEDITERRANEAN) (WKARAS2)

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Contents

i Executive summary

The Workshop on Age reading of European Sardine (*Sardinapilchardus*) (NE Atlantic and Mediterranean) [WKARAS 2] met to review the information on age determination, discuss the results of the 2017 otolith exchange, review the existing validation methods, clarify the interpretation of annual rings, and update the age reading protocol and a reference collection of well-defined otoliths.

The 2017 otolith exchange included images of otoliths' pairs collected from fish samples taken in 11 locations. Growth and reproduction were individually analysed by the participants for age attribution following a reference age reading protocol. R scripts based on Eltink's MS Excel spreadsheet (Eltink, 2000) following the recommendations of the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000) were used for age readings comparative analyses.

In order to clarify the causes of age reading discrepancies between readers, discussions based on joint analyses of projected images of selected otoliths among those used for the 2017 exchange were held during this 2019 workshop. Review of age reading criteria used for growth rings identification applied in each area was undertaken and age reading validations were discussed.

The use in each area of a reference collection of otoliths' images with ≥80% of age reading agreement between readers was discussed and pointed out as a suitable tool to improve age readings accuracy and to contribute for a higher agreement between readers in each area. As a contribution for the construction of the reference collections, a selection of images of otoliths from the 2017 exchange with ≥80% age reading agreement between the readers was undertaken during the joint discussion.

In order to assess age reading discrepancies and their causes in each area and the effects of the discussions held on the reading agreement between readers, a small age reading calibration exercise took place during the workshop, based on individual analysis through SmartDots of a sample of otoliths images selected from those used in the 2017 exchange. Overall the age reading agreement (PA), coefficient of Variation (CV) and Average Percentage Error (APE) obtained by advanced readers in relation to those achieved by their equivalent "experts"+"intermediate" in each area in the 2017 exchange, are not much different from each other. Despite the previous discussions on the *annulus* identification by image analysis during joint sessions, difficulties persisted mainly on the edge type classification and on the first growth ring identification.

A few recommendations come out from the discussions held during WKARAS2: exchanges should preferably be based on the structure analyses of samples of otoliths complemented by their images in SmartDots, the implementation in each area of routine otoliths age reading exchanges, regular age reading validation studies in each area and otoliths' images reference collections should be enriched by more quality images along time.

ii Expert group information

Expert group name	Workshop on Age Reading of European Sardine (Sardina Pilchardus) (NE Atlantic and Mediterranean) (WKARAS2)
Expert group cycle	Annual
Year cycle started	2019
Reporting year in cycle	1/1
Chair(s)	Eduardo Soares, Portugal
	Pedro Torres, Spain
Meeting venue(s) and dates	18-22 February 2019, Lisbon, Portugal (22 participants)

1 Terms of reference

An European Sardine Otoliths Exchange program was carried out in 2017 for inter-calibration between age readers of fisheries research laboratories in the N.E. Atlantic and the Mediterranean Sea areas. One of the main problems identified from the results analysis of that exchange (See Section 5 and Annex 1) was the low age reading agreement among readers for both areas, which averaged between 60 and 80%. This emphasized the need for an age reading workshop on European Sardine (*Sardina pilchardus*) (N.E. Atlantic and Mediterranean) [WKARAS 2] in support of ICES and GFCM fisheries advice. WKARAS 2 was chaired by Eduardo Soares, Portugal, and Pedro Torres, Spain and was held in Lisbon, Portugal, 18-22, February, 2019, to:

a) Review the results of the European Sardine Otoliths Exchange 2017 and identify causes of poor agreements between readers where apparent (Science plan code 5.2);

b) In light of TOR a) review and improve where necessary the sardine age determination criteria including annulus definition and validation techniques. Clarify the otoliths' annual growth rings identification, the methodologies applied and age reading validation techniques used on this species. Update the common age reading protocol and make specific guidelines for the improvement of age reading precision and the reduction of bias between readers and laboratories (Science plan code 5.2);

c) Create a reference collection of clearly-defined otoliths with a consensual age in a data base of digitized images for the Atlantic and the Mediterranean Sea (Science plan code 5.2);

d) Address the generic TORs adopted for workshops on age calibration (Science plan code 5.2).

Standardization of otoliths preparation procedures and of age reading criteria were therefore in the scope of this Workshop in order to increase the age reading data quality for the sardine stocks assessment in these areas.

WKARAS2 will report by 7 October 2019 for attention to ACOM, SCICOM and WGBIOP.

2 Agenda and participation

The WKARAS 2 meeting followed the next agenda:

Monday, 18-02-2019	**14:00 – 16:00**	– Welcome and start of the meeting; – Overview of ToRs, presentation of the agenda and its approval by the attendants; – Local and network arrangements; – Summary of preparation techniques of sardine otoliths at the different participating laboratories; – Age reading validation techniques in both areas.
	16:00 – 16:15	*Coffee break*
	16:15 – 17:30	– Presentation and discussion of the 2017 European sardine otolith exchange results: comparison of precision against modal age and bias; evaluation of levels of agreement among readers and laboratories; identification of causes of poor agreements between readers where apparent (ToRs a and b);
Tuesday, 19-02-2019	**09:00 – 11:00**	– Review of sardine age determination criteria in each area: Improvements (ToR b); – Otoliths' annual growth rings identification, methodologies applied and age reading validation techniques used in each area (ToR b);
	11:00 – 11:15	*Coffee break*
	11:15 – 13:00	– WKARAS 2 calibration exercise (via SmartDots) [to clarify readers' identification of the growth rings and to assess age readings agreement improvement among readers in each area];
	13:00 – 14:30	*Lunch break*
	14:30 – 16:00	– WKARAS 2 calibration exercise (via SmartDots) (cont'd);
	16:00 – 16:15	*Coffee break*
	16:15 – 17:30	– WKARAS 2 calibration exercise (via SmartDots) (cont'd);
Wednesday, 20-02-2019	**09:00 – 11:00**	– WKARAS 2 calibration exercise (via SmartDots) (conclusion);
	11:00 – 11:15	*Coffee break*
	11:15 – 13:00	– WKARAS 2 calibration exercise preliminary results presentation and discussion: – On-screen discussion of relevant otolith age readings from the workshop calibration exercise; – Identification of age reading persistent issues on sardine otoliths in each area;
	13:00 – 14:30	*Lunch break*
	14:30 – 16:00	– WKARAS 2 calibration exercise preliminary results presentation and discussion (cont'd);
	16:00 – 16:15	*Coffee break*
	16:15 – 17:30	– Update of common age reading protocol: guidelines for the improvement of age reading precision and for the reduction of bias between readers and laboratories in each area (ToR b);
Thursday, 21-02-2019	**09:00 – 11:00**	– Update of common age reading protocol (cont'd);
	11:00 – 11:15	*Coffee break*
	11:15 – 13:00	– Final Workshop's report structure and assignment of tasks among attendants;
	13:00 – 14:30	*Lunch break*
	14:30 – 16:00	– Creation of a sardine otoliths' reference collection for each area (clearly-defined otoliths with a consensual age in a data base of digitized images for each area) (ToR c);
	16:00 – 16:15	*Coffee break*
	16:15 – 17:30	– Sardine otoliths reference collection (cont'd);
Friday, 22-02-2019	**09:00 – 11:00**	– Recommendations based on the Workshop's results;
	11:00 – 13:00	– Future activities plan for enhancing age determination quality in sardine.
	13:00	End of meeting

WKARAS 2 participants

From the top left to right: Deniz Kukul, Ioannis Fytilakos, Sana El Arraf, Denis Gašparević, Andreia Silva, Konstantina Ofridopoulou, Raquel Milhazes, Erwan Duhamel, Moulay Hachem Idrissi, Delfina Morais, Geoffrey Bled De Fruit, Eduardo Soares, Célina Chantre, Hammou El Habouz, Isabel Loureiro, Pedro Torres, Cristina Bultó, Maria Sánchez, Iñaki Rico**.**

3 Review of information on otolith exchanges and workshops (ToR a)

Age determination of sardine was firstly based on the scales structure analysis. Standardization of age determination criteria and methods for this species in Center East Atlantic was established in 1978 and 1979 (FAO, 1978, 1979) A first reported seminar on Iberian Atlantic sardine ageing based on otoliths structure analysis was held in Vigo in 1997 (ICES, 1997). This was followed by three workshops in 2002 (Soares *et al* 2002.), 2005 (Soares *et al*, 2007) and 2011 (ICES, 2011). Preceding these workshops otolith age reading exchanges were carried out respectively in 2000, 2004 and 2010. All workshops made an effort to standardize otoliths preparation and age reading methods, preparing procedures manuals and reference collections of agreed age otoliths.

The first exchange and workshop held in 1997 had as priority to analyze and report the otolith structure and define the yearly growth pattern of otolith from different areas, and establish a protocol for age determination using diagrams and photographs to illustrate age reading criteria. A total of 9 participants (6 experts and 3 beginners) read about 250 otoliths from Portugal and Spain (ICES divisions 8c and 9a). The overall agreement was 55.8% for all readers and all areas but for the expert readers it was 70.9%. In particular excluding the 7+ years old fish, the PA for the experts increased to 78.5%. The main conclusions of the workshop was that otoliths coming from the southern area (ICES Subdivision 9a-CS) presented an overall structure which is different from those observed in otoliths from northern areas, especially in Subdivision 8c. To overcome this problem it was recommended to monitor annulus deposition patterns along the entire Portuguese and Spanish coasts throughout each year, study different growth patterns in adjacent areas (France and Morocco) and employed the daily ring counting technique to validate the annuli of the first age groups.

The second workshop was held in 2002 preceded by an otolith age reading exchange in 2000. Its objective was to discuss age reading differences in young and in old fish and establish criteria to improve the precision of age readings, identify the main difficulties in age readings of otoliths from southern areas, establish new criteria to improve ageing agreement and to check reading consistency within readers. A total of 12 readers from Portugal, Spain and France participated in the workshop. Otoliths images were from ICES Divisions 7, 8c and 9a. The overall agreement was 76.1% using all readers and all areas combined although the overall mean CV was higher on the youngest age groups (25% on age 1 and 24% on age 2). Disagreement in age readings of young (age groups 1 and 2) and old fish (from age group 4 onwards) and on otoliths from the southern areas (9a-South) were the main problems identified. Identification of the first annual ring was the main problem on younger ages. In older fish, discrimination of rings near the otolith edge caused most of the disagreements. These difficulties were more complicated in otoliths from the southern areas, due to the less clear structure and to the frequency of occurrence of false rings. Readers participating in the workshop outlined the need to perform studies of first ring measurements and edge type along the year and suggested to use of a higher magnification near the otolith edge to discriminate and count narrow closely packed rings near the otolith edge. They also advised to use the rostrum as the reading area for the otoliths from the southern area (9a-South).

After the 2004 otoliths exchange, a sardine age reading workshop was carried out in 2005 to evaluate readers' agreement and aging precision, to assess the extent of ageing difficulties identified in the 2004 exchange and to propose guidelines for their minimization. A total of 17 otolith readers from Portugal, Spain, France Greece and Morocco participated in this workshop. 555

otolith images from ICES divisions 7, 7c, 9a and Morocco were grouped into 10 sets according to the different areas. The overall agreement with modal age declined from northern to the southern areas; 72%, 66% and 25% PA for ICES divisions 8c, 9a and Morocco respectively. Within the Atlantic Iberian area, both the agreement among experienced readers and the CV (coefficient of variation) by age group declined in comparison to the last Workshop. Otoliths from the Mediterranean area generally showed low agreement levels (comparable to otoliths from southern Portugal) mainly due to the identification of the first annual ring. The workshop recommended the use of the diameter of the opaque core measured in juvenile fish otoliths as a gauge to help ageing older individuals. Agreement between readers from the Atlantic Iberian and the NW African areas was considerably low. Iberian readers assigned older ages to otoliths from the NW African areas while Moroccan readers assigned younger ages to the otoliths from the Iberian areas, indicating different age reading criteria. The high opacity of otoliths from the NW African areas raises serious difficulties to aging. The use of alternative preparation techniques, such as soaking in water/alcohol, was recommended to enhance growth rings visibility in these otoliths. The birthdate criterion and the associated interpretation of the otolith margin came up as important issues during the workshop. Off the West and South Iberian Peninsula, sardine has an extended spawning season (October-March). Individuals born in the start of the season may be classified in two different year-classes during their first year due to the ageing criteria which could confound year-class strength and bias the initial growth trajectory of successive cohorts. The Workshop participants agreed that changing the otolith margin convention for juveniles during the first semester of the year, would apparently solve the inconsistency of year class classification. However, participants alerted the need of a more detailed analysis of otoliths of juvenile fish and a broader discussion on this subject in other Working Groups to clarify the perception of all the problems involved and of the consequences for stock assessment of adopting any alternative birthdate margin convention.

An exchange of sardine otoliths was completed in 2010 in order to prepare material and data for the 2011 Workshop. There were 11 otolith readers from Portugal, Spain and France attending this workshop. The otolith exchange included a total of 300 otoliths images from ICES divisions 8c and 9a. The relative accuracy of sardine age determination was generally good: the average percentage of agreement with modal age was 77.0% and 75.2% for the 9a area and the 8c area, respectively.

Compared to the previous Workshop, relative accuracy of sardine age determination within the Iberian Stock area (Cantabrian Sea and South Iberian peninsula areas) has improved substantially and there is less evidence of bias (% of agreement increased ca. 20%) while precision increased in the Cantabrian Sea. The identification of the otolith edge and of the first annual ring were the main discrepancies between readers in sardine age determination like in the previous workshops. A study on otolith seasonal growth was presented during the workshop. The results showed that the seasonality of the otolith edge varies with fish age, with older individuals forming an opaque edge later in the year for a shorter period as observed in several other clupeids. Marginal growth was also different between the Bay of Biscay and the Portuguese waters, with a longer period of opaque growth in Age 1 individuals and more similar seasonal patterns for Ages 2-4 years in the former area. Also, a study on the attribution of age in years of young individuals was presented. Preliminary results from the analysis of daily growth rings of sardine juveniles in northern Portugal suggested that the diameter of the first annual ring was positively correlated with the fish length at the time of formation. Measurements of ring diameter indicated that the first translucent ring forms at a distance around 1.1 mm from the otolith nucleus, corresponding to a diameter around 2 mm. Participants decided to accommodated this information in the age reading protocol.

4 Review of the results of the European Sardine Otoliths Exchange 2017 and identification of causes of poor agreements between readers (ToR a)

A sardine otolith exchange was carried out in 2017. Since the last exchange was performed five years before (2011), it was important to check the age reading agreement between otolith readers from other areas and provide more accurate information about the level of agreement of current readers before the workshop. Also, this exchange would provide otolith images with the participants' readings to be discussed during the workshop. The report of this exchange is in the Annex 1 of this document.

This exchange was carried out in WebGR by otoliths image analysis by individual participants but several setbacks have arisen during this process, mainly related to issues on WebGR functionality due to the high number of the exchange otoliths images and of age readers with which the system was not able to handle. To overcome these difficulties and to achieve the main goals of the exchange, R scripts (R Core Team, 2017) based on Eltink MS Excel spreadsheet were developed.

A total of 31 readers from 10 laboratories of both areas participated in this exchange. 380 images of otoliths' pairs collected from fish samples taken in 11 locations (Annex 1 - Table 1) (Mediterranean and Atlantic areas) were individually analyzed by the participants for age attribution following a reference age reading protocol. Otolith readers were ranked as Experts, Intermediate and Trainees considering the years of experience estimating the age of Sardine (Annex 1 - Table 2).

The analyses were made combining Atlantic areas (8a, 8b, 8c and 9a) and Mediterranean areas (GSA01, GSA03, GSA06, GSA07, GSA09, GSA16, GSA22) together by readers expertise.

A low age reading agreement between readers and with modal age were found (in general PA ranged from 60 to 80%). This low age reading agreement level might be due to the use of otoliths' images in the Exchange as the only basis for the otoliths structures analyses and also to the frequent occurrence of low-quality images, which turned difficult the discrimination of the growth areas (*annuli*) in those cases. The use of only otoliths' images for age reading analyses in the exchanges seems quite limitative, as they do not give the 3D perception of otoliths' structure, not allowing the manipulation as that can be done with the otolith, to play with the light source intensity and position and also with the stereoscopic microscope focus and amplification in order to reach a reliable discrimination of *annuli*. On the other hand, in a number of images one could not be quite sure if their magnification actually corresponded to 20X as established as standard for the exchange, turning it difficult for the reader to locate the first *annulus* with confidence by measuring its reference diameter of ≈2 mm (radius ≈1 mm) in these images. Due to these results a Workshop was proposed.

A series of joint sessions of discussion on otoliths structure interpretation based on projected digi-tized images was held during the WKARAS 2 meeting and a small age reading calibration exercise was undertaken in order to make a new preliminary assessment of otoliths age reading agreement between readers. The extensive analyses of the results of WKARAS 2 small age reading calibration exercise is in Annex 2. The nineteen WKARAS 2 attendants and one age reader from IEO online have participated in this small age reading calibration exercise (Annex 2 - Table

2). It must be pointed out that only 15 readers out of the 31 which participated in the 2017 exchange have attended the WKARAS 2. The image samples analysis was individually undertaken by participants through SmartDots, during this small age reading calibration exercise (Annex 2 - Table 1).

To follow the nomenclature used in SmartDots, "Experts+Intermediate" were designated as "Advanced" while "Trainees" were called "Basic". SmartDots allows the indication of an Age Quality criteria. These criteria indicates the quality of the age reading, based on the difficulty level presented by the structures to the reader (AQ1: Easy to age with high precision; AQ2: Difficult to age with acceptable precision; AQ3: Unreadable or very difficult to age with acceptable precision.). In the extensive report of this exercise the AQ3 readings were not used (Annex DD). The following analysis the AQ3 were added in order to compare with the 2017 exchange results.

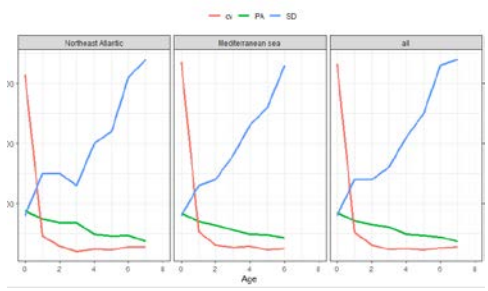
Table 4.1 – 2017 Exchange and WKARAS 2 small calibration exercise: comparison between Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percent Error (APE) for all and Advanced readers by each and the total area.

Area	2017 Exchange			WKARAS 2 Small calibration exercise			WKARAS 2 Small calibration exercise**AQ3 included		
	PA	CV	APE	PA	CV	APE	PA	CV	APE
Mediterranean Sea	66.7	104.8	60.4	65.6	72.4	43.8	66.2	70.2	44.1
N. E. Atlantic	64.9	57.9	37.1	72.8	50.5	26.6	73.4	51.0	28.5
Total	**66.2**	**92.2**	**54.1**	**69.7**	**59.8**	**33.9**	**70.3**	**59.2**	**35.1**
Advanced Readers (Experts+Intermediate)									
Mediterranean Sea	69.5	44.2	30.9	68.3	40.8	30.2	69.7	37.4	29.6
N. E. Atlantic	70.5	55.3	53.0	74.6	23.8	15.2	75.6	23.1	16.7
Total	**70.2**	**52.2**	**47.2**	**71.9**	**31.1**	**21.7**	**73.1**	**29.3**	**22.3**

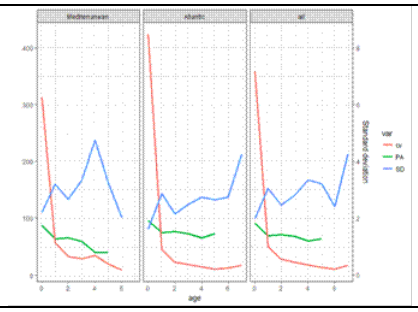
From the results of this small calibration exercise it can be concluded that no significant progress have been made in relation to the 2017 Exchange, i. e. general low age readings PA's and high CV's both between readers and modal ages still prevailed (Table 4.1 and Figure 4.1).

Nevertheless, from the image analyses joint sessions and from the discussions held during the workshop, it seems that most of readers, in particular the advanced ones, who attended the WKARAS 2 generally followed the same age reading criteria and the otoliths growth pattern in both areas when interpreting the otoliths' structures.

The main issues seem to be still the identification of the first growth ring and its discrimination from the checks, in some cases also the identification of the otoliths' edge type and the discrimination of marginal growth rings in older fish otoliths. The routinely use of reference otoliths image collections in each area of selected otoliths with a consensual age and of age validation studies regularly undertaken, would be useful in this context in order to improve age readings reliability and precision. Implementation of more regular otoliths exchanges for age readings calibration in each area and workshops for update of otoliths preparation and age reading standard procedures to be commonly followed by the researchers involved in the species age determination is also advisable.



2017 Exchange



WKARAS 2 Small calibration exercise

Figure 4.1 – 2017 Exchange and WKARAS 2 small calibration exercise: comparison between both events of Percentage of Agreement (PA), Coefficient of Variation (CV) and Standard Deviation (SD) for all readers by age group and area.

5 Age reading protocol: Guidelines (ToR b)

Readers agreed that age reading protocol should b based on the same rules as described in the previous sardine workshop (WKARAS, 2011) for the N.E. Atlantic areas.

Due to low age reading agreement between Atlantic and Mediterranean age readers, the ageing criteria were discussed and the readers decided to keep the first of January as the adopted reference birthdate. However the participants agreed that a validation study in these areas should be done.

This Workshop agreed that age readers should apply the following guidelines for preparation, observation and age determination of sardine:

1. Commonly, otoliths extracted from sampled fish are washed with fresh water, dried and mounted (with the sulcus down) on black plastic plaques glued with an inert resin in individual numbered cavities; In some cases, otoliths can also be observed in fresh water against black background. In this case however, 2 minutes maximum of otolith in water is advised (see point 8 below);

2. Otoliths are examined using a binocular microscope1 under reflected light with 20X magnification (opaque zones are then visible as white and hyaline zones dark). The magnification should be increased near the otolith edge to improve the discrimination of narrow rings in older individuals;

3. Sardine is aged by counting hyaline zones in the otolith; one year's growth ring (annulus) consists of one opaque zone and one hyaline zone.

4. Ring counts and edge type classification should preferably be done on the posterior (post-rostrum) region of the otolith where annual rings are generally clearer and otolith growth is larger;

5. In order to adopt a ring as an annulus it is recommended that the ring can be followed throughout the whole otolith contour. This rule must be applied specially for the first three annuli, in older specimens rings are very close together and become more difficult to follow;

6. The first of January is adopted as the reference birthdate;

7. For a fish caught in the first part of the year2 (prior to the growing period, i.e. opaque zone formation), the age corresponds to the number of hyaline zones present in the otolith (including the edge). For a fish caught in the second part of the year3 (after the opaque zone formation) the age corresponds to the number of hyaline zones completely formed (i.e. a hyaline edge is not counted);
8. A reference diameter of ≈2 mm (radius ≈1 mm) should be used to guide the identification of the first annual ring. This reference should be used in a flexible way, since the diameter of the

[1] i.e., a stereoscopic magnifier.

[2] 1st semester.

[3] 2nd semester.

first annual ring is proportional to the fish growth up to its formation;

9. For each otolith, the number of hyaline rings (excluding the edge), edge type, age and readability (1 - good, 2 - medium, 3 - difficult) should be recorded; false rings and other relevant characteristics of the otolith (e.g. crystallized otoliths) should be noted as well;

10. The percentage of opaque/hyaline edges should be calculated for each sample collected over the year to help determine the period of opaque edge deposition.

6 Otoliths Reference collection (ToR c)

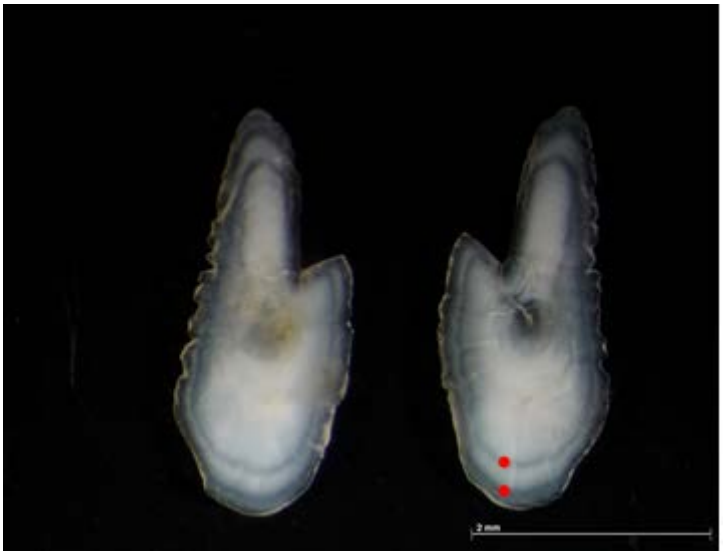
One of the aims of the WKARAS 2 was the creation of sardine otoliths' reference collection for each area (NE Atlantic and Mediterranean). This collection consists of clearly-defined otoliths images which age was consensually attributed, assembled in a data base to be easily accessed by age rea-ders. During the joint discussion sessions on 2017 Exchange otoliths' images, a preliminary version of a reference collection was prepared based on a selection of images of otoliths with ≥80% of age reading agreement between readers and modal age. It is foreseen that this preliminary collection, which is presented below, will be enriched and supplemented over time with new images selected from other age reading exchanges. All participants agreed that this will be a useful tool to help age readers to better identify the growth areas in the otoliths, improving their readings precision and increasing the percentage of age reading agreement between readers in each area.

N.E. Atlantic

ICES 8a

Date of Catch: 02-10-2014. TL=140 mm. Male. Age group 0

Date of Catch: 02-10-2014. TL=175 mm. Female. Age group 1.



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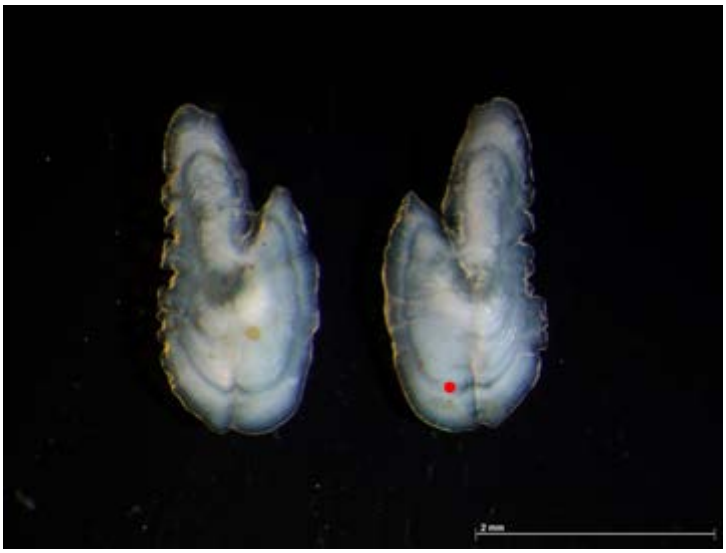
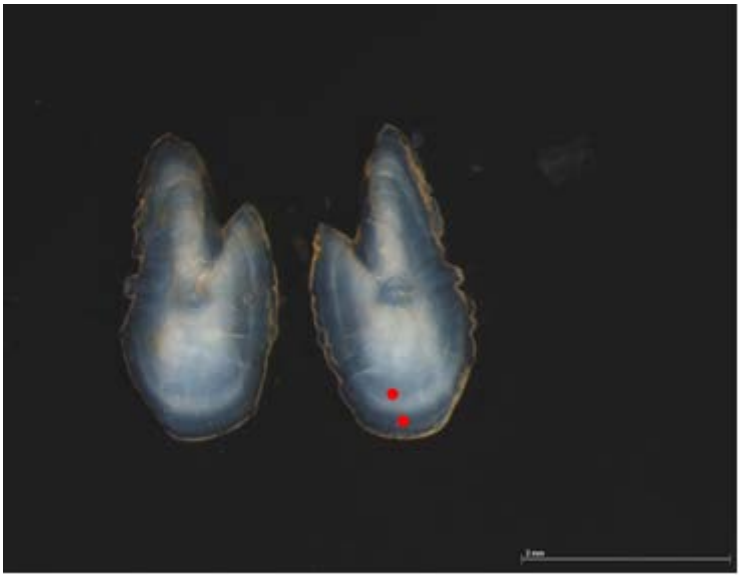
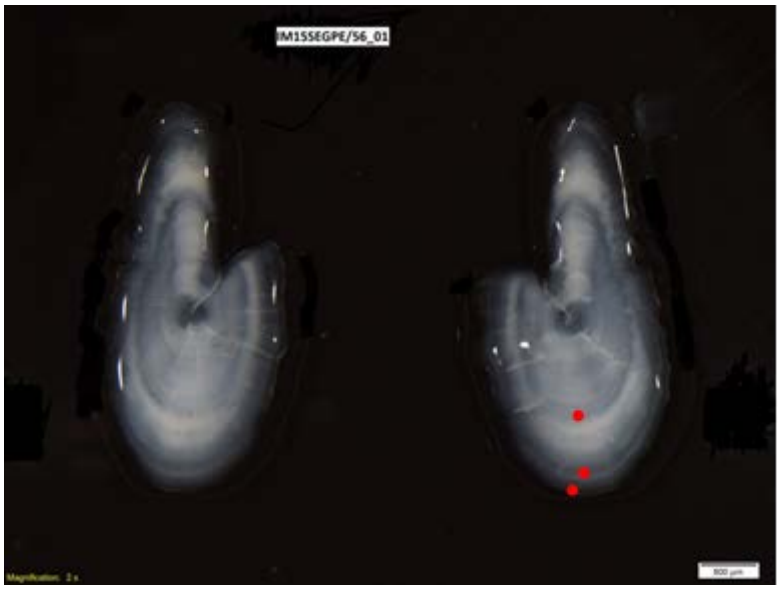


Date of Catch: 02-10-2014. TL=170 mm. Female. Age group 1.

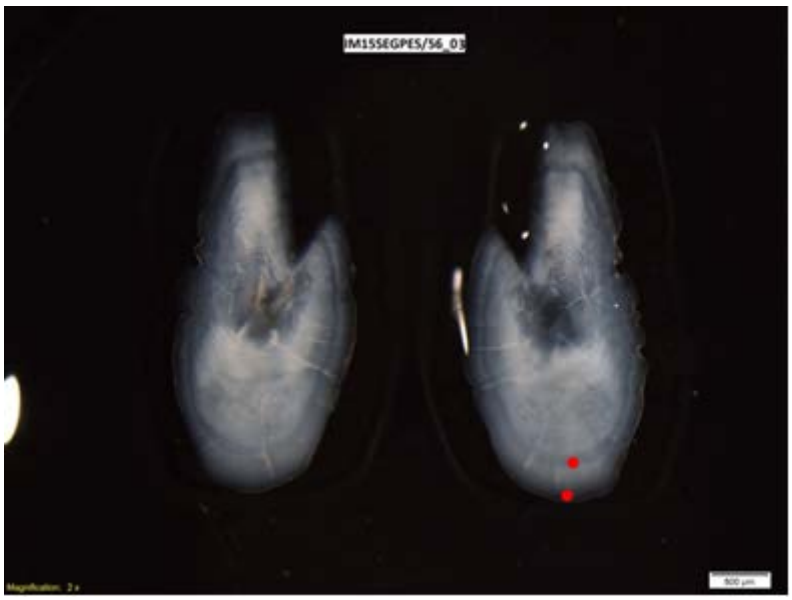


Date of Catch: 27-10-2014. TL=185 mm. Male. Age group 2.

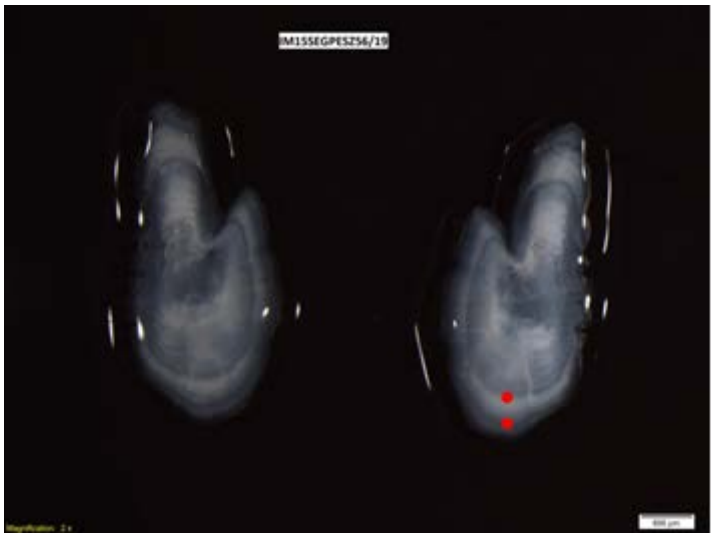
ICES 8b



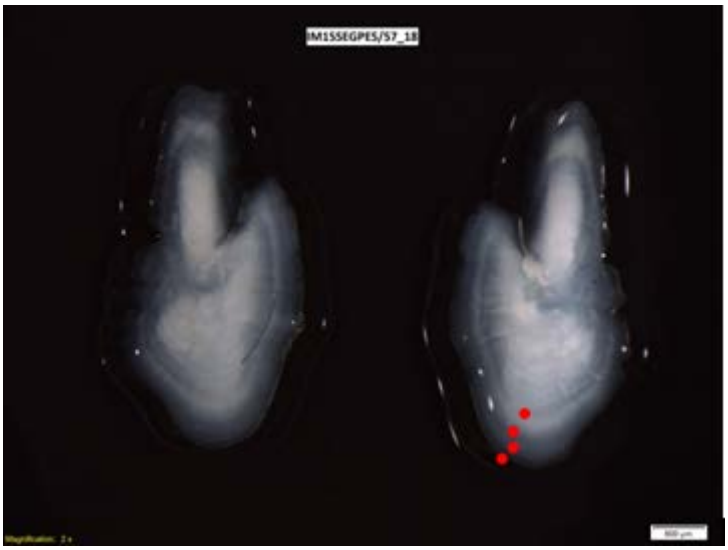
Date of Catch: 07-05-2015. TL=183 mm. Male. Age group 3.



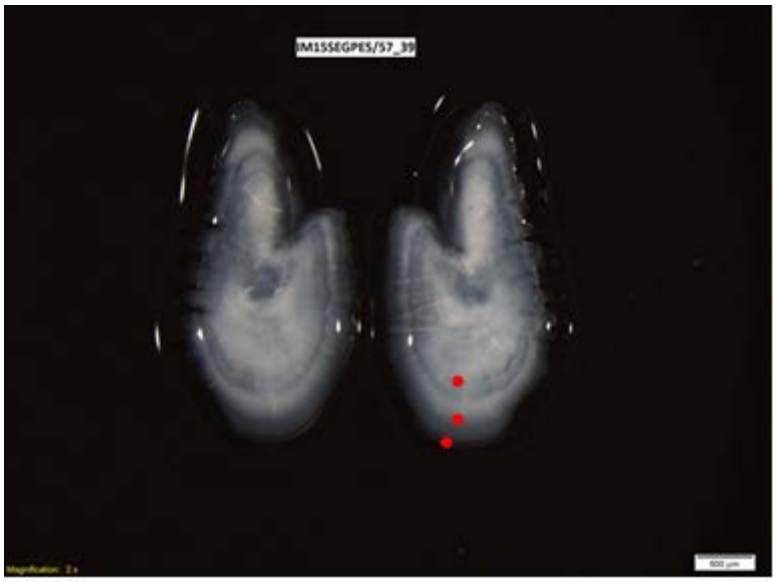
Date of Catch: 07-05-2015. TL=183 mm. Male. Age group 2.



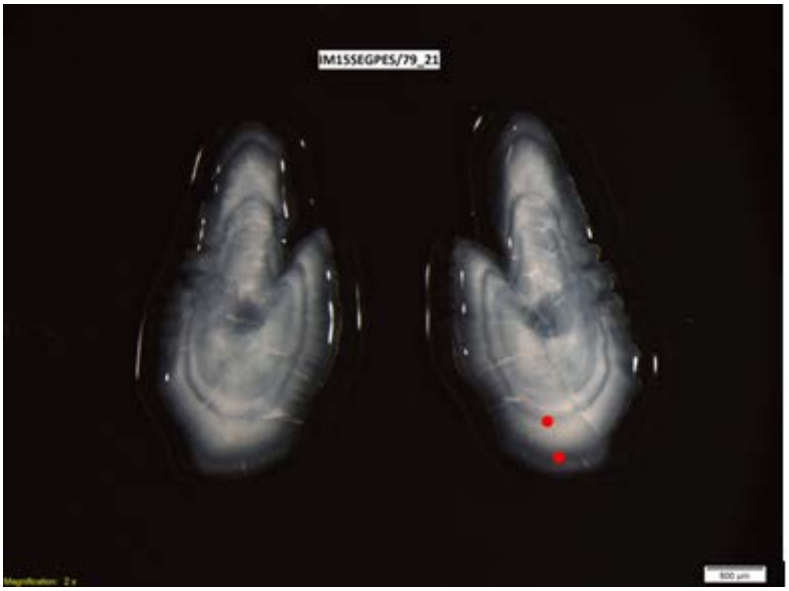
Date of Catch: 07-05-2015. TL=185 mm. Female. Age group 2.



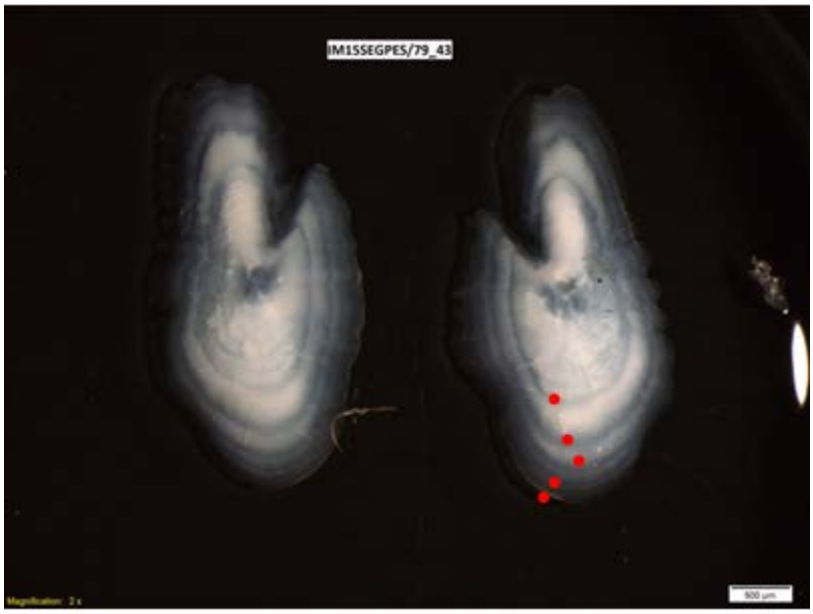
Date of Catch: 07-05-2015. TL=202 mm. Female. Age group 4



Date of Catch: 07-05-2015. TL=187 mm. Female. Age group 3

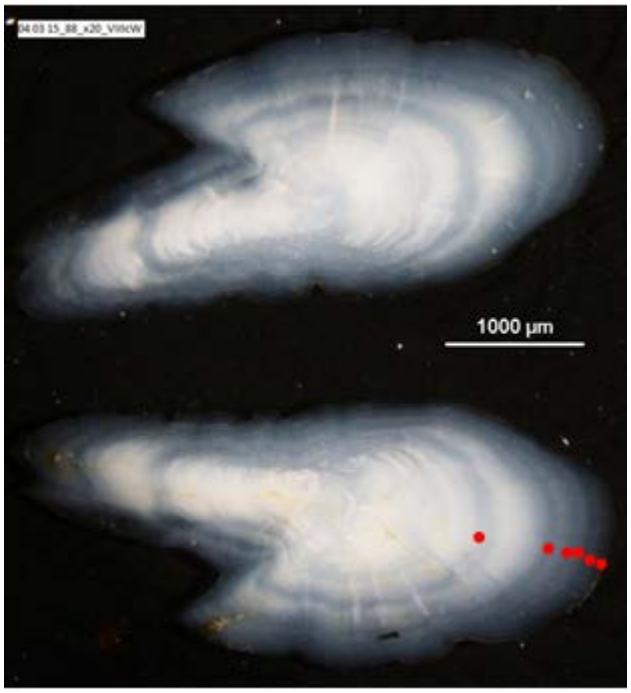


Date of Catch: 06-10-2015. TL=189 mm. Female. Age group 2.

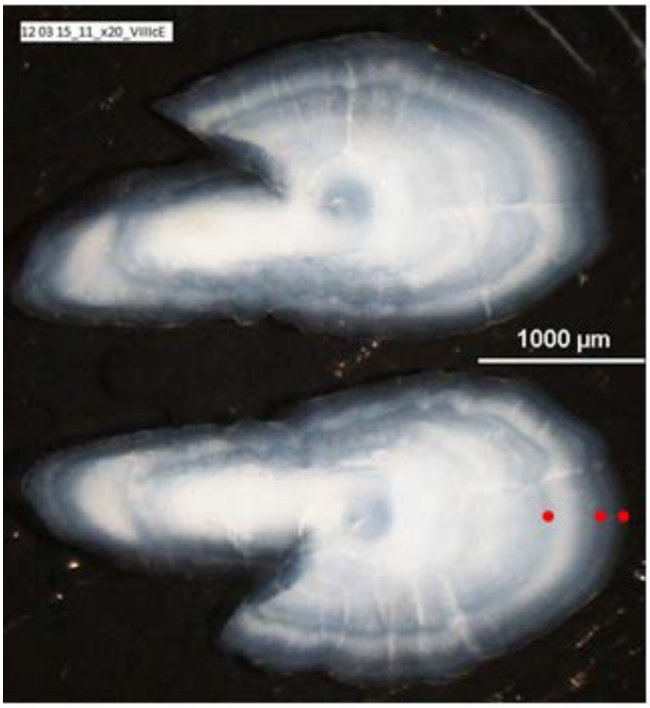


Date of Catch: 06-10-2015. TL=236 mm. Female. Age group 5.

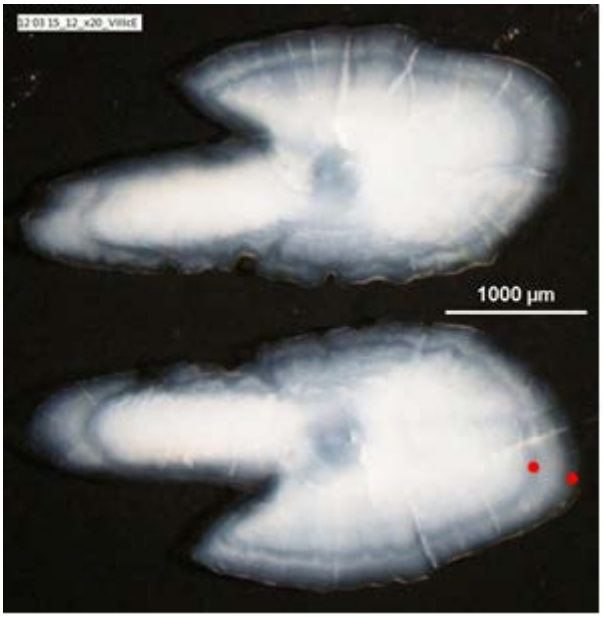
ICES 8c E & W



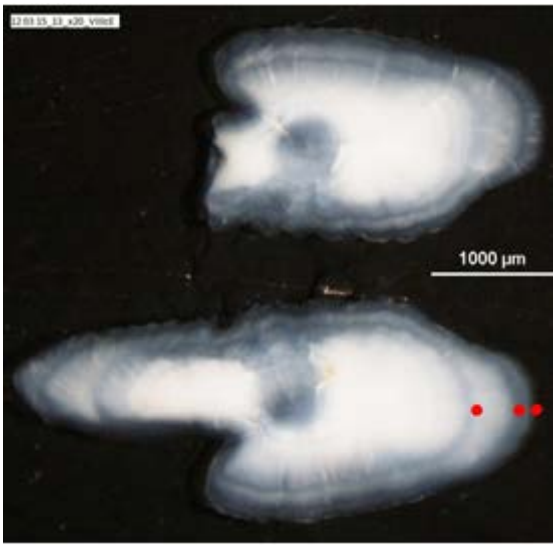
Date of Catch: 04-03-2015. TL=235 mm. Female. Age group 6.



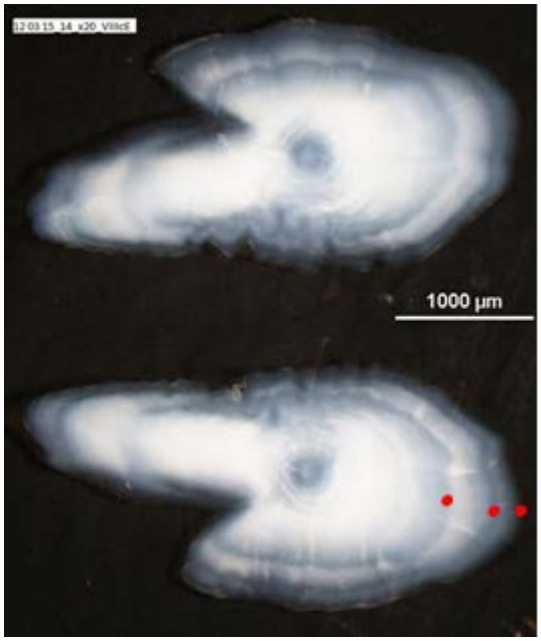
Date of Catch: 12-03-2015. TL=188 mm. Female. Age group 3



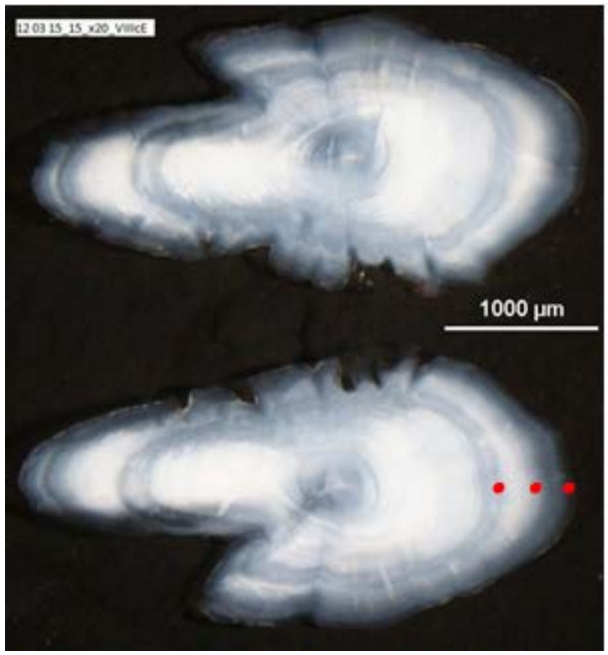
Date of Catch: 12-03-2015. TL=181 mm. Female. Age group 2.



Date of Catch: 12-03-2015. TL=200 mm. Female. Age group 3.

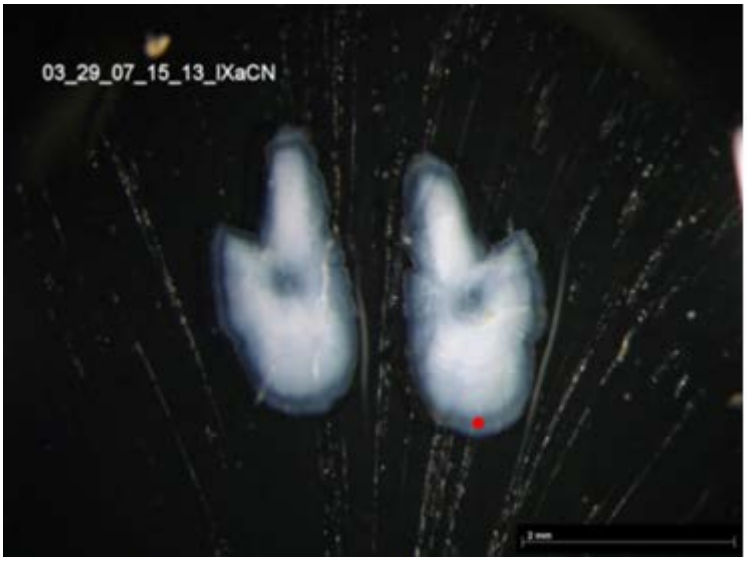


Date of Catch: 12-03-2015. TL=186 mm. Female. Age group 3.



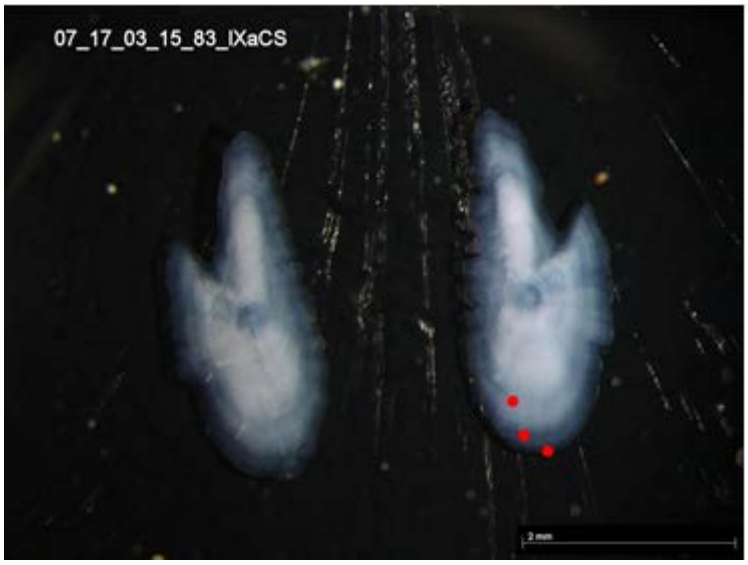
Date of Catch: 12-03-2015. TL=177 mm. Female. Age group 3.

ICES 9a



Date of Catch: 29-07-2015. TL=186 mm. Male. Age group 1.

Date of Catch: 27-08-2015. TL=143 mm. Female. Age group 0.



Date of Catch: 17-03-2015. TL=206 mm. Male. Age group 3

Mediterranean Sea

Otoliths images from FAO GSA09 [Ligurian and North Tyrrhenian Seas] with high enough age reading percentage of agreement between readers (≥80%) were not available within those used in the 2017 Exchange.

FAO GSA01

(Northern Alboran Sea)

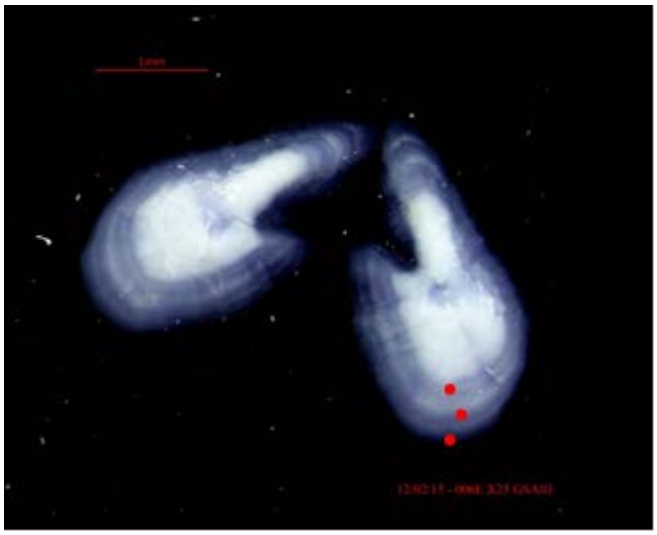
Date of Catch: 18-02-2015. TL=215 mm. Female. Age group 6.

Date of Catch: 21-09-2015. TL=191 mm. Female. Age group 3.

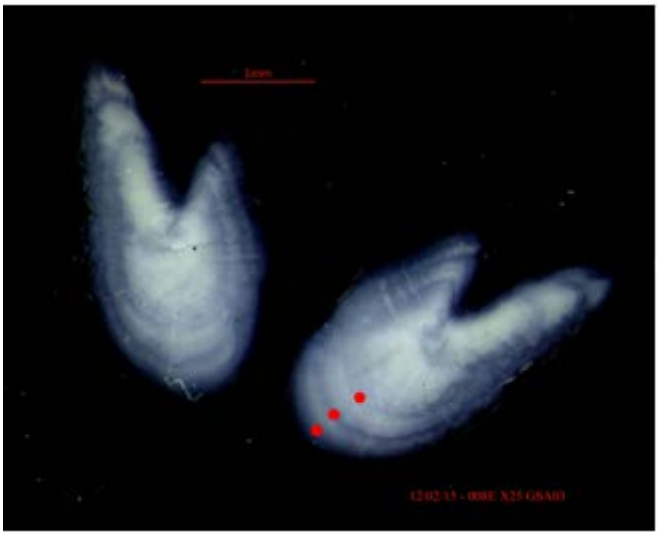
Date of Catch: 05-10-2015. TL=177 mm. Female. Age group 1.

FAO GSA03

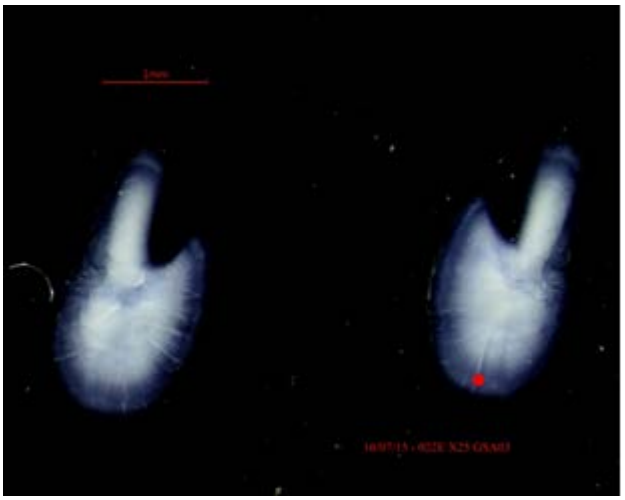
(Southern Alboran Sea)



Date of Catch: 12-02-2015. TL=170 mm. Male. Age group 3.



Date of Catch: 12-02-2015. TL=192 mm. Female. Age group 3.



Date of Catch: 16-07-2015. TL=140 mm. Male. Age group 1.

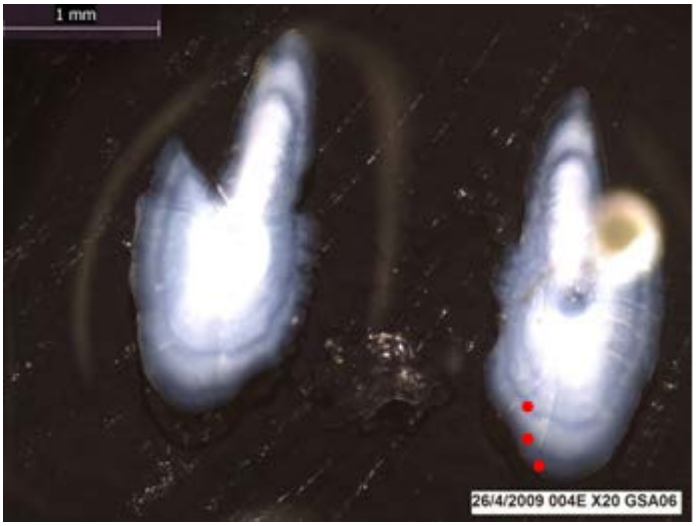
Date of Catch: 14-10-2015. TL=118 mm. Female. Age group 0.

Date of Catch: 04-11-2015. TL=109 mm. Female. Age group 0.

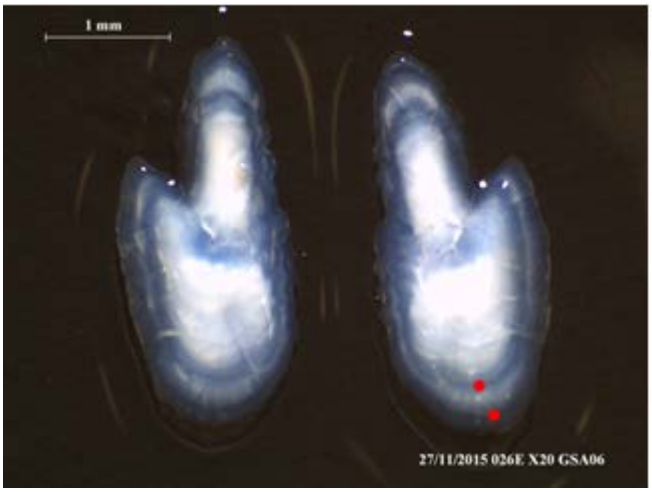
Date of Catch: 08-12-2015. TL=164 mm. Female. Age group 1.

FAO GSA06

(Northern Mediterranean Sea, Spain)

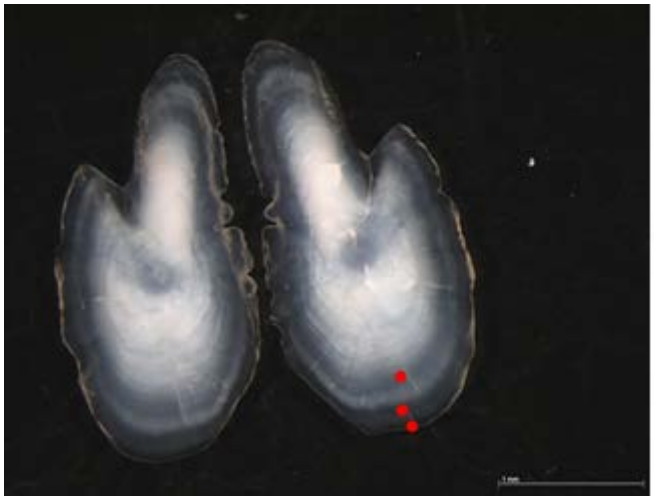


Date of Catch: 26-04-2009. TL=195 mm. Female. Age group 3.



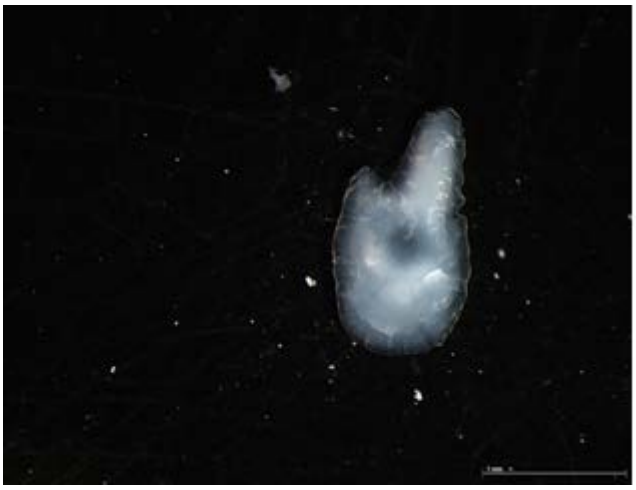
Date of Catch: 27-11-2015. TL=160 mm. Female. Age group 2.

FAO GSA07
(Gulf of Lions)



Date of Catch: 13-03-2015. TL=145 mm. Age group 3.
(Example of an old small fish in poor condition)

Date of Catch: 07-07-2015. TL=85 mm. Age group 0.

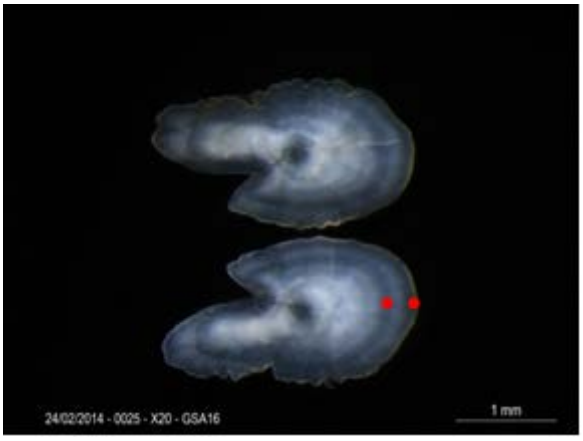


Date of Catch: 07-07-2015. TL=100 mm. Age group 0.

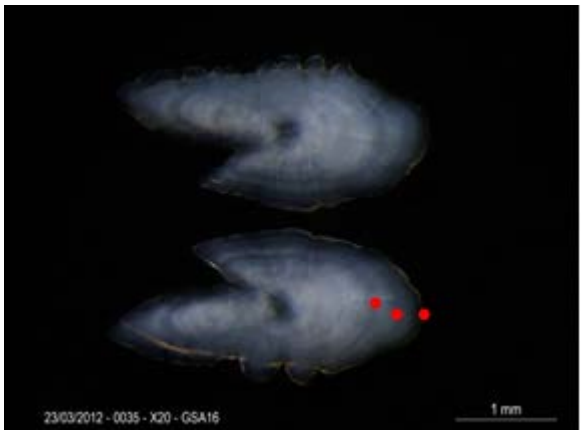
Date of Catch: 07-07-2015. TL=150 mm. Age group 2.

FAO GSA16
(South of Sicily)

Date of Catch: 06-06-2014. TL=104 mm. Age group 0.
(Exception to the convention criteria of January 1st reference birthdate.)



Date of Catch: 24-02-2014. TL=150 mm. Age group 2.



Date of Catch: 23-03-2012. TL=176 mm. Age group 3.

7 Otoliths sampling, preparation and age reading methods currently applied by participant research institutes

Methods for otoliths sampling, preparation and age reading applied by the participant research Institutes presented during the workshop are summarized in table 7.1.

At this point of discussion, it was noticed that in some areas, particularly in the Mediterranean, whole otoliths were observed after being hydrated by immersion in water, to turn more apparent the growth rings. Based on their experience, French colleagues pointed out that, when using this method special care should be taken in order to take a very short time (less than 2 minutes) to hydrate the otoliths, as they found that exceeding that time, the otoliths over hydrate, becoming more translucid and growth rings less apparent, misleading the reader on the age attribution to the otolith (figure 7.1). Thus it is advisable that this subject must be checked by readers who follow this otolith preparation for age reading, in order to avoid misleaded readings.

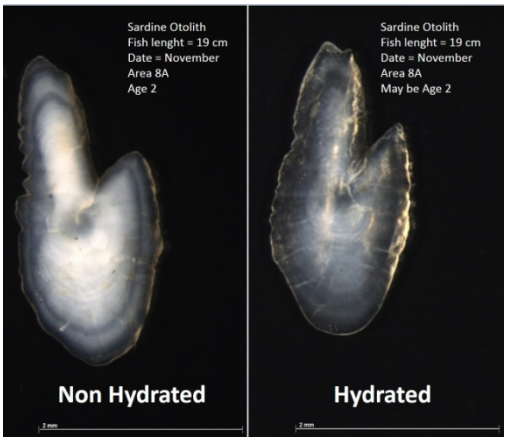


Figure 7.1 – Whole sardine otolith structure appearance: non hydrated (on the left) and hydrated after more than 2 minutes immersed in water (on the right). (Photo courtesy of Geoffrey Bled De Fruit, IFREMER, France).

Table 7.1 – Summary of methods for otoliths sampling, preparation for age reading applied by WKARAS 2 participant research institutes

Country	Institute	Otoliths sampling areas	Season/quarter	Otoliths' samples provenance	Otolith sampling scheme	Otoliths' preparation methods for observation	Type of observation light	Otolith cleaning process (water, alcohol, etc)	Observation-Medium (resin, water, other)	Age reading criteria	Use of Image analysis (Yes/No)	Age reading validation (Yes/No)
Portugal	Instituto Português do Mar e da Atmosfera (IPMA) (Portuguese Institute for Sea and Atmosphere)	9aCN, 9aCS, 9aS	Q1, Q2, Q3, Q4	Research surveys, commercial catches	Commercial catches sampling twice a month. 10 otolith pairs by fish length class (0.5 cm).	Whole otoliths embedded in transparent polyester resin (Entellan) mounted in cavities on black plastic plaques) (10 otoliths pairs per plaque).	Reflected	Water	Polyester transparent resin (Entellan)	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	No	Yes (Ré, 1984, 1986, Silva *et al*,, 2015)
Spain	Instituto Español de Oceanografía (IEO) (Spanish Institute of Oceanography)	8cE, 8cW, 9aN, 9aS, GSA 1, 2, 5 and 6.	Q1, Q2, Q3, Q4	Research surveys, commercial catches	Commercial catch sampling once a month. 40 otolith pairs collected by length class (0.5 cm). In Cadiz area and Alboran Sea (Mediterranean) 5 otolith pairs by length class (0.5 cm) respectively in each fortnightly and monthly samples.	Whole otoliths embedded in transparent Xylen substitute medium mounted in cavities on black plastic plaques) (10 otoliths pairs per plaque).	Reflected	Water	Xylen substitute medium	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	No	Yes (Alemany and Álvarez 1994, Álvarez and Alemany, 1997)……
Spain (Basque Country)	AZTI	8cE, 8cW	Q1, Q2, Q3, Q4	Research surveys, commercial catches	In each monthly sample, 40 otolith pairs by length class (0.5 cm)	Whole otoliths embedded in transparent Xylen substitute medium mounted in cavities on black plastic plaques) (10 otoliths pairs per plaque).	Reflected	Water	Xylen substitute medium	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	NO	No

Table 7.1 (cont'd)

Country	Institute	Otoliths sampling areas	Season/quarter	Otoliths' samples provenance	Otolith sampling scheme	Otoliths' preparation methods for observation	Type of observation light	Otolith cleaning process (water, alcohol, etc)	Observation Medium (resin, water, other)	Age reading criteria	Use of Image analysis (Yes/No)	Age reading validation (Yes/No)
Greece	Ελληνικό Κέντρο Θαλασσίων Ερευνών (ΕΛ.ΚΕ.Θ.Ε.) (Hellenic Centre for Marine Research [HCMR])	GSA 20 (Ionian Sea)	Q1, Q2, Q3, Q4	Research surveys, commercial catches.	Commercial catches: 4 otolith pairs by length class (0.5 cm) and sample.	Whole otoliths stored in freezer inside tagged plastic cases (<40°C). Otoliths observed immerged in freshwater in Petri cases under a stereoscopic magnifier against a black background.	Reflected	Water	Freshwater	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	Yes	No
	Fisheries Research Institute (FRI)	GSA 22 (Aegean Sea)	Q1, Q2, Q3, Q4	Research surveys, commercial catches.	In each monthly sample, 10 otolith pairs by length class (0.5 cm).	Whole otoliths stored dried in Eppendorf tubes. Otoliths observed immerged in freshwater in Petri cases under a stereoscopic magnifier against a black background.	Reflected	Water	Freshwater	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st.	Yes	No
Morocco	Institut National de Recherche Halieutique (INRH) (National Institute for Fisheries Research)	GSA 3	Q1, Q2, Q3, Q4	Research surveys, commercial catches	Commercial catches: 5 otolith pairs by length class (0.5 cm) and sample	Whole otoliths embedded in transparent polyester resin (Eukitt) mounted in cavities on black plastic plaques) (10 otoliths pairs per plaque).	Reflected	Water	Freshwater	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	No	No

Table 7.1 (cont'd)

Country	Institute	Otoliths sampling areas	Season/quarter	Otoliths' samples provenance	Otolith sampling scheme	Otoliths' preparation methods for observation	Type of observation light	Otolith cleaning process (water, alcohol, etc)	Observation Medium (resin, water, other)	Age reading criteria	Use of Image analysis (Yes/No)	Age reading validation (Yes/No)
France	Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) (French Research Institute for the Exploration of the Sea)	8a, 8b, GSA 7	Q1, Q2, Q3, Q4	Research surveys, commercial catches	Atlantic coast: in each monthly sample, 40 otolith pairs by length class (0.5 cm); Mediterranean coast: in each three-monthly sample, 20 otolith pairs by length class (0.5 cm).	Two preparation techniques for whole otoliths depending on time available for image acquisition: - Slow procedure: otoliths extracted from each sampled fish, washed with fresh water, dried and mounted on black plastic moulds glued with transparent polyester resin (Eukitt) in individual numbered cavities (PELGAS survey 3000 small pelagic fish otoliths read onboard); Fast procedure (safe for otoliths storage): Otoliths extracted, washed, dried and placed on black plastic plaques in individual numbered cavities filled with freshwater. Images are taken and otoliths are placed by pairs in individual numbered Eppendorf tubes stored in boxes. Procedure used in samples from fisheries regular samples and also in the PELMED research survey.	Reflected	Water	Freshwater	Hyaline ring counts and interpretation with catch date and reference birthdate January, 1st	Yes	No
Croatia	Institut za Oceanografiju i Ribarstvo (IZOR) (Institute of Oceanography and Fisheries)	GSA 17	1st Semester 2nd Semester	Research surveys, commercial catches	N/A	Otoliths collected, washed, dried and stored in vials. Immersed in alcohol for observation.	Reflected	Water	Alcohol	Hyaline ring counts and edge type	No	No

8 Address the generic ToRs adopted for workshops on age calibration (ToR d)

Details on the various methods of otoliths preparation, observation and age determination pres-ently carried out by the research institutes in both areas were summarized in table 7.1.

One of the main purposes of otoliths age reading exchanges and workshops is the standardization of methodologies, in order to improve age readings agreement between readers and the precision of age determination. In WKARAS 2 it was settled that the following guidelines approved at the 2011 WKARAS are suitable to be commonly applied in both areas:

1. Otoliths are examined using a stereoscopic magnifier under reflected light with 20X magnification (opaque zones are then visible as white and hyaline zones dark). The magnification should be increased near the otolith edge to improve the discrimination of narrow rings in older individuals;

2. Sardine is aged by counting hyaline zones in the otolith; one year's growth ring (*annulus*) consists of one opaque zone and one hyaline zone.

3. Ring counts and edge type classification should preferably be done on the posterior (post-rostrum) region of the otolith where annual rings are generally clearer and otolith growth is larger;

4. In order to adopt a ring as an *annulus* it is recommended that the ring can be followed throughout the whole otolith contour. This rule must be applied specially for the first three annuli, in older specimens rings are very close together and become more difficult to follow;

5. The first of January is adopted as the birthdate;

6. For a fish caught in the first part of the year (1st semester) (prior to the growing period, i.e. opaque zone formation), the age corresponds to the number of hyaline zones present in the otolith (including the edge). For a fish caught in the second part of the year (2nd semester) (after the opaque zone formation) the age corresponds to the number of hyaline zones completely formed (i.e. a hyaline edge is not counted);

7. For each otolith, the number of hyaline rings (excluding the edge), edge type, age and readability (1 - good, 2 - medium, 3 - difficult) should be recorded; false rings and other relevant characteristics of the otolith (e.g. crystallized otoliths) should be noted as well;

8. The percentage of opaque/hyaline edges should be calculated for each sample collected over the year to help determine the period of opaque edge deposition.

Currently a reference diameter of ≈2 mm (radius ≈1 mm) is used to guide the identification of the first annual ring in the N.E. Atlantic area, being advisable to use this reference in a flexible way, as the diameter of the first annual ring is proportional to the fish growth up to its formation. WKARAS 2 recommended that new validation studies should be regularly carried out in both areas in order to revise the first *annulus* identification (see point 10).

9 Recommendations for further cooperation, exchanges, workshops and other actions in relation to the age estimation of Sardine

From the discussions held and the exchanges results it was agreed that:

1. Age reading otolith exchanges should preferably be based on the structure analyses of samples of otoliths complemented by their images in SmartDots, for the growth rings identification by each reader, as a help for the analysis of results;

2. Whenever an age reading exchange only based on otoliths images takes place, not being possible to circulate among the readers the otoliths for observation, it is recommended to make sure that all the otoliths have been subjected to the same preparation and their images digitally acquired with the same image processing system under the same default settings in order to guaranty the same magnification and the best possible definition and quality of images;

3. The implementation in each area of routine otoliths age reading exchanges and of exchange of information between age readers, is also recommended as a contribution to the update of standard methodologies, to the improvement of age readings precision and of agreement between readers;

4. Regular age reading validation studies in each area, especially for the determination of first growth ring and for the false rings (checks) identification, should also be implemented in order to improve the age readings reliability and quality;

5. Otoliths' images reference collections consensually aged, started in this workshop for each area, should be enriched by more quality images along time, in order to build a comprehensive image database that can be accessed by readers, helping them to improve their readings reliability and precision;

6. It is also recommended that for otoliths mounting in plaques, the use of resins as Entellan or Eukit should be avoided known their toxicity to users. A less harmfull substance as PanReac Mounting Medium for substitutes of xylene for clinic diagnosis, should be used with this purpose.

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Annex 1: European Sardine Otoliths Exchange 2017 – Final Results

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Introduction

Following a WGHANSA - 2015 recommendation, WGBIOP-2015 have requested that an international otolith exchange for European sardine (*Sardina pilchardus*) (ICES Areas 7, 8, 9a and Mediterranean) would take place in 2016 co-coordinated by Eduardo Soares (IPMA – Lisboa, Portugal) and Pedro Torres (IEO – Malaga, Spain). This exchange had in mind to assess sardine otoliths age readings agreement between age readers of the Northeast Atlantic and of the Mediterranean Sea and the state of the art of the sardine age reading process in these two areas. Otoliths images sample sets from those areas were downloaded in WebGR to be analyzed by the participants.

Several setbacks have arisen during this process, mainly related to issues on WebGR functionality due to the high number of the exchange otoliths images and of age readers with which the system was not able to handle. To overcome these difficulties and to achieve the main goals of the exchange, R scripts (R Core Team. 2017) based on Eltink MS Excel spreadsheet (Eltink, 2000) following the recommendations of the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000) were developed and used for age readings comparative analyses and to assess the age reading agreement level amongst readers. This is a process somehow similar to what is currently used with SmartDots.

On the other hand WebGR site was deactivated with no previous notice, preventing us from accessing the images of the otoliths with the identification marks of growth rings by each reader that would aid the comparative analysis of the age readings results. Therefore, the age readings results analyses were only based on those data initially sent by the readers in MS Excel files to upload to the WebGR.

As a consequence of those difficulties, the accomplishment of the exchange results has been a time-consuming process, extending the execution of this task to 2017-2018.

SmartDots (now in Beta version) is being progressively adopted for exchanges of otoliths instead of WebGR, as it is assumed to be more suitable for that purpose.

Participants

A total of 31 readers from 10 laboratories of 16 areas of the Northeast Atlantic and the Mediterranean Sea participated in the European Sardine Otoliths Exchange 2017. A summary of their experience degree in that species age determination and indication of their geographical sampling area are shown in table 1.

Sardine age reading expertise level was based on the number of otoliths read of this species: Trainee (<2000 otoliths read); Intermediate (>2000 otoliths read) and Expert (>10000 otoliths read).

Table 1 – European Sardine Otoliths Exchange 2017 participants' references and their expertise level.

Reader N.	Reader name	Reader code name	Sardine Otoliths age reading expertise level	Area
1	Eduardo Soares	ES	Expert	9a
2	Andreia Silva	AS	Intermediate	9a
3	Delfina Morais	DM	Expert	9a
5	Isabel Loureiro	IL	Expert	8c+9a
6	Pedro Torres	PT	Expert	GSA1+GSA6
7	Pierluigi Carbonara	PC	Expert	GSA10+GSA19
8	Luca Lanteri	LL	Trainee	GSA9
9	Loredana Casciaro	LC	Intermediate	GSA10+GSA19
10	Michele Palmisano	MPAL	Intermediate	GSA10+GSA19
11	Salvatore Mangano	SM	Expert	GSA16
12	Maurizio Pullizi	MPUL	Expert	GSA16
13	Cristina Milani	CM	Trainee	GSA20+GSA22
15	Iñaki Rico	IR	Expert	8c
16	Andrea Massaro	AM	Trainee	GSA9
17	Mario Petrillo	MPET	Intermediate	GSA9
19	Gertrud Delfs	GD	Trainee	4 (North Sea)
20	Jean Louis Dufour	JD	Trainee	GSA7
21	Maria Barba	MB	Trainee	9a
22	Ester Herrera	EH	Trainee	GSA1
23	Jamal Settih	JS	Intermediate	GSA3
24	My Hachem Idrissi	MI	Intermediate	GSA3
25	Laaydi Jaber	LJ	Intermediate	Moroccan Atlantic Coast
26	Ana Ventero	AV	Expert	GSA1+GSA6
27	Ioannis Fytilakos	IF	Trainee	GSA20+GSA22
28	Gitta Hemken	GH	Trainee	4 (North Sea)
29	Denis Gašparević	DG	Intermediate	GSA17
30	Cristina Bultó	CB	Expert	GSA6
32	Raquel Milhazes	RM	Expert	9a
33	Erwan Duhamel	ED	Expert	8c
34	Geoffrey Bled Defruit	GB	Trainee	7
35	Célina Chantre	CC	Trainee	7

Material and methods

Otoliths images sample sets

A total of 380 images of otoliths' pairs were uploaded in WebGR and analyzed for age determination by each one of the participant readers. WebGR was, however, shut down, due to several issues that in many cases prevented the practical use of the system, and it is being replaced by SmartDots (still in Beta phase), which seems not to show the issues of the former system and is assumed to be more suitable for otoliths' ageing exchanges. Nevertheless, this change without previous notice from WebGR for SmartDots, with the shutdown of the former site, prevented us from accessing the images of the otoliths with the growth rings identification marks marked by

each reader that would aid the comparative analysis of the age readings results, leaving us with the only opportunity to base the analyses on the age records in excel files originally sent by email by the readers and which were also uploaded to the WebGR.

A summary list of the otoliths images samples by semester and areas is shown in table 2 and figure 1 shows the exchange otolith samples provenance areas.

Table 2 - European Sardine Otoliths Exchange 2017: overview of the otoliths image samples.

Area	Stra-tum	Semester	Fish total length (mm)		N. otoliths sampled
			min.	Max.	
NE Atlantic	8a	2	130	225	20
	8b	1	174	216	10
		2	171	236	10
	8c	1	170	237	10
		2	206	232	10
	9a	1	175	221	20
		2	109	230	20
	Total				**100**
Mediterranean Sea	GSA01	1	111	227	20
		2	115	236	20
	GSA03	1	129	199	20
		2	109	190	20
	GSA06	1	125	220	22
		2	105	185	18
	GSA07	1	115	155	20
		2	85	160	20
	GSA09	1	120	155	20
		2	110	170	20
	GSA16	1	104	176	20
		2	116	177	20
	GSA22	1	93	141	20
		2	101	154	20
	Total				**280**
Total					**380**

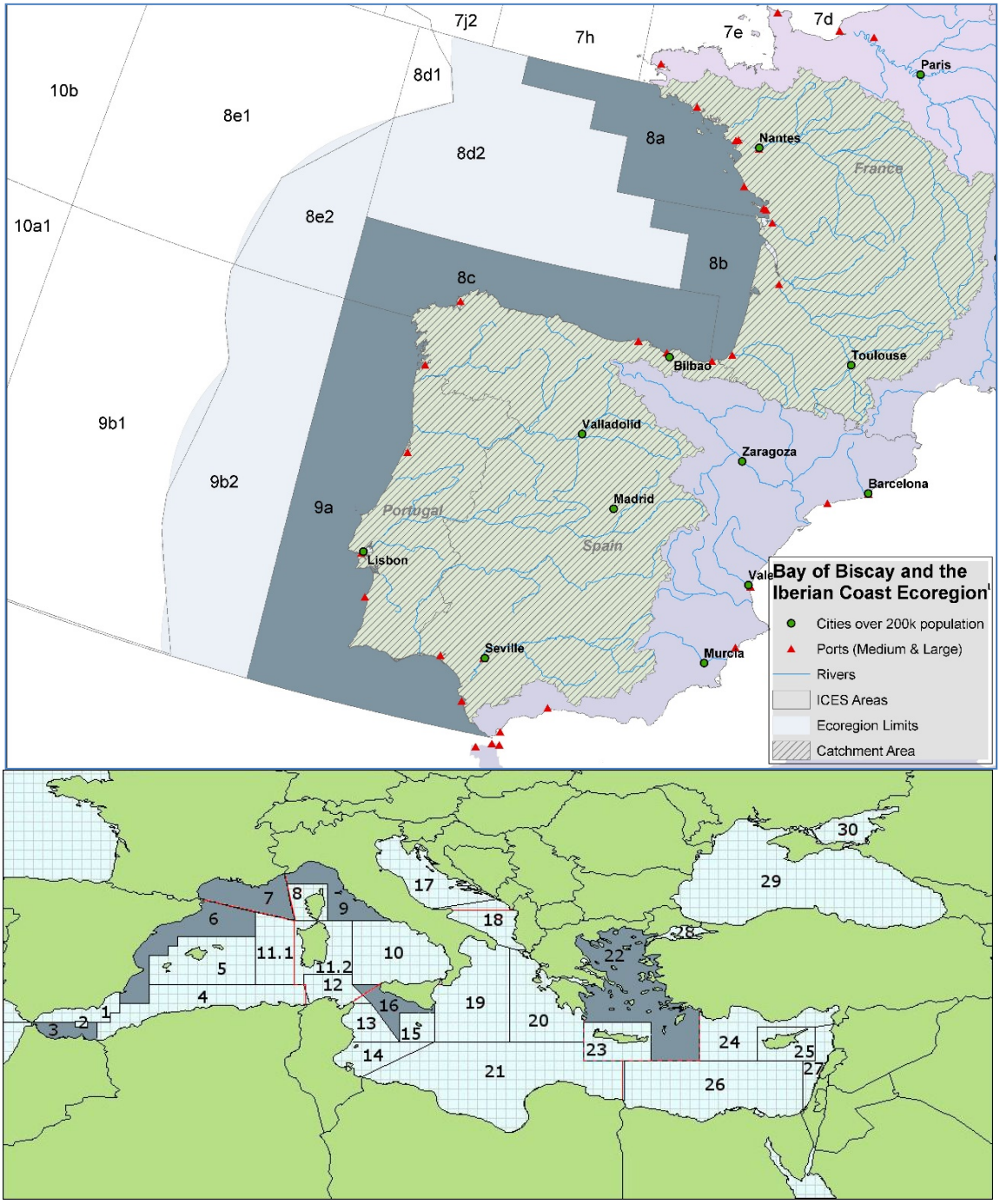

Figure 1 – European Sardine Otoliths Exchange 2017 - otolith samples provenance areas (dark grayish blue): A – NE Atlantic – ICES Areas of Bay of Biscay and Atlantic Iberian Coast (*http://www.ices.dk/explore-us/Action%20Areas/ESD/Pages/Bay-of-Biscay-and-the-Iberian-Coast-Ecoregion-description.aspx*); B – Mediterranean Sea – GFCM Geographical Sub-Areas (GSAs) (see table 1) (*http://www.fao.org/3/a-ax817e.pdf*).

Age Reading Protocol

Age reading protocol followed by all participants was made available to them in a working document uploaded to WebGR called "Guidelines for sardine otoliths picture samples preparation, observation and age determination criteria (adapted from ICES, 2011)" (see Annex of this document) which was based on the conclusions of the Workshop on Age Reading of European Atlantic Sardine (WKARAS) in 2011 (ICES, 2011).

Statistical analyses

R scripts based on Eltink's MS Excel spreadsheet (Eltink, 2000) following the recommendations of the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000) were used for age readings comparative analyses.

These analyses were undertaken according to the following scheme:

1. **All areas**: for all readers; for experts; for intermediate and for trainee readers;
2. **Northeast Atlantic**: for all readers; for experts; for intermediate and for trainee readers;
3. **Mediterranean Sea**: for all readers; for experts; for intermediate and for trainee readers.

Age Readings Analyses Results

The results are presented in tables and plots based on Eltink's, 2000 Excel spreadsheet, namely Percentage of Agreement (PA), Coefficient of Variation (CV), Average Percent Error (APE) and Age Bias Plots.

Age reader 33 was excluded from the analyses, as his readings were in disagreement with the remaining readers, probably due to issues that had happen with his observations (Figure 2).

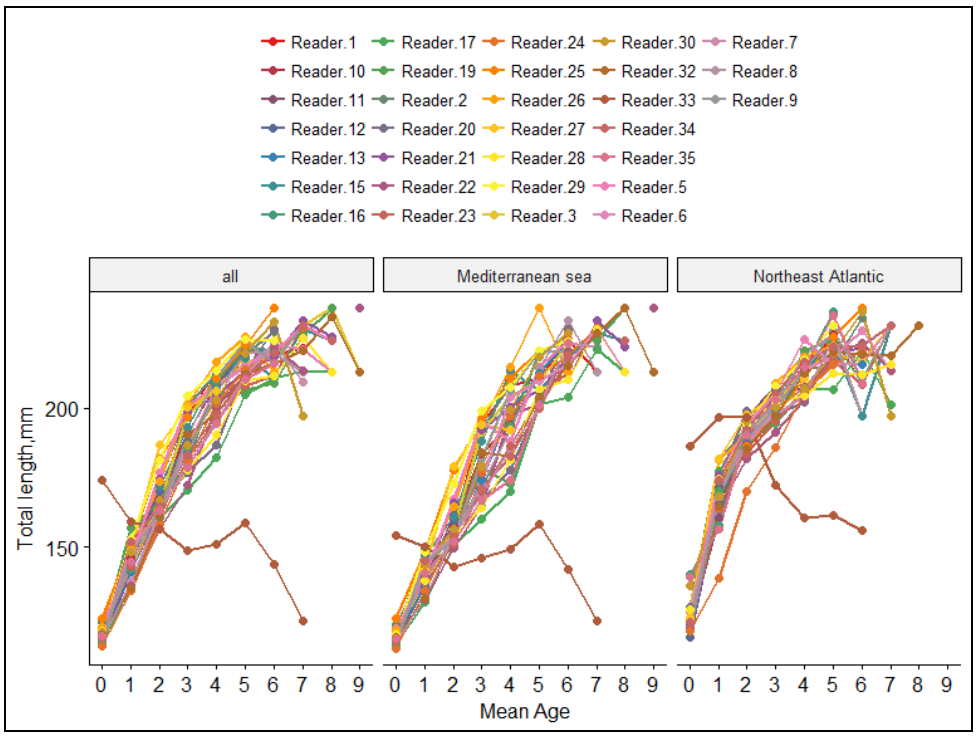

Figure 2 – Mean fish total length by age group by each reader by area (Age reader 33 diverges from the remaining readers).

Analysis of all readers by stratum and area

The results of the age readings precision analysis for all readers, including the mean percentage agreement (PA), coefficient of variation (CV) and average percentage error (APE) by stratum are presented in table 3 and figure 3.

Table 3 – All Readers: Mean Percentage Agreement (PA), Coefficient of Variation (CV) and Average Percentage Error of age readings by stratum and total area.

Area	Stratum	PA	CV	APE
NE Atlantic	8a	78.9	76.8	44.1
	8b	57.4	34.1	25.0
	8c	63.7	30.0	21.9
	9a	63.9	82.6	51.0
	Total	**64.9**	**57.9**	**37.1**
Mediterranean Sea	GSA01	61.5	67.1	46.9
	GSA03	72.8	118.7	74.0
	GSA06	67.4	45.6	31.6
	GSA07	60.0	142.0	77.7
	GSA09	56.2	54.2	38.5
	GSA16	77.4	67.2	43.4
	GSA22	76.0	234.3	124.4
	Total	**66.7**	**104.8**	**60.4**
Total Area		**66.2**	**92.2**	**54.1**

Whenever it was not possible to determine the modal age of an otolith, this was considered as the mean of age readings for that otolith.

In general terms, the average PA among all readers was low for both areas (64.9% for NE Atlantic, 66.7% for the Mediterranean Sea and a total average of 66.2%). PA ranged from 56.2% in stratum GSA09 (Mediterranean Sea) and 78.9% in stratum 8a (NE Atlantic). These values corresponded to high CVs, which ranged from 30.0% in stratum 8c (NE Atlantic) to 234,3% in stratum GSA22 (Mediterranean Sea). The odd CV high values exceeding 100% in strata GSA03, GSA07 and GSA22 (Mediterranean Sea) are due to a known issue of the CV calculation formula which is unable to generate correct values when the modal age is 0 and also when there are ages 0 assigned by some readers and ages other than 0 assigned by others to the same otoliths. Whenever PA is 100% and modal age is 0, the CV logically must be considered as 0. Concerning APE, it showed average high values for both areas (37.1% for NE Atlantic, 60.4% for the Mediterranean Sea and 54.1% for both). By Stratum, APE ranged from 21.9% (Stratum 8c) to 124.4% (Stratum GSA22). The high APE in this last stratum is also due to the 0 age issue already described for CV.

Analysis of expert readers by stratum and area

In Table 4, mean Percentages Agreement (PA), Coefficients of Variation (CV) and Average Percentage Error (APE) of experts' age readings by stratum, are shown.
The PA between expert readers was in average low for both areas (69.5% and 70.5%, respectively for NE Atlantic and the Mediterranean), ranging from 60.2% in stratum GSA09 to 80.1% in stratum GSA22 (both in the Mediterranean Sea). These values corresponded to high CV, which ranged from 22.3% in stratum 8b (NE Atlantic) to 83.2% in stratum GSA03 (Mediterranean Sea), showing an average of 44.2% in NE Atlantic, 55.3% in the Mediterranean and 52.2% for the total area. Concerning APE (average of 30.9% in NE Atlantic, 53.0% in the Mediterranean Sea and 47.2% for both areas together), it ranged from 16.8% (Stratum 8b) to 116.4% (GSA22).

Table 4 - Expert readers: Mean Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percentage Error (APE) of age readings by stratum and total area.

Area	Stratum	PA	CV	APE
NE Atlantic	8a	80.0	57.9	41.1

Area	Stratum	PA	CV	APE
	8b	66.3	22.3	16.8
	8c	64.3	23.9	20.2
	9a	68.4	58.4	38.3
	Total	**69.5**	**44.2**	**30.9**
Mediterranean Sea	**GSA01**	64.3	52.2	40.9
	GSA03	78.1	83.2	57.1
	GSA06	67.7	38.3	31.8
	GSA07	65.2	57.1	49.6
	GSA09	60.2	48.9	39.1
	GSA16	77.7	36.2	36.2
	GSA22	80.1	83.0	116.4
	Total	**70.5**	**55.3**	**53.0**
Total Area		**70.2**	**52.2**	**47.2**

Analysis of intermediate readers by stratum and area

Table 5 shows intermediates' age readings mean Percentages of Agreement (PA), Coefficients of Variation (CV) and Average Percentage Error (APE) by stratum.

Table 5 - Intermediate readers: Mean Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percentage Error (APE) of age readings by stratum and total area.

Area	Stratum	PA	CV	APE
NE Atlantic	**8a**	73.1	55.4	37.5
	8b	63.1	23.9	18.4
	8c	61.2	28.9	22.0
	9a	64.1	32.3	24.5
	Total	**65.1**	**34.5**	**25.4**
Mediterranean Sea	**GSA01**	62.5	62.2	45.0
	GSA03	69.6	86.5	59.5
	GSA06	65.6	37.1	29.0
	GSA07	66.2	79.6	54.6
	GSA09	55.9	64.0	50.9
	GSA16	70.3	69.8	47.2
	GSA22	80.3	116.9	86.4
	Total	**67.2**	**73.7**	**53.2**
Total Area		**66.7**	**63.4**	**45.9**

The PA between intermediate readers was in average low for both areas, as in the precedent cases (65.1% and 67.2%, respectively for NE Atlantic and the Mediterranean and 66.7% for both). PA ranged from 55.9% in stratum GSA09 to 80.3% in stratum GSA22 (both in the Mediterranean Sea). These values corresponded to high CV's, which ranged from 23.9% in stratum 8b (NE Atlantic) to 116.9% in stratum GSA22 (Mediterranean Sea), showing an average of 34.5% in NE Atlantic, 73.7% in the Mediterranean and 63.4% for the total area. Concerning APE (average of 25.4% in NE Atlantic, 53.2% in the Mediterranean Sea and 45.9% for both areas together), it ranged from 18.4% (8b) to 86.4% (GSA22).

Analysis of trainees by stratum and area

Table 6 shows trainees' age readings mean Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percentage Error (APE) by stratum.

Table 6 - Trainee readers: Mean Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percentage Error (APE) of age readings by stratum and total area.

Area	Stratum	PA	CV	APE
NE Atlantic	**8a**	87.7	48.6	28.8
	8b	56.8	33.6	25.2
	8c	72.3	27.4	18.7
	9a	65.9	61.8	46.1
	Total	**69.7**	**46.6**	**33.0**
Mediterranean Sea	**GSA01**	68.3	51.5	37.8
	GSA03	80.2	84.1	56.2
	GSA06	75.5	35.9	24.6
	GSA07	69.8	51.6	39.7
	GSA09	67.0	33.6	25.1
	GSA16	88.0	34.1	22.7
	GSA22	84.3	35.2	29.8
	Total	**76.2**	**46.6**	**33.7**
Total Area		**74.5**	**46.6**	**33.5**

The PA between trainee readers was in average low for both areas (69.7% and 76.2%, respectively for NE Atlantic and the Mediterranean and 74.5% for both areas). PA ranged from 56.8% in stratum 8b to 88.0% in stratum GSA16. These values corresponded to high CV, which ranged from 27.4% in stratum 8c (NE Atlantic) to 84.1% in stratum GSA03 (Mediterranean Sea), showing an average of 46.6% both in NE Atlantic as in the Mediterranean Sea and 46.6% for both areas together. Concerning APE (average of 33.0% in NE Atlantic, 33.7% in the Mediterranean Sea and 33.5% for both areas together), it ranged from 18.7% (8c) to 56.2% (GSA03).

Age readings comparative analysis of mixed readers, by expertise and area

Figure 3 shows the age reading comparative analysis results of mixed readers from both areas by expertise and area

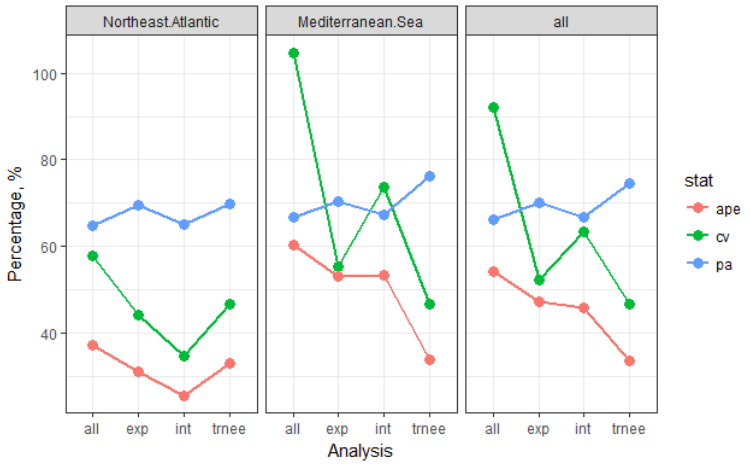

Figure 3 – PA, CV and APE for all readers mixed and by expertise level for each and the total area.

In the NE Atlantic, PA varied between □65% (intermediate readers) and □69.5% (experts and trainees). When all readers were considered, PA almost reached 64.5% in this area. These PA's corresponded to high CV values, which ranged from □34.5% (intermediate readers) to □46.5% (trainees). CV reached □57.5% when all readers were considered. Concerning APE, it ranged from □25% (intermediate readers) and □33% (trainees).

In the Mediterranean Sea, PA varied between □67% (experts) and □76% (trainees). When all readers were considered, PA reached □66.5%. These PA values corresponded to high CV's which ranged from □46.5% (trainees) to □74% (intermediate readers). APE ranged from □33.5% (trainees) to □53% (experts and intermediates), reaching □60% when all readers were considered.

For both areas combined, PA varied between □66.5% (intermediates) and □74.5% (trainees). When all readers were considered, PA reached □66%. These PA values corresponded to high CV's which ranged from □46.5% (trainees) to □63% (intermediate readers). When all readers were considered CV reached 92% in both areas combined. APE ranged from □33.5% (trainees) to 47% (experts), reaching □54% when all readers were considered.
In general, trainees showed higher PA's and lower CV's in both areas.

Age readings comparative analysis of NE Atlantic readers by expertise and area

Figure 4 shows the age reading comparative analysis results of NE Atlantic readers by expertise and area (Readers included in the analysis: Expert Readers: Reader.1, Reader.3, Reader.5, Reader.15, Reader.32; Intermediate Readers: Reader.2 and Reader.25; Trainee Readers: Reader.19, Reader.21, Reader.28 , Reader.34, Reader.35).

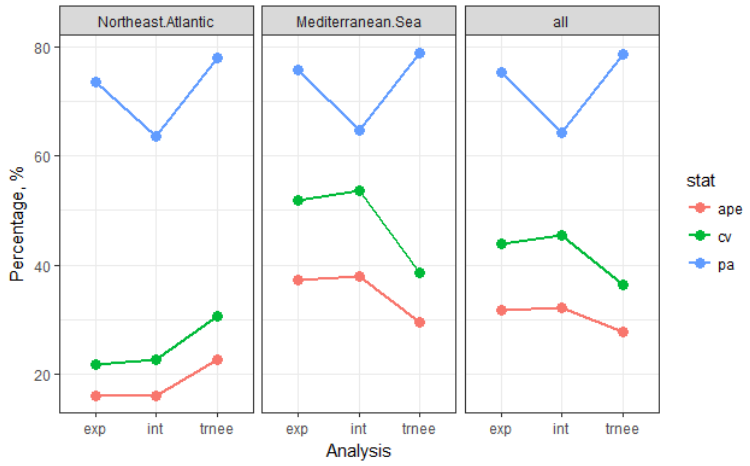

Figure 4 – PA, CV and APE for NE Atlantic age readers and by expertise level for each and the total area.

In the NE Atlantic and only considering readers of this area, PA varied between □63% (intermediate readers) and □80% (trainees). Trainees were followed by experts with □75% of PA. These PA values corresponded to CV that varied between □20% (experts) and □30% (trainees). Concerning APE, it ranged from <15% (experienced) to □22% (trainees).

In the Mediterranean Sea, PA varied between □65% (intermediate readers) and □80% (trainees). Experts followed trainees with a PA of □75%. CV ranged from □40% (trainees) and □72% (intermediate readers). APE varied between □30% (trainees) and □40% (intermediate readers).

For both areas combined, PA ranged from □64% (intermediate readers) to □79% (trainees). CV varied between □37% (trainees) and □45% (intermediate readers). APE ranged from □28% to 32% (intermediate readers).

The NE Atlantic trainees showed higher PA's in both areas followed by experts and a higher precision in the NE Atlantic area.

Age readings comparative analysis of Mediterranean Sea readers by expertise and area

Figure 5 shows the age reading comparative analysis results of Mediterranean Sea readers by expertise and area (Readers included in the analysis: Expert Readers: Reader.6, Reader.7, Reader.11, Reader.12, Reader.26, Reader.30; Intermediate Readers: Reader.9, Reader.10, Reader.17, Reader.23, Reader.24, Reader.29; Trainee Readers: Reader.8, Reader.13, Reader.16, Reader.20, Reader.22 ,Reader.27).

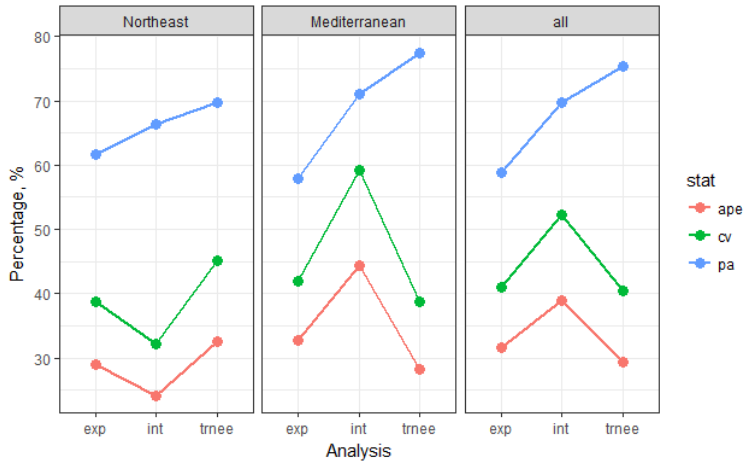

Figure 5 – PA, CV and APE for Mediterranean Sea age readers and by expertise level for each and the total area.

In the NE Atlantic, considering only readers from Mediterranean Sea area, PA varied between 62% (experienced) and 70% (trainees). CV ranged from 33% (intermediate readers) and 45% (trainees). APE ranged from 24% (intermediate readers) and 33% (trainees).

In the Mediterranean Sea, in this case, PA varied between 58% (experienced) and 77% (trainees). CV ranged from 38% (trainees) to 60% (intermediate readers). APE ranged from 28% (trainees) to 45% (intermediate readers).

For both areas combined, PA ranged from 59% (experienced) to 75% (trainees). CV varied between 40% (experienced and trainees) and 53% (intermediate). APE ranged from 29% (trainees) and 39% (intermediate).
The Mediterranean trainees showed higher PA's in both areas followed by intermediates and a higher precision in the Mediterranean area.

Relative bias

The minimal requirement for age reading's consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated can be non-parametrically tested with a one sample Wilcoxon signed rank test.
Tables 7, 8 and 9 show the results of the age readings' inter-reader bias respectively for NE Atlantic, the Mediterranean Sea and both areas combined (under diagonal), the age reading's percentage agreement (PA) between each two readers (above diagonal) and the reader against modal age bias tests (tables' bottom line).

Table 7 - Inter-reader bias test and reader against modal age bias test of sardine otoliths for North East Atlantic Area (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

	Reader.1	Reader.2	Reader.3	Reader.5	Reader.6	Reader.7	Reader.8	Reader.9	Reader.10	Reader.11	Reader.12	Reader.13	Reader.15	Reader.16	Reader.17	Reader.19	Reader.20	Reader.21	Reader.22	Reader.23	Reader.24	Reader.25	Reader.26	Reader.27	Reader.28	Reader.29	Reader.30	Reader.32	Reader.34	Reader.35
Reader.1		61	62	62	60	66	66	72	73	44	46	64	62	53	58	60	55	57	47	44	32	56	60	50	64	53	59	48	59	58
Reader.2	*		58	66	51	55	58	52	66	45	43	62	61	44	46	62	60	67	36	40	25	46	69	56	62	63	59	45	59	49
Reader.3	-	-		63	68	60	57	64	75	49	48	68	66	49	58	58	56	71	48	39	29	50	57	52	60	60	61	53	61	52
Reader.5	-	-	-		52	54	71	63	67	56	58	65	57	54	47	72	65	63	35	31	20	45	62	66	62	70	60	43	67	61
Reader.6	-	*	-	*		60	54	65	69	44	43	67	65	46	50	49	46	65	51	46	32	50	48	50	55	55	63	60	53	48
Reader.7	-	**	-	*	-		69	71	79	50	52	73	65	56	65	55	61	58	49	44	29	51	51	55	66	58	71	56	61	61
Reader.8	-	-	-	-	-	-		68	71	60	64	76	61	58	58	62	70	59	43	42	24	48	65	62	65	65	68	48	67	59
Reader.9	-	*	-	-	-	-	-		81	56	61	74	68	55	64	63	58	64	49	47	33	52	59	59	61	63	68	57	67	64
Reader.10	-	-	-	-	-	-	-	-		58	59	84	77	56	61	73	65	72	52	47	31	57	61	57	73	71	73	61	75	69
Reader.11	*	-	-	-	*	**	-	*	-		89	57	49	48	47	57	54	45	35	31	12	44	53	55	44	57	51	44	56	57
Reader.12	*	-	-	-	*	**	-	*	-	-		59	51	42	47	59	56	44	37	32	16	43	55	58	45	57	53	43	53	54
Reader.13	-	-	-	-	-	-	-	-	-	-	-		71	47	58	63	64	69	46	45	26	56	58	57	72	70	76	61	72	68
Reader.15	-	-	-	-	-	-	-	-	-	-	-	-		43	56	63	57	66	53	45	25	47	55	53	62	66	61	58	67	58
Reader.16	-	*	-	-	-	-	-	-	-	-	-	-	-		45	53	43	48	28	29	18	34	47	53	44	54	51	36	47	50
Reader.17	-	**	*	**	-	-	**	-	*	**	**	*	**	**		45	46	53	48	46	33	49	44	44	53	45	60	50	48	51
Reader.19	-	-	-	-	*	*	-	*	-	-	-	-	-	-	**		67	65	35	37	21	45	61	54	62	71	58	48	73	59
Reader.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-		57	33	39	25	50	56	54	65	66	63	44	60	56
Reader.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-		41	42	29	54	61	53	68	64	61	50	64	58
Reader.22	**	**	**	**	*	*	**	*	**	**	**	**	**	**	-	**	**	*		38	40	52	31	34	38	33	44	50	39	35
Reader.23	-	**	*	**	-	-	**	-	*	**	**	*	*	**	-	**	**	-	-		41	40	41	35	36	37	40	42	41	35
Reader.24	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*	**	**	**	-	*		35	17	17	27	18	25	30	23	20
Reader.25	-	**	-	**	-	-	-	-	-	**	**	-	-	-	-	**	*	-	-	-	**		39	37	56	46	48	44	43	42
Reader.26	*	-	-	-	*	**	-	*	-	-	-	-	-	-	**	-	-	-	**	**	**	**		59	59	58	53	43	65	51
Reader.27	**	-	*	-	**	**	**	**	**	-	-	**	-	**	**	-	*	**	**	**	**	**	-		53	69	58	46	51	43
Reader.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	**	*	**	-	-	**		63	66	50	66	56
Reader.29	*	-	-	-	*	**	-	*	-	-	-	-	-	-	**	-	-	-	**	**	**	**	-	-	-		61	50	67	59
Reader.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	**	*	**	-	-	*	-	-		56	64	59
Reader.32	-	**	-	**	-	-	-	-	-	**	**	-	-	-	-	**	*	-	-	-	**	-	**	**	-	**	-		54	46
Reader.34	-	-	-	-	-	*	-	-	-	-	-	-	-	-	**	-	-	-	**	**	**	*	-	-	-	-	-	*		70
Reader.35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-	**	**	**	-	-	**	-	-	-	-	-	
modal.age	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	**	*	**	-	-	*	-	-	-	-	-	-

In NE Atlantic area, reader against modal age bias test showed a low occurrence of bias, except for readers 17, 23 and 27 (*) and 22 and 24 (**). Inter-reader bias test, showed a relatively low occurrence of sign of bias, except in particular for readers 17, 22, 23, 24, 25, 26, 27, 29 and 32, which showed occurrence of signs of bias when tested against the other readers. Relatively to PA's between each two readers they ranged from 12% (Reader 11 against 24) and 89% (Reader 11 against 12).

Table 8 - Inter-reader bias test and reader against modal age bias test of sardine otoliths for Mediterranean Sea area (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

	Reader.1	Reader.2	Reader.3	Reader.5	Reader.6	Reader.7	Reader.8	Reader.9	Reader.10	Reader.11	Reader.12	Reader.13	Reader.15	Reader.16	Reader.17	Reader.19	Reader.20	Reader.21	Reader.22	Reader.23	Reader.24	Reader.25	Reader.26	Reader.27	Reader.28	Reader.29	Reader.30	Reader.32	Reader.34	Reader.35
Reader.1		61	63	71	51	49	60	58	64	51	50	58	58	57	40	52	55	65	50	48	43	53	60	62	50	59	51	53	52	49
Reader.2	-		70	63	56	52	68	64	68	61	58	62	63	60	43	61	56	66	52	43	37	44	62	57	57	53	57	53	59	52
Reader.3	-	-		70	56	51	61	61	66	55	54	59	61	55	41	54	57	70	54	46	44	47	62	56	55	52	56	61	51	48
Reader.5	-	-	-		57	48	62	59	70	56	54	60	61	55	41	56	57	72	49	46	49	49	71	71	54	71	58	56	53	49
Reader.6	-	-	-	-		56	65	73	70	58	59	61	67	58	50	69	57	57	57	52	57	49	57	51	68	63	78	58	65	59
Reader.7	**	**	**	**	**		71	66	63	66	71	72	51	67	63	60	68	47	61	44	40	35	50	47	68	42	59	43	62	66
Reader.8	**	*	-	**	-	-		81	82	72	71	82	60	77	59	77	80	62	63	50	42	41	63	55	77	55	71	52	74	74
Reader.9	*	-	-	*	-	**	-		84	68	68	76	70	73	52	77	70	60	59	56	52	46	64	52	72	64	76	53	76	69
Reader.10	-	-	-	-	-	**	-	-		69	65	79	73	71	53	80	73	69	60	59	53	46	68	61	73	66	75	56	73	69
Reader.11	*	-	-	**	-	**	-	-	-		86	72	54	64	53	64	67	55	57	46	43	34	57	55	68	51	66	44	66	63
Reader.12	**	**	*	**	-	-	-	-	*	-		73	52	67	56	63	68	50	60	44	40	32	56	54	67	49	65	44	64	65
Reader.13	**	**	*	**	-	-	-	-	*	-	-		62	69	57	71	77	61	61	51	44	39	58	56	75	51	65	49	69	70
Reader.15	-	-	*	-	-	**	**	**	*	**	**	**		54	42	70	53	68	45	55	51	54	61	59	58	73	65	51	60	54
Reader.16	**	**	**	**	*	-	-	-	*	-	-	-	**		57	65	72	52	59	45	38	38	55	50	68	49	61	46	68	67
Reader.17	**	**	**	**	**	*	**	**	**	**	**	**	**	**		52	63	43	60	39	38	31	39	40	65	34	50	40	58	61
Reader.19	-	-	-	-	-	**	-	-	-	-	-	*	-	*	**		67	60	53	55	49	48	58	52	71	62	74	48	76	70
Reader.20	**	**	**	**	**	-	-	*	**	*	-	-	**	-	*	**		52	59	44	35	38	53	48	71	50	61	46	69	72
Reader.21	-	-	-	-	-	**	**	*	-	**	**	**	-	**	**	-	**		51	48	51	48	68	68	61	62	56	57	54	48
Reader.22	**	**	**	**	**	-	-	*	**	*	-	-	**	-	*	**	-	**		48	47	37	49	47	58	39	55	52	50	51
Reader.23	-	-	-	*	-	*	-	-	-	-	-	-	*	-	**	-	-	-	-		55	48	48	44	47	56	54	41	50	46
Reader.24	*	-	-	**	-	-	-	-	-	-	-	-	**	-	**	-	-	*	-	-		40	46	47	43	54	52	48	45	39
Reader.25	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**		49	48	37	59	49	35	41	37
Reader.26	-	-	-	-	-	**	**	*	-	**	**	**	-	**	**	-	**	-	**	*	**	**		65	53	69	59	49	52	52
Reader.27	-	*	**	-	**	**	**	**	**	**	**	**	-	**	**	*	**	-	**	**	**	**	-		48	65	52	48	47	46
Reader.28	**	**	*	**	*	-	-	-	*	-	-	-	**	-	**	*	-	**	-	-	-	**	**	**		50	67	49	70	70
Reader.29	*	**	**	*	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**	**	**	*	*	-	**		62	43	59	50
Reader.30	-	-	-	-	-	**	-	-	-	-	-	-	*	*	**	-	**	-	**	-	-	**	-	**	*	**		51	69	63
Reader.32	**	**	*	**	*	-	-	-	*	-	-	-	**	-	**	*	-	**	-	-	-	**	**	**	-	**	*		42	40
Reader.34	**	*	-	**	-	*	-	-	-	-	-	-	**	-	**	-	-	**	-	-	-	**	**	**	-	**	-	-		76
Reader.35	**	**	**	**	**	-	-	*	**	*	-	-	**	-	**	**	-	**	-	-	-	**	**	**	-	**	**	-	-	
modal.age	*	-	-	*	-	**	-	-	-	-	-	-	**	-	**	-	*	*	**	-	-	**	*	**	-	**	-	-	-	*	

In the Mediterranean Sea area, reader against modal age bias test showed a low occurrence of bias, except for readers 5, 20, 21, 26 and 35 (*), 7, 17, 25, 27 and 29 (**). Inter-reader bias test, showed a strong occurrence of sign of bias, except in particular for readers, 1, 2, 3, 5, 6 and 10, which showed less signs of bias when tested against the other readers. Relatively to PA's between each two readers they ranged from 31% (Reader 17 against 25) and 84% (Reader 9 against 10).

Table 9 - Inter-reader bias test and reader against modal age bias test of sardine otoliths for both areas combined (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

	Reader.1	Reader.2	Reader.3	Reader.5	Reader.6	Reader.7	Reader.8	Reader.9	Reader.10	Reader.11	Reader.12	Reader.13	Reader.15	Reader.16	Reader.17	Reader.19	Reader.20	Reader.21	Reader.22	Reader.23	Reader.24	Reader.25	Reader.26	Reader.27	Reader.28	Reader.29	Reader.30	Reader.32	Reader.34	Reader.35
Reader.1		61	63	69	54	53	62	62	66	49	49	60	59	56	44	54	55	63	49	47	40	54	60	59	53	57	53	52	54	51
Reader.2	-		67	64	55	53	65	61	67	56	54	62	62	56	44	61	57	66	48	42	34	44	64	57	59	56	58	51	59	51
Reader.3	-	-		68	59	54	60	62	68	53	52	62	62	53	46	55	56	70	52	44	40	48	61	55	56	54	57	59	54	49
Reader.5	-	-	*		55	49	64	60	69	56	55	61	60	55	43	60	59	70	46	42	41	48	69	69	56	70	58	53	56	52
Reader.6	-	-	-	*		57	62	71	70	54	55	62	66	55	50	64	54	59	55	51	51	49	55	51	65	61	74	58	62	56
Reader.7	**	**	**	**	**		70	68	67	62	66	72	54	64	63	59	66	50	58	44	37	39	50	49	67	46	62	47	62	65
Reader.8	*	**	-	**	-	*		77	79	69	69	81	60	72	58	73	77	62	58	48	37	43	64	57	74	58	70	51	72	70
Reader.9	-	*	-	**	-	*	-		83	64	66	76	69	68	56	73	67	61	56	54	47	47	62	54	69	64	73	54	73	68
Reader.10	-	-	-	-	-	**	-	-		66	64	81	74	67	55	78	71	70	58	56	47	49	66	60	73	68	75	57	74	69
Reader.11	-	*	-	**	-	**	-	-	-		87	68	53	59	52	62	64	52	51	42	35	36	56	55	61	53	62	44	63	62
Reader.12	*	**	-	**	-	**	-	-	-	-		69	52	60	53	62	65	49	54	41	33	35	55	55	61	51	62	44	61	62
Reader.13	*	**	-	**	-	-	-	-	-	-	-		64	63	57	69	73	63	57	49	39	43	58	56	74	56	68	52	70	70
Reader.15	-	-	*	-	*	**	**	**	*	**	**	**		51	46	68	54	67	47	53	44	52	59	57	59	71	64	52	62	55
Reader.16	**	**	*	**	-	-	-	-	*	*	-	-	**		54	62	64	51	51	41	32	37	53	51	61	50	59	44	63	63
Reader.17	**	**	**	**	**	**	**	**	**	**	**	**	**	**		50	58	46	56	41	37	36	40	41	62	37	53	42	55	58
Reader.19	-	-	-	-	-	**	*	-	-	-	-	*	-	**	**		67	61	49	50	42	47	59	52	69	65	70	48	75	67
Reader.20	**	**	*	**	-	-	-	-	*	*	-	-	**	-	**	**		53	52	43	33	41	54	50	69	54	62	46	67	68
Reader.21	-	-	-	-	-	**	*	-	-	-	**	**	-	**	**	-	**		49	46	45	50	66	64	63	62	57	55	57	51
Reader.22	**	**	**	**	**	-	**	**	**	**	**	*	**	*	-	**	-	**		45	45	41	44	43	53	37	52	51	47	46
Reader.23	*	**	-	**	-	-	-	-	*	*	-	-	**	-	**	*	-	*	-		51	46	46	41	44	51	50	42	47	43
Reader.24	**	**	**	**	*	-	*	*	**	**	**	-	**	-	-	**	-	**	-	-		38	38	39	39	44	45	43	39	34
Reader.25	**	**	**	*	**	**	**	**	**	**	**	**	*	**	**	**	**	**	**	**	**		46	45	42	55	49	37	42	38
Reader.26	-	-	*	-	**	**	**	**	*	**	**	**	-	**	**	-	**	-	**	**	**	-		63	55	66	57	47	55	51
Reader.27	*	*	**	-	**	**	**	**	**	**	**	**	-	**	**	**	**	*	**	**	**	-	-		49	66	53	47	48	45
Reader.28	**	**	*	**	-	-	-	-	*	*	-	-	**	-	**	**	-	**	-	-	-	**	**	**		53	66	49	69	67
Reader.29	**	*	**	-	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**	**	**	-	-	-	**		62	45	61	52
Reader.30	-	-	-	*	-	**	-	-	-	-	-	-	-	*	**	-	*	-	**	*	**	**	*	**	*	**		52	67	62
Reader.32	**	**	*	**	-	-	-	-	*	*	-	-	**	-	**	**	-	**	-	-	-	**	**	**	-	**	*		45	41
Reader.34	-	*	-	**	-	**	-	-	-	-	-	-	**	-	**	-	-	-	**	-	*	**	**	**	-	**	-	-		74
Reader.35	**	**	**	**	-	-	-	-	**	**	-	-	**	-	**	**	-	**	-	-	-	**	**	**	-	**	*	-	-	
modal.age	-	*	-	**	-	**	-	-	-	-	-	-	*	-	**	-	-	-	**	-	**	**	**	**	-	**	-	*	-	*	

In both areas combined, reader against modal age bias test showed a general significant presence of bias, except for readers 1, 3, 6, 8-13, 16, 19-21, 23, 28, 30 and 34 which presented no bias. Inter-reader bias test, showed a high occurrence of sign of bias, except in particular for readers, 1 - 6, which showed less signs of bias when tested against the other readers. Relatively to PA's between each two readers they ranged from 32% (Reader 24 against 16) and 87% (Reader 11 against 12).

Age Bias Plots

Figures 6a to 6c show the age bias plots of all age readers' readings exchange results against modal age for each area and for both areas combined.

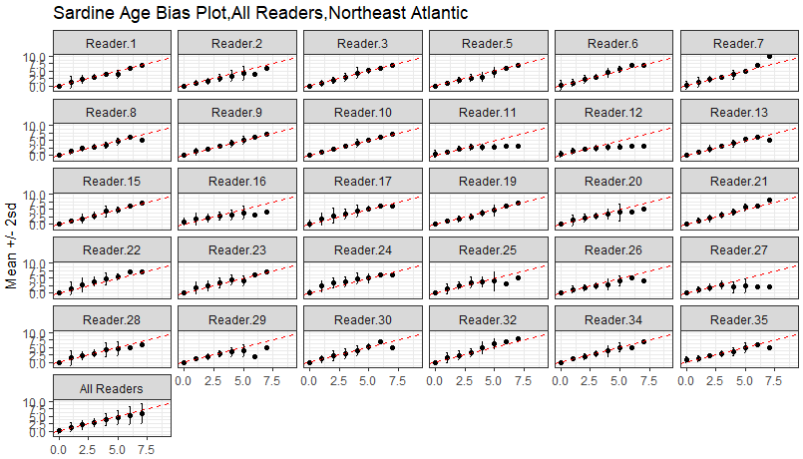

Figure 6a – Age bias plots of individual age readers against modal age for NE Atlantic area.

In general, there was a good reading agreement with modal age by readers in the NE Atlantic area. Although readers 11, 12, 16, 20, 25, 26, 27 and 29 underestimated age group 4 onwards, and readers 19, 21, 28 and 32 overestimated age group 5 onwards.

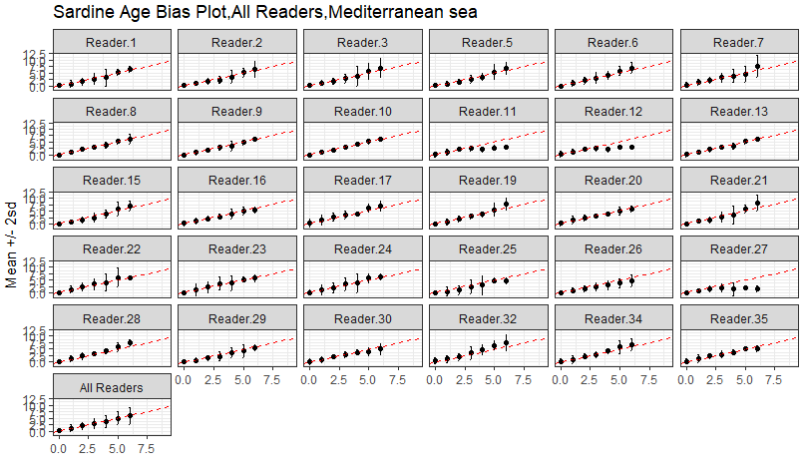

Figure 6b – Age bias plots of individual age readers against modal age for Mediterranean Sea area.

In the Mediterranean Sea, all readers considered have also shown a good reading agreement with modal age. Readers 11, 12 and 27 underestimated age group 4 onwards.

Figure 6c – Age bias plots of individual age readers against modal age and for both areas combined.

In both areas combined, in general readers also showed a good agreement with modal age. Readers 11, 12 13, 16, 16 and 27 underestimated older age groups (age groups 4-5 onwards).

Figures 7a to 7c show the age bias plots of all experienced age readers' readings exchange results against modal age for each area and for both areas combined.

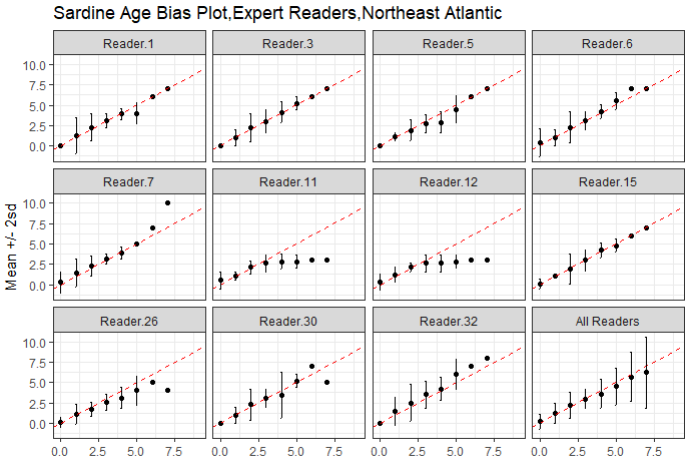

Figure 7a – Age bias plots of individual experienced age readers against modal age, for the NE Atlantic area.

Experienced readers have shown a good agreement with modal age in NE Atlantic area, although in older age groups they have shown an age underestimation trend (age group 6 onwards). Readers 7 and 32 have tended to overestimate older age groups (age group 7 onwards). Readers 11, 12, 26 and 30 had tended to underestimate older age groups (age group 6-7 onwards), although the last one overestimated age group 6.

Figure 7b – Age bias plots of individual experienced age readers against modal age, for the Mediterranean Sea area.

In the Mediterranean Sea area, experienced readers tended to underestimate older fish (age group 7 onwards), although they showed a good agreement with modal age for the remaining age groups. Readers 11, 12, 26 and 30 tended to underestimate older age groups (age group 5 onwards).

Figure 7c – Age bias plots of individual experienced age readers against modal age, for both areas combined.

For both areas combined, in general experienced readers underestimated older ages (age group 6 onwards). Readers 11, 12, 26 and 30 particularly showed this trend.

Figures 8a to 8c show the age bias plots of all intermediate age readers' readings exchange results against modal age for each area and for both areas combined.

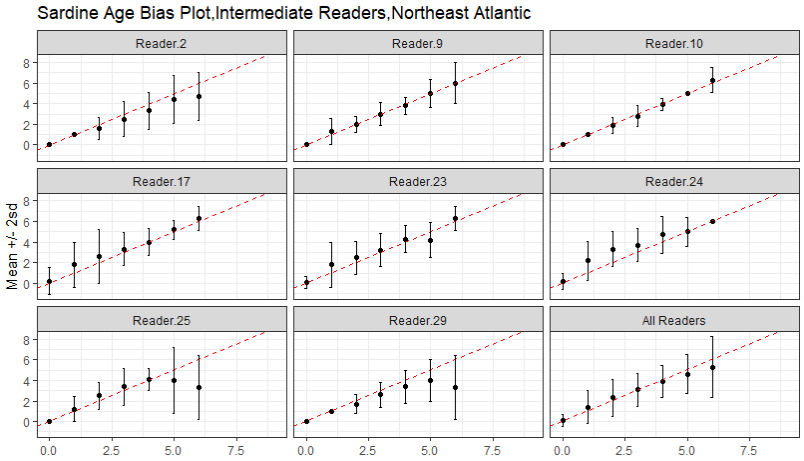

Figure 8a – Age bias plots of individual intermediate age readers against modal age, for the NE Atlantic area.

Intermediate readers in general showed a trend to underestimate older ages (age group 5 onwards) in NE Atlantic, particularly readers 2, 25 and 29. Readers 17, 23 and 24 tended to overestimate the age of younger fish (age groups 0 to 4).

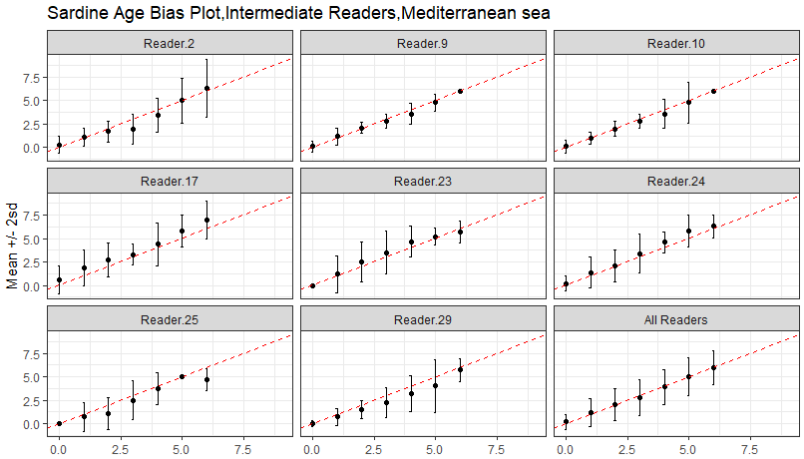

Figure 8b – Age bias plots of individual intermediate age readers against modal age, for the Mediterranean Sea area.

Intermediate readers in general showed a good agreement with modal age in the Mediterranean Sea area. Individually, readers 2, 25 and 29 tended to underestimate age of older fish (age group 3 onwards) and reader 17 tended in general to overestimate age of all fish.

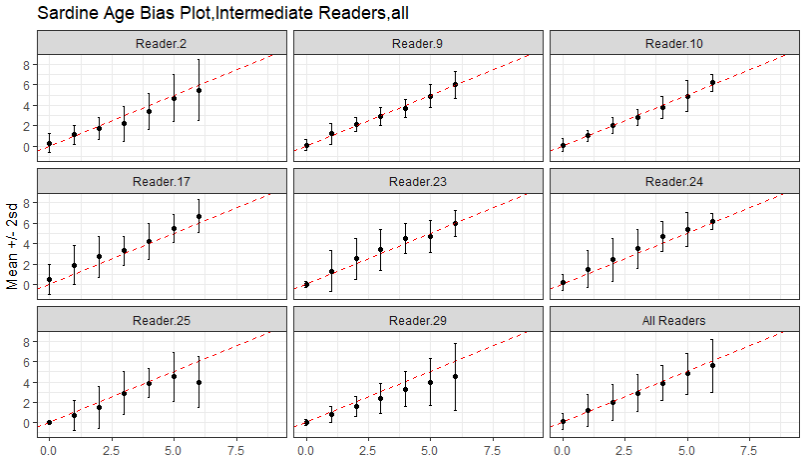

Figure 8c – Age bias plots of individual intermediate age readers against modal age, for both areas combined.

For both areas combined, intermediate readers in general showed a good agreement with modal age. Readers 25 and 29 tended to underestimate age of old fish (age group 4-5 onwards).

Figures 9a to 9c show the age bias plots of all trainee age readers' readings exchange results against modal age for each area and for both areas combined.

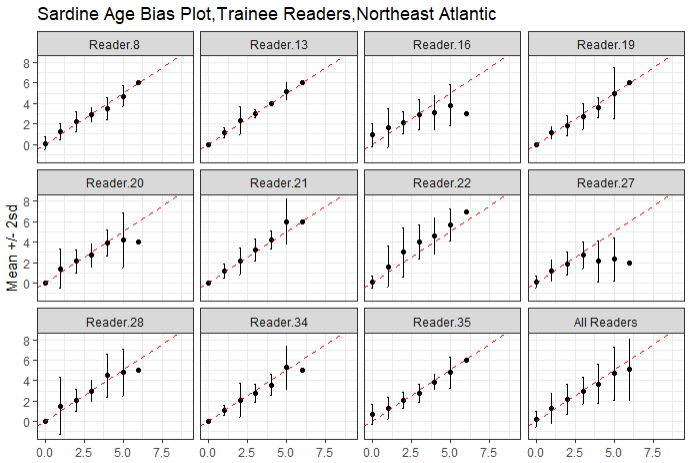

Figure 9a – Age bias plots of individual trainee age readers against modal age, for the NE Atlantic area.

Trainee readers tended to underestimate older fish ages in NE Atlantic. Individually, readers 16, 20, 27, 28 and 34 showed this trend (age group 5 onwards) while reader 22 showed the opposite trend, overestimating the age of most of the fish (age group 1 onwards).

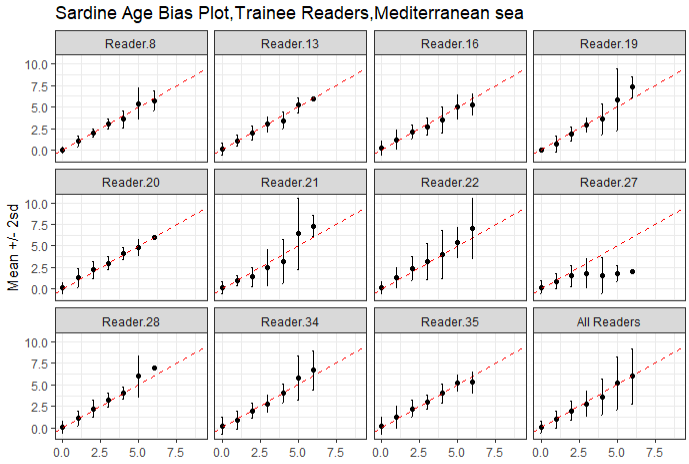

Figure 9b – Age bias plots of individual trainee age readers against modal age, for the Mediterranean Sea area.

In the Mediterranean Sea area trainees showed a general good agreement with the modal age. Individually, readers 19, 21, 22, 28 and 24 tended to overestimate ages of older fish (age group 5 onwards), while reader 27 showed the opposite trend (age group 3 onwards).

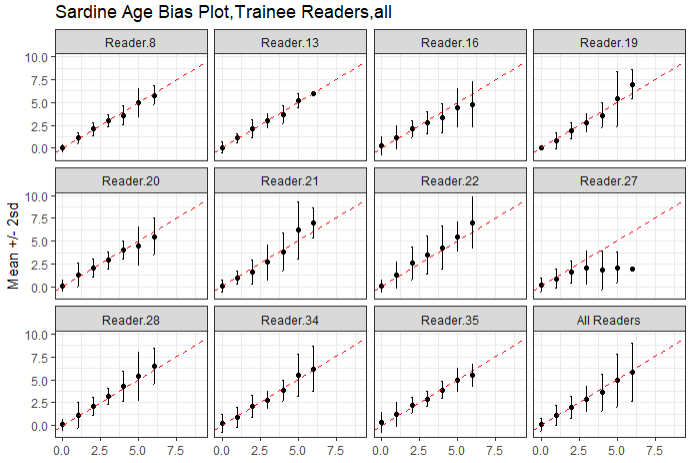

Figure 9c – Age bias plots of individual trainee age readers against modal age, for both areas combined.

In both areas combined, generally readers showed a good agreement with modal age, although individually reader 27 showed an underestimation trend to underestimate the older fish age (age group 3 onwards).

General conclusions and considerations

1. With so many readers from different areas involved in the 2017 Exchange, to compare their age readings and to reach to a conclusion is a rather difficult task, as many different factors which may influence their outcome may be at stake;
2. The main thing that comes to light from this exercise is that there was a general low age reading agreement with modal age and among readers in both areas (PA ranged from 60% to 80%), even if only readers of each area were considered for their respective area data analyses;
3. One part of the causes of this low agreement level may be the use of only otoliths' images in the Exchange, as the only observation basis used for the analyses and also their low quality in innumerous cases. On the other hand use of images is limitative, as they do not allow a manipulation as that can be done with the real otolith, not giving the 3D perception of its structure and with which the observer has the possibility to play with

the light source intensity and position and also with the stereoscopic microscope focus and amplification allowing to reach a reliable discrimination of the growth areas (*annuli*);

4. Different age reading criteria individually applied by readers, which could not be adequate to the interpretation of otoliths growth pattern in the areas involved, might also contribute to such a low age readings agreement;
5. Difficulties in the otolith margin type and the *annuli* identification were impossible to verify due to the unavailability of access to the Exchange 2017 images in the WebGR with the identification marks made by each reader;
6. In order to contribute for the reliability and precision improvement of age readings, reference otoliths collection of known age (with ≥80% of age readers' agreement), should be used in each area.
7. Clear and updated otoliths' preparation and age reading protocols should also be used in each area as common references for the readers;
8. Also, regular otoliths age readings exchanges and workshops in each area are advisable to be undertaken in order to assess the agreement level among readers, to identify possible issues with the readings and their causes and find ways to solve them in order to improve their reliability and precision. For this purpose otoliths and their scanned images should be used.

References

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ICES, 2011. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14-18 February 2011, Lisbon, Portugal. ICES CM 2011/ACOM: 42, REF. PGCCDBS. 87 p.

R Core Team. 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. Internet: https://www.R-project.org/.

European Sardine (*Sardina pilchardus*, Walbaum 1792) Otolith Exchange 2017

Annex

Guidelines for sardine otoliths picture samples preparation, observation and age determination criteria (adapted from ICES, 2011)

1. Samples of 40-60 otolith (sagittae) pairs collected in each area will be selected for taking digitized pictures to be used in the exchange according to Table I. These otoliths aim to represent the range of fish lengths/ages sampled in each area by semester;
2. Measurements are done according to Figure 1. The diameter or radius of the first hyaline ring is the distance up to the end of the previous opaque zone;
3. Otoliths are mounted with the sulcus acusticus down on black plastic plaques glued with a transparent resin in individual numbered cavities;
4. Otolith calibrated digitized pictures will be taken with an image processing system using a PC with a dedicated software and a high definition video camera connected to a binocular microscope with 20X magnification under reflected light. Opaque zones are visible as white and hyaline (translucent) ones as dark. Magnification value and the fishing date must be recorded in the picture. A reference calibrated length scale (1 mm) will also be included in each picture (Fig. 2);
5. Ageing of sardines is based on the counting of hyaline zones in the otolith. One year's growth ring (annulus) consists of one opaque zone and one adjacent hyaline zone;
6. Growth ring counts and edge type classification should preferably be done on the posterior (post-rostrum) region of the otolith where annual rings are generally clearer and otolith growth is larger;
7. In order to adopt a ring as an annulus it is recommended that the ring can be followed throughout the whole otolith contour. This rule must be applied specially for the first two annuli, as in older specimens rings are very close together becoming more difficult to follow all over its contour (usually from age 2 they are more distinguishable on the post-rostrum);
8. The first of January is adopted as the birthdate;
9. For a fish caught in the first semester of the year (prior to the growing period, i.e. opaque zone formation), the age corresponds to the number of hyaline zones present in the otolith (including the edge). For a fish caught in the second semester (after the opaque zone formation) the age corresponds to the number of hyaline zones completely formed (i.e. a hyaline edge is not counted);
10. A reference diameter of ≈2 mm (radius ≈1 mm) should be used as a guide to the identification of the first annual ring. This reference should be used in a flexible way, since the diameter of the first annual ring is proportional to the fish growth up to its formation;

11. From each otolith picture observation, the number of hyaline rings (true annual growth rings, excluding the edge), edge type, age and readability (1 - good, 2 - medium, 3 - difficult) should be recorded. False rings and other relevant characteristics of the otolith (e.g. crystallized otoliths) should be annotated as well. All these data will be recorded in a separate excel sheet which template will be provided to the participants. Growth rings will be identified by each reader by marks in the photos in the WebGR.

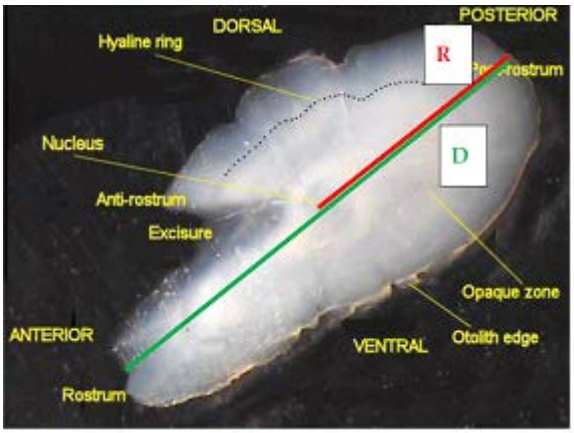

Figure 1 – Main structural areas of a sardine otolith. The lines show axis of measurement of otolith/ring diameter (D) and radius (R).

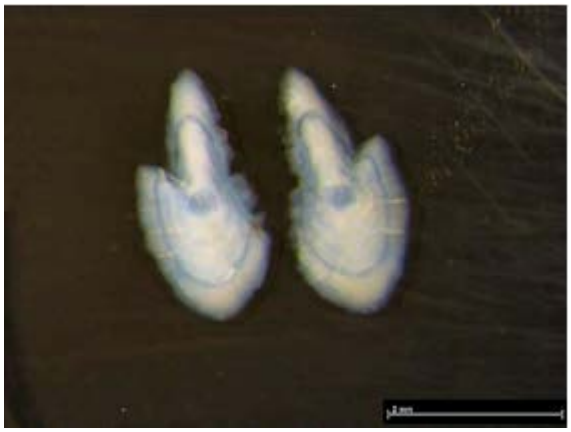

Figure 2 – Calibrated digitized picture of sardine otolith pair (with the *sulcus acusticus* face down) taken with an image processing system using a PC with a dedicated software and a high definition video camera connected to a binocular microscope with 20X magnification and under reflected light.

References

ICES, 2011. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14-18 February 2011, Lisbon, Portugal. ICES CM 2011/ACOM: 42, REF. PGCCDBS. 87 p.

Annex 2: Report of WKARAS 2 small age reading calibration exercise

Introduction

During WKARAS 2 meeting a small age reading calibration exercise was undertaken in order to make a new preliminary assessment of otoliths age reading agreement between readers in the aftermath of joint sessions of discussion on otoliths structure interpretation based on projected digitized images held during the Workshop. For the results analyses, readers expertise designation was similarly based on the criteria used in the 2017 Exchange, but "Experts" were this time designated as "Advanced" while "Trainees" and "Intermediates" were put together in one group called "Basic", following the nomenclature used in SmartDots. For each expertise group data analysis it was only used the specific modal age for that group, i. e., for advanced readers readings analysis it was only considered modal age of these readers, for basic readers it was only considered their modal age, for all readers readings analysis was considered the mixed modal age.

Age Quality indicating the quality of the age reading, based on the difficulty level presented by the structures to the reader (AQ1: Easy to age with high precision; AQ2: Difficult to age with acceptable precision; AQ3: Unreadable or very difficult to age with acceptable precision.) was also annotated by readers. In the following analyses AQ3 was not included as it happened in the 2017 Exchange.

For the age readings comparative analyses, R scripts based on Eltink's MS Excel spreadsheet (Eltink, 2000) following the recommendations of the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000) were used as in the 2017 Exchange.

Material and methods

A total of 139 selected images of otoliths pairs from both areas previously used in 2017 Exchange (79 from N. E. Atlantic and 60 from the Mediterranean Sea) were individually analyzed by the age readers through SmartDots, during this small age reading calibration exercise (Tables 1 and 2).

Table 1 – Samples identification

Division	Semester	Min.length	Max.length	N.samples	Area
8a	2	130	225	20	Atlantic
8b	1	174	216	10	Atlantic
8b	2	171	236	10	Atlantic
8c	1	170	237	10	Atlantic
8c	2	206	232	10	Atlantic
9a	1	175	221	20	Atlantic
9a	2	109	230	20	Atlantic
GSA01	1	111	227	20	Mediterranean
GSA01	2	115	236	20	Mediterranean
GSA03	1	129	199	20	Mediterranean
GSA03	2	109	190	20	Mediterranean
GSA06	1	125	220	22	Mediterranean
GSA06	2	105	185	18	Mediterranean

GSA07	1	115	155	20	Mediterranean
GSA07	2	85	160	20	Mediterranean
GSA09	1	120	155	20	Mediterranean
GSA09	2	110	170	20	Mediterranean
GSA16	1	104	176	20	Mediterranean
GSA16	2	116	177	20	Mediterranean
GSA22	1	93	141	20	Mediterranean
GSA22	2	101	154	20	Mediterranean

Readers followed the same common age reading protocol used during the 2017 Exchange for their age assignments to the observed otoliths and also had in mind the discussions previously held during the Workshop's joint sessions on otoliths structure interpretation.

Similarly to what happened in the 2017 Exchange, the age reading data of this exercise were subjected to statistical comparative analyses using R scripts based on Eltink's MS Excel spreadsheet (Eltink, 2000) following the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000).

The SmartDots analysis only reported the all age readers and all areas combined results. To compare with the previous exchange and make the analysis of a) advanced readers by areas and b) trainee readers by areas, the results were analysed using R scripts based on Eltink MS Excel spreadsheet (Eltink, 2000). The recommendations of the "Guidelines and tools for age reading comparisons" (Eltink et al., 2000) were followed. Like the previous exchange, the high number of the exchange otoliths images and age readers makes difficult to use the Eltink MS Excel spreadsheet (Eltink, 2000) R Scripts were developed and used for age readings comparative analyses and to assess the age reading agreement level amongst readers, in a process somehow similar to what is currently used with SmartDots.

Modal ages (most frequent age among readers) for each otolith was calculated and cases with more than one mode, the average age was used instead.

Percentage of agreement (PA), Coefficient of Vatiation (CV) Average Percentage Error (APE) and Relative Bias (RB) were obtain for each otolith read between the different readers.

Percentage Agreement

$$Percentage\,of Agreement\,(PA) = 100 \times \left(\frac{no.of\ readers\,agreeing\,with\,modal\,age}{total\ no.of\ readers} \right)$$

Coefficient of Variation (CV)

The cv's are calculated per reader and modal age.

$$Coefficient\,of\ Variation\,(CV) = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - x_j)2}{R\text{-}1}}}{x_j}$$

where R is the number of times each fish is aged, X_{ij} is the i^{th} age determination of the j^{th} fish, X_j is the mean age calculated for the jth fish.

Average Percentage Error (APE)

APE was calculated based on the method outlined by Beamish & Fournier (1981). This method is not independent of fish age and thus provides a better estimate of precision. The average percentage error is calculated per image as:

$$Average\,Percentage\,Error\,(APE_j) = 100 \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|X_{ij} + X_j\right|}{X_j}$$

where xij is the ith age determination of the jth fish, xj is the average age calculated for the jth fish and R is the number of times each fish was aged.

Relative Bias (RB)

To test the age readings consistency, the absence of bias between two readers or between a reader and the modal age was analysed with the one-sample Wilcoxon signed rank test (Zar, 1984).

The average values of the above indices were calculated by areas. Moreover the combined weighted mean and a rank value were added per reader, where the reader with the lowest weighted mean was assigned with a rank and so forth (in the situation of ties between two weighted means will every tied element be assigned to the lowest rank. This is the procedure for all ties methods when assigning ranks).

Fish Growth Analysis

Biological information was provided with the otolith images. The fish total length was used along with age to provide a measure of fish growth. This data was used to establish growth curves for each reader by area.

Participants

The nineteen WKARAS 2 attendants and one age reader from IEO online have participated in this small age reading calibration exercise. The following table shows the participants list, their age reading expertise level, reader code designations and their rank in this exercise as well.

Table 2 – Participant readers list

Reader Name	Expertise Level	2017 exchange participant
Cristina Bultó (R0)	1 (Advanced)	Yes
Geoffrey Bled-Defruit (R10)	1 (Advanced)	Yes
Erwan Duhamel (R12)	1 (Advanced)	Yes
Isabel. Loureiro (R16)	1 (Advanced)	Yes
Raquel Milhazes (R18)	1 (Advanced)	Yes
Delfina Morais (R20)	1 (Advanced)	Yes
Iñaki Rico (R28)	1 (Advanced)	Yes
Eduardo Soares (R34)	1 (Advanced)	Yes
Pedro Torres (R36)	1 (Advanced)	Yes
Ana Ventero (online) (R38)	1 (Advanced)	Yes
Sana El Arraf (R40)	0 (Basic)	No

María Sanchez Barba (R42)	0 (Basic)	Yes
Célina Chantre (R44)	0 (Basic)	Yes
Ioannis Fytilakos (R48)	0 (Basic)	Yes
Denis Gašparević (R50)	1 (Advanced)	Yes
Hammou El Habouz (R52)	1 (Advanced)	No
Moulay Hachem Idrissi (R58)	0 (Basic)	No
Konstantina Ofrydopoulou (R62)	0 (Basic)	No
Denis Kukul (R64)	0 (Basic)	No
Andreia Silva (R74)	0 (Basic)	Yes

Age Readings Analyses Results

For the analyses the Age Quality AQ3 otoliths age readings were not considered. Samples' fish total length distribution (Figure 1) shows two modes in the Mediterranean Sea area (10-16 cm and 16-22 cm) and three in the N. E. Atlantic (10-11 cm, 13-15 cm and 17-23 cm). In the N. E. Atlantic average fish length was higher than in the Mediterranean. The N. E. Atlantic shows bigger fish length, mainly ranging from 17 cm to 23 cm comparatively with Mediterranean areas that mainly shows fish length between 8 cm to 17 cm.

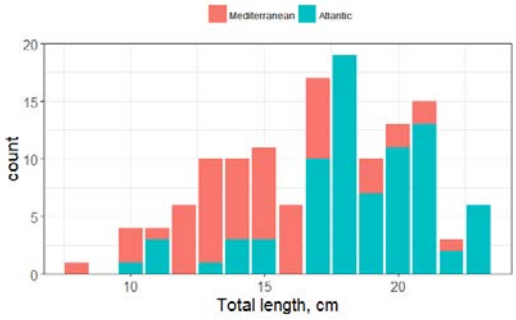

Figure 1 – Samples' fish total length distribution by areas.

Mean fish total lengths at age individually obtained by readers showed similar pattern with an increasing trend as age advances (Age groups 0 to 7), ranging between around 11.5-14.0 cm and 19.0-22.0 cm (Figure 2).

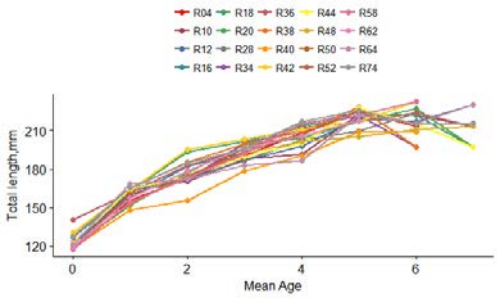

Figure 2 – Fish mean total length at age.

All readers age readings analyses

When all readers are globally considered (mixed readers from both areas), average age reading percentage agreement between readers and the modal age is relatively low in both areas

with high coefficients of variation (CV) and average percent errors (APE) (Table 3 and Figure 3).

Table 3 – Percentage of Agreement (PA), Coefficient of Variation (CV) and Average Percent Error (APE) for all readers by each and the total area.

Area	PA	CV	APE
Mediterranean Sea	65.6	72.4	43.8
N. E. Atlantic	73.4	50.5	26.6
Total	**69.7**	**59.8**	**33.9**

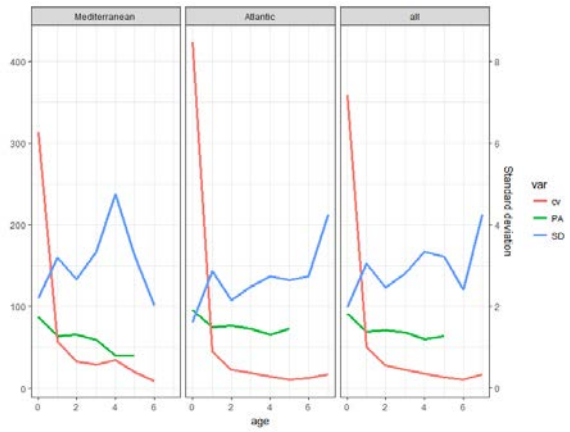

Figure 3 – Coefficient of Variation (CV), Percentage of Agreement (PA) and Standard Deviation (SD) plotted against Modal Age in each area and both areas combined.

Figure 4 shows PA, CV and APE by areas, expertise level and all readers mixed. Average PA was higher in all areas between advanced readers, corresponding to lower CV and APE.

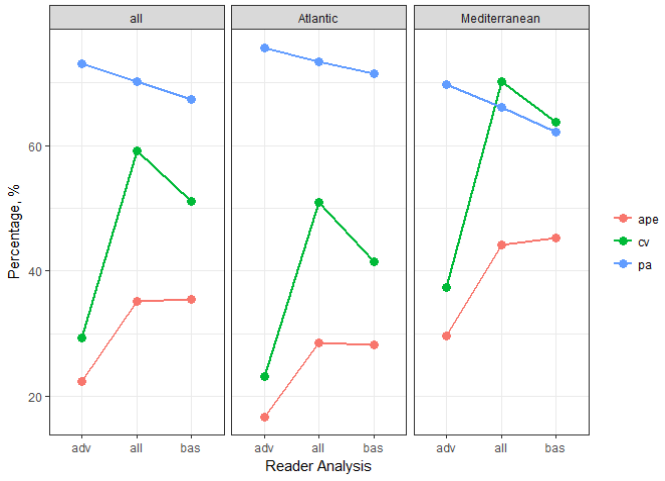

Figure 4 – PA, CV and APE by areas, expertise level and all readers mixed.

Tables 4 to 6 show the age readings frequencies by reader, age group and area. In general, most of the readings were mainly concentrated in age groups 0 to 3 in both areas.

Table 4 – Mediterranean Sea: Age readings frequency of each reader by age group and total.

Reader	Age Group							Total
	0	1	2	3	4	5	6	

R04	9	13	19	15	2	2	-	60
R10	8	11	19	16	4	-	1	59
R12	8	10	19	16	3	2	1	59
R16	9	23	16	6	2	1	-	57
R18	16	20	5	3	2	1	1	48
R20	8	28	14	2	-	1	1	54
R28	9	11	18	15	5	1	1	60
R34	11	10	28	8	1	2	-	60
R36	28	11	9	8	1	2	1	60
R38	11	17	15	3	1	-	-	47
R40	5	5	17	18	9	4	2	60
R42	18	31	6	3	1	1	-	60
R44	9	11	22	13	2	-	1	58
R48	10	19	13	12	3	1	1	59
R50	10	7	25	9	1	1	-	53
R52	9	16	19	13	2	1	-	60
R58	9	13	18	13	1	1	-	55
R62	11	21	19	7	1	1	-	60
R64	9	3	20	17	9	1	1	60
R74	11	19	14	6	-	-	1	51

Table 5 – N. E. Atlantic: Age readings frequency of each reader by age group and total.

Reader	Age Group								Total
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
R04	8	11	19	26	10	3	2	-	79
R10	7	11	20	22	5	3	3	2	73
R12	7	16	19	20	9	3	3	1	78
R16	8	12	25	18	4	4	-	1	72
R18	8	17	18	22	4	4	2	1	76
R20	7	9	15	21	8	3	3	1	67
R28	7	16	19	20	9	4	2	2	79
R34	7	13	26	20	6	6	1	-	79
R36	12	14	15	21	9	4	2	2	79
R38	8	14	24	2	4	2	1	-	55
R40	3	4	17	22	17	11	4	-	78
R42	11	17	20	19	5	5	2	-	79
R44	8	10	25	22	4	5	-	1	75
R48	2	9	17	22	9	7	4	2	72
R50	7	11	21	19	4	1	-	-	63
R52	7	11	19	26	10	5	-	-	78
R58	7	11	15	27	9	3	2	-	74
R62	7	9	24	24	8	7	-	-	79
R64	8	9	17	29	9	4	2	1	79
R74	8	21	19	16	6	2	-	4	76

Table 6 – Total area: Age readings frequency of each reader by age group and total.

Reader	Age Group								Total
	0	1	2	3	4	5	6	7	
R04	17	24	38	41	12	5	2	-	139
R10	15	22	39	38	9	3	4	2	132
R12	15	26	38	36	12	5	4	1	137
R16	17	35	41	24	6	5	-	1	129
R18	24	37	23	25	6	5	3	1	124
R20	15	37	29	23	8	4	4	1	121
R28	16	27	37	35	14	5	3	2	139
R34	18	23	54	28	7	8	1	-	139
R36	40	25	24	29	10	6	3	2	139
R38	19	31	39	5	5	2	1	-	102
R40	8	9	34	40	26	15	6	-	138
R42	29	48	26	22	6	6	2	-	139
R44	17	21	47	35	6	5	1	1	133
R48	12	28	30	34	12	8	5	2	131
R50	17	18	46	28	5	2	-	-	116
R52	16	27	38	39	12	6	-	-	138
R58	16	24	33	40	10	4	2	-	129
R62	18	30	43	31	9	8	-	-	139
R64	17	12	37	46	18	5	3	1	139
R74	19	40	33	22	6	2	1	4	127

Tables 7 to 9 show, for each and both areas together, the coefficients of variation (CV) of age readings (%) of each and all readers by age group, the CV weighted mean by each and for all readers and the readers' ranking according to their readings agreement with modal age. The odd CV high values exceeding 100%, especially in age group 0, are due to the known issue of the CV calculation formula related to the occurrence of age 0 in modal age already referred in Annex 3 of this report.

Table 7 – Mediterranean Sea: Coefficient of variation (CV) of each reader by age group. Weighted mean (%) by each and for all readers and their ranking.

Reader	Age Group								Weighted mean (%)	Ranking
	0	1	2	3	4	5	6	7		
R04	316.2	50.6	20.2	14.2	20.2	-	-	-	75.8	18
R10	210.8	43.6	19.3	12.3	20.2	-	-	-	55.9	7
R12	210.8	46.0	19.3	19.5	20.2	-	-	-	58.0	8
R16	210.8	36.5	29.9	19.9	84.9	-	-	-	62.6	10
R18	316.2	80.0	48.7	38.7	-	-	-	-	110.6	20
R20	210.8	23.5	35.6	33.3	-	-	-	-	62.7	11
R28	316.2	43.8	20.2	13.9	15.7	-	-	-	73.7	15
R34	-	45.0	21.4	20.9	20.2	-	-	-	23.3	3
R36	-	77.0	88.2	89.6	141.4	-	-	-	69.6	14
R38	-	53.5	35.5	19.2	-	-	-	-	28.7	4
R40	151.2	47.9	26.7	23.2	15.7	-	-	-	51.1	6

R42	-	80.0	51.1	50.1	70.7	-	-	-	49.0	5
R44	316.2	48.5	12.1	16.7	-	-	-	-	74.3	16
R48	210.8	69.8	42.9	33.1	60.6	-	-	-	75.3	17
R50	316.2	50.5	12.5	15.9	28.3	-	-	-	79.8	19
R52	316.2	30.4	17.1	9.9	20.2	-	-	-	68.6	13
R58	-	31.8	17.1	0.0	-	-	-	-	13.7	1
R62	-	26.7	29.1	20.9	47.1	-	-	-	21.6	2
R64	241.5	48.2	21.0	15.1	0.0	-	-	-	62.4	9
R74	210.8	40.6	39.5	21.4	-	-	-	-	68.2	12
All Readers	312.5	57.0	32.8	28.6	34.2	19.4	9	-	239.7	-

Table 8 – N. E. Atlantic: Coefficient of variation (CV) of each reader by age group. Weighted mean (%) by each and for all readers and their ranking.

Reader	Age Group								Weighted mean (%)	Ranking
	0	1	2	3	4	5	6	7		
R04	-	45.6	19.3	11.8	9.1	12.4	-	0.0	18.3	7
R10	-	36.1	16.1	10.4	20.9	10.8	-	0.0	16.7	5
R12	-	0.0	20.2	17.3	8.6	10.8	-	10.9	11.6	2
R16	-	46.8	17.6	19.5	15.6	0.0	-	-	20.7	10
R18	-	40.8	29.1	14.9	21.5	10.8	-	38.6	22.3	12
R20	-	36.7	25.5	15.9	11.4	10.2	-	-	18.6	8
R28	-	0.0	20.2	17.0	10.9	10.8	-	0.0	11.4	1
R34	264.6	80.9	20.2	29.8	18.9	12.4	-	47.1	56.8	20
R36	-	49.0	34.3	29.6	10.9	10.8	-	0.0	28.0	17
R38	-	63.1	35.5	11.8	28.6	26.6	-	-	27.0	16
R40	97.5	51.3	26.4	23.3	16.8	12.4	-	0.0	33.9	18
R42	-	51.8	33.4	21.2	16.5	0.0	-	12.9	26.2	15
R44	-	46.7	11.8	15.2	15.0	0.0	-	23.6	18.7	9
R48	173.2	79.3	24.7	16.7	11.2	10.2	-	0.0	34.9	19
R50	-	32.5	0.0	15.0	13.3	NA	-	-	12.6	3
R52	-	46.3	17.7	11.8	9.1	0.0	-	15.7	18.0	6
R58	-	26.6	20.2	11.8	9.1	0.0	-	-	14.0	4
R62	-	51.8	28.5	20.5	15.3	0.0	-	0.0	24.4	14
R64	-	54.3	20.8	13.2	13.4	0.0	-	10.9	21.0	11
R74	-	27.7	24.8	26.4	30.2	28.6	-	0.0	23.3	13
All Readers	423.5	44.5	22.9	18.7	14.0	10.9	12.4	17.1	152.5	-

Table 9 – Total Area: Coefficient of variation (CV) of each reader by age group. Weighted mean (%) by each and for all readers and their ranking.

Reader	Age Group								Weighted mean (%)	Ranking
	0	1	2	3	4	5	6	7		
R04	412.3	49.8	19.8	12.4	11.1	10.5	0.0	0.0	71.5	16
R10	282.3	40.7	17.8	11.8	21.4	16.3	0.0	0.0	55.3	8
R12	282.3	45.8	19.8	19.1	11.8	9.5	0.0	10.9	56.6	9
R16	282.3	42.2	24.4	19.5	30.2	10.5	0.0	-	61.0	10
R18	412.3	61.9	38.4	20.5	20.1	9.5	0.0	38.6	87.9	20

R20	282.3	31.5	36.2	26.8	11.4	10.5	0.0	-	64.1	13
R28	412.3	40.6	20.6	16.2	11.2	16.3	0.0	0.0	70.8	15
R34	412.3	65.0	20.7	27.4	18.2	10.5	0.0	47.1	80.2	19
R36	-	62.3	60.4	49.6	37.2	9.5	0.0	0.0	45.7	6
R38	-	56.4	34.8	15.2	24.8	28.9	-	-	28.0	2
R40	124.8	49.7	26.3	23.5	15.7	10.5	0.0	0.0	40.9	5
R42	-	65.0	46.4	35.6	30.9	10.5	12.9	12.9	38.9	4
R44	412.3	48.9	12.1	16.3	15.3	22.2	12.9	23.6	73.3	17
R48	190.0	78.2	34.3	23.0	22.0	28.3	0.0	0.0	53.9	7
R50	412.3	46.7	8.8	15.1	24.5	20.2	0.0	-	78.9	18
R52	412.3	40.6	17.7	11.3	11.1	10.5	0.0	15.7	68.8	14
R58	-	29.2	19.8	9.8	11.7	10.5	12.9	-	15.4	1
R62	-	54.2	31.5	22.3	20.6	22.2	0.0	0.0	28.3	3
R64	319.8	55.4	20.6	14.6	11.8	0.0	0.0	10.9	62.0	11
R74	282.3	33.9	31.7	24.8	30.9	36.0	28.3	0.0	63.9	12
All Readers	358.7	50.9	27.8	22.0	18.0	13.0	10.7	17.1	189.6	-

Tables 10 to 13 show 2X Standard Deviation (SD) by each one and all readers by age group and area.

Table 10 – Mediterranean Sea: 2X Standard Deviation for each reader by age group.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	0.630	1.450	0.83	0.850	1.410	-	-	-
R10	0.840	1.280	0.86	0.780	1.410	-	-	-
R12	0.840	1.470	0.86	1.300	1.410	-	-	-
R16	0.840	0.730	0.99	1.030	4.240	-	-	-
R18	0.630	1.000	1.20	2.070	-	-	-	-
R20	0.840	0.500	1.02	1.330	-	-	-	-
R28	0.630	1.260	0.92	0.900	1.410	-	-	-
R34	0.000	1.240	0.83	1.040	1.410	-	-	-
R36	0.000	1.150	2.25	3.280	7.070	-	-	-
R38	0.000	0.990	1.04	0.840	-	-	-	-
R40	3.330	2.520	1.40	1.590	1.410	-	-	-
R42	0.000	1.000	1.08	1.590	2.830	-	-	-
R44	0.630	1.490	0.47	1.030	-	-	-	-
R48	0.840	1.770	1.57	1.870	4.240	-	-	-
R50	0.630	1.550	0.49	0.880	1.410	-	-	-
R52	0.630	0.680	0.69	0.580	1.410	-	-	-
R58	0.000	0.730	0.69	0.000	-	-	-	-
R62	0.000	0.500	0.97	1.040	2.830	-	-	-
R64	1.930	2.050	1.00	1.030	0.000	-	-	-
R74	0.840	0.700	1.26	1.070	-	-	-	-
All Readers	1.099	1.597	1.33	1.676	2.381	1.625	1.014	-

Table 11 – N. E. Atlantic: 2X Standard Deviation for each reader by age group.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	0.0000	1.030	0.86	0.720	0.710	1.15	-	0.000

R10	0.0000	0.920	0.68	0.600	1.770	1.15	-	0.000
R12	0.0000	0.000	0.83	1.010	0.710	1.15	-	1.410
R16	0.0000	1.070	0.66	1.080	1.070	0.00	-	-
R18	0.0000	0.820	0.97	0.830	1.670	1.15	-	4.240
R20	0.0000	0.930	1.12	0.970	0.980	1.15	-	-
R28	0.0000	0.000	0.83	1.020	0.930	1.15	-	0.000
R34	0.7600	2.370	0.83	1.610	1.410	1.15	-	4.240
R36	0.0000	0.920	1.37	1.700	0.930	1.15	-	0.000
R38	0.0000	1.140	1.04	0.490	2.000	2.31	-	-
R40	2.5100	2.200	1.46	1.770	1.510	1.15	-	0.000
R42	0.0000	0.830	1.15	1.130	1.280	0.00	-	1.410
R44	0.0000	1.120	0.49	0.840	1.070	0.00	-	2.830
R48	1.1500	2.930	1.10	1.100	1.040	1.15	-	0.000
R50	0.0000	0.750	0.00	0.870	1.000	NA	-	-
R52	0.0000	1.230	0.77	0.720	0.710	0.00	-	1.410
R58	0.0000	0.580	0.92	0.720	0.710	0.00	-	-
R62	0.0000	1.660	1.24	1.250	1.070	0.00	-	0.000
R64	0.0000	1.450	0.97	0.820	1.070	0.00	-	1.410
R74	0.0000	0.520	0.87	1.340	2.560	3.06	-	0.000
All Readers	0.8123	1.434	1.08	1.239	1.365	1.32	1.373	2.133

Table 12 – All areas: 2X Standard Deviation for each reader by age group.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	0.4900	1.29	0.850	0.750	0.840	1.000	0.000	0.000
R10	0.6600	1.11	0.770	0.710	1.750	1.630	0.000	0.000
R12	0.6600	1.19	0.850	1.170	0.940	1.000	0.000	1.410
R16	0.6600	0.90	0.860	1.060	1.940	1.000	0.000	-
R18	0.4900	0.98	1.140	1.140	1.560	1.000	0.000	4.240
R20	0.6600	0.72	1.310	1.460	0.980	1.150	0.000	-
R28	0.4900	0.99	0.890	1.000	0.970	1.630	0.000	0.000
R34	0.4900	1.85	0.830	1.450	1.350	1.000	0.000	4.240
R36	0.0000	1.05	1.980	2.510	2.900	1.000	0.000	0.000
R38	0.0000	1.03	1.020	0.640	1.790	2.310	-	-
R40	2.9400	2.38	1.420	1.720	1.410	1.000	0.000	0.000
R42	0.0000	0.92	1.290	1.640	2.160	1.000	1.410	1.410
R44	0.4900	1.34	0.490	0.940	1.070	2.000	1.410	2.830
R48	0.8800	2.40	1.390	1.450	1.930	2.830	0.000	0.000
R50	0.4900	1.26	0.350	0.870	1.630	1.410	0.000	-
R52	0.4900	0.99	0.740	0.680	0.840	1.000	0.000	1.410
R58	0.0000	0.65	0.850	0.590	0.880	1.000	1.410	-
R62	0.0000	1.36	1.210	1.280	1.400	2.000	0.000	0.000
R64	1.5000	1.93	0.970	0.930	0.940	0.000	0.000	1.410
R74	0.6600	0.61	1.070	1.260	2.540	3.420	2.830	0.000
All Readers	0.9951	1.52	1.228	1.405	1.669	1.603	1.203	2.133

Tables 13 to 15 show mean ages obtained by each and all readers by age group in each and both areas combined. Reader R40 show an age overestimation trend in younger ages in both areas.

Table 13 – Mediterranean Sea: Mean age by age group for each and all readers.

Reader	Age Group							
	0	1	2	3	4	5	6	7
R04	0.1	1.44	2.06	3.00	3.5	5.0	5.0	-
R10	0.2	1.47	2.22	3.17	3.5	4.0	6.0	-
R12	0.2	1.60	2.22	3.33	3.5	5.0	6.0	-
R16	0.2	1.00	1.65	2.60	2.5	4.0	5.0	-
R18	0.1	0.62	1.23	2.67	4.0	5.0	6.0	-
R20	0.2	1.06	1.44	2.00	-	5.0	6.0	-
R28	0.1	1.44	2.28	3.25	4.5	4.0	6.0	-
R34	0.0	1.38	1.94	2.50	3.5	5.0	5.0	-
R36	0.0	0.75	1.28	1.83	2.5	5.0	6.0	-
R38	0.0	0.92	1.46	2.20	4.0	3.0	-	-
R40	1.1	2.62	2.61	3.42	4.5	5.0	6.0	-
R42	0.0	0.62	1.06	1.58	2.0	4.0	5.0	-
R44	0.1	1.53	1.94	3.08	3.0	3.0	6.0	-
R48	0.2	1.27	1.83	2.83	3.5	3.0	6.0	-
R50	0.1	1.54	1.94	2.78	2.5	4.0	5.0	-
R52	0.1	1.12	2.00	2.92	3.5	4.0	5.0	-
R58	0.0	1.14	2.00	3.00	3.0	4.0	5.0	-
R62	0.0	0.94	1.67	2.50	3.0	3.0	5.0	-
R64	0.4	2.12	2.39	3.42	4.0	5.0	6.0	-
R74	0.2	0.87	1.60	2.50	3.0	3.0	6.0	-
All Readers	0.2	1.30	1.90	2.70	3.3	4.2	5.6	-

Table 14 – N. E. Atlantic: Mean age by age group for each and all readers.

Reader	Age Group							
	0	1	2	3	4	5	6	7
R04	0.00	1.13	2.22	3.04	3.88	4.67	5.0	6.0
R10	0.00	1.27	2.12	2.90	4.25	5.33	6.0	7.0
R12	0.00	1.00	2.06	2.92	4.12	5.33	6.0	6.5
R16	0.00	1.14	1.88	2.76	3.43	5.00	5.0	7.0
R18	0.00	1.00	1.67	2.79	3.88	5.33	6.0	5.5
R20	0.00	1.27	2.20	3.05	4.29	5.67	6.0	7.0
R28	0.00	1.00	2.06	3.00	4.25	5.33	6.0	7.0
R34	0.14	1.47	2.06	2.71	3.75	4.67	5.0	4.5
R36	0.00	0.93	2.00	2.88	4.25	5.33	6.0	7.0
R38	0.00	0.90	1.46	2.06	3.50	4.33	4.0	6.0
R40	1.29	2.14	2.78	3.79	4.50	4.67	6.0	6.0
R42	0.00	0.80	1.72	2.67	3.88	5.00	6.0	5.5
R44	0.00	1.20	2.06	2.78	3.57	5.00	5.0	6.0
R48	0.33	1.85	2.22	3.29	4.62	5.67	6.0	7.0
R50	0.00	1.15	2.00	2.90	3.75	3.00	5.0	-
R52	0.00	1.33	2.17	3.04	3.88	5.00	5.0	4.5
R58	0.00	1.08	2.28	3.04	3.88	5.00	6.0	6.0
R62	0.00	1.60	2.17	3.04	3.50	5.00	5.0	5.0

R64	0.00	1.33	2.33	3.08	4.00	5.00	6.0	6.5
R74	0.00	0.93	1.76	2.55	4.25	5.33	4.0	7.0
All Readers	0.10	1.20	2.10	2.90	4.00	5.10	5.5	6.1

Table 15 – All areas: Mean age by age group for each and all readers.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	0.06	1.29	2.14	3.08	3.75	4.75	5.0	6.0
R10	0.12	1.35	2.22	3.00	4.25	5.00	6.0	7.0
R12	0.12	1.32	2.16	3.05	4.12	5.25	6.0	6.5
R16	0.12	1.10	1.78	2.71	3.50	4.75	5.0	7.0
R18	0.06	0.81	1.41	2.55	3.62	5.25	6.0	5.5
R20	0.12	1.19	1.89	2.82	4.00	5.50	6.0	7.0
R28	0.06	1.23	2.22	3.08	4.38	5.00	6.0	7.0
R34	0.06	1.42	2.00	2.68	3.75	4.75	5.0	4.5
R36	0.00	1.16	2.05	3.08	4.25	5.25	6.0	7.0
R38	0.06	1.00	1.54	2.21	3.38	4.00	4.5	5.5
R40	1.18	2.42	2.68	3.74	4.50	4.75	6.0	6.0
R42	0.00	0.71	1.38	2.34	3.62	4.75	5.5	5.5
R44	0.06	1.39	2.05	2.89	3.50	4.50	5.5	6.0
R48	0.18	1.58	2.08	3.29	4.25	5.00	6.0	7.0
R50	0.06	1.39	1.97	2.84	3.62	4.00	5.0	5.5
R52	0.06	1.23	2.00	3.03	4.00	4.75	5.0	4.5
R58	0.00	1.03	2.05	3.03	4.00	4.75	5.5	5.5
R62	0.00	1.26	1.95	2.87	3.38	4.50	5.0	5.0
R64	0.24	1.74	2.41	3.21	4.00	5.00	6.0	6.5
R74	0.12	0.94	1.68	2.53	4.25	4.75	5.0	7.0
All Readers	0.10	1.30	2.00	2.90	3.90	4.80	5.5	6.1

Tables 16 to 19 and Figure 5 show mean fish length at age by reader in each and in both areas combined. In general, average fish total lengths at age were higher in the N.E. Atlantic than in the Mediterranean Sea.

Table 16 – Mediterranean Sea: Mean fish length (mm) at age by reader and modal age.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	114	144	155	169	206	218		
R10	111	149	156	168	174		215	
R12	111	145	154	168	158	216	215	
R16	116	145	171	168	192	215		
R18	126	152	178	199	201	220	215	
R20	116	145	167	193	211	220	215	
R28	114	153	148	168	188	165	215	
R34	116	152	154	187	200	218		
R36	116	150	155	173	188	210	215	
R38	121	149	164	188	165	215		
R40	107	130	141	160	180	193	192	
R42	126	155	186	202	220	215		

R44	114	150	156	170	183		215	
R48	122	144	156	179	191	200	215	
R50	113	141	157	180	220	215		
R52	112	143	160	176	192	215		
R58	120	142	160	176	192	215		
R62	116	149	166	180	200	215		
R64	113	143	155	164	163	220	215	
R74	124	153	157	177			215	
Modal age	115	149	158	174	165	220	215	

Table 17 – N. E. Atlantic: Mean fish length (mm) at age by reader and modal age.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	132	168	190	202	209	231	214	
R10	125	181	186	201	206	223	224	214
R12	125	174	194	201	211	223	218	230
R16	128	182	189	201	224	227		214
R18	128	179	196	202	219	222	226	197
R20	125	172	187	201	207	216	231	214
R28	125	174	193	200	211	221	228	214
R34	134	168	190	205	213	224	197	
R36	125	168	195	200	210	221	228	214
R38	136	182	196	212	220	229	197	
R40	138	171	170	193	196	214	217	
R42	138	180	198	203	209	217	232	
R44	132	179	190	201	208	228		197
R48	122	173	189	200	202	206	205	214
R50	125	174	191	203	214	226	197	
R52	133	168	189	202	210	228		
R58	148	168	187	200	215	218	232	
R62	125	177	188	201	206	218		
R64	130	177	193	195	209	222	216	230
R74	132	177	202	199	217	226		216
Modal age	125	175	191	201	213	224	235	214

Table 18 – All areas: Mean fish length (mm) at age by reader and modal age.

Reader	Age Group							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
R04	122	155	173	190	208	225	214	
R10	117	164	172	189	192	223	222	214
R12	117	163	174	186	198	220	217	230
R16	122	158	182	193	215	225		214
R18	127	163	191	202	213	222	222	197
R20	120	153	177	200	208	217	227	214
R28	119	165	172	186	203	210	224	214
R34	123	161	171	200	211	222	197	
R36	119	159	175	190	206	217	224	214
R38	128	166	183	204	209	226	197	
R40	119	148	155	179	190	209	209	

R42	130	164	195	203	211	217	232	
R44	122	164	174	190	201	228	215	197
R48	122	153	175	192	200	206	207	214
R50	118	162	172	196	215	223	197	
R52	122	154	174	193	207	226		
R58	133	153	172	192	211	217	232	
R62	120	157	178	196	206	218		
R64	121	168	172	183	186	222	216	230
R74	127	165	182	191	217	226	215	216
Modal age	119	162	174	193	207	223	225	214

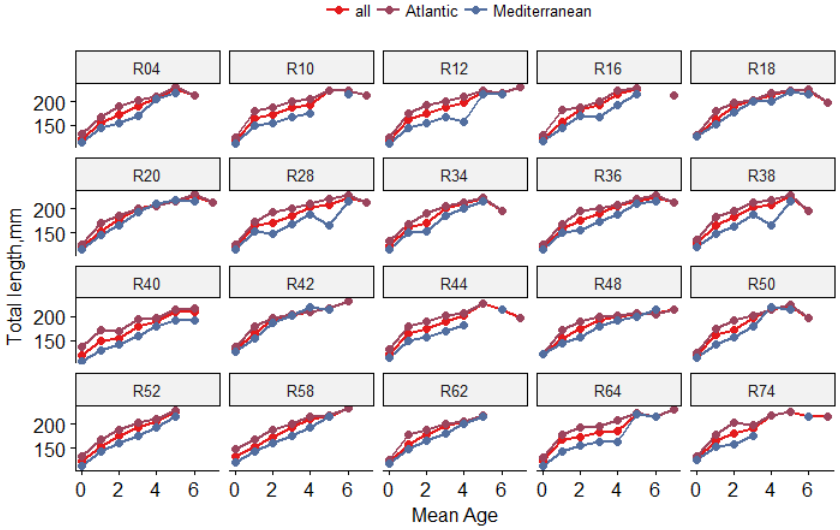

Figure 5 - Fish mean total length at age by reader and area.

Table 19 shows the summary of the average PA, CV and Bias by area and age group. PA in both areas ranged between around 39% (Mediterranean Sea, age group 4) and 96% (N. E. Atlantic, age group 0). Highest PA's were found for age group 0 (87.4% in the Mediterranean and 95.6% in the N. E. Atlantic). CV's which were generally higher in younger age groups (age groups 0 to 3) in both areas, ranged from 9.0% (Age group 6) to 57% (Age group 1) both in the Mediterranean Sea area. Bias was higher in older age groups.

Table 19 – Percentage of agreement (PA), coefficient of variation (CV) and bias by area and age group.

Age Group	Area								
	Mediterranean Sea			N. E. Atlantic			All Areas		
	PA	CV	Bias	PA	CV	Bias	PA	CV	Bias
0	87.4	312.5	0.2	95.6	423.5	0.1	90.7	358.7	0.1
1	63.8	57.0	0.3	74.3	44.5	0.2	68.8	50.9	0.3
2	65.3	32.8	-0.1	76.5	22.9	0.1	70.9	27.8	0.0
3	59.5	28.6	-0.3	72.8	18.7	-0.1	68.5	22.0	-0.1
4	39.4	34.2	-0.7	65.1	14.0	0.0	60.4	18.0	-0.1
5	40.0	19.4	-0.8	72.4	10.9	0.1	64.1	13.0	-0.2
6	-	9.0	-0.4	-	12.4	-0.5	-	10.7	-0.5
7	-	-	-	-	17.1	-0.9	-	17.1	-0.9

Figures 6 to 8 show the age bias plots of each and all age readers' readings results against modal age for each area and both areas together.

In general there was a good age reading agreement of readers with modal age in all areas, although a few readers showed a slight trend to underestimate ages of older fish (mainly R34, R38, R44, R50, R52 and R62).

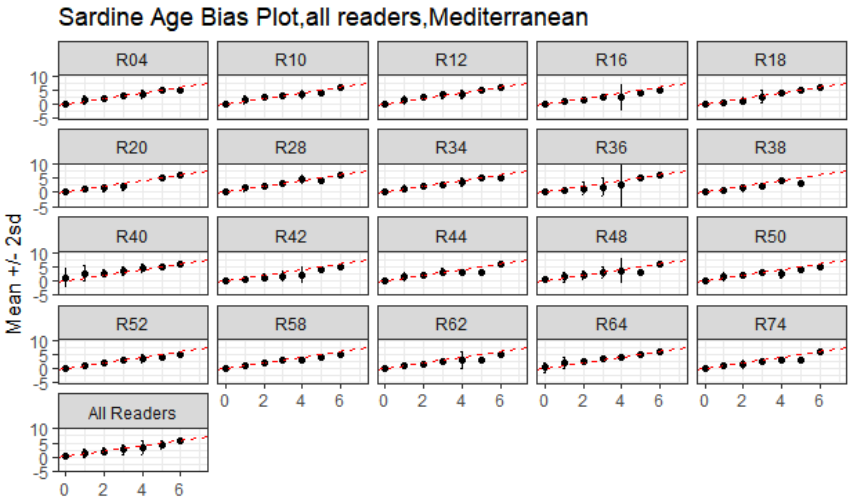

Figure 6 – Mediterranean Sea: all readers age bias plots.

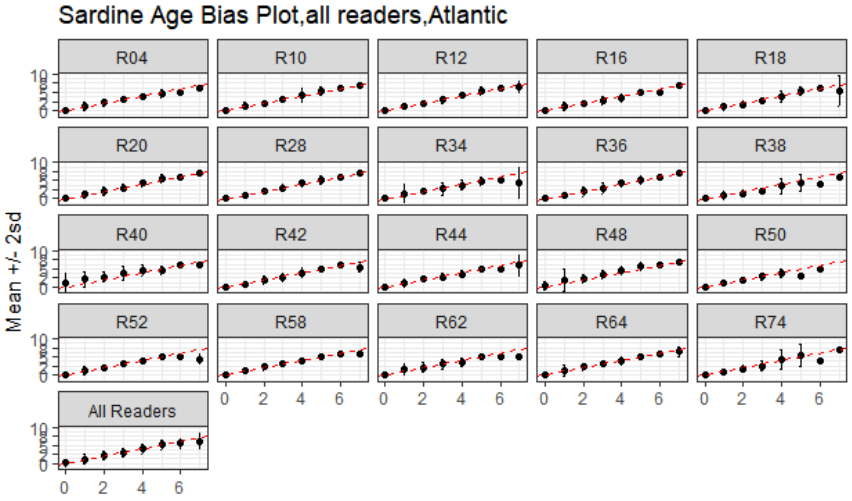

Figure 7 – N. E. Atlantic: all readers age bias plots.

Figure 8 – All areas: all readers age bias plots.

Tables 20 to 22 show the results of the age readings' inter-reader bias tests for all readers respectively for the Mediterranean Sea, the N. E. Atlantic and both areas combined (under diagonal), the age read-ing's percentage agreement (PA) between each two readers (above diagonal) and each reader against modal age bias tests (tables' bottom line).

In the Mediterranean Sea area, reader against modal age bias test showed in general a low occurrence of bias, except for readers R20, R38 (*) and R18, R36, R40, R42 and R64 (**). Inter-reader bias test re-sults show the occurrence of sign of bias between most of readers. PA's between each two readers ranged from 13% (R18 against R40) and 92% (R52 against R58).

Table 20 – Mediterranean Sea: Inter-reader bias test and reader against modal age bias test of sardine otoliths for both areas combined (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

Reader	R04	R10	R12	R16	R18	R20	R28	R34	R36	R38	R40	R42	R44	R48	R50	R52	R58	R62	R64	R74	Modal age
R04		67	67	58	32	42	68	70	52	37	42	32	77	53	60	72	68	65	58	50	78
R10	-		85	57	32	40	78	55	52	37	47	33	73	50	62	72	67	50	63	42	72
R12	-	-		53	28	38	82	52	57	30	52	27	70	48	60	72	67	47	62	40	70
R16	-	*	**		43	60	52	47	50	52	23	52	55	58	48	68	65	60	40	45	67
R18	**	**	**	*		53	32	37	42	35	13	58	33	37	28	37	35	47	27	48	48
R20	**	**	**	-	-		42	45	43	48	15	55	45	52	35	50	45	58	27	53	57
R28	-	-	-	*	**	**		58	55	33	45	30	73	55	57	65	63	52	62	43	72
R34	-	-	-	-	**	*	-		47	45	33	40	63	52	65	63	62	67	48	48	68
R36	**	**	**	*	-	-	**	**		30	32	42	50	48	47	57	55	48	43	45	60
R38	**	**	**	-	-	-	**	*	-		22	45	37	37	32	43	40	55	27	42	47
R40	**	**	*	**	**	**	*	**	**	**		10	38	32	33	37	35	27	45	20	42
R42	**	**	**	**	-	*	**	**	-	-	**		32	42	33	42	40	50	18	52	42
R44	-	-	-	-	**	**	-	-	**	**	**	**		55	58	68	67	62	58	53	75
R48	-	-	-	-	**	-	-	-	**	-	**	**	-		43	48	47	55	43	40	50
R50	-	-	-	-	**	**	-	-	**	*	**	**	-	-		65	63	57	43	38	62
R52	-	-	-	-	**	*	-	-	**	*	**	**	-	-	-		92	67	48	50	85
R58	-	-	-	-	**	*	-	-	**	*	**	**	-	-	-	-		63	45	47	80
R62	*	*	**	-	*	-	*	-	*	-	**	**	-	-	-	-	-		37	55	73
R64	*	-	-	**	**	**	-	**	**	**	-	**	*	**	**	**	*	**		25	53
R74	*	**	**	-	-	-	**	-	-	-	**	*	*	-	*	-	-	-	**		60
Modal age	-	-	-	-	**	*	-	-	**	*	**	**	-	-	-	-	-	-	**	-	

Table 21 – N. E. Atlantic: Inter-reader bias test and reader against modal age bias test of sardine otoliths for both areas combined (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

Reader	R04	R10	R12	R16	R18	R20	R28	R34	R36	R38	R40	R42	R44	R48	R50	R52	R58	R62	R64	R74	Modal age
R04		67	71	56	59	56	70	66	71	33	53	68	70	51	59	76	75	71	72	58	78
R10	-		72	57	61	57	77	54	72	28	43	67	70	57	57	67	68	63	75	59	77
R12	-	-		56	70	66	84	61	76	30	43	75	70	59	65	75	72	57	71	67	84
R16	-	-	-		57	42	61	58	54	34	28	63	65	47	58	58	54	67	57	56	67
R18	-	-	-	-		54	68	61	66	33	29	72	63	49	56	62	62	57	57	67	71
R20	-	-	-	-	-		65	43	65	20	35	54	49	49	48	54	54	42	54	47	63
R28	-	-	-	-	-	-		57	84	27	42	68	66	61	61	68	71	56	76	70	84
R34	-	-	-	-	-	-	-		57	37	38	59	66	43	61	66	59	65	49	62	63
R36	-	-	-	-	-	-	-	-		25	42	63	61	54	56	65	68	54	75	62	77
R38	**	**	*	*	*	**	**	*	*		15	33	35	19	25	32	30	34	32	35	32
R40	**	**	**	**	**	**	**	**	**	**		35	35	33	32	43	44	37	46	27	43
R42	-	-	-	-	-	-	-	-	-	-	**		71	51	62	67	67	66	62	71	75
R44	-	-	-	-	-	-	-	-	-	*	**	-		52	68	68	62	72	66	65	75
R48	-	*	*	**	**	-	*	**	*	**	-	**	**		51	59	56	51	56	47	63
R50	-	-	-	-	-	-	-	-	-	-	**	-	-	**		63	58	59	57	59	68
R52	-	-	-	-	-	-	-	-	-	**	**	-	-	-	-		86	68	68	62	81
R58	-	-	-	-	-	-	-	-	-	**	**	-	-	-	-	-		61	72	61	80
R62	-	-	-	-	-	-	-	-	-	**	**	-	-	-	-	-	-		62	54	68
R64	-	-	-	-	-	-	-	-	-	**	**	*	-	-	*	-	-	-		56	75
R74	-	-	-	-	-	-	-	-	-	-	**	-	-	**	-	-	-	-	*		70
Modal age	-	-	-	-	-	-	-	-	-	**	**	-	-	*	-	-	-	-	-	-	

In the N. E. Atlantic area, reader against modal age bias test showed a low occurrence of bias, except for readers R38 and R40 (**). Inter-reader bias test results show less occurrence of sign of bias between most of readers than in the Mediterranean Sea area. PA's between each two readers ranged from 15% (R38 against R40) and 86% (R52 against R58).

Table 22 – All areas: Inter-reader bias test and reader against modal age bias test of sardine otoliths for both areas combined (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)). Age reading's percentage agreement (PA) between each two readers (above diagonal).

Reader	R04	R10	R12	R16	R18	R20	R28	R34	R36	R38	R40	R42	R44	R48	R50	R52	R58	R62	R64	R74	Modal age
R04		67	69	57	47	50	69	68	63	35	48	53	73	52	60	74	72	68	66	55	78
R10	-		78	57	48	50	78	55	63	32	45	53	71	54	59	69	68	58	70	52	75
R12	-	-		55	52	54	83	57	68	30	47	54	70	55	63	73	70	53	67	55	78
R16	*	*	*		51	50	57	53	53	42	26	58	60	52	54	63	59	64	50	51	67
R18	*	**	**	-		54	53	50	55	34	22	66	50	44	44	51	50	53	44	59	61
R20	-	-	-	-	-		55	44	55	32	27	55	47	50	42	53	50	49	42	50	60
R28	-	-	-	*	*	-		58	71	29	43	52	69	58	59	67	68	54	70	58	78
R34	-	-	-	-	-	-	-		53	40	36	51	65	47	63	65	60	65	49	56	65
R36	*	*	*	-	-	-	*	-		27	37	54	56	52	52	61	63	52	61	55	70
R38	**	**	**	*	-	*	**	**	-		18	38	36	27	28	37	35	43	29	38	38
R40	**	**	**	**	**	**	**	**	**	**		24	37	32	32	40	40	32	45	24	42
R42	**	**	**	-	-	*	**	**	-	-	**		54	47	50	56	55	59	43	63	60
R44	-	-	-	-	*	-	-	-	-	**	**	**		53	64	68	64	68	63	60	75
R48	-	-	-	**	**	*	-	-	**	**	**	**	-		47	55	52	53	50	44	58
R50	-	-	-	-	-	-	-	-	-	**	**	*	-	*		64	60	58	51	50	65
R52	-	-	-	*	*	-	-	-	*	**	**	**	-	-	-		88	68	60	57	83
R58	-	-	-	*	*	-	-	-	*	**	**	**	-	-	-	-		62	60	55	80
R62	-	-	-	-	-	-	-	-	-	**	**	**	-	-	-	-	-		51	55	71
R64	-	-	-	**	**	**	-	**	**	**	**	**	*	-	**	*	-	**		42	65
R74	**	**	**	-	-	-	**	-	-	-	**	-	*	**	-	*	*	-	**		65
Modal age	-	-	-	-	-	-	-	-	-	**	**	**	-	-	-	-	-	-	*	-	

In both areas combined, reader against modal age bias test showed a low occurrence of bias, except for readers R38, R40 and R42 (**) and R64 (*). Inter-reader bias test results show an occurrence of sign of bias between most of readers. PA's between each two readers ranged from 18% (R38 against R40) and 88% (R52 against R58).

Annex 3: Contributions to the Workshop

During the workshop, 7 presentations were performed. The list of these presentations which can be accessed in the WKARAS 2 sharepoint by the Workshop participants, is the following:

Sardine Age Determination in Adriatic Sea. By Gasparevic, D., Institute of Oceanography and Fisheries. Split. Croatia. Presentation to WKARAS 2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Denis Gasparevic).

Otolith Preparation and Age Reading Techniques. By Fytilakos, I., Hellenic Center of Marine Research (HCMR). Presentation to WKARAS 2, 18-22 February, 2019. Lisbon, Portugal. (Presented by Ioannis Fytilakos).

Techniques de Préparation et Lecture des Otolithes *Sagitae* de la Sardine Européenne : *Sardina pilchardus*. By Hammou El Habouz, Centre Régional à l'Agadir, Laboratoire des Ressources Halieutiques (INRH, Agadir, Morocco). Presentation to WKARAS 2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Hammou El Habouz).

European Sardine Otoliths Exchange 2017 Results. By Soares, E., Torres, P. and Silva, A. V., Portuguese Institute of Sea and Atmosphere (IPMA, Portugal) and Instituto Español de Oceanografia (IEO, Spain). Presentation to WKARAS2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Eduardo Soares).

Otolith Images from France. By Bled Defruit, G., Centre Manche - Mer du Nord (IFREMER, Boulogne Sur Mer, France). Presentation to WKARAS 2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Geoffrey Bled Defruit).

Review of the Information on Age Determination, Otolith Exchanges and Validation Techniques of Atlantic Sardine (*Sardina pilchardus*). By Silva, A. V., Torres, P. and Soares, E.. Portuguese Institute of Sea and Atmosphere (IPMA, Portugal). Presentation to WKARAS 2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Andreia V. Silva).

An overview on the **Assessment of *Engraulis encrasicolus, Sardina pilchardus and Sardinella aurita* Fisheries Resources in the Mediterranean (GSA 01, 06 and 17 areas), with a particular focus on sardine otoliths preparation and age reading methodology,** (previously presented to the General Fisheries Commission for the Mediterranean, GFCM) By Torres, P., Instituto Español de Oceanografía (IEO, Spain). Presentation to WKARAS 2, Lisbon, Portugal, 18-22 February, 2019. (Presented by Pedro Torres).

Annex 4: List of participants

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(*) participation online in small exchange.

Annex 5: Resolutions

WKARAS2 – Workshop on Age reading of European Sardine (*Sardina pilchardus*) (NE Atlantic and Mediterranean)

2017/2/EOSG24 A **Workshop on Age reading of European Sardine (*Sardina pilchardus*) (NE Atlantic and Mediterranean)** (WKARAS2), chaired by Eduardo Soares, Portugal, and Pedro Torres, Spain, will be held in Lisbon, Portugal, 18-22, February, 2019, to:

a) Review the results of the European Sardine Otoliths Exchange 2017 and identify causes of poor agreements between readers where apparent (Science plan code 5.2);
b) Determine causes of inconsistencies in age determination and improve where necessary the sardine age determination criteria including annulus definition and validation techniques. Clarify the otoliths' annual growth rings identification, the methodologies applied and age reading validation techniques used on this species. Update the common age reading protocol and make specific guidelines for the improvement of age reading precision and the reduction of bias between readers and laboratories (Science plan code 5.2);
c) Create a reference collection of clearly-defined otoliths with a consensual age in a data base of digitized images for the Atlantic and the Mediterranean Sea (Science plan code 5.2);
d) Address the generic TORs adopted for workshops on age calibration (Science plan code 5.2).

Standardization of otoliths preparation procedures and of age reading criteria will be therefore in the scope of this Workshop in order to increase the age reading data quality for the sardine stocks assessment in these areas.

WKARAS2 will report by 2019 for the attention of WGBIOP and ACOM.

Supporting information

Priority	Age determination is essential in fish stock assessment for the estimation of rates of mortality and growth. Thus, it is fundamental to get reliable age readings in order to contribute to accurate assessments. Therefore, a sardine otolith exchange program was carried out in 2017 for inter-calibration between age readers of fisheries research labs in NE Atlantic and Mediterranean Sea areas. One of the main problems identified from the results analysis of the European Sardine Otoliths Exchange 2017 was the low age reading agreement among readers for both areas, which averaged between 60 and 80%. This emphasized the need for an age reading workshop on European Sardine (WKARAS 2) in support of ICES fisheries advice. The aim is to convene age readers of both areas in order to review the exchange results and the sardine age determination criteria, clarify the otoliths' annual growth rings identification, the methodologies applied, to update the common age reading protocol and to define a reference collection of well-defined otoliths for this species for each area..
Scientific justification	Currently there is excessive uncertainty around aging of European Sardine between readers within stock and between readers of different stocks. This uncertainty could negatively affect the quality of ICES fisheries advice and needs to be addressed if possible.
Resource requirements	No specific resource requirement beyond the need for members to prepare for and participate in the meeting.
Participants	The Workshop is foreseen to be attended by researchers from Portugal, Spain, France, United Kingdom, Germany, Italy, Greece, Croatia, Morocco and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to advisory committees	ACOM
Linkages to other committees or groups	WGBIOP
Linkages to other organizations	There is a direct link with the EU DCF.