ICES WKARHOM3 REPORT 2018

ECOSYSTEM OBSERVATION STEERING GROUP

ICES CM 2018/EOSG:28

REF ACOM & SCICOM

Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus, T. mediterraneus* and *T. picturatus*) (WKARHOM3)

5–9 NOVEMBER 2018

Livorno, Italy

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International Council for the Exploration of the Sea
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1 Executive summary

Based on the previous work of WKARHOM2 (2015), the Working Group on Biological Parameters (WGBIOP 2017) identified the need for a new otolith exchange followed by an age reading Workshop. The Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus, T. mediterraneus and T. picturatus*) (WKARHOM3) was held in Livorno (Italy) from the 5th to the 9th November 2018. The meeting was co-chaired by Alba Jurado-Ruzafa (Spain), Kélig Mahé (France) and Pierluigi Carbonara (Italy), and included fifteen age readers from nine countries. The objectives of this workshop were to review the current methods of ageing *Trachurus* species, to evaluate the new precision of ageing data of *Trachurus* species and to update guidelines, common ageing criteria and reference collections of otoliths. The exchange results showed a low value of percentage of agreement from 45.1% to 59.1% for the three *Trachurus* species. The Coefficient of Variation was lower for *T. trachurus* (17.3–32.2) than for the other *Trachurus* species (60.1-73.4) because the sampled specimens were older for this species than for the two other species. With feedback from the readers present at the exchange and the discussion during the WKARHOM3 meeting, the main cause of age determination error for *T. trachurus* was identified as otolith preparation techniques (whole/slice). However, for the three *Trachurus* species, there are several difficulties in age determination: identification of the first growth annulus, presence of many false rings (mainly in the first and second annuli) and the interpretation and identification of the edge characteristics (opaque/ translucent). The second reading was performed during the workshop with 50 images per each species. Each reader read only the images of the species that is read in their laboratory. The percentage of agreement between readers increased to 70.6% with a CV of 18.4 for *T. trachurus* and to 67.8% with a CV of 31.7 for *T. mediterraneus*. Finally, this group reached an agreement on defining an ageing guideline and a reference collection presented in this report and the aim is to employ these tools for all laboratories.

2 Administrative details

Expert Group name

Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (WKARHOM3)

Chair(s)

Alba Jurado-Ruzafa (Spain), Kélig Mahé (France) & Pierluigi Carbonara (Italy)

Meeting venue

Livorno, Italy

Meeting dates

5–9 November 2018

3 Terms of Reference

WKARHOM3 - Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel

2016/2/SSGIEOM18

The **Workshop** on **Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus, T. mediterraneus* and *T. picturatus*) [WKARHOM3]**, chaired by Alba Jurado, Spain, Pierluigi Carbonara, Italy and Kélig Mahé, France, will meet in Livorno (Italy), 5-9 November 2018, to:

a) Review information on age determination, otolith exchanges and validation study on these species
b) Clariy the position of the first annulus with the images analysis for three species
c) Evaluae the effect of different schemes of ageing particularly the date of birth for *Trachurus mediterraneus*
d) Cotinue the guidelines and common ageing criteria;
e) Develop existing reference collections of otoliths;
f) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

WKARHOM3 will report by 13 June 2018 for the attention of ACOM-SCICOM.

Supporting information

Priority:	Essential. Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Age data are provided by different countries and are estimated using international ageing criteria. It is necessary to continue to clarify this guideline of age interpretation. Therefore, an appropriate otolith exchange programme will be carried out in 2017 for the purpose of inter-calibration between ageing labs. Results of this otolith exchange will be discussed during WKARHOM3.
Scientific justification and relation to action plan:	The aim of the workshop is to identify the current ageing problems between readers and standardize the age reading procedures in order to improve the accuracy and precision in the age reading of this species.
Resource requirements:	No specific resource requirement beyond the need for members to prepare for and participate in the meeting.
Participants:	In view of its relevance to the DCF, and ICES WG, the Workshop try to join international experts on growth, age estimation and scientists involved in assessment in order to progress towards a solution. Participants should announce their intention to participate in the WK no later than two months before the meeting.
Secretariat facilities:	None
Financial:	
Linkages to advisory committees:	ACOM/WGBIOP
Linkages to other committees or groups:	WGBIOP
Linkages to other organisations:	There is a direct link with the EU DCF.

4 List of participants

The Workshop on Age reading of *Trachurus* species (WKARHOM3) was held in Livorno in 2018. Fifteen age readers from nine countries participated in this workshop, which concerned three *Trachurus* species (Table. 1). The agenda is presented in the Annex 1.

Table 1. Participants list of WKARHOM3 workshop with for each reader, the preparation method used to ageing.

Surname, Name	Country, Affiliation	e-mail	2015 workshop	*T. trachurus*	*T. mediterraneus*	*T. picturatus*	Stock assessment Reader
Carbonara, Pierluigi (chair)	Italy, COISPA	carbonara@coispa.it	Yes	Whole otolith/Slice	Whole otolith	Whole otolith	Yes
Casciaro, Loredana	Italy, COISPA	casciaro@coispa.eu	No	Whole otolith	Whole otolith		
Defruit, Geoffrey Bled	France, IFREMER	geoffrey.bled.defruit@ifremer.fr	No	Slice			
Delfs, Gertrud	Germany, Thünen-Institut	gertrud.delfs@thuenen.de	Yes	Slice			Yes
Diaz, Justine	Norway, IMR	justine.diaz@hi.no	No	Burnt broken otolith			
Dijkman-Dulkes, André	Netherlands, IMARES	andre.dijkman@wur.nl	No	Slice			Yes
Dimitriadis, Giannis	Greece, FRI	gdimitriadis@inale.gr	No	Whole otolith	Whole otolith		
Dueñas-Liaño, Clara	Spain, IEO-Santander	clara.duenas@st.ieo.es	Yes	Whole otolith/Slice			Yes
Ferreira, Maria Joao	Portugal, IPMA	mjferreira@ipma.pt	No	Whole otolith/Slice			
Hemken, Gitta	Germany, Thünen-Institut	gitta.hemken@thuenen.de	No	Slice			
Jurado-Ruzafa, Alba (chair)	Spain, IEO-Canary Islands	alba.jurado@ieo.es	Yes			Whole otolith	
López, Eduardo	Spain, IEO-Vigo	eduardo.lopez@ieo.es	Yes	Whole otolith/Slice	Whole otolith		
Mahé, Kélig (chair)	France, IFREMER	kelig.mahe@ifremer.fr	Yes				
Massaro, Andrea	Italy, CIBM	andrea.massaro@aplysia.it	Yes	Whole otolith	Whole otolith	Whole otolith	Yes
Mullins, Eugene	Ireland, Marine Institute	eugene.mullins@marine.ie	Yes	Slice			Yes

Six of the readers send the ageing data to the stock assessment concerning only the *T. trachurus*.



From the top to the bottom, from left to right: André Dijkman-Dulkes, Eugene Mullins, Giannis Dimitriadis, Pierluigi Carbonara, Alba Jurado-Ruzafa, Andrea Massaro, Kélig Mahé, Eduardo López, Geoffrey Bled Defruit, Clara Dueñas Liaño, Gertrud Delfs, Gitta Hemken, Justine Diaz, Maria Joao Ferreira & Loredana Casciaro.

In memoriam - Kirsti Børve Eriksen

Kirsti participated in the WKARHOM1 and WKARHOM2 meetings in Santa Cruz de Tenerife in 2015. We have an excellent memory of her.



5 Review information on age determination, otolith exchanges and validation study on these species Background

5.1 Otolith exchanges and Workshops

There have been previous age reading workshops on horse mackerel, three including the three species (Table 2). Based on the results of last otolith exchange and the workshop in 2015, the Working Group on Biological Parameters (WGBIOP) (ICES, 2017) identified the need for another exchange and age reading workshop on *Trachurus* species (WKARHOM3). This workshop was hosted by CIBM (Centro Interuniversitario di Biologia Marina) (Livorno, Italy) on 5-9 November 2018.

Table 2. Past *Trachurus* species otolith exchanges and workshops.

Year	Exchange/Workshop	Species	References
1996	Exchange	*T. trachurus*	•Report of the horse mackerel otolith workshop (ICES, 1999)
1999	Workshop	*T. trachurus*	
2006	Both	*T. trachurus*	•Report of the Horse Mackerel Exchange and Workshop 2006 (Bolle *et al*., 2011)
2012	Both	*T. trachurus* *T. mediterraneus* *T. picturatus*	•Report of the **WKARHOM1** (ICES, 2016)
2015	Both	*T. trachurus* *T. mediterraneus* *T. picturatus*	•Report of the Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel Otolith Exchange 2015 (Mahé *et al*., 2015) •Report of the **WKARHOM2** (ICES, 2015)
2018	Both	*T. trachurus* *T. mediterraneus* *T. picturatus*	-

5.2 WORKING DOCUMENTS presented during WARHOM3

During the first day of WKARHOM3 meeting, several presentations were presented by some participants to review the studies or preparation methods of otoliths concerning these three species of *Trachurus*:

- WD1: Alba Jurado-Ruzafa. Workshops on Age reading of horse mackerel, Mediterranean horse mackerel and Blue jack mackerel (*Trachurus trachurus, T. mediterraneus and T. picturatus*). ICES WKARHOM Background.
- WD2: Loredana Casciaro and Pierluigi Carbonara. Ageing analysis of *Trachurus mediterraneus* and *T. trachurus.*
- WD3: Giannis Dimitriadis. Biology sampling methodology for *Trachurus trachurus and T. mediterraneus*. Greek DCF.
- WD4: Justine Diaz. Horse mackerel in the Norwegian Sea.
- WD5: Clara Dueñas, Eduardo López, Ana Antolínez and Begoña Villamor. Age determination methodology of Horse mackerel (*Trachurus trachurus*) and Mediterranean horse mackerel (*Trachurus mediterraneus*) in the Santander and Vigo labs of IEO (BIOPEL project).
- WD6: Andrea Massaro. Life-history of *Trachurus trachurus, T. mediterraneus and T. picturatus* in GSA 9: Liguarian and North Thyrrhenian Sea.

- WD7: Pierluigi Carbonara and Loredana Casciaro. Semi-direct and indirect age validation for the horse mackerel in South Adriatic Sea (Central Mediterranean).
- WD8: Alba Jurado-Ruzafa and M. Teresa G. Santamaría. Growth studies based on otoliths of *Trachurus picturatus*. Indirect validation and challenges.
- WD9: Alessandro Ligas. Stock assessment of Atlantic horse mackerel in GSAs 9, 10 & 11.

5.3 Validation studies

Age validation should be a necessary step in all growth studies in order to improve accuracy and precision as well as to provide unbiased data for stock-assessment models.

In validation studies, two aspects shall be determined: (1) the increments are laid down according to a periodicity that can be related to a regular time-scale (precision); (2) the age estimation structure has a consistent interpretable pattern (absolute age) of increments (accuracy) (Campana, 2001; Panfili *et al*., 2002). Both aspects have been poorly addressed in studies on Trachurus species.

For the *T. trachurus* Waldron and Kerstan 2001 in the South-East and North-East Atlantic proposed a validation based on the Marginal increment analysis and daily ring.

For the T. picturatus Garcia *et al*. (2015) reported in Azores Island a semi-direct validation (Marginal Increment Analysis) on the deposition periodicity of the annulus. Moreover for the Madeira area Vasconcelos *et al*. (2006) report a validation of the coherency in the ageing criteria by partial radii measurement. Finally Jurado-Ruzafa & Santamaría (in press) for the Canary Island report the validation of deposition periodicity of the annuli through qualitative (Marginal analysis in term of monthly opaque trend) and quantitative (Marginal Increment Analysis) approach (Figure 1).

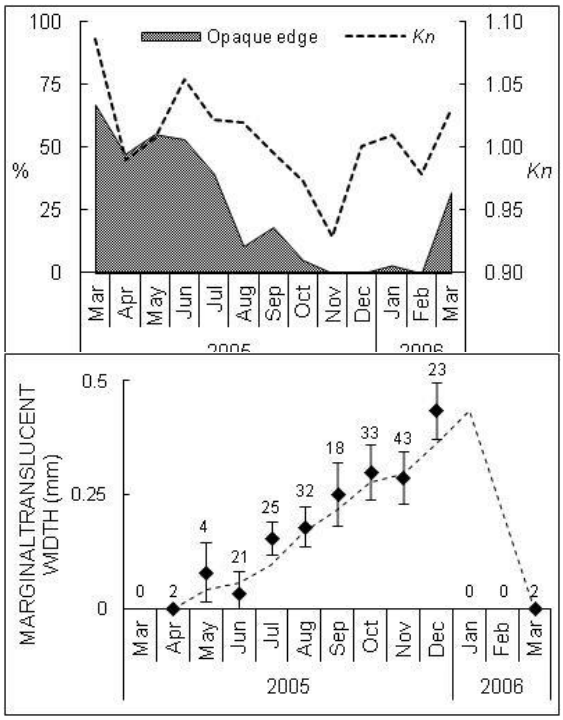


Figure 1. Monthly percentages trend of the opaque edge (left) and monthly mean width of the translucent edge (right) for the *T. picturatus* in Canary Islands (Jurado-Ruzafa & Santamaría, in press).

Moreover Jurado-Ruzafa & Santamaría (in press) reported the frequency distribution partial radii measurements in order to validate the coherency of the ageing criteria adopted (Figure 2).

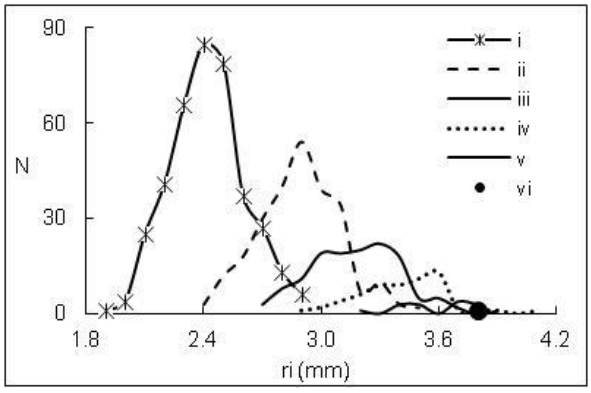


Figure 2. Radii measurements by annulus for the *T. picturatus* in Canary Islands (Jurado-Ruzafa & Santamaría, in press).

For *the T. mediterraneus,* no data were found in the literature.

During the workshop some examples of the validation study on the *T. trachurus* in Mediterranean basin based on the indirect and semi-direct methods were presented. The deposition of the one annulus was showed in the Adriatic and Ligurian-North Tyrrhenian (Figures 3 & 4)

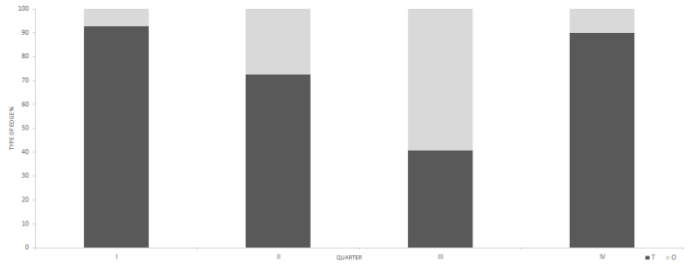


Figure 3. Edge trend for the *T. trachurus* in the Ligurian and North Tyrrhenian Sea.

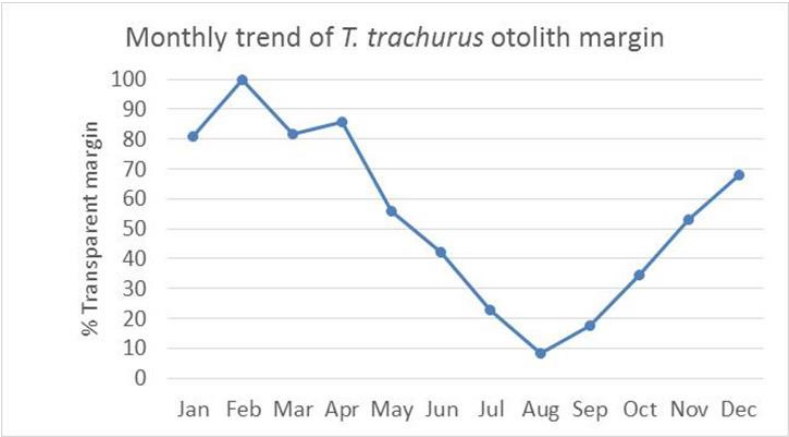


Figure 4. Monthly trend for the otolith edge of the *T. trachurus* in the Adriatic Sea.

Moreover the back-calculation results (Table 3 & Figure 5) were compared with the mean length of the mode (Bhattacharya method) from the winter Length Frequency Distribution (LFD) Survey (GRUND 2009).

Table 3. Back-calculation results for the *T. trachurus* in the Adriatic Sea.

		Rings											
N° Growth Increment	N° Specimens	1°	2°	3°	4°	5°	6°	7°	8°	9°	10°	11°	12°
1	143	72.64											
2	63	73.41	131.24										
3	250	73.18	131.25	188.15									
4	126	75.70	138.03	192.95	233.69								
5	68	80.76	140.24	190.90	229.97	258.02							
6	54	81.23	139.26	191.32	231.37	258.16	282.38						
7	28	77.06	142.38	194.46	231.03	259.05	280.65	301.79					
8	11	80.73	142.93	195.91	239.08	272.47	295.60	316.05	333.38				
9	7	76.43	144.43	194.61	234.33	266.09	287.86	306.40	320.32	334.22			
10	3	86.97	148.71	198.84	231.80	283.14	273.77	291.46	307.43	317.14	328.42		
11	1	79.49	147.63	191.43	225.50	249.84	269.31	313.11	317.98	327.71	347.18	371.51	
12	1	88.37	154.73	215.99	251.72	282.35	302.77	328.29	353.81	374.23	384.44	404.86	420.17
Tot. Number		755	612	549	299	173	105	23	12	5	3	1	1
Mean (cm)		75.09	136.36	191.24	232.40	260.00	283.49	305.63	326.24	332.74	343.38	388.19	420.17
Mean Increment (cm)			136.36	54.89	41.16	27.60	23.49	22.14	20.61	6.50	10.64	44.81	31.99
SD		11.35063	15.74223	17.824352	20.49372	23.1162	24.46939	20.83815	24.26462	23.7542	26.48223		
CV		15.12	11.54	9.32	8.82	8.89	8.63	6.82	7.44	7.14	7.71		

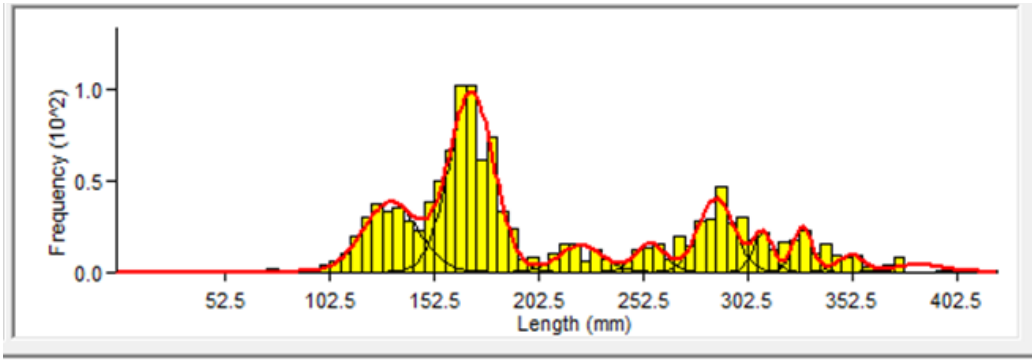


Figure 5. Length Frequency Distribution from the winter survey.

This analysis provided an indirect validation of the detected age group (Carbonara *et al.,* 2018). The winter survey LFD was used in this analysis because the winter period seems to represent an age class (Table 4).

Table 4. Comparison (t-test) between the mean back-calculated length and the mean length of the mode (LFD).

Back-calculation				Bhattacharya method				t-Test
Mean Length	S.D.	N° specimen		Mean Length (mm)	S.D.	N° specimen		P-value
75.09	11.35	755	1° ring					
136.36	15.74	612	2° ring	133.87	19.59	252	1° mode	0.0502
191.24	17.82	549	3° ring	189.35	19.78	755	2° mode	0.0759
232.40	20.49	299	4° ring	230.62	7.32	164	3° mode	0.2826
260.00	23.12	173	5° ring	258.36	5.22	42	4° mode	0.6465
283.49	24.47	105	6° ring	287.51	8.99	174	5° mode	0.0511
305.63	20.84	23	7° ring	310.03	4.69	50	6° mode	0.1580
326.24	24.26	12	8° ring	328.87	4.67	57	7° mode	0.4414

This analysis show as the first ring back-calculated at a total length of 75.09 mm not have any correspondence in the LFD mode. While the others back calculated rings and mode not presented significant difference (t-test $p>0.05$). In term of accuracy, Campana (2001) indicated the analysis of discrete length modes as a robust approach to validating the interpretation of annuli.

The comparison of the growth curves from the otolith reading and LFD analysis from Medits survey (ELEFAN and Bhattacharys methods) did not show any statistical differences. This result could represent an indirect validation (Campana, 2001; Panfili *et al.,* 2002) of the otolith age estimation criteria (Figure 6).

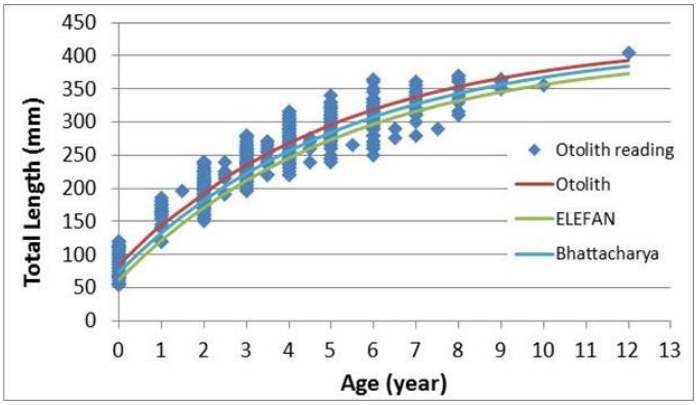


Figure 6. Growth curves comparison from otolith reading ($L\infty$= 427.03 mm, k= 0.192 $year^{-1}$, t0= -1.147 year) ELEFAN ($L\infty$ = 409.5 mm, k= 0.188 $year^{-1}$, t0= -0.875 year) and Bhattacharya $L\infty$ = 421.47 mm, k= 0.186 $year^{-1}$, t0= -1.032 year).

6 First reading during exchange 2018

6.1 SmartDots experience

The SmartDots program functioned reasonably well for viewing and annotation of otolith images. The WKARHOM3 participants know that it is a new application and its use will be better in the upcoming years.

As other ageing groups, the WKARHOM3 participants discussed around the use of SmartDots and their feedback had identified several points:

- The WKARHOM3 participants consider that it is necessary to read the otolith with the fish length information. Moreover, when the data were uploaded, there was mismatch between the total length and the images for several individuals and consequently, the view of incorrect total length of fish could influence the results of the exchange.
- The WKARHOM3 participants consider that it was too difficult to use one common reference axis because all readers do not agree on the position of this axis. During the discussion around the annotations, it was not possible to compare some annotations along the same axis. It is preferable to set a limited area or zone of reading to have the comparable distance between different reading axes.
- The WKARHOM3 participants would like that the order of the images was carried out according to the preparation method (whole otolithvs.sliced), and after, by catch month to group all images by different quarters (Q1, Q2, Q3 and Q4).
- The WKARHOM3 participants would appreciate a guideline of exchange including instructions about how to produce the images set explaining and how to name the images, in order to reduce problems during their upload.
- The WKARHOM3 participants would like one unique identification number and a unique annotation colour for each reader participating in several events during the same workshop.
- The WKARHOM3 participants would like that if a reader identifies the quality level as AQ3, this reading will be automatically eliminated to the analysis.

6.2 Results of exchange 2018

Three reports have been sent to the co-chairs of WKARHOM3, but after discussing the results, the participants of WKARHOM3 decided to use the raw data with the Eltink spreadsheet (Eltink, 2000) to modify some parameters before producing the analysis:

- To present the data for one method according to the preparation method of otolith and the semester
- To modify the data with AQ3 precision level which often showed "age group 0" by no data

Mean precision of age is defined as the variability in the age reading estimates. For individual fish the Coefficient of Variation (CV) and percentage agreement to modal age was calculated. This measure of precision is independent of the closeness to the true age (ICES, 2007). The spreadsheet was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink *et al.* (2000).

Modal ages were calculated for each otolith read, with percentage agreement, mean age and precision coefficient of variation as a definition (for each otolith):

- percentage of agreement = 100x(no. of readers agreeing with modal age/total no. of readers).
- precision c. v. = 100x(standard deviation of age readings/mean of age readings).

6.2.1 All readers

For all *Trachurus* species, the percentage of agreement showed a low value from 45.1% to 59.1%. The results by semester showed the same trend for three species with better agreement during semester 2 than during semester 1. For *T. trachurus*, the trend appeared to be reversed for slides. The Coefficient of Variation was lower for *T. trachurus* than other *Trachurus* species because the sampled specimens were older for this species than two other species (Table 5).

Table 5. Results of *Trachurus* exchange in 2018 for all readers according to the species, the otolith preparation and semester.

Species	latin name	Otolith preparation	Sampling semester	Sampling number	Range of age	Readers number	Percentage of agreement	Coefficient of Variation
Blue Jack mackerel	*Trachurus picturatus*	whole otolith	1	*25*	0–5	11	59.1	73.4
			2	*23*	0–3	11	54.5	66.3
Horse mackerel	*Trachurus trachurus*	slide	1	*38*	3–21	14	45.1	17.3
			2	*25*	1–13	14	55	20.7
Horse mackerel	*Trachurus trachurus*	whole otolith	1	*64*	0–9	14	52.8	31.6
			2	*34*	0–12	14	46.1	32.2
Mediterranean Horse mackerel	*Trachurus mediterraneus*	whole otolith	1	*29*	0–7	11	50.3	72.6
			2	*50*	0–10	11	46.4	60.1

6.2.2 Stock assessment readers

There were only several readers who participated to provide the data for stock assessment for *Trachurus trachurus*. The results showed a little more precision between readers than for all readers (Table 6).

Table 6. Results of *T. trachurus* exchange in 2018 for all readers according to the species, the otolith preparation and semester.

Species	Latin name	Otolith preparation	Sampling semester	Sampling number	Range of age	Readers number	Percentage of agreement	Coefficient of Variation
Horse mackerel	*Trachurus trachurus*	slide	1	*38*	3–21	14	48.4	18.7
			2	*25*	1–13	14	58.8	20.6
Horse mackerel	*Trachurus trachurus*	whole otolith	1	*64*	0–9	14	62.4	25.6
			2	*34*	0–12	14	52	26.7

6.3 Identify causes of age determination error

With the feedback from readers at the exchange and the discussion during the WKARHOM3 meeting, several causes of age determination error were identified:

1) Otolith preparation techniques (whole/sliced; immersion in distilled water/seawater/tap water/thymol/resin...)

2) Otolith reading method (transmitted/ reflected light)

3) Difference in the used date of birth : 1st January in Atlantic ocean for *Trachurus trachurus* and *Trachurus picturatus vs.* 1st July in Mediterranean Sea for *Trachurus mediterraneus*

4) Various magnifications of images

Moreover, some difficulties in age determination were identified:

5) Identification of the first *annulus*

6) Presence of many false rings, mainly in the first and second *annuli*.

7) Overlapping of rings from 4th-5th *annuli*

8) Identification of the edge nature (opaque/ translucent)

7 Second reading during the workshop 2018

To create a second set, several images were chosen randomly from the exchange in 2018. The set of images for *T. trachurus* was created from whole otoliths and sliced because the readers use both techniques. For each species, only the concerned species to the reader, had participated to the second reading. The percentage of agreement between readers increased to 70.6% with a CV to 18.4 for *T. trachurus* and to 67.8% with a CV to 31.7 for *T. mediterraneus* (Table 7).

Table 7. Results of *T. trachurus* second reading in 2018 for all readers according to the species, the otolith preparation and semester.

Species	Latin name	Otolith preparation	Sampling number	Range of age	Readers number	Percentage of agreement	CV
Horse mackerel	*Trachurus trachurus*	slide/whole otolith	*49*	0–14	9	70.6	18.4
Mediterranean Horse mackerel	*Trachurus mediterraneus*	whole otolith	*44*	0–7	3	67.8	31.7

8 Update age reading protocols for each species

8.1 Horse mackerel (Trachurus trachurus)

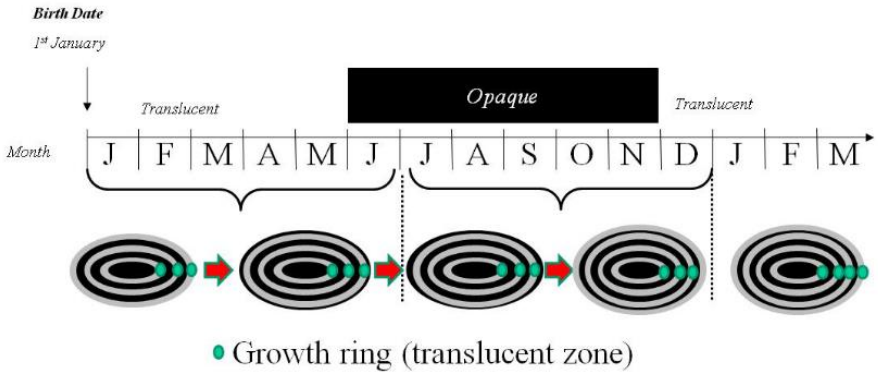
8.1.1 Otolith sampling

This group recommends that both sagittal otoliths (left & right otoliths) are sampled for each fish. For additional information on sampling and otolith preparation, see Annex 1 (Review of procedures for otolith preparation and analysis).

Note: If sectioning otoliths, it is important to keep the blades sharp for a good cut, resulting in good ring observation. In addition, brushing the slides with baby oil improves the readability greatly.

8.1.2 Diagram of otolith interpretation

The following (Figure 7) is a schematic interpretation of the growth development of *T. trachurus* from its birthday on 1st January following it through to age. The translucent zones are used to determine the age, and towards the end of the year the translucent zone is developing, but should not be counted as a fully developed ring until the 1st January.



Semesters	Edge of otolith	Age
1	Opaque edge (on first quarter) Translucent edge New opaque edge (on May or June)	N+1 N N
2	Opaque edge Translucent edge (on November or December)	N N-1

Figure 7.Schematic interpretation of the growth development of the annual zones over the course of a year. The arrows indicate the development of growth rings (translucent and opaque zones) during the year.

An annulus is characterized by the brightest contrast between the preceding translucent and the subsequent opaque zone deposited in the following year. An annulus should be traceable on the whole otolith or the slice, with the exception of the dorso-medial surface of the rostrum. In a sliced otolith, problems may arise in the area of the *sulcus acusticus* and the dorso-medial direction on the medial side.

8.1.3 Important Guidelines to follow when interpreting the age

- Preference of source of light: the readers preferably use reflected light and black background particularly when image processing is used.
- Magnification: the same magnification is recommended to compare the size of growth rings between some otoliths because the widths of consecutive annual growth zones should decrease with increasing age. Be careful with the magnification when reading by stereomicroscope, as a high magnification can cause overestimation of the age with the mistake between false rings and annuli. To identify many annuli on the edge for the older fish, it is possible to zoom in the edge area only.
- Image characteristics: the readers recommend to use only calibrated images (with bar of calibration, pretreatments of images could induce bias due to different size of otoliths; Figure 8) and to see the entire slice or the entire whole otolith to follow the annulus around the whole otolith (for only the first four annuli) or the annulus of each side of the *sulcus*. Both whole otoliths should be present on the image. Moreover, the interpretation area of otolith must be clean and without problems due to the preparation (bubble in resin).

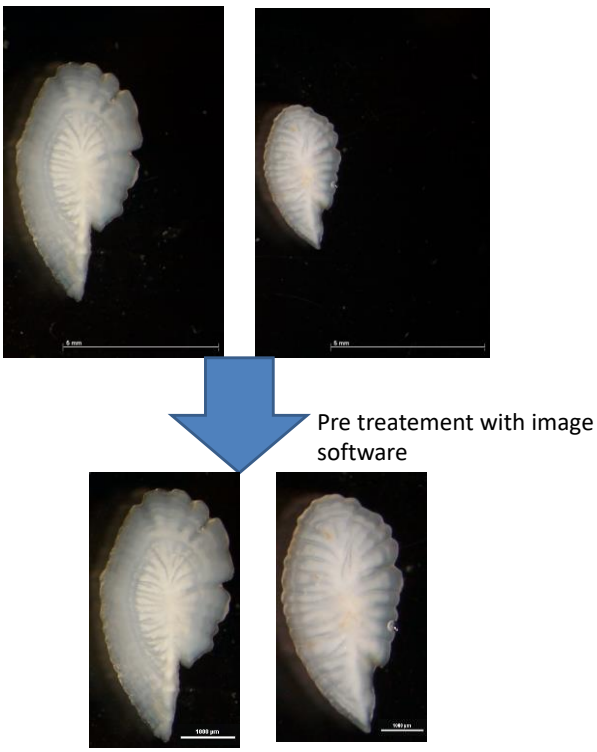


Figure 8. Example of the pretreatments of images could induce bias due to different size of otoliths.

- First four growth annuli: on whole otolith, it is possible to follow them all around the nucleus. On sliced otoliths, it is possible to follow them on each side of the *sulcus*.
- Usually the first 3 or 4 annuli are broad and then become more compact and thinner as the fish gets older and growth slows down. The distance between the annuli should decrease gradually with age.

- Extra data such as fish length, sex, maturity, catch area, and catch date should all be considered when assigning an age.
- Relevant data of the otolith interpretation (size, distance of annulus...) from the fish sampled at the same time should be available in case of doubt when assigning fish age.

8.1.4 Observation of whole otolith or slice

Whole otoliths are analysed under a stereomicroscope with reflected light against a black background. The best orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down (Figure 9). In this way the dark rings could be counted in the posterior area as translucent growth rings. *Rostrum* region is used as the confirmation area.

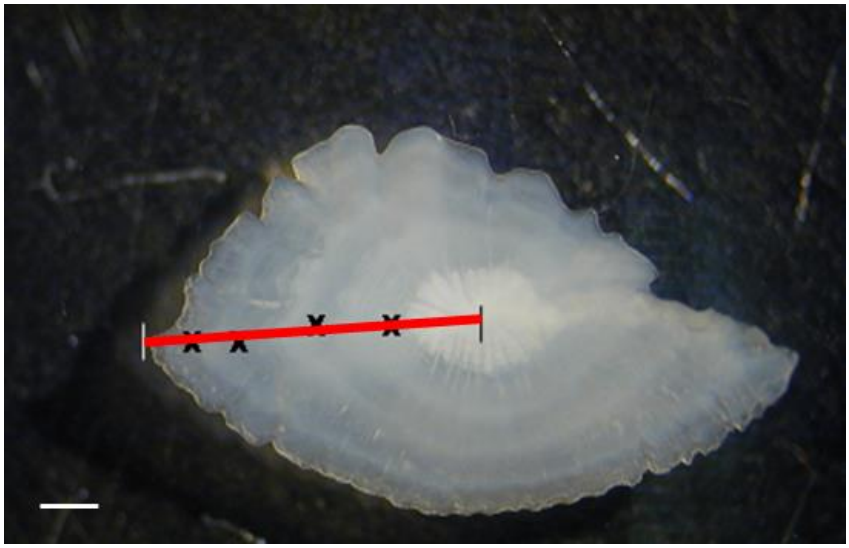


Figure 9: Whole otolith of *T. trachurus* with the main reading axis (red line).

Sliced otoliths are analysed under a stereomicroscope. The best otolith orientation for the analysis is from the core area and along each side of *sulcus acusticus*, up or down depending on the preference of the reader (Figure 10). In this way the dark rings can be counted as translucent growth rings.

Changing the focus can get rid of false or split rings, resulting in the true rings becoming more visible. Adjusting the light intensity and direction can also be helpful.

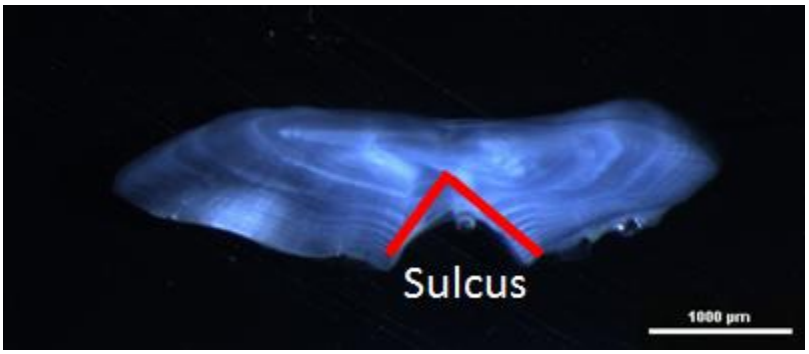


Figure 10. Slice otolith of *T. trachurus* with the main reading axis along each side of *sulcus* (red line).

8.1.5 Quality of images

The readers recommend to only use calibrated images to see the entire sliced or both whole otoliths to follow the annulus around the whole otolith (for only the first four annuli) or the annulus of each side of the sulcus. Both whole otoliths should be present on the image. Examples of some images with the acceptable quality are presented in Figure 11.

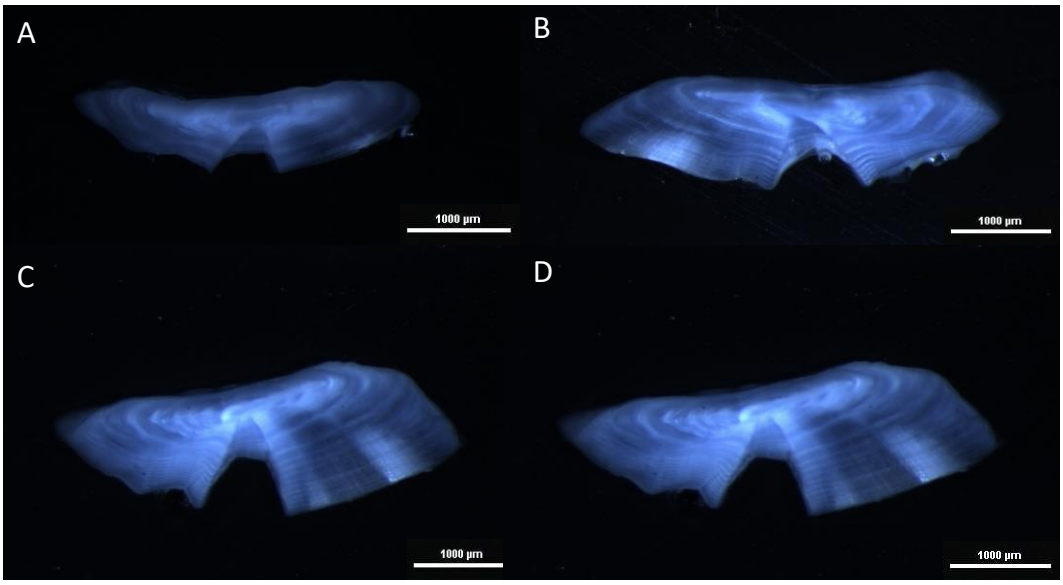


Figure 11. Calibrated image examples of sliced otoliths of *T. trachurus* with acceptable quality because there is good contrast and brightness. Moreover, the otoliths are sliced through the *nucleus*.

In comparison, some examples of sliced images without the acceptable quality are presented in the Figure 12.

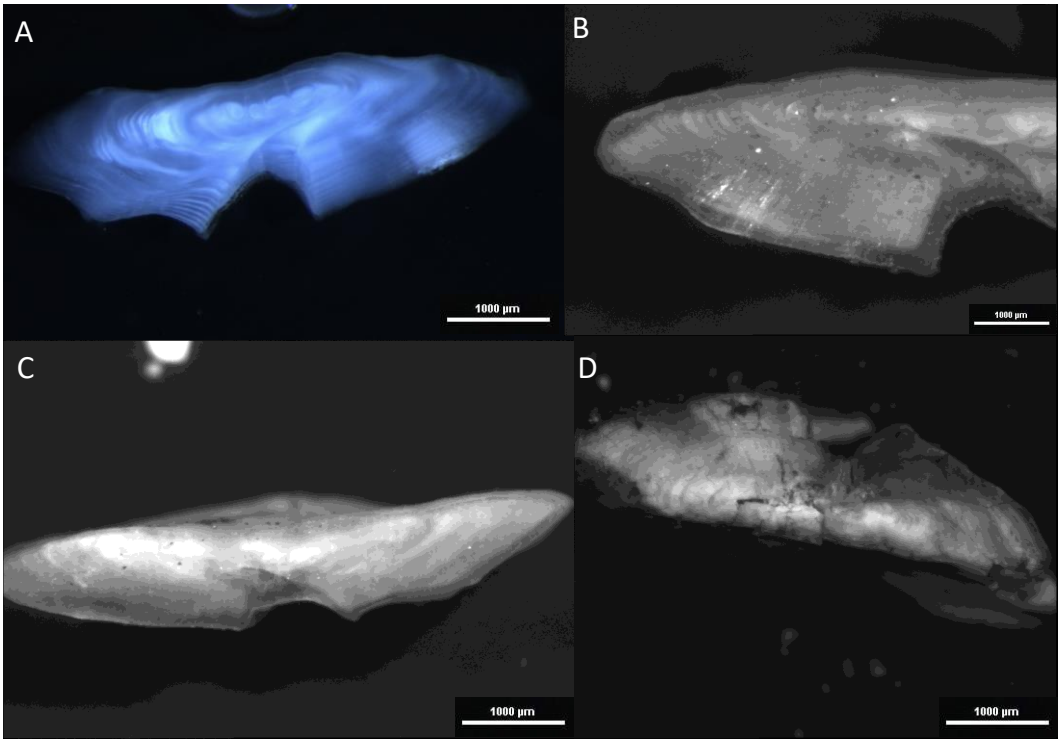


Figure 12. Calibrated image examples of otolith slices of *T. trachurus* without the acceptable quality, the slice was not through the nucleus (A.), the entire sliced otolith was not observable (B.),brightness is set too high (C.) and the preparation is too dirty (D.).

8.2 Mediterranean horse mackerel (*Trachurus mediterraneus*)

8.2.1 Introduction

Mediterranean horse mackerel, *T. mediterraneus* (Steindachner, 1758), is distributed throughout the Mediterranean, the Black Sea, and the north-eastern Atlantic (Tortonese, 1975; Whitehead *et al*., 1986; Fischer *et al*., 1987). Biological data about Mediterranean horse mackerel are very limited for the entire Mediterranean region (Arneri and Tangerini, 1984; Alegria Hernandez, 1988) and information on ageing accuracy is lacking. By contrast, horse mackerel, which is more abundant in the eastern Atlantic, has attracted much more scientific interest (Belan, 1971; Macer, 1977; Farina Perez, 1983; Kuderskaya, 1983; Arruda, 1984; Kerstan, 1985; Venediktova, 1985; Wysokinski, 1985; Arruda, 1987).

In general, horse mackerel otoliths are very difficult to be read in older fish because they become thick with age (Macer, 1977; Alegria Hernandez, 1984; Kerstan, 1985; Eltink and Kuiter, 1989; Karlou-Riga and Sinis, 1997).

Because of ageing difficulties several otolith exchange programmes and workshops have taken place in recent years in an attempt to reach agreement on a common way of annuli interpretation (Eltink, 1985; ICSEAF, 1986; Marecos, 1986; Borges, 1989; Eltink and Kuiter, 1989; ICES, 1991, ICES 2012). Although the age interpretation for horse mackerel has been much improved, for Mediterranean horse mackerel the ageing appears to have many problems.

Similarly to *T. trachurus*, the interpretation of the ageing of *T. mediterraneus* otoliths is difficult, mostly for the older specimens where age determination is particularly imprecise. For the otoliths of *T. mediterraneus* there are specific problems to assign the age

to younger specimens too and in particular to interpret the first two true annulus (Karlou-Riga, 2000), indeed, the characteristic of the detection of a ring around the otolith also on the rostrum zone is not always helpful.

8.2.2 Storage

After the extraction, otoliths are washed in order to remove the organic material, than dried and stored in eppendorf

8.2.3 Preparation and interpretation

The otoliths are analysed to the binocular microscope with reflected light against a black background. The best otolith orientation for the analysis is with the distal surface turned up and the proximal surface (*sulcus acusticus*) down (Figure 13). In this way the dark rings could be counted in the posterior area as translucent growth rings (slow growth). The opaque zone (white – fast growth) plus a dark ring is considered as an annual increment (annulus).

Rostrum region is used as the confirmation area.

Distance from the core to each ring is measured in posterior area (post-rostrum) along a axis from to core to the posterior edge of distal face (Figure 14).

Otolith is immersed in seawater to be analysed. Usually the otoliths of *T. mediterraneus* need the clarification phase before the age analysis. Time depends on the otolith size and on laboratories approach (from 3 hours to 24 hours). To increase contrast between dark and opaque rings, otoliths can be toasted in an oven for minimum 24 hours and 190-200 ºC of temperature, depending the size of the samples, to achieve the best growth rings alternation (Figure 15)

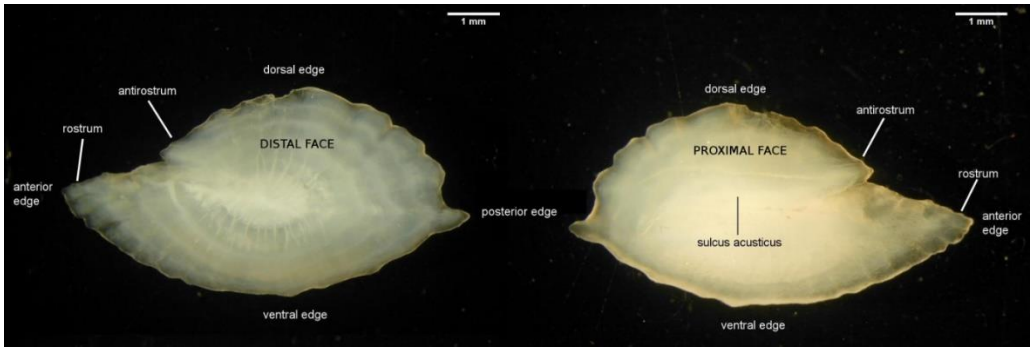


Figure 13. Proximal and distal face of *T. mediterraneus* otolith.

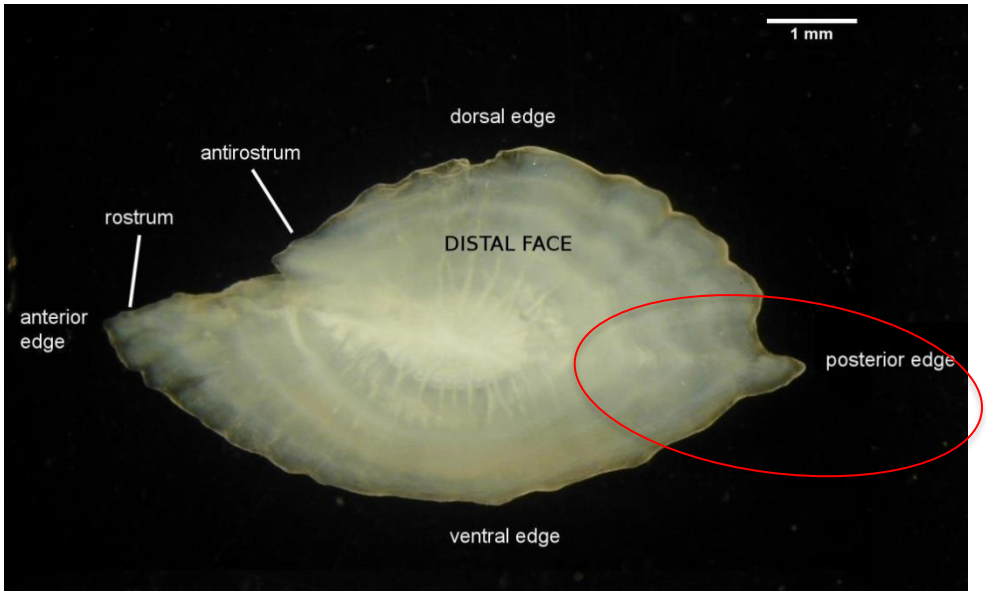


Figure 14. Post-rostrum area is used to calculate distance from the core to each ring



Figure 15. Toasted otolith of *T. mediterraneus*

8.2.4 Ageing

About the ageing criteria, for the Mediterranean areas, the birthday is set at the 1st of July, according with the spawning period (Vietti *et al*., 1997; Karlou-Riga *et al.,* 2000). The criteria to age the otoliths reported in Table 8 take in count the time of annulus formation (generally transparent ring during winter and spring months; opaque area during summer and autumn months), the capture date, the otolith edge and the spawning period.

In the specimens caught during the second part of the year, if a transparent ring is observed at the edge of the otolith it is not counted as annual ring. Indeed, the opaque edge is not still formed, but the birthday is passed. If a transparent ring is observed at the edge of the otolith at the first semester of the year, the ring on the edge it is not considered as annual ring because the birthday is not reached.

For the specimens with opaque edge caught in the first part of the year the age correspond to the number of the transparent rings, though the transparent ring at the edge is not still formed. For the specimens caught in the second part of the year with the opaque edge, the age correspond to the transparent rings (Figure 16)

Table 8. The age interpretation criteria to age the otoliths of *T. mediterraneus* . Birth date: 1 July. N is the number of the transparent rings.

DATE CAPTURE	OTOLITHS EDGE	AGE
1January–30 June	Transparent	N -1
	Opaque	N-1
1July–31 December	Transparent	N-1
	Opaque	N

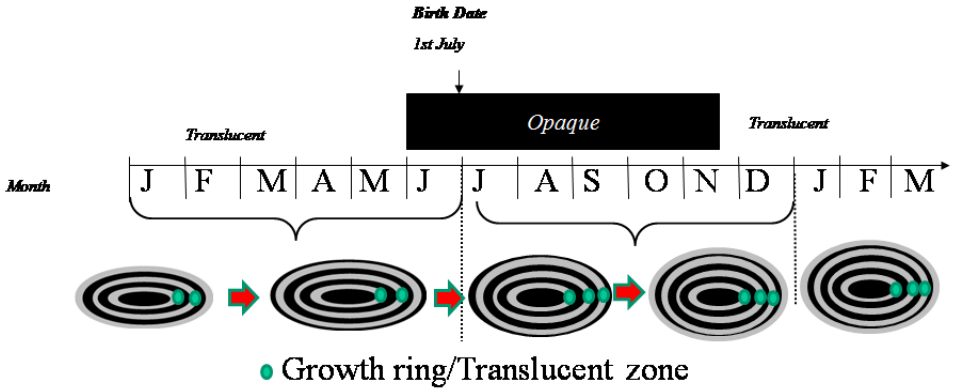


Figure 16. Scheme age for *T. mediterraneus*. Birth date: 1 July

Table 9 reports the criteria to age *T. mediterraneus* considering 1st January as birth date (adopted for Atlantic areas).

In the specimens caught during the second part of the year, if a transparent ring is observed at the edge of the otolith it is not counted as annual ring. If a transparent ring is observed at the edge of the otolith at the first semester of the year, the ring on the edge is not considered as annual ring.

For the specimens with opaque edge caught in the first part of the year the age correspond to the number of the transparent rings, though the transparent ring at the edge is not still formed. For the specimens caught in the second part of the year with the opaque edge, the age correspond to the transparent rings (Figure 17)

Table 9. The age interpretation criteria to age the otoliths of *T. mediterraneus*. Birth date: 1 January. N is the number of the transparent rings.

DATE CAPTURE	OTOLITHS EDGE	AGE
1 January-30 June	Transparent	N
	Opaque	N
1July-31 December	Transparent	N-1
	Opaque	N

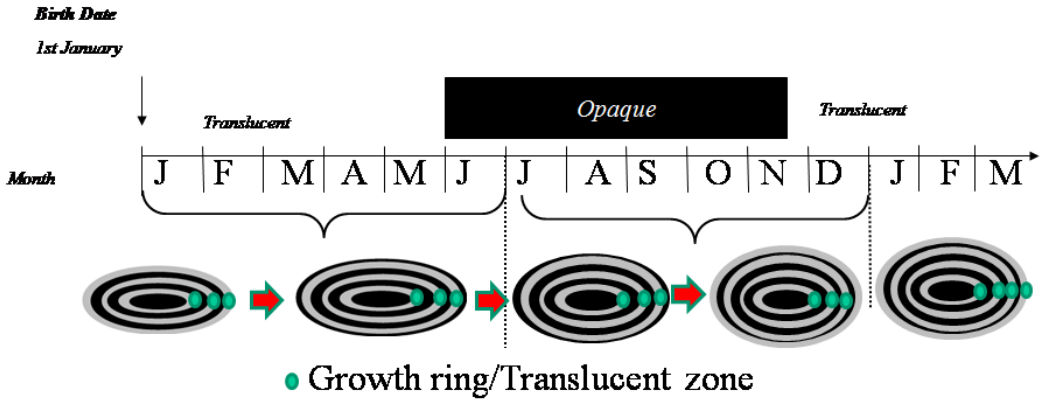


Figure 17. Scheme age for *T. mediterraneus*. Birth date: 1 January

8.2.5 False and true annuli

As reported in Karlou-Riga (2000), before the first winter ring some false rings are laid down. Indeed, the small specimens (5-8 cm) caught during summer and autumn months, from the spring-summer spawning, present a transparent edge (Figure 18). This is a false ring probably laid down when the juveniles changed the environment and the diet.

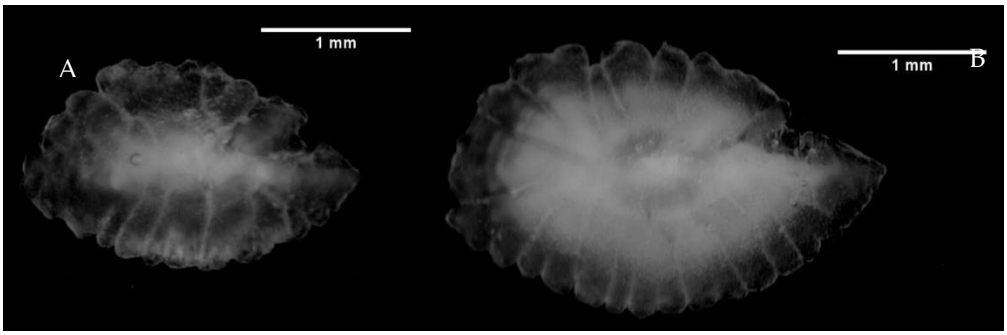


Figure 18. The otoliths from small specimens (A 5 cm and B 7.5 cm total length) caught respectively (A) during the summer (29/07/2011) and (B) the autumn (06/10/2011).

The measure of these otoliths is about 2 mm (0.95 mm radius) and the trace of this false ring at the similar measurement is visible also in the otoliths of older specimens (Figure 19).

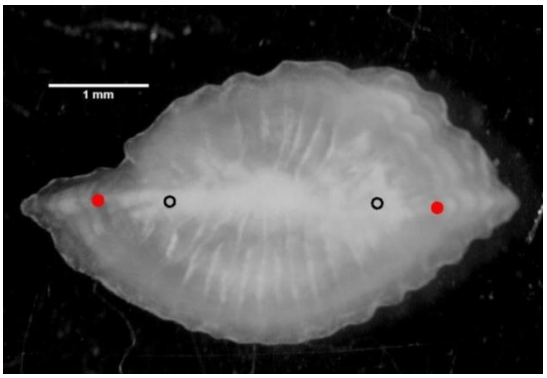


Figure 19. Specimen of *T. mediterraneus* with 14.5 cm of total length caught in the summer (28/07/2011). The open black circle is a false ring the red one the first winter ring

The first winter true ring is laid down subsequently and specimens with total length around 12-14 cm caught in the winter and the early spring months present on the edge a transparent ring more evident than the one before, with a measurement on the radius of about 1.5 mm (whole otolith measure about 3.5 mm) and a false ring close to the edge (Figure 20).

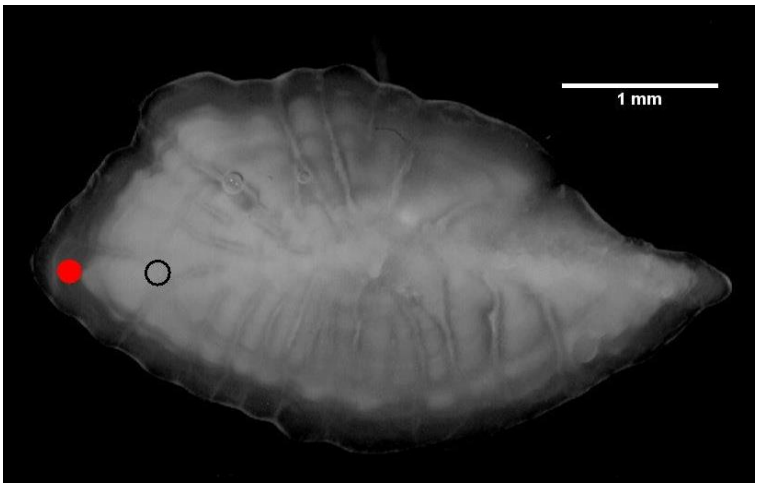


Figure 20. Otolith of *T. mediterraneus* of specimen with 12.5 cm of total length caught in the early spring (12/05/2011). The open black circle is a false ring the red one the first winter ring

Sometimes the first true ring appears not exclusively as a single ring. Indeed, Karlou-Riga *et al*. (2000) distinguish 4 types of otoliths based on the morphology of the first winter ring (Figure 21).

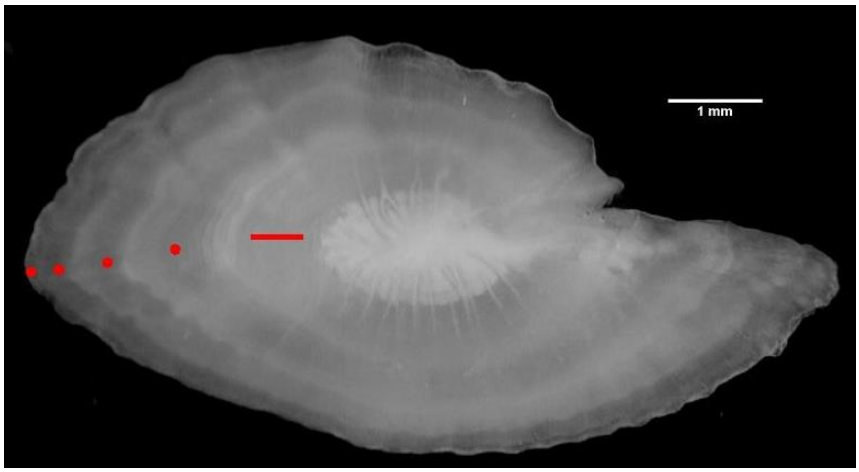


Figure 21. The specimens (female 29 cm total length caught on the 15/05/2011) with first winter ring as a transparent zone because the false rings are jointed with the first true ring. The red spot represent the winter ring; the red line represent the first winter

After the first winter ring, other false ring could be laid down during the second year of life (Figure 22). This could be the trace of the reaching of first maturity. Indeed Vietti *et al*. (1997) report for the North Adriatic Sea the first maturity at 2 years old with 15.6 and 16 cm as the smaller mature specimens respectively for male and female.

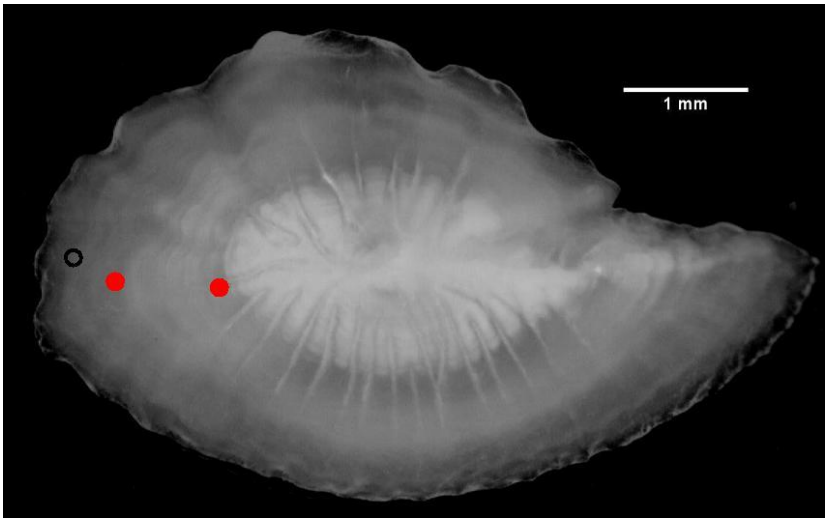


Figure 22. Otolith of a female with 20.5 cm of total length and the gonads in a post reproductive stage caught during early winter (15/10/2011). The open black circle is a false ring the red one the true winter ring.

After the second winter ring, the deposition pattern of the winter band (transparent – black one) appear regular with a reduction of its distance (Figure 23).

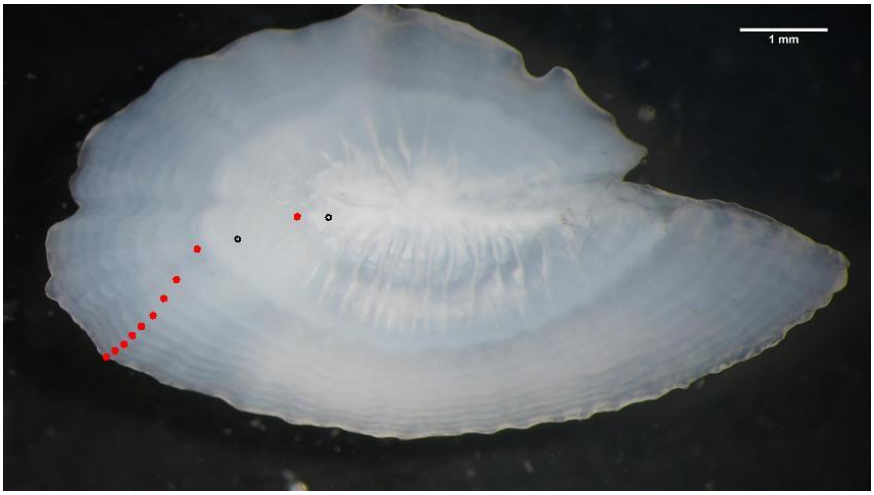


Figure 23. Otolith of *T. mediterraneus* male with 35.5 cm caught in the winter (24/03/2011). The open black circles represent the false rings while the red dots represent the true winter rings

8.3 Blue jack mackerel (*Trachurus picturatus*)

8.3.1 INTRODUCTION

General information about the species, including geographical distribution and biological aspects, was presented in the previous WKARHOM (ICES, 2015).

8.3.2 Whole otoliths

The age determination technique for *T. picturatus* is carried out on whole otoliths. Annuli are counted preferentially on the *nucleus* to the *postrostrum* area (Figure 24).

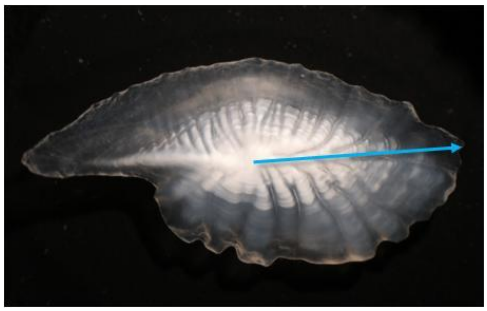


Figure 24. Preferred reading area in the otolith of *T. picturatus*

When possible, both whole otoliths are immersed in distilled water during, at least, 1 hour. Otoliths are observed under a compound microscope with reflected light and dark background with *sulcus acusticus* placed downwards, so dark (translucent/late summer-winter ring) and white (opaque/spring-beginning summer ring) rings could be seen in alternate positions. They are not mounted or embedded prior to the examination because it may be necessary to lift them up and to view them from different angles. It is not recommended to use high magnification (10x). The direction of the light relative to the otolith surface also needs to be varied and the use of transmitted light is not recommended.

8.3.3 Annulus identification

- It is commonly agreed that one opaque and one translucent zone constitute an annual growth zone (AGZ) in blue jack mackerel otoliths (Isidro, 1990; Jesus, 1992; Pereira, 1993; Vasconcelos *et al.*, 2006), being proved that one annulus is laid down yearly (Garcia *et al.*, 2015, Jurado-Ruzafa and Santamaría, *in press*) (Figure 25). It is essential to base the annuli identification consistently in the same criteria for all the otoliths.

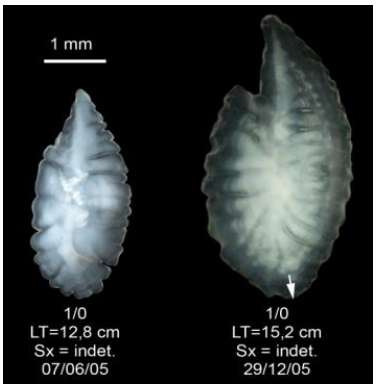


Figure 25. Otoliths of *T. picturatus* corresponding to fish younger than 1 year (Age class 0). Type of edge can be identified (left: opaque; right: translucent). White arrow indicates the translucent ring width.

- In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). Especially for the first annulus, AGZ should be traceable on the whole otolith, with the exception of the dorso-medial surface of the rostrum. However, in most cases, it does not occur.

- In general, the widths of consecutive AGZ decrease with increasing age. Counting annuli after in specimens older than 3 (when growth rate decreases) is more difficult because of an overlap each other.
- It has been identified a high presence of false rings, mainly in the first annulus. Counting each of these well visible rings will result in an overestimation of the age (Figure 26).

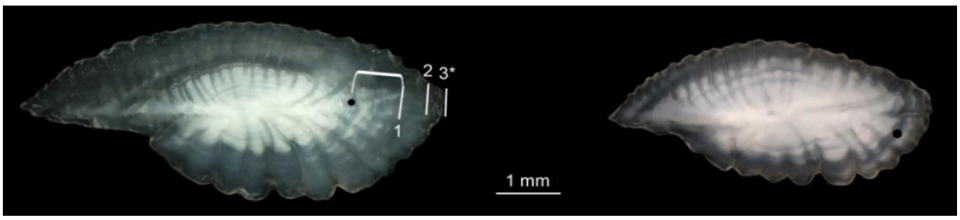
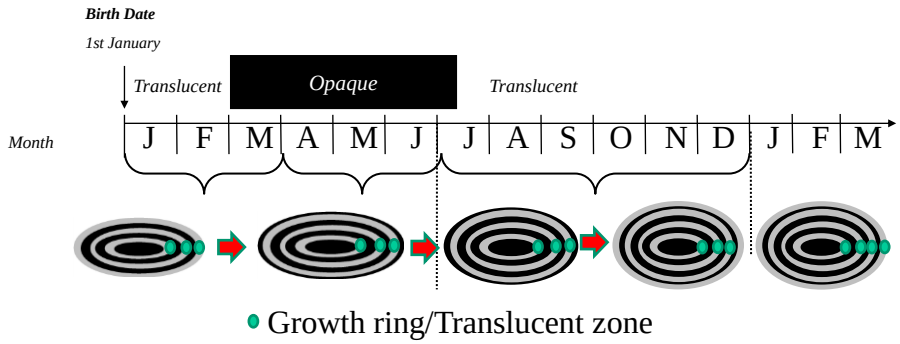


Figure 26. Black dots indicate false rings in the first annulus of *T. picturatus* otoliths from the Canary Islands. Otolith on the left is 3 years old, and the otolith on the right is 1 year old.

8.3.4 Reading criteria

General adopted criteria for the otolith increments interpretation of *T. picturatus* are:

- Birth date: 1st January (based on the reproductive cycle studies, along the Macaronesian archipelagos, the species spawns during winter to early spring (Faria and Vasconcelos, 2008; Jurado-Ruzafa and Santamaría, 2013; Garcia *et al.*, 2015).
- Growth pattern scheme: the age assignment is not only depending on the number of annuli, but also on the edge type related to the catch date and the birth date considered (Figure 27):
    - If fish was caught during the year with an opaque zone on the otolith edge, the age assigned will be equal to the number of rings observed minus one.
    - If fish was caught in the first quarter with a translucent ring on the otolith edge, the age assigned will be equal to the number of annual rings observed.
    - The otoliths with translucent edge of fish caught in the second quarter of the year (*) have to be examined carefully and assessed by the reader, based on the width of this increment. It has to be determined whether this translucent ring corresponds to the finalisation of the annulus of the previous year, or to the new translucent ring of the year.
    - If fish was caught in the second semester with translucent otolith edge, the age assigned will be equal to the number of annual rings observed minus 1.



Quarter	Edge of otolith	Age
1	Translucent Opaque	N N-1
2	Translucent Opaque	N-1*
3-4	Translucent Opaque	N-1

Figure 27. Scheme of the growth pattern considered for otolith age assignment for *T. picturatus*. (*) explained in the text

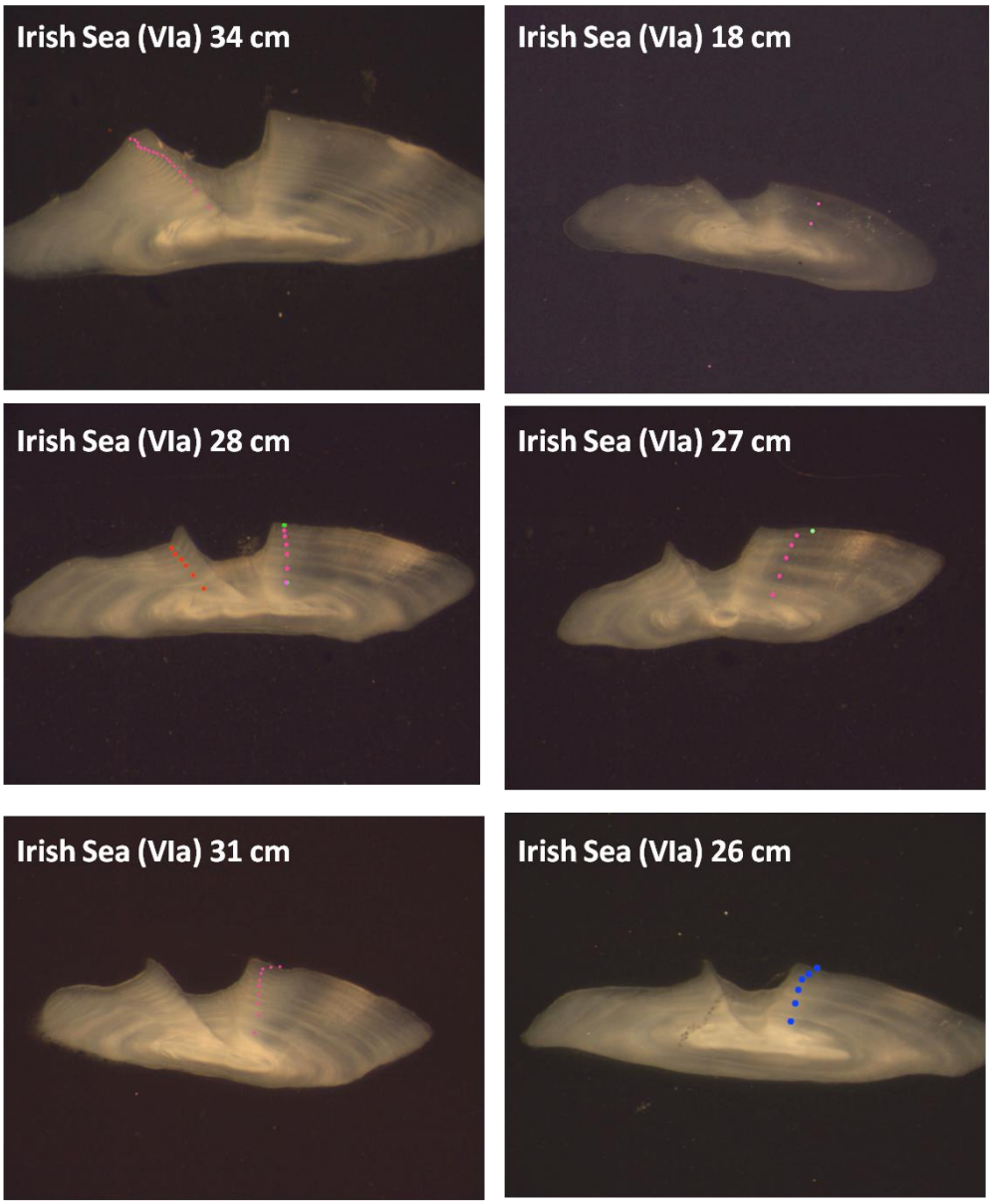
8.3.5 Age determination coherency

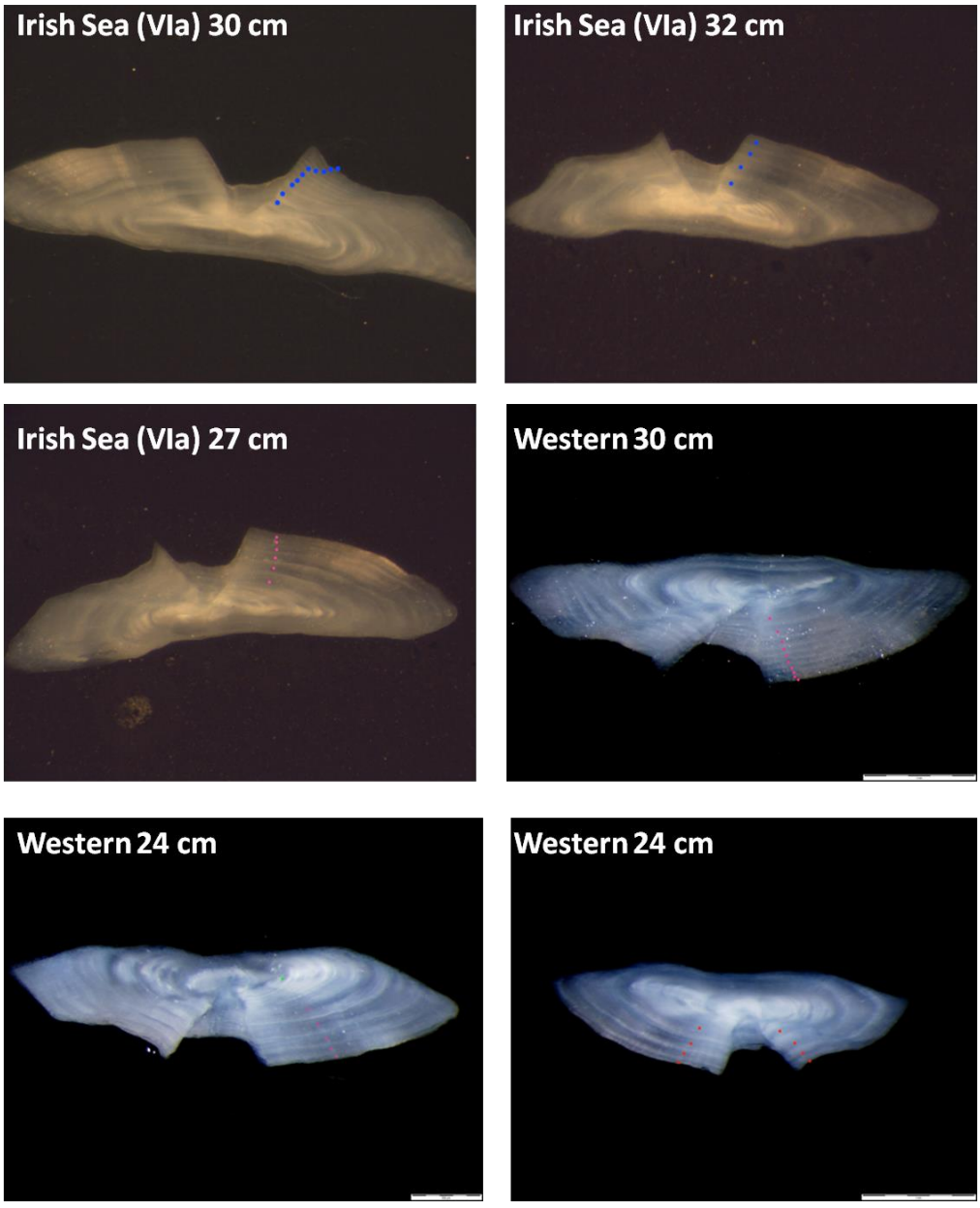
Age determination coherency has been assessed through radii measurement analysis in studies carried out for the species inhabiting waters of Madeira (Vasconcelos *et al.*, 2006) and the Canary Islands (Jurado-Ruzafa and Santamaría, *in press*). In both cases, the frequency distribution of otolith radii by age class and partial annuli radii (respectively) was in agreement with the seasonal regularity of the growth pattern considered for the species.

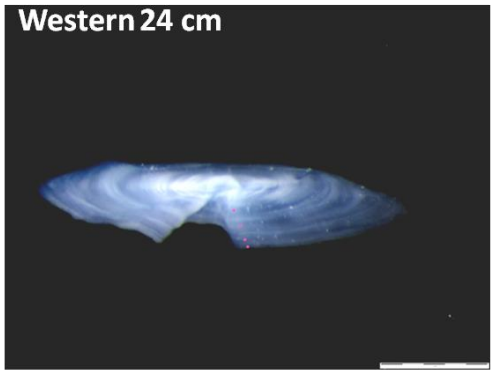
9 Update otoliths reference collections and a database of otoliths images

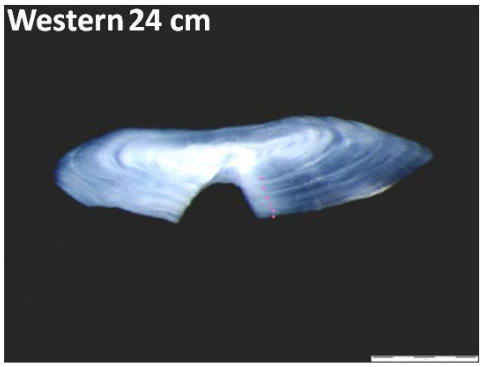
9.1 Horse mackerel (Trachurus trachurus)

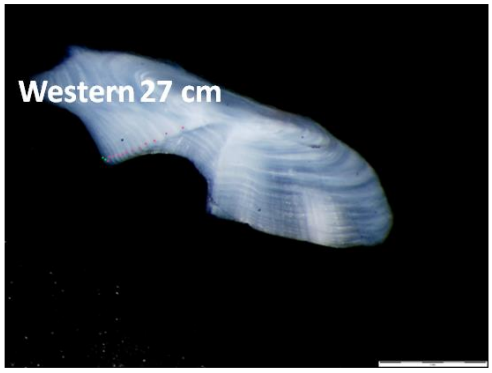
Slices

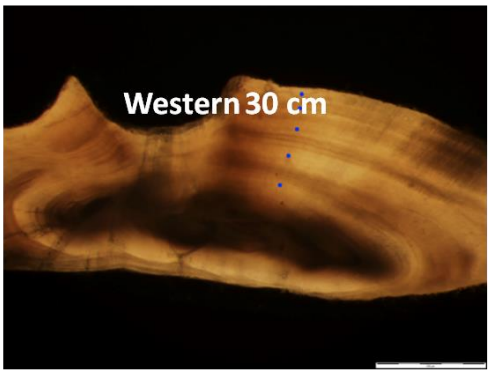


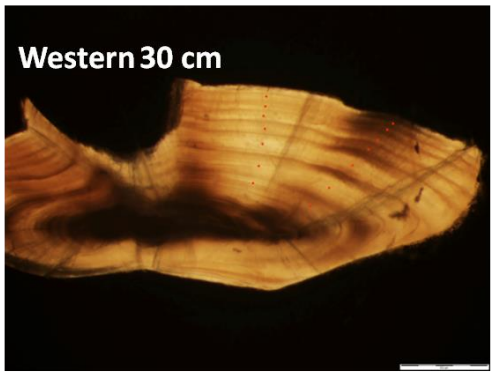

Irish Sea (VIa) 30 cm
Irish Sea (VIa) 32 cm
Irish Sea (VIa) 27 cm
Western 30 cm
Western 24 cm
Western 24 cm

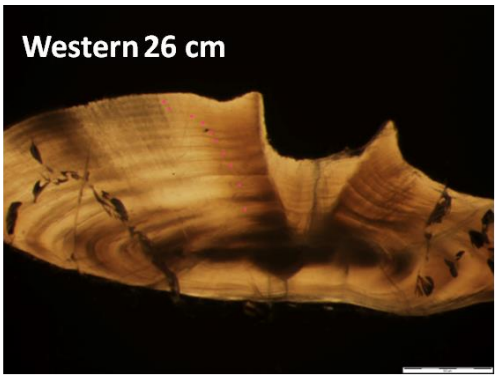

Western 24 cm


Western 24 cm


Western 27 cm


Western 30 cm

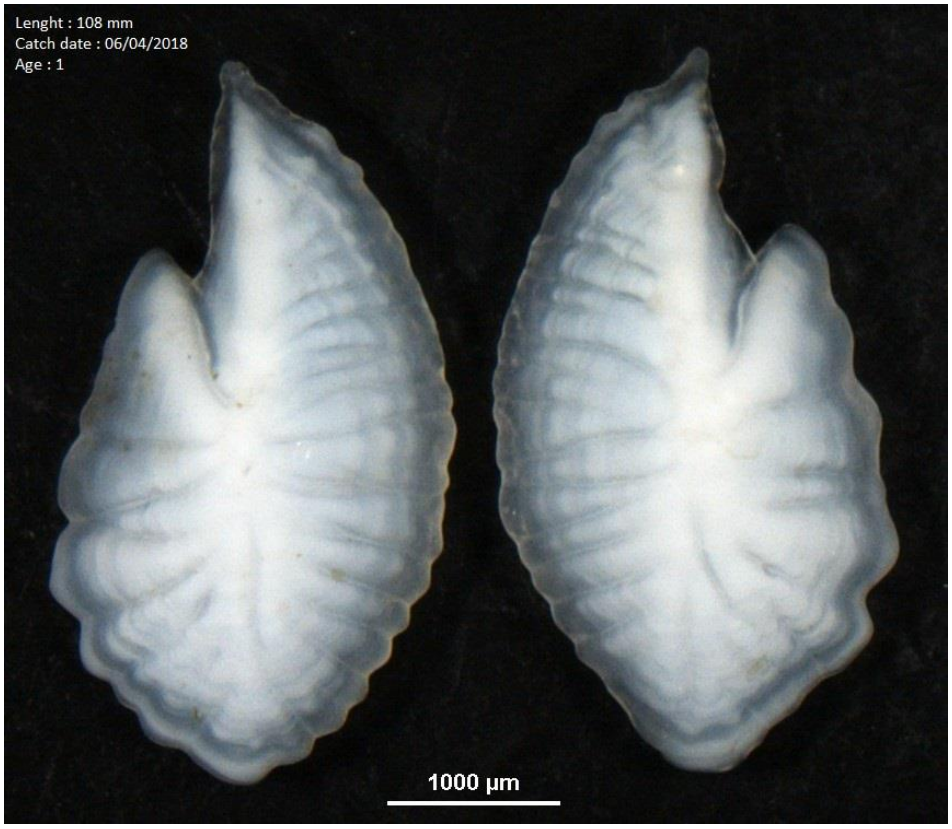

Western 30 cm


Western 26 cm


Lenght : 279 mm
Catch date : 15/01/2015
Age : 7
Area : 6A


Lenght : 260 mm
Catch date : 15/11/2015
Age : 3
Area : 6A

Whole otoliths



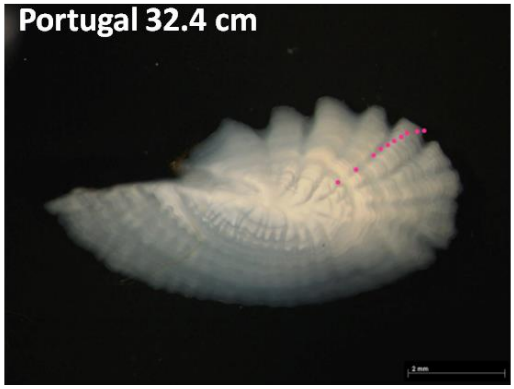


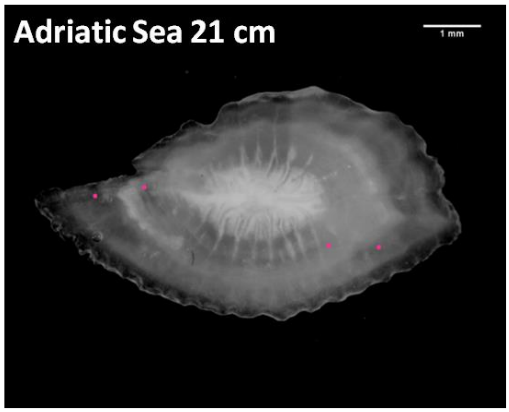

Lenght : 180 mm
Semester : 2
Age : 1


Lenght : 238 mm
Catch date : 08/11/2017
Age : 3
1000 µm

9.2 Mediterranean horse mackerel (*Trachurus mediterraneus*)

Whole otoliths

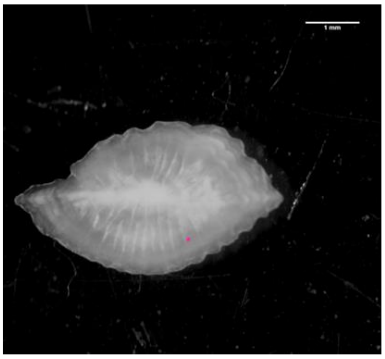




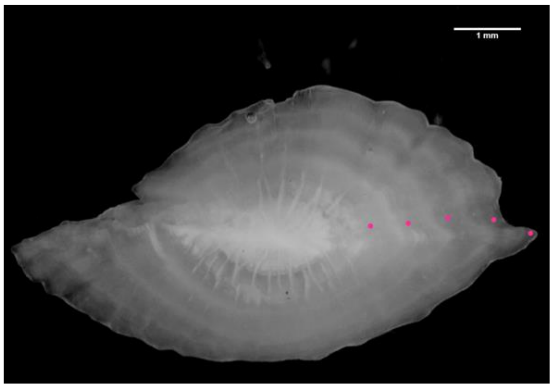
Adriatic Sea 35.5 cm



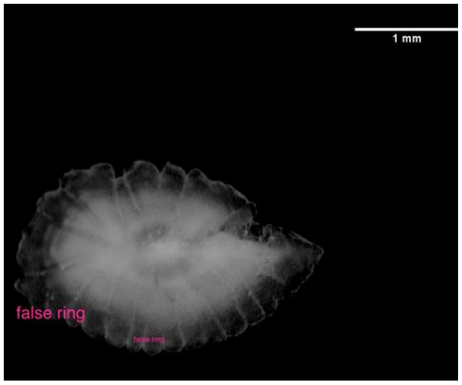
Adriatic Sea 15.5 cm



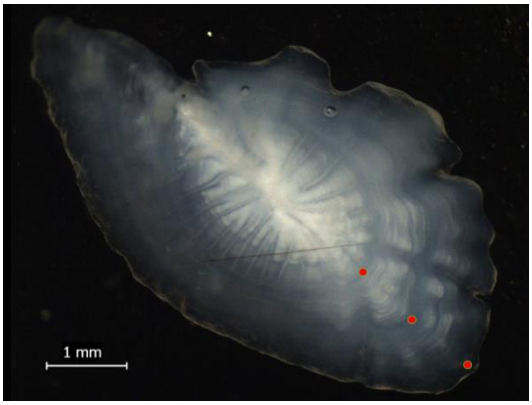
Adriatic Sea 27.5 cm



Adriatic Sea 7.5 cm

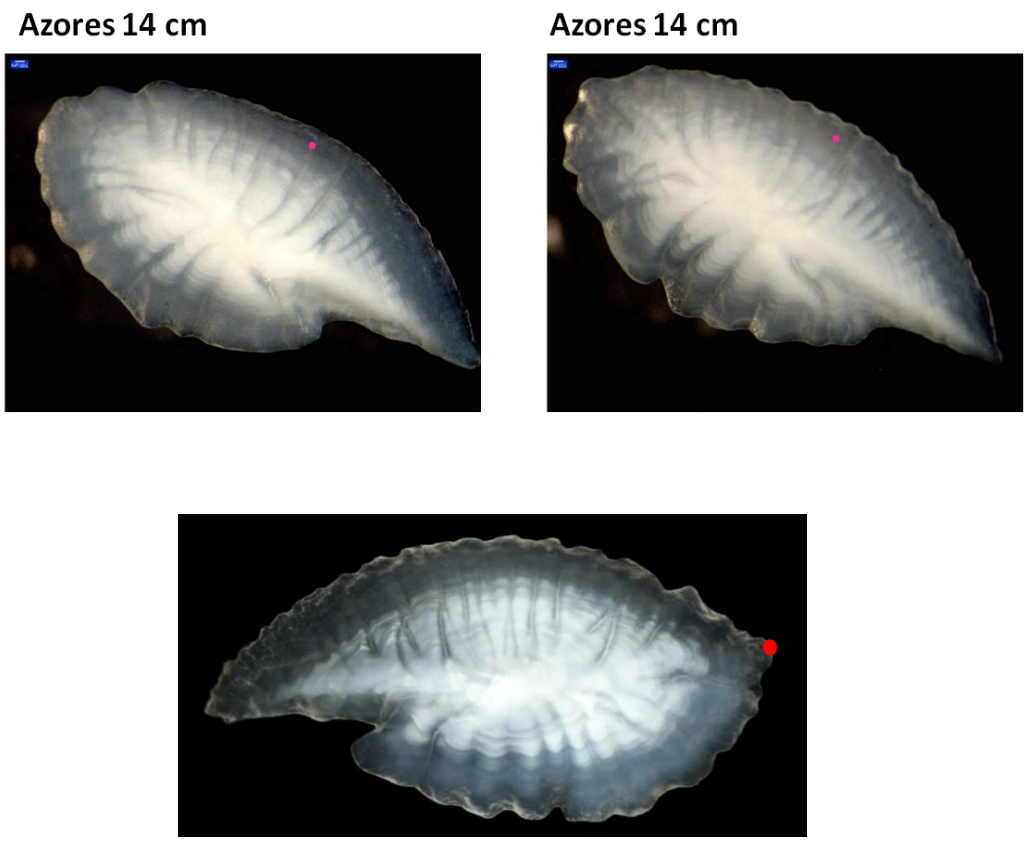


Area GSA 9 – 21.5 cm



9.3 Blue jack mackerel (*Trachurus picturatus*)

Whole otoliths



Canary Islands, Age class 1 (catch month: march). TL: 17.0 cm



Canary Islands, Age class 2 (caught month: september; last annulus is unfinished). TL: 19.8 cm



Canary Islands, Age class 3 (caught month: july; last annulus is unfinished). TL: 24.8 cm



Canary Islands, Age class 4 (caught month: july; last annulus is unfinished). TL: 27.2 cm

10 Recommendations

RECOMMENDATIONS	ADDRESSED TO
WKARHOM3 recommends to develop the SmartDots (see the dedicated section) as the official tool for the exchange and for training purposes on the *Trachurus* species	WGBIOP; WGSMART
WKARHOM3 recommends the new full exchange in 2021 for the *T. trachurus, T. mediterraneus, T. picturatus*	WGBIOP
WKARHOM3 recommends to implement the validation study of ageing (semi-direct, indirect methods) in order to solve the inconsistency for the ageing analysis on the species addressed (first growth increment, false rings)	National Ageing Coordinators
WKARHOM3 recommends to use the updated guideline and reference images by species for the ageing analysis	National Ageing Coordinators; WGBIOP
WKARHOM3 recommends to only use calibrated images (with calibration bar, pretreatments of images could induce bias to different sized otoliths), as this was not adhered to in the current exchange.	National Ageing Coordinators, WGBIOP

11 Recommendations for the next Exchange of *T. trachurus*

- 1st of January is the used birthdate in all areas
- 200 otoliths of *Trachurus trachurus* for the next exchange
- Approximately 100 otoliths from Atlantic Sea and 100 otoliths from the Mediterranean Sea
- For the Atlantic, we request 40 images from the following areas:
    - ICES Areas 8 and 9: 20 whole otoliths (<27 cm fish length), 20 sliced (>27 cm fish length)
    - ICES Areas 6 and 7: 40 sliced otoliths
    - GSA 20 and 22: 40 whole otoliths
    - GSA 9, 10, 18, and 19: 20 whole otoliths (<30 cm fish length), 20 sliced (>30 cm fish length)
- Of these images, half should be from the first semester and the other half from the second semester.
- Various fish lengths from each sampling area is recommended, fish sex information is not necessary
- Before photographing, sliced otoliths should be treated with baby oil, and the whole otoliths should sit in water for 3–24 hours
- The magnification should be a set value for all submitted images regardless of otolith size
- Only high quality images should be submitted to the exchange
- Each reader chose his reading axis

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Annex 1: Agenda

ICES WKARHOM3 - 3rd Workshop on Age Reading of horse mackerel, Mediterranean horse mackerel and blue jack mackerel (*Trachurus trachurus*, *Trachurus mediterraneus*, *Trachurus picturatus*)

The meeting will start at 14.30 of 5th November 2018

and will end on 9th November at 13.00

Meeting place: CIBM Scoglio della Regina viale Italia 6

Livorno Italy

Monday 5th November 2018

14.30-15.00

Welcome of the participants, Round Table of participants and Approval of the agenda

15.00-16.00

Presentation from each lab/participant about biology, otolith preparation methods, ageing criteria and ageing scheme

16.00-16.30 Coffee break

16.30-17.00

Presentation from each lab/participant about biology, otolith preparation method, ageing criteria and ageing scheme

17.00-18.00

ToR a: Validation studies input from participants

Tuesday 6th November 2018

9.30-10.30

Presentation of Exchange results

10.30-11.00 Coffee break

11.00-12.00

Discussion of results on Exchange exercise

12.00-13.30 Lunch break

13.30-15.00

ToR b: Clarify the position of the first annulus with the images analysis for the three species

15.00-16.00

ToR c: Evaluate the effect of different schemes of ageing, particularly the date of birth for *T. mediterraneus*

16.00-16.30 Coffee break

16.30-18.00

ToR c: Evaluate the effect of different schemes of ageing, particularly the date of birth for *T. mediterraneus*

Wednesday 7th November 2018

9.30-10.30

Reading exercise

10.30-11.00 Coffee break

11.00-12.00

Reading exercise

12.00-13.30 Lunch break

13.30 – 16.00

Reading exercise

16.00-16.30 Coffee break

16.30-18.00

ToR d: Continue the guidelines and common ageing criteria (work in subgroups: *T. trachurus, T. mediterraneus, T. picturatus*)

Thursday 8th November 2018

9.30-10.30

ToR d: Continue the guidelines and common ageing criteria (work in subgroups: *T. trachurus, T. mediterraneus, T. picturatus*)

10.30-11.00 Coffee break

11.00-12.00

ToR d: Continue the guidelines and common ageing criteria (work in subgroups: *T. trachurus, T. mediterraneus, T. picturatus*)

12.00-13.30 Lunch break

13.30-15.00

Plenary session

15.00-16.00

Presentation and Discussion on the results of 2nd reading

16.00 - 16.30 Coffee break

16.30-17.00

Presentation and Discussion on the results of 2nd reading

17.00-18.00

ToR e: Develop existing reference collections of otoliths

Friday 9th November 2018

9.30-10.30

Plenary session

Recommendations and conclusion

10.30-11.00 Coffee break

11.30-12.00

Planning of future work

Any other Business and meeting closure

Annex 2: Review of procedures for otolith preparation and analysis

Trachurus trachurus

Portugal

IPMA

10 individuals by length class are selected for complete biological sampling in the main ports of landing this species: North -Matosinhos, Central - Peniche and South-Portimão. In the case of scientific surveys, 10 individuals are analysed per length class (5 males and 5 females) and by coast area (North, Centre and South). In both cases, biological sampling includes the extraction of the otoliths. After collected otoliths are washed with freshwater and clean to remove any remains of organic tissues that may hinder your observation.

Each pair of otoliths is then stored in a paper bag properly identified with the sample number, place and date of collection, the number of sampled, the length of the fish, the sex and the state of maturation.

Subsequently, the otoliths selected for reading from fish with total length ≤ 26cm are dipped in water for a period at minimum 24 hours to highlight the rings. After that, they are observed in the baby oil (before clean with a soft paper) under a binocular microscope, with reflect light against a dark background (reflected light opaque areas appear white and the hyaline areas appear dark.)

The otoliths of horse mackerel with total length > 27cm are cleaned with alcohol and marked with a pencil trace in its nucleus. Are positioned in a perforated plate, in the order which will occupy on each line of the mould where will be placed with the inside face down in the moulds specially prepared for the effect containing approximately 42 otoliths. In order to keep a record of the exact origin of the otoliths, a sheet of reading ages is filled (Figure a2.1), as these are placed on the board.

SPECIES _____ _____ TRAINER _____

BLOCK N° _____ READER _____

DATE OF INSTALLATION DATE OF READING

#/PORTO	DATE	NOBS	CT	BLOCK	QUEUE	POSITION	AGE	BOARD	OBS.
					1	1			
					1	2			
					1	3			
					1	4			
					1	5			
					1	6			
					1	7			
					2	1			
					2	2			
					2	3			
					2	7			

Figure a2.1. Partial Aspect of a record sheet and ages of Otoliths

The next step is mounting in epoxy resin (*Epoxy Resin SP106*®). An anti-adherent substance is applied to interior surfaces, so as to facilitate subsequent removal of resin blocks (Figure a2.2).

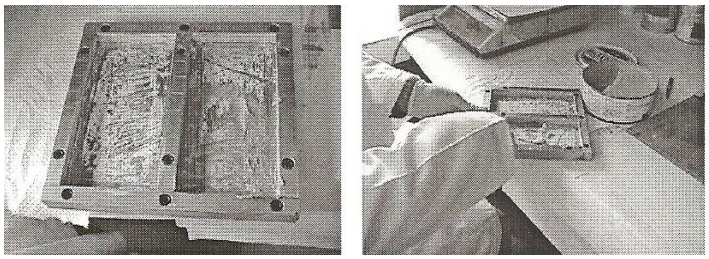
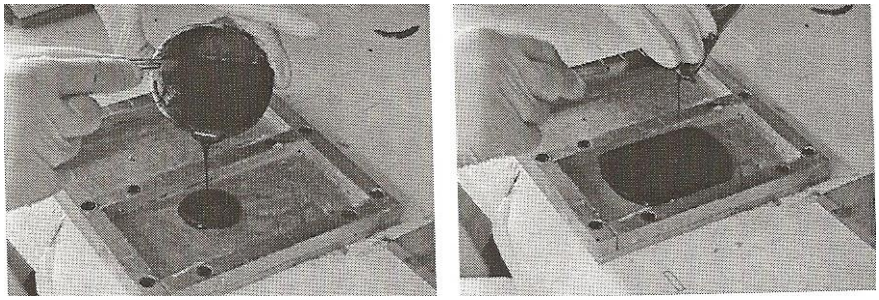


Figure a2.2. Non-stick application in the mould (taken from the manual preparation of otoliths of hake)

To prepare a first layer of resin (resin 20gr, 4gr of catalyst, 0.6gr dye black), to be made about 12–24h in advance, in order to allow for their total drying and prevent the otoliths sinking (Figure. a2.3).



Figurea2.3. Filling the mould with resin (taken from the manual preparation of otoliths of hake)

The preparation of the second layer of resin (10gr resin, catalyst, 2gr 0.3gr dye black) is made on the day that assembling of the otoliths, and is poured over the first ever made and dry. This layer acts as a glue to attach the otoliths and you have to pay special attention to the drying time of this layer (approximately 1h after preparation, but it depends on the room temperature and air humidity), when going from the viscous liquid resin, will start the assembly of otoliths.

Two spaghettis pasta are then placed in the mould to act as a reference in the identification and placement of otoliths (Figure a2.4). The first tag is placed in parallel and as close as possible to the right side of the mold and the second, joining the upper left and lower right.

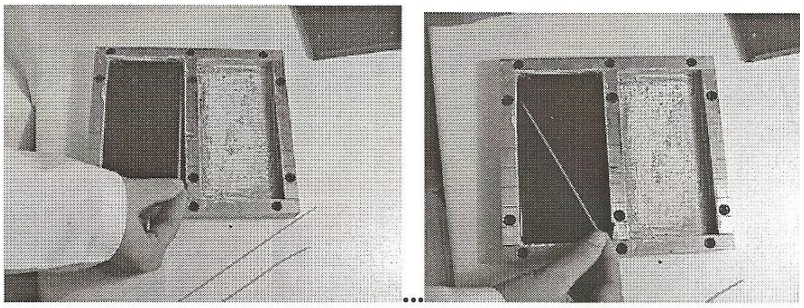


Figure a2.4. Inserting the reference mould marks (taken from the manual preparation of otoliths of hake)

The placement of the otoliths in each row, follows the order recorded on the sheet. This step is performed with the help of a camera attached to a monitor that lets you view the correct positioning of the otolith (Figure a2.5).

During assembly whenever necessary, the otoliths should be tying up so that they are in a straight line.

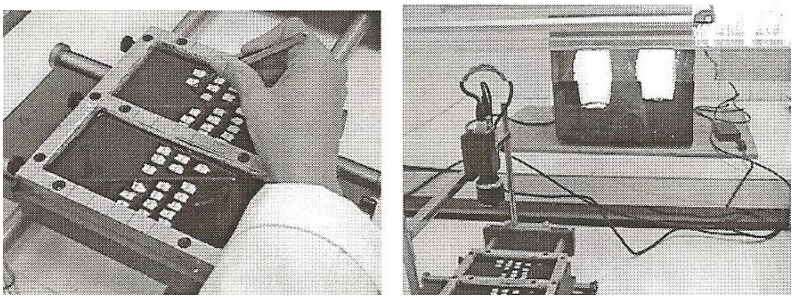


Figure a2.5. Monitor as supporting the placing of otoliths in the mould (taken from the manual preparation of otoliths of hake)

The preparation of the third layer of resin (30gr resin, catalyst 6gr) serves to cover all otoliths after they have been positioned and addition of the dye is dry around of 24h.

It is extremely important that the core stay perfectly aligned and centered on each line in the template, in order to ensure that the section passes at the core.

After dry and hardened, the resulting resin blocks are cut on a cutting device *Labcut250 Abrasive Cutting Machine,* fitted with a disc of high hardened rotation with diamond dust.

Each block is placed on the machine and cut separately and the transparent resin surface facing up.

The relative placement of each row of otoliths to the disk is carried out through a sight on the prop table (Figure. a2.6), three cuts per line (one before, another at the core level and the third, later) to ensure getting a cut passing through the core of the otolith.

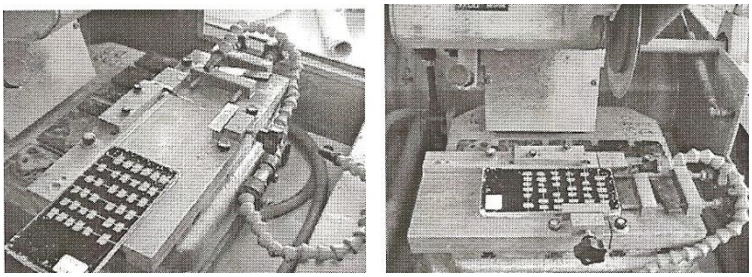


Figure a2.6. Installing the block cutting machine (taken from the manual preparation of Otoliths of hake)

The sections obtained are then glued on sheets of glass 26x76 mm with glue *DPX mountant for Histology* (Figure a2.7) and observed with binocular magnifier under transmitted light.



Figure a2.7. Sections glued on glass slides

Norway

IMR

Sampling and storing of the otoliths

The Norwegian horse mackerel fishery includes three stocks: western, southern and North Sea. We receive a small quantity of samples (6-10) the past few years from fish landings. Of these, about 300 otoliths are read every year, 30 specimens from each sample. Sampling is conducted in the last quarter of the year. Both sagittae otoliths are collected from each fish. They are washed thoroughly immediately after collection in order to remove the organic material from the surface and are left to dry for at least 24 hours.

Equipment and preparation of the otoliths

Before the reading one of the otoliths is broken transversely across the short axis through the nucleus. The fractured surface of the anterior half of the broken otolith is polished using wet sandpaper, nr. P600. The rostrum is broken off and the polished part is then burnt over a Bunsen burner for a few seconds while constantly in motion. To clarify the ring structure, these otoliths are carefully charred until darkish brown (Møllller Christensen, 1964) (see photos below). The treated otolith is mounted in plas-

ticine and brushed with baby oil on the break. The otoliths are read under a stereomicroscope using direct light, preferably an intensive cold-light source. The translucent rings in the burnt otolith are counted in the large ventral lobe near the *sulcus acusticus*.







Quality control

The otolith quality is given a rating of 1, 2 or 3; with 1 referring to a legible otolith and age can be given with certainty, 2 refers to age is given with some uncertainty, and 3 referring to an illegible or missing otolith and, thus, no age is given. Otoliths given legibility quality 1 and 2 are used in stock assessment. There is a manual for otolith preparation, but not for reading the age. There is currently one experienced *T. trachurus* reader at the IMR and a second reader is currently in training.

The Netherlands

Wageningen Marine Research

Samples

- Samples from commercial trawlers, they take the sample right out of the total catch.
- Samples from ICES areas IV, VI, VII, VIII.
- 1 sample is between the 20 and 25kg, that is up to more than 200 fishes.
- All fishes are measured to the cm below.
- Out of that sample there will be picked 25 fishes to get gutted.
- The 25 fishes are represented for the whole sample.

The cutting

- Measuring in mm.

- Weight in gram.
- Male or female.
- Take both of the otoliths
- Otoliths are cleaned in freshwater and stored dry

Making the otolith readable

- The otolith is embedded in black polyester raisin block.
- Than it is cut in the middle to make a slide
- The slide is now in the black polyester so we have a black background
- The black stroke (which contains 5 or 10 otoliths) is glued to a small plate of glass
- Each glass plate contains 25 otoliths.
- The plates are stored in a hard covered box

The reading

- Under a binocular, magnification maximum 38.
- The black polyester strokes with the otoliths in them are oiled with baby oil.
- There is a separate reflecting light source.
- The otoliths is given a birth year as well as a quality number
- Quality 1 = sure of the age
- Quality 2 = not sure of the age
- Quality 3 = not readable, no age, this is not going in to the ices assessment groups.

Ireland

Marine Institute

Area: ICES areas VI and VII

Otolith Preparation

- Both otoliths are removed washed, dried and stored in plastic trays
- Otoliths from all sizes of fish mounted in resin and sectioned to 0.5mm sections
- Full mounting and sectioning method outlined in WKARHOM2 2015 report

Reading Procedure

- Sections are brushed with baby oil then read using reflected light at magnification 30–35X

Digitizing Image Procedure

- Otoliths are viewed and aged using a camera and monitor but no digitizing is used unless for age training or an otolith exchange

Quality Assessment

- Primary age reader reads all the otoliths from commercial and survey samples
- Second and Third age readers currently being trained read 20% of Primary age readers survey readings
- After more training 2nd and 3rd age readers will move on to read 20% of the commercial samples and will then graduate to reading a % of otoliths allocated by the primary reader each year
- Age QC % agreement produced and age differences are discussed and agreed between readers

Validation techniques

- No validation studies have to date been carried out on this species.

Greece

Fisheries Research Institute (FRI)

Sampling

The annual sample goal for *T. trachurus* is 400 specimens, only from GSA 22 (Aegean Sea), while for *T. mediterraneus* the annual goal is 800 specimens from GSA 22 and GSA 20 (Eastern Ionian Sea). The annual sampling obligations are, whenever it is possible, equally divided between Fisheries Research Institute (FRI) and Hellenic Center for Marine Research (HCMR). The sample is also temporally stratified, by distributing the sample uniformly through the four annual quarters. The major proportion of the sample is collected from the commercial catches and discards of bottom trawls and purse-seines, by on board observers. A minor proportion of the sample is originated from experimental surveys (MEDITS etc.) and rarely from small scale fisheries. 12 sagittae otolith pairs are collected by 12 specimens per length class (length class range: 10mm). Although the otoliths are taken by a random subsample per length class, the collection aims to cover as many length classes per quarter as it is possible.

Storage

In laboratory, both otoliths are extracted. They are washed in freshwater, cleaned from organic material, dried and stored in Eppendorf tubes.

Equipment and preparation

The preparation for photographing the otoliths is being done under the same protocol for *T. trachurus* and *T. mediterraneus*. The otoliths pair is immersed in solution of freshwater and 70% alcohol. The whole otolith is photographed by a Nikon SMZ18 stereomicroscope (Figure a2.8) with magnification 1x or 2x, using direct intensive light against a dark background.



Figure a2.8. The Nikon SMZ18 stereomicroscope used to take pictures in FRI

Interpretation

The images are imported in Image Pro Plus version 4.5 Software for age reading. The orientation of the otolith is horizontal. The length from the nucleus to the edge of the otolith as well as the length from the nucleus to every annual ring is measured and the annual rings are marked with points (Figure a2.9). It has to be noted that for these species the translucent growth zone represent the slow growth period of their annual life cycle. Date of birth for *T. trachurus* is defined the 1st of January and for *T. mediterraneus*, the 1st of July according to WKARHOM 1 and 2 reports. Particular attention is given to the type of zone that is found in the edge of the otolith as well as to the capture date. Finally, the estimated age is imported into the database in the form of N+.

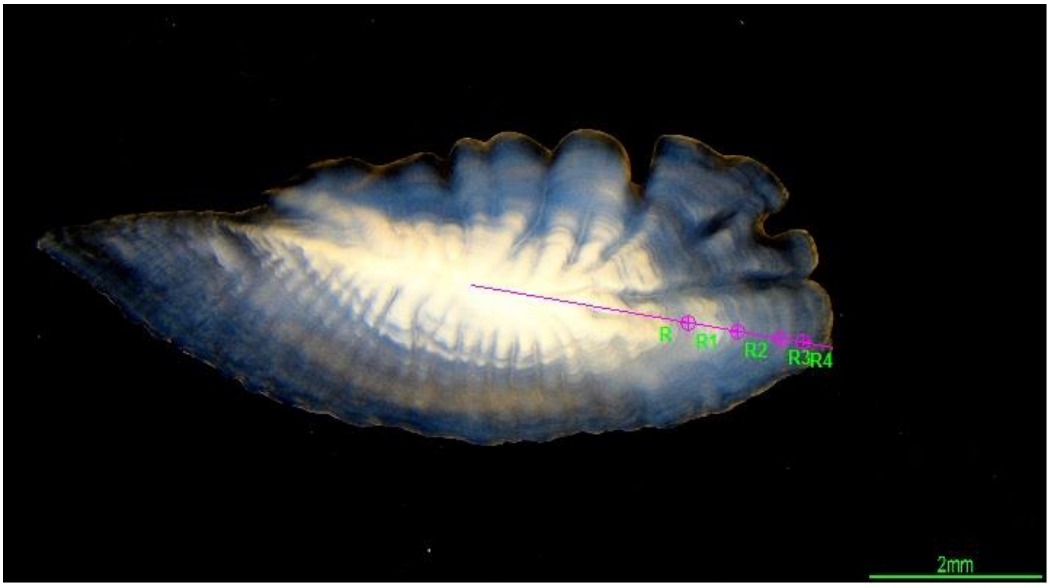


Figure a2.9. Imported image into Image Pro Plus software

Quality Control

FRI is exclusively responsible for the age reading of *T. trachurus* and *T. mediterraneus*. FRI has 2 readers for each species with high and medium experience. The otolith sets

are read by the readers and their results are analysed and discussed. FRI doesn't apply any method for age validation due to incomplete time-series. No manual for age reading is available on FRI.

Spain

IEO-Santander and Vigo

Sampling

Horse mackerel is being monitored for the Data Collection Framework since 1982. Stocks and sampled geographical areas are presented in Figure a2.10.

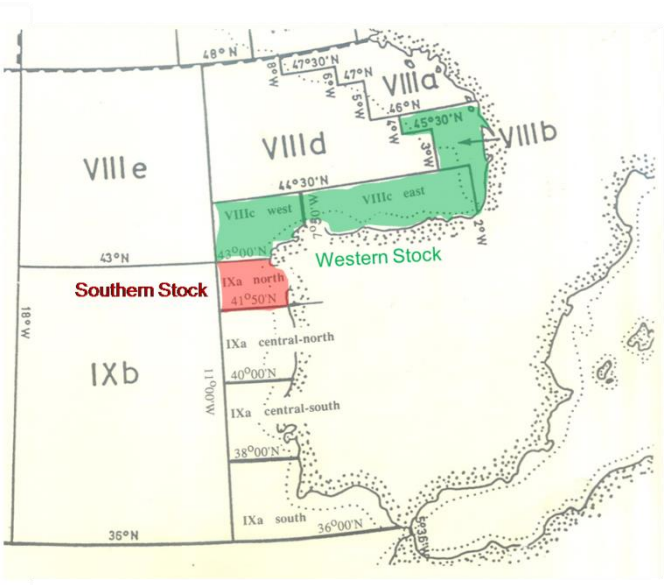


Figure a2.10. *T. trachurus* southern stock (ICES Division 9a North); *Trachurus trachurus* Western stock (ICES Subdivisions 8c East and 8c West)

Calcified structure (otoliths) preparation

Otoliths are extracted from each sampled fish and stored in plastic microtubes (until 2011 pink envelopes were used).

For fish smaller than 26 cm, both otoliths are placed, in freshwater, seawater, distilled water or in alcohol with glycerin, to read them under a microscope (Nikon SMZ1500).

For fish equal or bigger than 26cm, the whole otoliths are embedded in polyester resin (pre-accelerated polyester resin, black colorant and catalyst) in an aluminium mould. The resin blocks containing the embedded otoliths are removed from the moulds and cut into thin sections (0.5 mm) following the dorso-ventral plane of the otolith.

The cutting machine (Figure a2.11) is a high speed saw machine that permits to obtain multiple sections (OTO-LABCUT 230F Floor Standing Abrasive Cutting Machine complete with sectioning fixture and diamond wafering blade, Benetec Limited.). The resulting sections are stuck in glass slides, with the proper label (Figure a2.12).



Figure a2.11. OTO-LABCUT 230F Floor Standing Abrasive Cutting Machine



Figure a2.12. Resulting labeled sections

Digitalization, processing and analysis of images

Images processing and analysis is used for validation studies and for otolith exchanges. Images are taken with a camera Nikon - Digital Sight DS-5M -12V.5V.18V (TV lens 0.55x DS Nikon) using the image analysis software NIS-Elements-D 3.0 in several formats (Figure a2.13): TIFF files (*.tif), JPEG files (*.jpg), Also jp2 (jpeg2000) files, JASC PAINT SHOP PRO (*.psp).



Figure a2.13. Digitalization system used in the laboratories of Santander and Vigo of the IEO (left), and an example of an image of a sections otolith (right).

Quality control

A specific experienced reader ages the otoliths twice, on two separate occasions. The readings for a given otolith are accepted only if they are coincident. When there is a discrepancy between the two readings, a third reading is carried out. Unreadable otoliths are rejected.

Ageing criteria described in ICES Workshops are followed (ICES, 1999; 2006; 2012 and 2015).

An age length key is elaborated by month, quarter, semester and year. Mean lengths-at-age and the corresponding standard deviations are calculated.

Germany

Thünen Institute of Sea Fisheries in Hamburg, Germany (vTI-SF)

Sampling and storing of calcified structures

Horse mackerel from commercial catches and surveys are sampled by the Institute of Sea Fisheries within the EU data collection framework. Sampling takes mostly place in the first and the fourth quarter of the year. Usually, of every 1cm length class 10 sagittae otolith pairs are taken during the trips by ICES division and stored in paper envelopes. Each envelope is labelled with cruise number, station, area, fish species, length, weight, sex, maturity and catch date.

In the laboratory one otolith is used for slicing whereas the other otolith is stored dry in Eppendorf tubes. After reading the sliced otolith sections are glued on glass plates which are then also stored. Altogether, 800–1000 horse mackerel otoliths are taken and read on a yearly basis by Germany.

Equipment and preparation of calcified structures

Usually, every left otolith is taken for slicing. The right otoliths are stored in Eppendorf tubes in boxes with all haul and fish information (see paragraph above). Each left otolith is prepared by marking the nucleus with a pencil (see Irish description for details).

Under a fume hood a rubber casting mould with marks for the otoliths is half filled with epoxy casting resin (GTS polyester–resin (VossChemie, 35–40% Styrol) with 6ml MEKP-hardener). Depending on the size of the otoliths up to 50 otoliths can be embedded in one mould. The otoliths are laid onto the resin bed according to their marks. To fix the otoliths this is done before the resin is completely hardened. Then the casting mould is filled completely with resin. After hardening of the resin the block with the embedded otoliths is placed into a device for series cuts (Figure a2.14).The block is then halfautomatically cut by a wet abrasive cut-off machine producing 0.5mm thick slices through the nucleus of the otoliths (Figure a2.15).

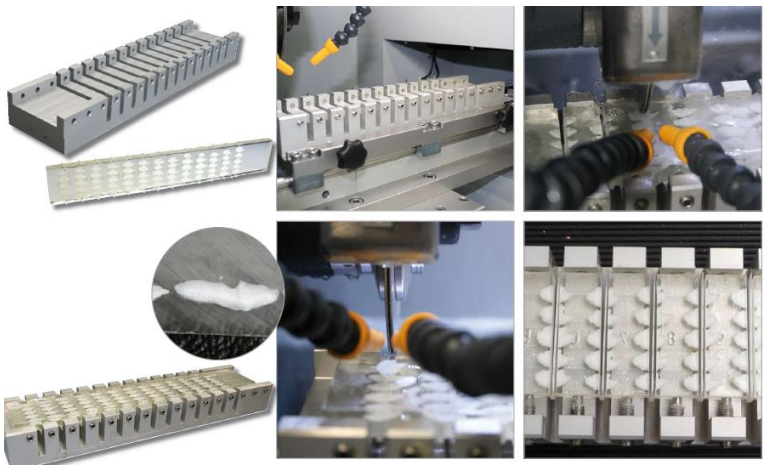


Figure a2.14. Device for series cuts of otoliths (Picture by ATM GmbH)



Figure a2.15. Otolith sawing machine used by the Institute of Sea Fisheries

The slide is interpreted under binocular with the black background.

Quality control

Germany has one experienced *Trachurus trachurus* age reader and is currently training a second. The experienced age reader has participated in several international otolith validation exchanges and workshops. Detailed manuals of each processing step (sampling on commercial vessels, embedding the otoliths in resin etc.) are available in German language.

Italy

COISPA and CIBM

Sampling

In COISPA and CIBM Institute are collected the otoliths of *T. trachurus* the sampling is carried out mostly during two research and monitoring projects: MEDITS trawl survey in the late spring early summer and CAMPBIOL landing monitoring project (Data Collection Framework, EU Reg. 1543/2000, 1639/2001 and 1581/2004) through all year.

The sampling areas are the South Adriatic sea (MEDITS and CAMPBIOL), Central-Southern Tyrrhenian sea (MEDITS and CAMPBIOL), Western Ionian sea (CAMPBIOL), Ligurian and North Tyrrhenian sea (MEDITS and CAMPBIOL) following the FAO classification respectively GSA (GFCM-Geographical Sub-Areas) 18, 10, 19 and 9

Regarding methodologies used, each laboratory follow the guidelines report in Carbonara *et al*., 2018

Storage

After the extraction the otoliths are washed in order to remove the organic material dried and after stored in a coded plastic tube.

Equipment

The whole otoliths are analysed by the binocular microscope. The thin slices are analysed by the optical microscope. The thin sections are performed by the Buehler Isomet low speed or Struers minitom cutting machine. The diamond blade mounted on the cutting machine is the Buehler series 15HC n. 11-4244 diameter 100 mm thickness 0.25 mm. Two component epoxy resin Buehle EPO-KWICK (resin and hardener) are used to embed the otoliths and the resin Entellan Merck are used to mount the thin slices on the glass slides.

Preparation and interpretation of Trachurus trachurus otoliths

One of the pair of otoliths (usually the left) are placed in immersion in seawater to be clarified before the analysis. The time of the immersion depending of the size of the fish usually for the otoliths of juveniles specimens (< 20 cm of total length) one or two minute, between 20 and 30 cm of total length no more of two hours and for the specimens with total length > 30 cm about 4 hours. The otoliths are analysed by the binocular microscope in seawater (clarification medium) with reflected light against the black background.

Quality control

The same structure are read by two readers and are accepted only the coincident estimation. The percentage of agreement and CV are also analysed. The readings of not expert readers are accepted when the percentage of agreement with the expert readers is > 80%.

The measures taken under the microscope (distance from the core to each ring) are intercalibrated with defined measures.

Trachurus mediterraneus

Information about sampling, equipment, otolith preparation and quality control by laboratory is described in the reports of WKARHOM 1 and 2 (ICES, 2015, 2016). Laboratories revised and updated information, when necessary.

Spain

IEO-Santander and Vigo

Sampling

Mediterranean horse mackerel is being monitored since 2011, however, since 2015, there is not data collection framework requirement and samples are only collected during scientific surveys. Sampled geographical area is presented in Figure a2.16.

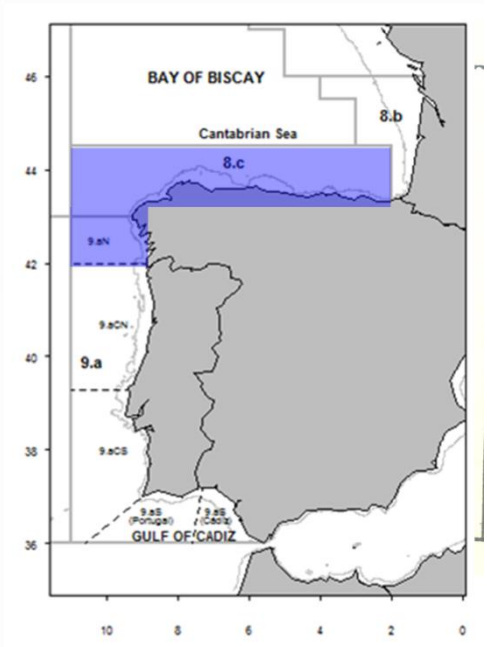


Figure a2.16. *T. mediterraneus* is distributed in ICES Division 8c (Subdivision 8c East and 8c West) and 9a North. Map author: Carmen Hernández-Parras

Calcified structure (otoliths) preparation

Otoliths are extracted from each sampled fish and stored in plastic microtubes (until 2011 pink envelopes were used).

For fish smaller than 27 cm, the whole both otoliths are analysed under a binocular microscope, with reflected light on a black background.

For fish equal or bigger than 27cm, the otoliths are burnt in a muffle at 190-200ºC during 24 hours, and they are hydrated for reading (Figure a2.17).

In both cases, readings are occasionally performed from the screen in live using a Leica camera connected to the stereomicroscope. And, when necessary, measurements are taken from pictures taken through this system with Leica software.

A first reading is made with seawater, leaving them a minimum 4-6 hours hydrating and a second reading with distilled water, with the same hydration time, validating the highest reading.



Figure a2.17. Burnt otolith of *T. mediterraneus* with a total length of 368 mm

Quality control

The criteria and interpretation of growth rings in the otoliths of *T. mediterraneus* is currently being developed.

Italy

COISPA and CIBM

Sampling

In COISPA and CIBM Institute are collected the otoliths of *T. mediterraneus* and the sampling are carried out mostly during two research and monitoring projects: MEDITS trawl survey in the late spring early summer and CAMPBIOL landing monitoring project (Data Collection Framework, EU Reg. 1543/2000, 1639/2001 and 1581/2004) through all year.

The sampling areas are the South Adriatic sea (MEDITS and CAMPBIOL), Central-Southern Tyrrhenian sea (MEDITS and CAMPBIOL), Western Ionian sea (CAMPBIOL), Ligurian and North Tyrrhenian sea (MEDITS and CAMPBIOL) following the FAO classification respectively GSA (GFCM-Geographical Sub-Areas) 18, 10, 19 and 9

Regarding methodologies used, each laboratory follow the guidelines report in Carbonara *et al*., 2018

Storage

After the extraction the otoliths are washed in order to remove the organic material dried and after stored in a coded plastic tube.

Equipment

The whole otoliths are analysed by the binocular microscope. The thin slices are analysed by the optical microscope. The thin section are performed by the Buehler Isomet low speed or Struers minitom cutting machine. The diamond blade mounted on the cutting machine is the Buehler series 15HC n. 11-4244 diameter 100 mm thickness 0.25 mm. Two component epoxy resin Buehle EPO-KWICK (resin and hardener) are used to embed the otoliths and the resin Entellan Merck are used to mount the thin slices on the glass slides.

Preparation and interpretation of Trachurus mediterraneus otoliths

To the ageing of *T. mediterraneus* is followed the same protocol of the *T.trachurus* with some modification. Regard the preparation usually the otoliths of Mediterranean horse mackerel don't need the clarification phase before the age analysis except for the bigger specimens (> 30 cm) where the very short (5-10 minutes) permanence in the seawater could be necessary.

Quality control

The same structure are read by two readers and are accepted only the coincident estimation. The percentage of agreement and CV are also analysed. The readings of not expert readers are accepted when the percentage of agreement with the expert readers is > 80%.

The measures taken under the microscope (distance from the core to each ring) are intercalibrated with defined measures.