WORKSHOP 4 ON AGE READING OF HORSE MACKEREL, MEDITERRANEAN HORSE MACKEREL, AND BLUE JACK MACKEREL *(T. TRACHURUS, T. MEDITERRANEUS, AND T. PICTURATUS)* (WKARHOM4)

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WORKSHOP 4 ON AGE READING OF HORSE MACKEREL, MEDITERRANEAN HORSE MACKEREL, AND BLUE JACK MACKEREL *(T. TRACHURUS, T. MEDITERRANEUS, AND T. PICTURATUS)* (WKARHOM4)

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Contents

i Executive summary

Based on the previous work from WKARHOM3 (ICES, 2018), the Working Group on Biological Parameters (ICES, 2020) 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) (WKARHOM4) had several main objectives; to review the current protocols of ageing Trachurus species, to update the advances in the validation of the ageing criteria (i.e. the annual deposition of one annulus, coherency of the interpretation), to evaluate the new precision of ageing data of Trachurus species and to update guidelines, common ageing criteria and reference collections of otoliths. An online otolith exchange was performed using SmartDots during 2021 and 2022, and results including the three Trachurus species were published in advance of the meeting, showing a low Percentage of Agreement (PA) both when considering all the readers (44-55%) and the advanced readers only (52-54%) (Massaro and Jurado-Ruzafa, 2022). For T. trachurus the Coefficient of Variation (CV) was lower for the sliced samples (22-18%) than for whole otoliths samples (44-38%). Readers participating in the exchange, following discussion during the WKARHOM4 meeting, agreed that the main cause of age determination error for T. trachurus was due to the different otolith preparation techniques (whole/sliced). These differences reflect the stunted growth and compactness of the annuli in older specimens (from the 4th-5th annuli onwards). Anyway, 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 exercise was performed during the workshop organized in four different events (i.e. Trachurus trachurus whole otoliths -135 images-, T. trachurus sliced otoliths -95 images- T. mediterraneus whole otoliths -150 images- and T. picturatus whole otoliths –121 images). For T. trachurus no enhancement among readers' precision was observed in all cases, in terms of PA and CV. Conversely, for T. mediterraneus and T. picturatus a noticeable improvement in terms of PA and decrease of CV occurred compared to the pre-workshop exchange. Finally, this group updated the ageing guidelines and a reference collection of images for all the species, with the aim to employ these tools for all laboratories.

ii Expert group information

Expert group name	Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (WKARHOM)
Year cycle started	2022
Reporting year in cycle	1/1
Chair(s)	Andrea Massaro, Italy
	Alba Jurado-Ruzafa, Spain
Meeting venue(s) and dates	14-18 November 2022, Lisbon, Portugal (37 participants)

1 Introduction

1.1 *Trachurus* species addressed in WKARHOM4

Genus *Trachurus* belongs to the Family Carangidae and is composed of numerous medium-pelagic species with commercial interest, sustaining artisanal and industrial fisheries worldwide (Fischer et al., 1981). In the North-, Central-East Atlantic and the Mediterranean Sea, this group is mainly represented by *T. trachurus*, *T. mediterraneus* and *T. picturatus*, with varying geographical distribution and abundance, although with quite overlapping among them depending on the area (Fig. 1.1).

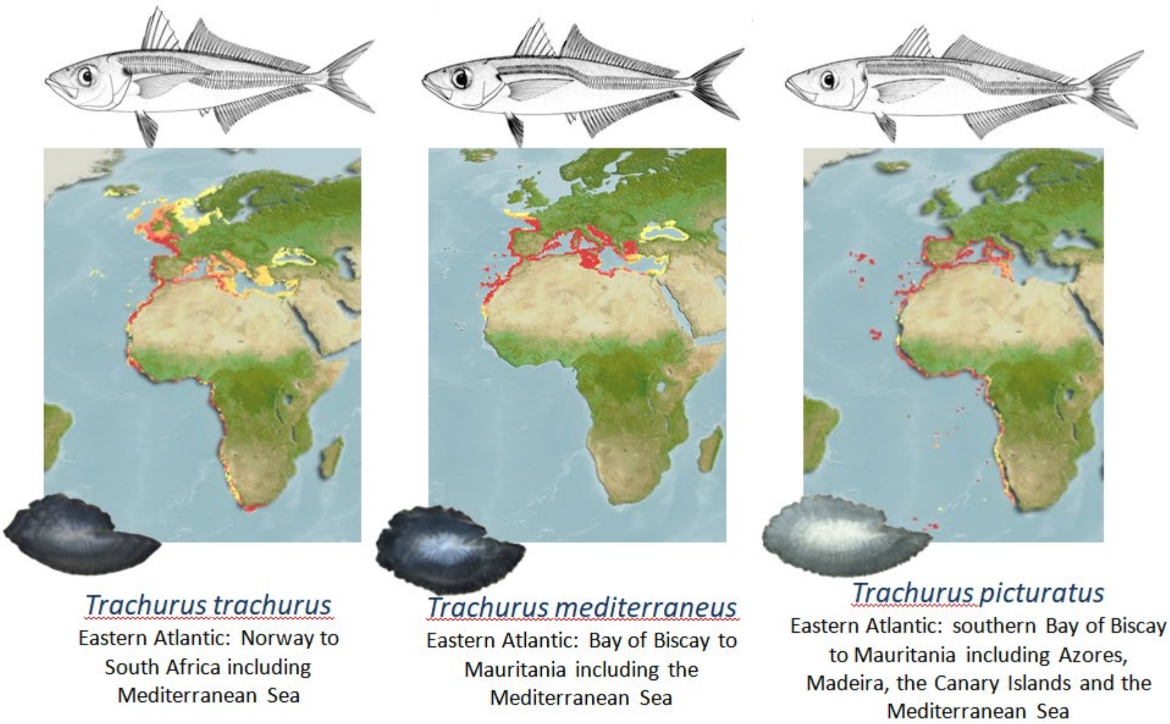


Figure 1.1. Geographical distribution of the *Trachurus* species addressed in the present otolith exchange (Froese and Pauly, 2022; Fischer et al., 1987)

1.2 Background for the Workshops on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) (WKARHOM4)

The biological knowledge of these species is also variable and different among areas (ICES, 2018), but it is widely agreed that readability of the *Trachurus* otoliths is quite difficult. There are regional differences, and age interpretation is more difficult in the southern areas of their distribution limits, where seasonal climate oscillations are lower. In order to provide appropriate management advice, age determination is essential in fish stock assessment to estimate the rates of mortalities and growth, thus, ageing procedures must be reliable. However, otolith preparation and age reading methods might differ considerably between countries. Organizing otolith exchanges on a regular basis and age reading workshops is recommended if serious problems are found, to agree and to update the criteria used among countries. With this aim, an expert group met in 1996 to agree common criteria in the ageing process of horse mackerel. This was the actual origin of the activities which led to the creation of the ICES WKARHOM (Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) in 2012 (Table 1.1).

Table 1.1 Summary of the otolith exchanges and workshops on *Trachurus* species in the ICES framework.

Year	Exchange/ Workshop	Species	References
1996 1999	Exchange Workshop	*T. trachurus*	Report of the horse mackerel otolith workshop (ICES, 1999)
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*	Report of the WKARHOM3 (ICES, 2018)

The WKARHOM3 participants (Livorno, 2018) recommended:

To develop the SmartDots as the official tool for the exchange and for training purposes on the *Trachurus* species (addressed to WGBIOP; WGSMART)

To organize a new full exchange in 2021 for the *T. trachurus, T. mediterraneus, T. picturatus* (addressed to WGBIOP)

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) (addressed to National Ageing Coordinators)

1. To use the updated guideline and reference images by species for the ageing analysis (addressed to National Ageing Coordinators; WGBIOP)
2. To only use calibrated images (with calibration bar, pre-treatments of images could induce bias in the interpretation of different sized otoliths), as this was not adhered to in the current exchange (addressed to National Ageing Coordinators, WGBIOP)

2 First reading exercise previous to WKARHOM4 (2021-2022)

Following the second WKARHOM3-recommendation, an otolith Exchange was organized during 2021 (which finalized in February 2022), including 45 readers from 21 laboratories belonging to 10 countries (including samples from the Atlantic archipelagos of Madeira, Azores and The Canary Islands) interested in evaluating their ageing criteria and to inter-calibrate with other laboratories/countries involved in ageing procedures along the geographical distribution of these species, both in the Atlantic Ocean and the Mediterranean Sea (Massaro and Jurado-Ruzafa, 2022).

Readers were instructed to follow the WKARHOM3 schemes and protocols when completing the current otolith exchange annotations on SmartDots. Precision measures were analysed by species in order to evaluate the necessity of a 4th WKARHOM meeting, both for all readers and the advanced ones.

The Percentage of Agreement achieved (PA<60% in all the cases) is considered to be low and arise the necessity of a new inter-calibration meeting.

2.1 Results and conclusions of the otolith exchange 2021-22

One detailed report about the activity and the results is available in the SmartDots website (https://smartdots.ices.dk/ViewListEvents), for the three events created with this purpose (362, 387 and 388, one by species) (Massaro and Jurado-Ruzafa, 2022). The summarized indices for the precision are presented in Table 2.1.

Table 2.1. Summary of the overall results for the previous otolith Exchange. CV: Coefficient of Variation (%); PA: Percentage of Agreement (%); APE: Averaged Percentage Error (%)

	All readers			Advanced readers		
Species - preparation	**CV**	**PA**	**APE**	**CV**	**PA**	**APE**
Trachurus trachurus-whole	44	46	32	38	52	-
Trachurus trachurus-sliced	22	44	15	18	50	-
Trachurus mediterraneus-whole	44	49	32	55	54	-
Trachurus picturatuss-whole	54	55	35	-	-	-

Some highlights were provided as conclusions because general recommendations provided during the previous WKARHOM meetings were not considered, both when taking the otolith images and when annotating finished annuli. For example:

⇒ it was of great importance to use the same magnification when taking photos for the same "otoliths set", to be able to compare size and partial annuli radii. It was a source of age determination error (even intra-reader) that was repeatedly reported. However, the use of different magnitudes for pictures taken for the some "otoliths sets" was observed.

⇒ although clear instructions had been provided, some readers did not mark annuli at the end of the translucent ring. Therefore, distance measures and the subsequent analyses obtained using these data were not reliable.

The assignment of the edge type constituted a critical point highlighted by some readers, due to its difficult recognition. Likewise, the axis selected by coordinators for annotating each annulus was not always the preferred one that readers had chosen to mark. However, it is the only way to establish comparable measurements and to analyse and detect clear differences on the application of the ageing criteria.

2.2 Analysis of age reading variability in the otolith exchange 2021-22 of horse mackerel (by Carbonara, P., A. Massaro and A. Jurado-Ruzafa)

Principal component analysis (PCA) is a multivariate statistical technique used to extract the important information from a multivariate data set. PCA has already been applied to identify the most informative variables influencing the differences in the ageing data of red mullet (Carbonara et al., 2019). Hence, the following analyses aimed to find the main sources of bias among readings done on *T. trachurus* during the otolith exchange performed during 2021-22, previously to the WK.

The qualitative and quantitative variables used on the whole otolith data set of *T. trachurus* are reported in the Table 2.2.

Table 2.2. Data used in the Principal Component Analysis (*T. trachurus*, whole otoliths)

Quantitative variables	Qualitative variables
Length	Sex
Catch date	Area
Age	Experience
Modal age	
Percentage of Agreement	

In the PCA performed on the otolith whole dataset, the first two PCs were retained, accounting for 83.8% of the total variability. The first principal component (PC1) was significantly correlated with all five original variables (Length, Catch date, Age, Modal age, PA_%) (Table 2.3). Among the qualitative variables the significant correlation with PC1 were showed for the sex and area, while the reader experience, is not significantly correlated with PC1 (Fig. 2.1). Similar results were obtained for the PC2 (Table 2.3, Fig. 2.1).

Table 2.3. Results of the Principal Component Analysis for the first and second principal component (*T. trachurus*, whole otoliths)

Dimension	Variable	Correlation score	p-value
Dimension 1	Length_mm	0.8865623	<0.001
	Catch date	0.3448885	<0.001
	Age	0.9098341	<0.001
	Modal age	0.9220697	<0.001
	PA_%	-0.6768455	<0.001
	CV_%	-0.4594495	<0.001
	APE_%	-0.2171305	<0.001
Dimension 2	Length_mm	-0.02737022	0.08
	Catch date	0.2055272	<0.001
	Age	0.10092	<0.001
	Modal age	0.1247232	<0.001
	PA_%	-0.4993188	<0.001
	CV_%	0.8374766	<0.001
	APE_%	0.9516212	<0.001

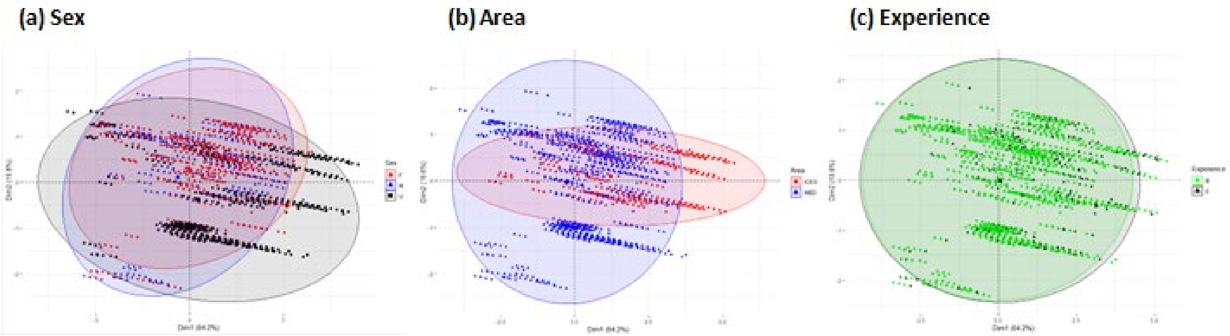


Figure 2.1. Confidence ellipses drawn around the levels of the categorical variables considered in the PCA, considering the variables sex (Male Female and combined, area of sample origin (Mediterranean and Atlantic) and reader experience (basic and expert).

The qualitative and quantitative variables used on the sliced otolith data set of *T. trachurus* are reported in the Table 2.4.

Table 2.4. Data used in the Principal Component Analysis (*T. trachurus*, sliced otoliths)

Quantitative variables	Qualitative variables
Length	Sex
Catch date	Area
Age	Experience
Modal age	
Percentage of Agreement	
Coefficient of Variation	
Average Percentage of Error	

In the PCA performed on the otolith whole dataset, the first two PCs were retained, accounting for 74.6% of the total variability. The first principal component (PC1) was significantly correlated with all seven original variables (Length, Catch date, Age, Modal age, PA_%, CV, APE) (Table 2.5). Among the qualitative variables, significant correlation with PC1/PC2 was shown for the sex and area, while the reader experience, is not significantly correlated with PC1 (Fig. 2.2). Similar results were obtained for the PC2 (Table 2.5).

Table 2.5. Results of the Principal Component Analysis for the first and second principal component (*T. trachurus*, sliced otoliths)

Dimension	Variable	Correlation score	p-value
Dimension 1	Length_mm	0.8865623	<0.001
	Catch date	0.3448885	<0.001
	Age	0.9098341	<0.001
	Modal age	0.9220697	<0.001
	PA_%	-0.6768455	<0.001
	CV_%	-0.4594495	<0.001
	APE_%	-0.2171305	<0.001
Dimension 2	Length_mm	-0.02737022	0.08
	Catch date	0.2055272	<0.001
	Age	0.10092	<0.001
	Modal age	0.1247232	<0.001
	PA_%	-0.4993188	<0.001
	CV_%	0.8374766	<0.001
	APE_%	0.9516212	<0.001

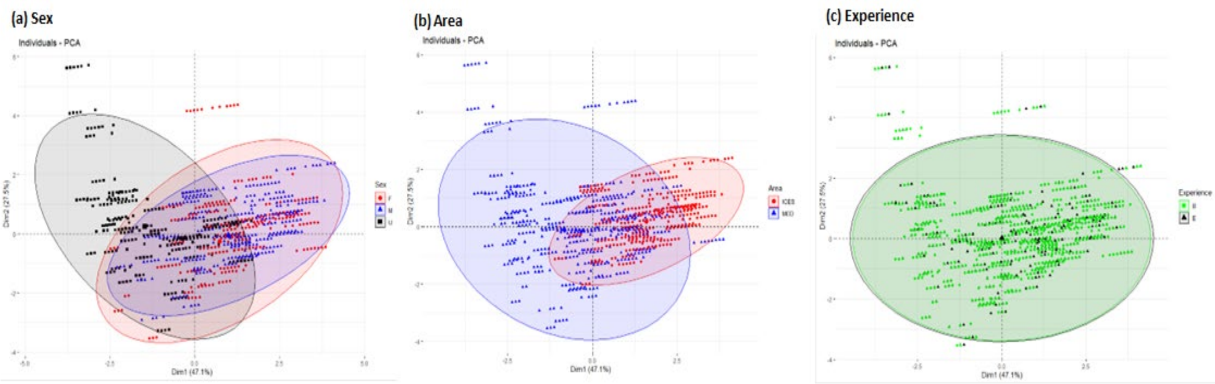


Figure 2.2. Confidence ellipses drawn around the levels of the categorical variables considered in the PCA, considering the variables sex (Male Female and combined, area of sample origin (Mediterranean and Atlantic) and reader experience (basic and expert)

The qualitative and quantitative variable used on the whole and sliced otolith data-set of *T. trachurus* are reported in the Table 2.6.

Table 2.6. Data used in the Principal Component Analysis (*T. trachurus*, whole and sliced otoliths)

Quantitative variables	Qualitative variables
Length	Sex
Catch date	Area
Age	Experience
Modal age	Method
Percentage of Agreement	

In the PCA performed on the otolith whole dataset, the first two PCs were retained, accounting for 83.5% of the total variability. The first principal component (PC1) was significantly correlated with all five original variables (Length, Catch date, Age, Modal age, PA_%, CV, APE) (Table 2.7). Among the qualitative variables, significant correlation with PC1/PC2 was found for the sex, area and preparation method while the reader experience, is not significantly correlated with PC1 (Fig. 2.3). Similar results were obtained for the PC2 (Table 2.7, Fig. 2.3).

Table 2.7. Results of the Principal Component Analysis for the first and second principal component (*T. trachurus*, whole and sliced otoliths)

Dimension	Variable	Correlation score	p-value
Dimension 1	Length_mm	0.9224208	<0.001
	Catch date	-0.1603367	<0.001
	Age	0.9287489	<0.001
	Modal age	0.9284221	<0.001
	PA_%	-0.6735595	<0.001
Dimension 2	Length_mm	-0.0004317	NS
	Catch date	0.9265539	<0.001
	Age	-0.07061187	<0.001
	Modal age	-0.12577853	<0.001
	PA_%	-0.49188687	<0.001

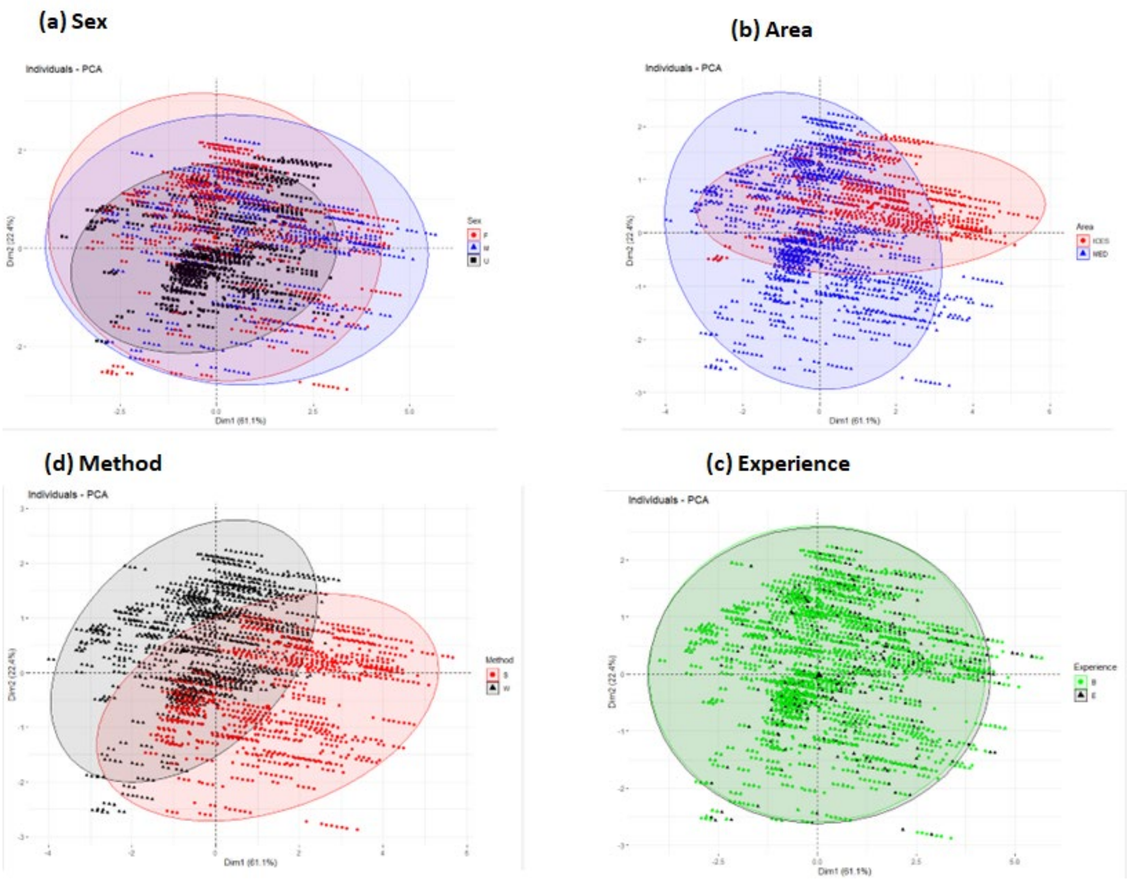


Figure 2.3. Confidence ellipses drawn around the levels of the categorical variables considered in the PCA, considering the variables sex (Male Female and combined, area of sample origin (Mediterranean and Atlantic), reader experience (basic and expert) and preparation method (whole and sliced)

In summary, the results show that for whole and sliced otoliths, the factors significantly linked to the data variability (i.e. ageing differences) seem to be the catch date, origin area and sex rather than the readers experience. Moreover, considering together the whole and sliced samples' sets, the factors significantly linked to the data variability seem to be the catch date, origin area, sex and preparation method rather than the readers experience.

3 Review and updated information on age determination, otolith exchange and validation study on *Trachurus* species

3.1 Presentations during the meeting

ToR *a* was addressed by the presentation of updated information by the participants.

P01: *Trachurus trachurus* (IFREMER). Authors: Bled-Defruit, G. and S. Telliez. (Abstract in Annex 3)

P02: Age determination methodology of Horse mackerel (*Trachurus trachurus*) in the Cantabrian Sea (ICES Division 27.8.c) and Galician waters (ICES Subdivision 27.9.a.n). Authors: Dueñas-Liaño, C., I. Maneiro, E. López, C. Hernández and J. Valtierra

P03: IMR Norway Updated Otolith Protocol. Author: Diaz, J. (Abstract in Annex 3)

P04: WMR – Age reading of horse mackerel (*Trachurus trachurus*). Author: Dijkman, A., N. van der Meeren, T. Huijer and U. Beier.

P05: Life history of *Trachurus trachurus*, *Trachurus mediterraneus* and *Trachurus picturatus* in GSA 9 (Liguarian and North Thyrrenian Sea). Authors: Russo, L. and A. Massaro. (Abstract in Annex 3)

P06: HCMR protocols for age determination for *Trachurus trachurus* and *Trachurus mediterraneus*. Authors: Nikiforidou, V. and A. Legaki. (Abstract in Annex 3)

P07: Review on *Trachurus picturatus* in East Atlantic waters: achievements and challenges. Author: Jurado-Ruzafa, A. (Abstract in Annex 3)

P08: Blue jack mackerel in Azorean waters. Advances by DRP. Author: Cruz, R.

P09: *Trachurus trachurus and Trachurus picturatus - sampling and protocols for* age determination - IPMA (Portugal). Authors: Correia, G., M. J. Ferreira and M. Felício. (Abstract in Annex 3)

P10: Otolith preparation and interpretation for horse mackerel ageing process in GSA 11. Author: Bellodi, A. (Abstract in Annex 3)

P11: Ageing analysis of *Trachurus mediterraneus* and *Trachurus trachurus* in the GSAs 18 and 19 (South Adriatic Sea and Western Ionian Sea). Authors: Casciaro, L. and P. Carbonara.

P12: Marine Institute Ireland: Sampling and ageing protocols for Horse mackerel *Trachurus trachurus.* Mullins, E. and S. O'Connor. (Abstract in Annex 3)

P13: Biology sampling methodology for *Trachurus trachurus* and *Trachurus mediterraneus* (Greek DCF). Authors: Dimitriadis, G. and T. Sioulas. (Abstract in Annex 3)

P14: Contribution to the development of a methodology for reading the age of the European horse mackerel *Trachurus trachurus* (Linnaeus, 1758) from thin sections of "sagitae" otoliths. Author: El Habouz, H. (Abstract in Annex 3)

P15: Analysis of age Reading variability in horse mackerel otolith Exchange. Authors: Carbonara, P., A. Massaro and A. Jurado-Ruzafa. (summarized in sub-section 2.3)

In addition to some abstracts of the listed presentations, Annex 3 includes new or updated protocols used by the participating labs to age *Trachurus* species based on the otoliths.

3.2 Validation studies

A detailed revision of the state of art was presented in the previous WK (ICES, 2018).

An update for *T. trachurus* in Moroccan waters was presented by INRH, based on a back-calculation analysis performed on sliced otoliths (abstract included in Annex 3).

Finally, a recent scientific paper on the age and growth of *T. picturatus* in Portuguese mainland waters, present an indirect validation of the coherency in the ageing criteria used during the otoliths' interpretation (Neves et al., 2022), as other previous studies in other areas.

4 Second reading exercise during the WKARHOM4 meeting

Adhoc exercises were proposed to the participants to evaluate whether the discussions and agreements achieved during the meeting were assumed by the participating readers (Table 5.1).

Table 5.1. Summary of the exercises proposed during the WKARHOM4 meeting

Species-preparation	n. of participants (Basic+Advanced)	n. samples
Trachurus trachurus-whole	20+7	135
Trachurus trachurus-sliced	19+8	95
Trachurus mediterraneus-whole	7+2	150
Trachurus picturatus- whole	4+1	119

As in the previous exchange, readings were carried out without any information about fishes' size. On the one hand, some of the participants would prefer to know the fish size during the exchange. On the other hand, the overall group prefer to keep the fish lengths unknown to avoid directed bias produced by the logical process of looking for an age probable for a given size.

In addition, for *Trachurus trachurus*, expertise levels were set based on the preparation method because some advanced readers only interpret ages from otoliths whole or sliced. In these cases, they were considered basic readers for the other preparation.

4.1 Summary of the results and discussion

Overall results from the four activities are presented in Table 5.2.

Table 5.2. Mean weighted values of Coefficient of variation (CV, %), Percentage of Agreement (PA, %) and Averaged Percentage Error (APE, %) obtained from the four *adhoc* otolith exchanges.

	All Readers			Advanced Readers		
Species - preparation	**CV**	**PA**	**APE**	**CV**	**PA**	**APE**
Trachurus trachurus-whole	49	51	32	45	53	-
Trachurus trachurus-sliced	18	47	12	14	51	-
Trachurus mediterraneus- whole	36	61	24	25	76	-
Trachurus picturatus- whole	40	64	30	-	-	-

For ***Trachurus trachurus* – whole otoliths**, when considering all the readers (basic and advanced expertise), the PA increased from 46% to 51%, CV increased from 44% to 49%, and APE remained unchanged. For advanced readers, PA only increased by one percentage point (from 52% to 53%) with a CV augmentation from 38% to 45%. Analysing the PA trend related with modal age, although advanced readers showed a higher value of PA in the modal age < 3 compared to the basics, the general trend for both expertise levels decreased from class 3 onwards (Table 5.3). This was also reflected in the recognition of the first growth rings.

For ***Trachurus trachurus* – sliced otoliths**, the PA slightly increased for all readers (from 44% to 47%), and practically stayed the same (from 52% to 51%) when only considering the advanced readers. CV decreased in both expertise levels (18% and 14%). As regards the PA by modal age, advanced readers showed a high PA (>70%) until modal age 7, followed by a general decrease onwards (Table 5.4). High variability in annuli radii occurred for all the modal ages. In general, the identification of the first annulus (< 3 years) did not seem to cause any particular problems, in contrast, the compactness process in older age classes seem to increase error (bias) among readers (Table 5.4 – Table 5.5).

For ***Trachurus mediterraneus***, the PA noticeably increased for both expertise levels, from 49% to 61% for all readers, and from 54% to 76% for advanced readers (only 2 readers). CV showed a clear decrease for all readers (from 44% to 36%) and for advanced readers (from 55% to 25%). The PA values by modal aged reached 75% for modal age 0 and 1, and decreased onwards for all readers. Conversely, for advanced readers, the PA remained constant above 70% for all modal ages (Table 5.5). A higher variability in annuli radii was observed from annulus 4 onwards.

Lastly, for ***Trachurus picturatus***, the analysis was carried out for all readers, because only one advance reader participated in the exercises. The PA noticeably increased from 55% to 64%, CV and APE decreased from 54% to 40% and from 35% to 30%, respectively. For all modal ages, PA values remained above 60%, except for the modal age 7 (50%) (Table 5.6). The analysis of the evolution of the annuli radii showed a low variability in first rings, while the high variability observed from the 2nd annuli, reflected identification difficulties.

To conclude, the augmentation of the agreement expected in *T. trachurus* considering different expertise levels based on the otolith preparation methods were not observable in the results obtained in the *adhoc* exercise after the agreements discussed in the WK. Conversely, some improvements were observed, mainly in *T. mediterraneus* and *T. picturatus*.

Finally, additional information about the samples, the readers participating in each reading exercise and summarized results are provided in the following sub-sections.

Table 5.3. Trachurus Trachurus (whole otoliths)

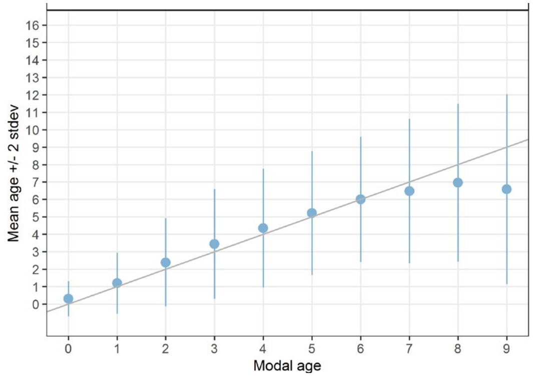
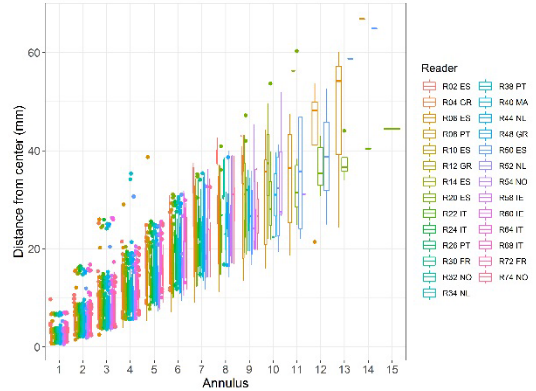
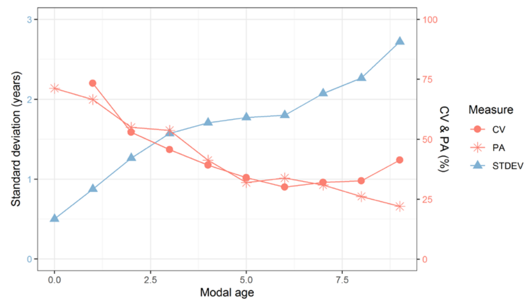
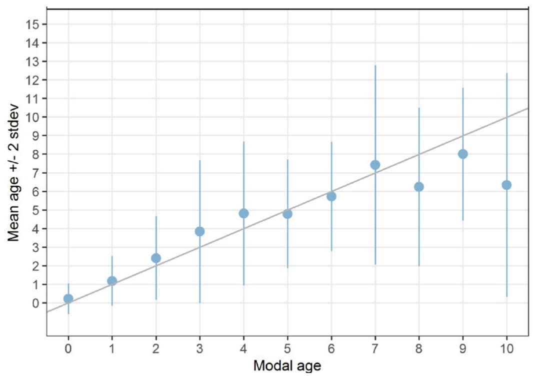
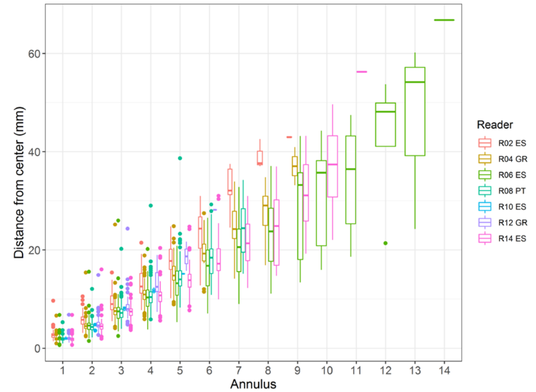
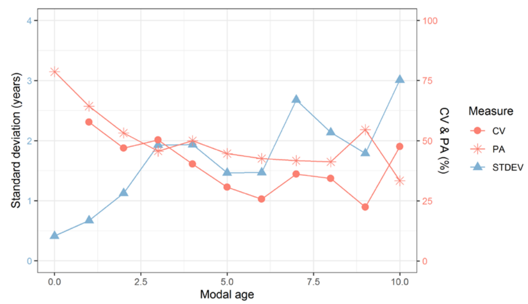
	Age bias by modal age	Radii (mm) by annulus	CV, PA and STDEV by modal age
All readers			
Advanced readers			

Table 5.4. ***Trachurus Trachurus*** **(sliced otoliths)**

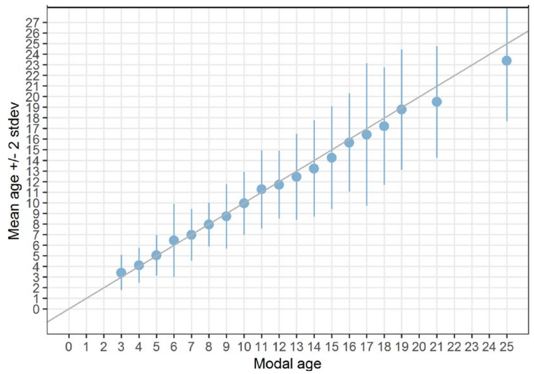
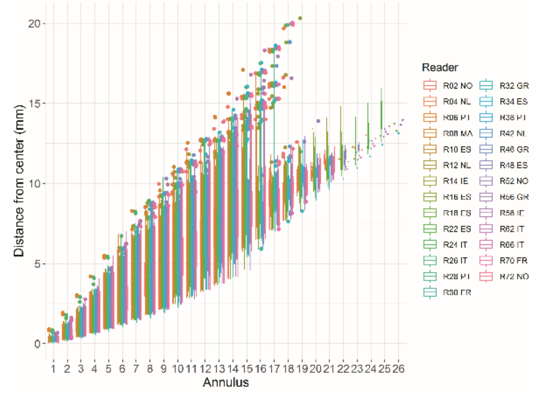
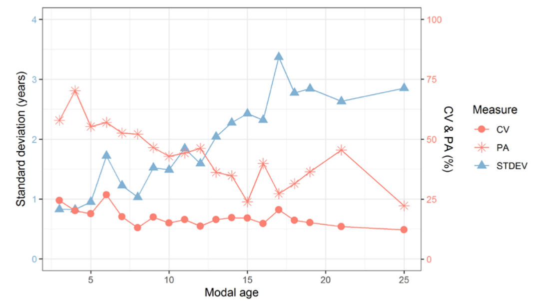
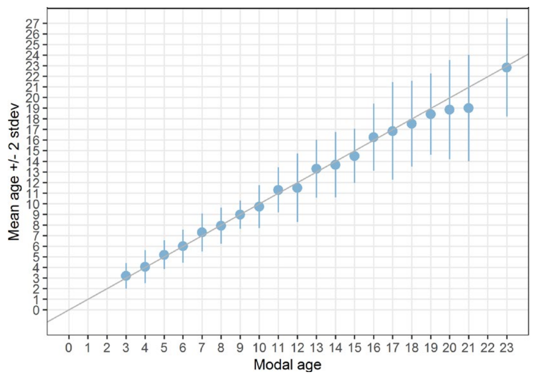
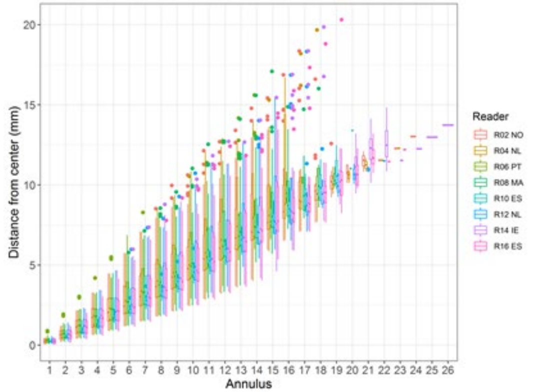
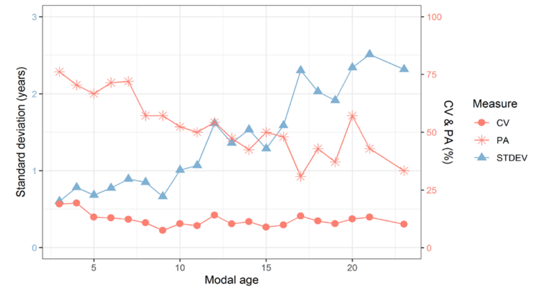
	Age bias by modal age	Radii (mm) by annulus	CV, PA and STDEV by modal age
All readers			
Advanced readers			

Table 5.5. ***Trachurus mediterraneus*** **(whole otoliths)**

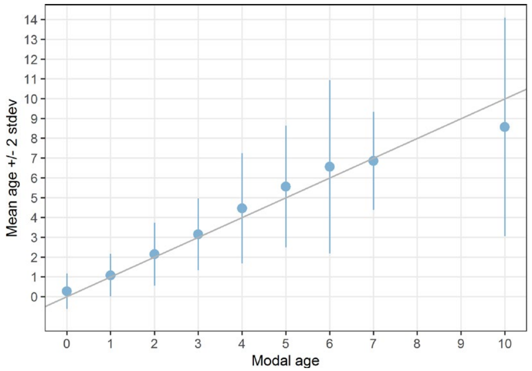
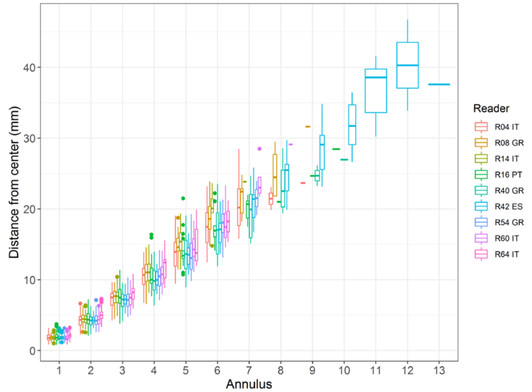
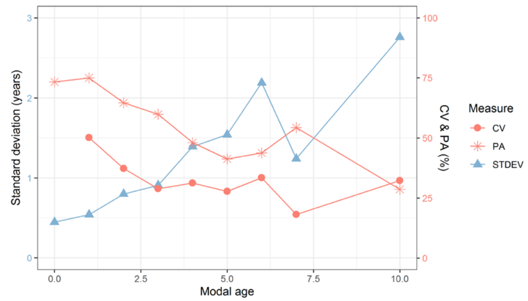
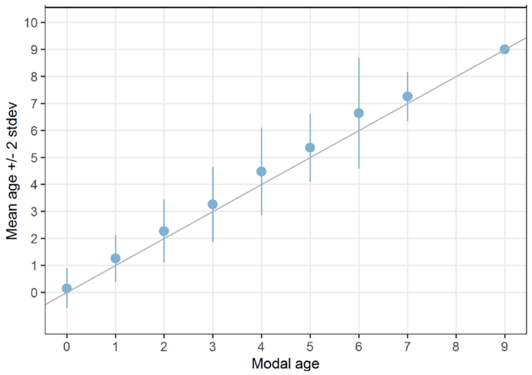
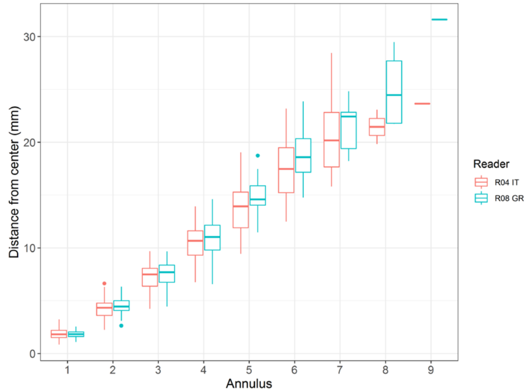
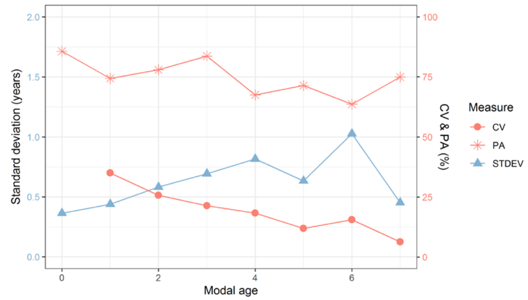
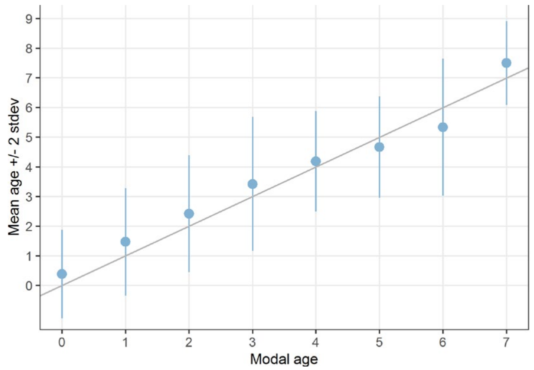
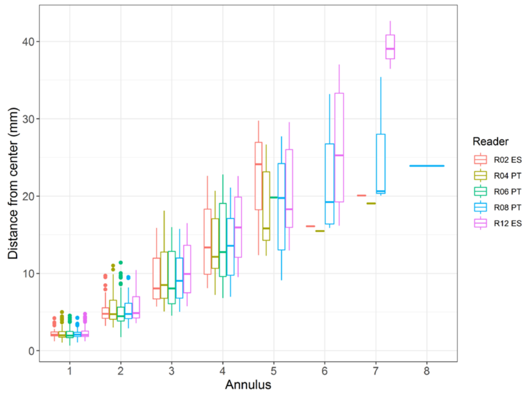
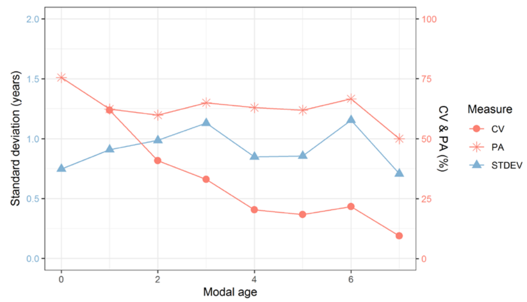
	Age bias by modal age	Radii (mm) by annulus	CV, PA and STDEV by modal age
All readers			
Advanced readers			

Table 5.6. *Trachurus picturatus* (whole otoliths)

<table>
<tr><th></th><th>Age bias by modal age</th><th>Radii (mm) by annulus</th><th>CV, PA and STDEV by modal age</th></tr>
<tr><td>All readers</td><td>
</td><td>
</td><td>
</td></tr>
</table>

4.2 Trachurus trachurus – whole otoliths

Table 5.7. Overview of samples used for the *Trachurus trachurus* – whole otoliths exchange.

Year	ICES area	Quarter	Number of samples	Modal age range	Length range (mm)
2016	11.1	1	6	1-4	135-225
2016	11.1	2	8	1-3	150-205
2016	11.1	3	3	0	95-115
2017	7.0	4	10	2-7	230-320
2017	11.1	1	3	1	110-140
2018	7.0	1	15	3-4	220-260
2018	9.0	4	3	4-9	340-380
2019	9.0	1	10	3-7	205-370
2019	9.0	2	4	3-7	310-335
2019	9.0	3	8	2-4	160-210
2019	9.0	4	17	1-9	110-350
2019	11.1	1	1	3	240
2020	9.0	1	10	1-8	135-375
2020	9.0	2	3	0-4	65-360
2020	9.0	3	1	5	395
2020	9.0	4	3	2-5	130-310
2020	11.1	1	4	2-4	175-215
2020	11.1	3	3	5	270-320
2021	9.0	1	7	1-6	120-300
2021	9.0	2	6	0-2	45-125
2021	9.0	3	4	0-1	65-85
2021	9.0	4	4	2-3	90-155
2021	11.1	4	2	1-2	140-160

Table 5.8. Readers overview.

Reader code	Expertise	SmartUser
R02 ES	Advanced	Acosta
R04 GR	Advanced	Dimitriadis
R06 ES	Advanced	Duenas
R08 PT	Advanced	Ferreira
R10 ES	Advanced	LopezE
R12 GR	Advanced	Nikiforidou
R14 ES	Advanced	Rico
R20 ES	Basic	Arevalo
R22 IT	Basic	Bellodi
R24 IT	Basic	Casciaro
R26 PT	Basic	Correia
R30 FR	Basic	Defruit
R32 NO	Basic	DiazJ
R34 NL	Basic	Dijkman
R38 PT	Basic	Felicio
R40 MA	Basic	Hamou
R44 NL	Basic	Huijer
R48 GR	Basic	Legaki
R50 ES	Basic	Maneiro
R52 NL	Basic	MeerenN
R54 NO	Basic	Meissner
R58 IE	Basic	Mullins
R60 IE	Basic	OConnorS
R64 IT	Basic	Pesci
R68 IT	Basic	RussoL
R72 FR	Basic	Telliez
R74 NO	Basic	Tonheim

4.2.1 All readers

The weighted average percentage agreement based on modal ages for all readers is 51%, with the weighted average CV of 49% and APE of 32%.

Table 5.9. Coefficient of Variation (CV, %) of all readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	R20 ES	R22 IT	R24 IT	R26 PT	R30 FR	R32 NO	R34 NL	R38 PT	R40 MA	R44 NL	R48 GR	R50 ES	R52 NL	R54 NO	R58 IE	R60 IE	R64 IT	R68 IT	R72 FR	R74 NO	all
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
1	-	43	50	68	0	51	63	93	70	44	43	47	56	65	72	43	57	100	54	65	63	48	36	27	0	50	48	**73**
2	41	32	56	36	-	32	60	75	21	32	31	38	29	38	48	29	41	48	50	66	57	29	25	21	39	41	39	**53**
3	33	26	61	22	23	19	35	52	19	17	29	29	19	28	32	14	29	42	49	41	52	25	21	9	21	24	23	**46**
4	50	19	42	20	-	16	36	41	20	28	23	32	34	36	30	14	21	41	45	19	25	28	23	20	34	16	27	**39**
5	27	17	27	13	-	20	15	38	27	22	27	24	15	37	18	20	24	30	14	-	14	17	36	19	23	17	13	**34**
6	16	11	30	7	-	20	24	18	14	15	21	16	25	24	18	17	22	13	27	-	14	16	24	21	27	18	23	**30**
7	33	28	41	9	-	35	16	20	12	24	35	0	7	53	8	16	0	14	11	-	7	13	23	20	15	17	7	**32**
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**33**
9	28	0	7	0	-	-	0	20	20	47	0	-	-	79	-	13	28	-	39	-	-	9	35	47	47	-	-	**41**
Weighted Mean	**37**	**27**	**48**	**30**	**15**	**28**	**42**	**58**	**29**	**28**	**30**	**33**	**31**	**40**	**41**	**23**	**34**	**50**	**44**	**55**	**48**	**29**	**27**	**19**	**25**	**30**	**31**	**49**

Table 5.10. Percentage agreement (PA, %) of all readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	R20 ES	R22 IT	R24 IT	R26 PT	R30 FR	R32 NO	R34 NL	R38 PT	R40 MA	R44 NL	R48 GR	R50 ES	R52 NL	R54 NO	R58 IE	R60 IE	R64 IT	R68 IT	R72 FR	R74 NO	all
0	100	50	100	100	100	100	100	60	83	50	33	100	83	67	100	100	100	83	100	83	0	33	0	33	17	100	50	**71**
1	27	67	81	40	100	77	68	23	71	82	68	82	77	68	64	68	59	50	77	25	58	73	65	90	100	81	73	**67**
2	35	61	67	17	0	53	55	29	84	65	72	58	52	71	48	52	50	48	58	44	43	55	74	65	45	55	55	**55**
3	25	50	59	21	50	79	29	26	69	75	68	63	64	67	33	71	46	52	32	50	31	61	61	93	39	65	50	**54**
4	26	57	26	57	0	27	22	9	37	52	32	50	33	59	53	78	35	43	30	60	11	52	48	78	13	64	16	**41**
5	33	50	17	36	0	0	33	33	40	58	33	42	43	58	60	33	9	17	0	-	25	33	33	60	0	25	30	**32**
6	17	17	17	83	-	0	50	50	20	50	17	67	17	67	33	17	50	67	17	-	0	33	0	60	0	50	40	**34**
7	25	25	0	50	-	0	0	25	0	50	0	100	75	33	25	0	100	25	0	-	0	75	25	0	0	25	75	**31**
8	0	100	0	0	-	0	100	0	0	0	0	100	100	-	0	0	0	100	0	-	-	0	0	0	0	0	100	**26**
9	50	100	50	0	-	0	100	50	0	0	0	0	0	0	0	0	50	0	0	-	-	0	0	0	0	0	100	**22**
Weighted Mean	**32**	**55**	**52**	**39**	**50**	**51**	**45**	**26**	**61**	**64**	**52**	**64**	**56**	**64**	**50**	**59**	**48**	**47**	**43**	**45**	**35**	**54**	**52**	**72**	**38**	**61**	**50**	**51**

Table 5.11. Relative bias all readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	R20 ES	R22 IT	R24 IT	R26 PT	R30 FR	R32 NO	R34 NL	R38 PT	R40 MA	R44 NL	R48 GR	R50 ES	R52 NL	R54 DE	R58 IE	R60 IE	R64 IT	R68 IT	R72 FR	R74 NO	all
0	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.40	0.17	0.50	0.67	0.00	0.17	0.33	0.00	0.00	0.00	0.17	0.00	0.50	1.00	0.67	1.00	0.67	0.83	0.00	0.50	**0.30**
1	-0.64	0.38	-0.19	0.25	0.00	-0.05	0.09	1.41	0.10	0.00	0.36	-0.09	0.23	0.27	-0.18	0.23	0.50	-0.05	-0.14	1.19	0.68	0.36	0.35	0.10	0.00	-0.19	0.36	**0.20**
2	-0.68	0.42	0.33	1.48	-1.00	0.13	0.29	2.16	-0.03	0.32	0.21	-0.06	0.37	0.26	0.64	0.61	0.30	0.19	0.55	0.94	1.57	0.45	0.23	0.35	-0.42	-0.28	0.55	**0.37**
3	-0.79	0.79	1.00	1.29	-0.50	-0.18	0.79	3.78	-0.23	0.04	0.04	-0.11	0.32	0.00	0.76	0.29	0.38	0.33	1.43	0.80	2.08	0.54	-0.25	0.07	-0.61	-0.23	0.58	**0.46**
4	-0.52	0.65	1.78	0.67	1.00	-0.77	1.52	2.61	-0.74	-0.43	-0.36	0.27	0.28	0.36	0.53	0.17	0.74	0.22	1.87	0.60	0.78	1.13	-0.57	-0.35	-1.48	-0.41	0.89	**0.39**
5	-0.25	0.75	2.42	0.45	-1.00	-1.64	0.92	2.50	-0.80	-0.50	-0.83	0.00	0.86	-0.33	-0.10	-0.25	0.91	0.33	3.08	-	1.00	1.17	-1.50	-0.60	-2.25	-0.50	0.90	**-**
6	-1.17	1.17	2.17	-0.17	-	-2.17	1.33	0.00	-1.00	-0.67	-0.33	0.17	1.33	-0.33	0.67	-1.33	1.00	0.50	2.33	-	2.33	1.00	-1.83	-0.80	-3.17	-0.75	0.60	**-**
7	-1.75	-0.25	2.50	-0.50	-	-3.00	2.00	2.00	-2.33	-1.00	-2.67	0.00	0.25	-2.00	-0.75	-2.00	0.00	-0.25	1.75	-	2.50	0.50	-1.50	-2.00	-3.75	-1.25	0.25	**-**
8	-5.00	0.00	1.00	-1.00	-	-4.00	0.00	2.00	-3.00	1.00	-4.00	0.00	0.00	-	-2.00	-2.00	2.00	0.00	1.00	-	-	1.00	-4.00	-3.00	-5.00	1.00	0.00	**-**
9	-1.50	0.00	0.50	-2.00	-	-5.00	0.00	1.50	-5.50	-4.50	-5.00	-2.00	-1.00	-4.50	-4.00	-3.50	-1.50	-1.00	2.00	-	-	-1.50	-5.00	-4.50	-6.00	-4.00	0.00	**-**
Weighted Mean	**-0.70**	**0.57**	**0.97**	**0.69**	**-0.30**	**-0.54**	**0.70**	**2.29**	**-0.42**	**-0.13**	**-0.18**	**-0.02**	**0.37**	**0.02**	**0.27**	**0.06**	**0.47**	**0.19**	**1.19**	**0.91**	**1.33**	**0.64**	**-0.38**	**-0.13**	**-1.01**	**-0.34**	**0.59**	**0.35**

For each pair that is being compared, the differences between the readings per image are found and the frequency of each occurring difference is presented in the next table.

Table 5.12. Inter reader bias test (i.e. probability of bias between readers and with modal age). - = no sign of bias (p>0.05), * = possibility of bias (0.01<p<0.05), * * = certainty of bias (p<0.01)

Comparison	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	R20 ES	R22 IT	R24 IT	R26 PT	R30 FR	R32 NO	R34 NL	R38 PT	R40 MA	R44 NL	R48 GR	R50 ES	R52 NL	R54 DE	R58 IE	R60 IE	R64 IT	R68 IT	R72 FR	R74 NO
R02 ES	-	**	**	**	-	-	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	-	**	**
R04 GR	**	-	-	-	*	**	-	**	**	**	**	**	-	**	-	**	-	**	**	*	**	-	**	**	**	**	-
R06 ES	**	-	-	-	-	**	-	**	**	**	**	**	*	**	-	**	*	**	-	**	**	-	**	**	**	**	-
R08 PT	**	-	-	-	-	**	-	**	**	**	**	**	-	**	*	**	-	**	*	-	**	-	**	**	**	**	-
R10 ES	-	*	-	-	-	-	*	*	-	-	-	-	*	-	-	*	-	*	*	*	-	*	-	-	-	-	*
R12 GR	-	**	**	**	-	-	**	**	-	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	-	**
R14 ES	**	-	-	-	*	**	-	**	**	**	**	**	-	**	-	**	*	**	**	**	**	-	**	**	**	**	-
R20 ES	**	**	**	**	*	**	**	-	**	**	**	**	**	**	**	**	**	**	**	-	-	**	**	**	**	**	**
R22 IT	**	**	**	**	-	-	**	**	-	**	*	**	**	**	**	**	**	**	**	**	**	**	-	**	**	-	**
R24 IT	**	**	**	**	-	**	**	**	**	-	-	-	**	-	**	*	**	*	**	**	**	**	*	-	**	**	**
R26 PT	**	**	**	**	-	**	**	**	*	-	-	-	**	-	**	*	**	*	**	**	**	**	*	-	**	**	**
R30 FR	**	**	**	**	-	**	**	**	**	-	-	-	**	-	*	-	**	-	**	**	**	**	*	-	**	**	**
R32 NO	**	-	*	-	*	**	-	**	**	**	**	**	-	*	-	*	-	-	**	**	**	-	**	**	**	**	*
R34 NL	**	**	**	**	-	**	**	**	**	-	-	-	*	-	-	-	**	-	**	**	**	**	**	-	**	**	**
R38 PT	**	-	-	*	-	**	-	**	**	**	**	*	-	-	-	-	-	-	**	**	**	*	**	**	**	**	*
R40 MA	**	**	**	**	*	**	**	**	**	*	*	-	*	-	-	-	**	-	**	**	**	**	**	**	**	**	**
R44 NL	**	-	*	-	-	**	*	**	**	**	**	**	-	**	-	**	-	*	**	**	**	-	**	**	**	**	-
R48 GR	**	**	**	**	*	**	**	**	**	*	*	-	-	-	-	-	*	-	**	**	**	**	**	*	**	**	**
R50 ES	**	**	-	*	*	**	**	**	**	**	**	**	**	**	**	**	**	**	-	**	*	**	**	**	**	**	*
R52 NL	**	*	**	-	*	**	**	-	**	**	**	**	**	**	**	**	**	**	**	-	-	*	**	**	**	**	**
R54 DE	**	**	**	**	-	**	**	-	**	**	**	**	**	**	**	**	**	**	*	-	-	**	**	**	**	**	**
R58 IE	**	-	-	-	*	**	-	**	**	**	**	**	-	**	*	**	-	**	**	*	**	-	**	**	**	**	-
R60 IE	**	**	**	**	-	**	**	**	-	*	*	*	**	**	**	**	**	**	**	**	**	**	-	**	**	-	**
R64 IT	**	**	**	**	-	**	**	**	**	-	-	-	**	-	**	**	**	*	**	**	**	**	**	-	**	**	**
R68 IT	-	**	**	**	-	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	-	**	**
R72 FR	**	**	**	**	-	-	**	**	-	**	**	**	**	**	**	**	**	**	**	**	**	**	-	**	**	-	**
R74 NO	**	-	-	-	*	**	-	**	**	**	**	**	*	**	*	**	-	**	*	**	**	-	**	**	**	**	-
Modal age	**	**	**	**	-	**	**	**	**	-	-	-	**	-	*	-	**	-	**	**	**	**	**	-	**	**	**

4.2.2 Advanced readers

Table 5.13. Coefficient of Variation (CV, %) of all advanced readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	all
0	-	-	-	-	-	-	-	**-**
1	-	39	40	53	0	33	56	**58**
2	38	29	57	30	0	28	53	**47**
3	41	32	66	24	0	14	45	**50**
4	41	27	47	17	-	15	36	**40**
5	51	8	27	16	16	23	15	**31**
6	24	14	17	13	-	15	11	**26**
7	24	9	47	11	-	28	31	**36**
8	17	16	7	17	-	20	0	**34**
9	28	0	7	0	-	-	0	**22**
10	-	-	-	-	-	-	-	**48**
Weighted Mean	**39**	**27**	**47**	**27**	**3**	**22**	**41**	**45**

Table 5.14. Percentage agreement (PA, %) of all advanced readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	all
0	100	30	90	90	100	90	70	**79**
1	45	52	81	44	100	82	73	**64**
2	53	53	53	23	100	62	63	**53**
3	38	48	41	14	100	93	34	**46**
4	50	50	38	62	-	53	50	**50**
5	25	83	33	58	50	8	58	**45**
6	25	50	12	86	-	0	88	**43**
7	50	50	50	50	-	0	50	**42**
8	0	67	67	0	-	0	100	**41**
9	50	100	50	0	-	0	100	**55**
10	0	0	100	0	-	0	100	**33**
Weighted Mean	**46**	**53**	**52**	**41**	**90**	**62**	**60**	**53**

Table 5.15. Relative bias of all advanced readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R02 ES	R04 GR	R06 ES	R08 PT	R10 ES	R12 GR	R14 ES	all
0	0.00	0.70	0.10	0.10	0.00	0.10	0.30	**0.19**
1	-0.45	0.52	0.10	0.61	0.00	0.18	0.14	**0.16**
2	-0.50	0.63	0.50	1.41	0.00	0.17	0.47	**0.38**
3	-0.62	0.90	1.85	1.68	0.00	-0.10	1.45	**0.74**
4	-0.19	1.06	2.12	0.54	-	-0.47	1.62	**-**
5	-1.17	0.17	1.00	0.17	-0.50	-1.75	0.33	**-0.25**
6	-0.62	0.38	1.12	-0.29	-	-2.50	0.25	**-**
7	-1.00	0.50	3.50	-0.50	-	-2.00	2.00	**-**
8	-4.67	-0.67	0.33	-2.00	-	-4.50	0.00	**-**
9	-1.50	0.00	0.50	-2.00	-	-5.00	0.00	**-**
10	-7.00	-5.00	0.00	-4.00	-	-6.00	0.00	**-**
Weighted Mean	**-0.67**	**0.59**	**1.00**	**0.69**	**-0.10**	**-0.50**	**0.73**	**0.34**

4.3 *Trachurus trachurus* – sliced otoliths

Table 5.16. Overview of samples used for the *Trachurus trachurus* – sliced otoliths exchange.

Year	ICES area	Quarter	Number of samples	Modal age range	Length range (mm)
2014	4	1	2	6-13	265-365
2015	4	3	2	10-14	325-335
2015	4	4	8	3-11	285-335
2016	4	1	1	16	395
2016	4	3	15	4-17	275-665
2017	4	1	10	3-14	235-375
2017	7	4	11	3-16	230-370
2018	7	1	15	3-5	220-260
2019	17	1	9	13-18	350-410
2019	17	2	7	11-18	355-395
2019	17	3	9	9-25	325-430
2019	17	4	6	11-19	345-405

Table 5.17. Readers overview.

Reader code	Expertise	SmartUser
R02 NO	Advanced	DiazJ
R04 NL	Advanced	Dijkman
R06 PT	Advanced	Ferreira
R08 MA	Advanced	Hamou
R10 ES	Advanced	LopezE
R12 NL	Advanced	MeerenN
R14 IE	Advanced	Mullins
R16 ES	Advanced	Rico
R18 ES	Basic	Acosta
R22 ES	Basic	Arevalo
R24 IT	Basic	Bellodi
R26 IT	Basic	Casciaro
R28 PT	Basic	Correia
R30 FR	Basic	Defruit
R32 GR	Basic	Dimitriadis
R34 ES	Basic	Duenas
R38 PT	Basic	Felicio
R42 NL	Basic	Huijer
R46 GR	Basic	Legaki
R48 ES	Basic	Maneiro
R52 NO	Basic	Meissner
R56 GR	Basic	Nikiforidou
R58 IE	Basic	OConnorS
R62 IT	Basic	Pesci
R66 IT	Basic	RussoL
R70 FR	Basic	Telliez
R72 NO	Basic	Tonheim

4.3.1 All readers

The weighted average percentage agreement based on modal ages for all readers is 47%, with the weighted average CV of 18% and APE of 12%.

Table 5.18. Coefficient of Variation (CV, %) of all readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	R18 ES	R22 ES	R24 IT	R26 IT	R28 PT	R30 FR	R32 GR	R34 ES	R38 PT	R42 NL	R46 GR	R48 ES	R52 NO	R56 GR	R58 IE	R62 IT	R66 IT	R70 FR	R72 NO	all
3	15	16	24	22	-	16	14	55	-	15	15	0	0	22	15	28	-	33	15	13	14	19	11	0	21	15	16	**24**
4	0	13	13	10	-	10	10	28	-	31	0	14	24	7	13	7	-	12	14	19	9	22	9	17	14	7	0	**20**
5	11	8	7	18	-	16	12	18	-	21	8	19	17	20	14	14	-	15	8	10	21	20	14	8	29	14	0	**19**
6	34	41	0	22	-	33	37	29	-	13	17	0	0	34	9	14	-	9	0	34	17	35	17	0	15	33	9	**27**
7	7	12	7	7	-	15	13	0	-	16	14	7	14	7	7	7	-	16	13	12	10	26	7	7	30	10	12	**18**
8	10	5	12	0	-	12	7	10	-	7	6	11	7	10	11	6	-	6	12	10	6	15	5	11	25	9	5	**13**
9	8	9	9	11	-	9	4	6	-	4	8	8	17	6	24	13	0	0	9	15	4	25	7	14	19	7	4	**17**
10	9	9	13	5	-	17	7	12	-	20	7	6	18	5	9	10	-	6	20	6	5	37	7	11	-	5	5	**15**
11	10	14	12	8	13	7	17	8	-	14	5	0	5	10	8	9	13	13	24	15	15	21	4	0	-	9	10	**16**
12	7	7	5	10	18	13	7	6	-	17	7	7	7	7	12	4	-	13	14	9	4	18	6	10	-	6	7	**14**
13	5	13	13	11	-	9	4	6	-	17	11	12	14	9	12	5	-	8	15	4	3	20	8	23	-	8	7	**16**
14	4	9	11	8	-	5	6	9	-	4	9	7	17	5	3	6	10	7	13	5	9	14	8	7	25	7	0	**17**
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**17**
16	6	4	11	6	16	7	10	11	-	17	7	7	18	8	3	9	-	7	13	10	6	15	6	4	-	5	3	**15**
17	5	0	7	7	8	10	7	5	-	25	-	4	9	8	7	12	-	7	25	10	9	35	6	6	28	3	7	**21**
18	3	8	8	8	4	6	7	5	-	7	4	9	10	2	4	6	-	7	10	10	10	16	5	10	-	5	3	**16**
Weighted Mean	**8**	**11**	**11**	**10**	**12**	**11**	**10**	**15**	**-**	**16**	**8**	**9**	**13**	**10**	**11**	**10**	**8**	**11**	**13**	**12**	**9**	**22**	**8**	**10**	**21**	**9**	**5**	**18**

Table 5.19. Percentage agreement (PA, %) of all readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R02NO	R04NL	R06PT	R08MA	R10ES	R12NL	R14IE	R16ES	R18ES	R22ES	R24IT	R26IT	R28PT	R30FR	R32GR	R34ES	R38PT	R42NL	R46GR	R48ES	R52NO	R56GR	R58IE	R62IT	R66IT	R70FR	R72NO	all
3	40	50	83	50	-	80	33	17	-	0	67	100	100	50	67	33	-	67	67	83	33	67	17	100	60	67	60	**58**
4	100	69	73	77	-	85	77	38	-	31	100	85	54	92	69	92	-	62	69	69	85	15	85	77	0	92	100	**70**
5	67	83	86	43	-	50	71	29	-	29	83	71	20	67	57	43	0	43	86	71	43	29	43	86	0	43	100	**55**
6	75	25	100	25	-	50	50	75	-	25	33	100	100	75	50	50	-	50	100	75	67	0	25	100	0	50	67	**57**
7	60	60	75	60	-	75	20	100	-	20	60	80	60	60	40	60	-	60	40	20	60	0	60	80	0	60	60	**53**
8	40	80	60	100	-	20	60	40	-	60	80	40	40	40	80	40	-	80	60	40	40	25	20	40	0	60	80	**52**
9	50	50	20	67	100	33	17	67	-	83	60	50	0	67	33	50	100	100	33	67	0	0	67	17	0	67	83	**47**
10	50	25	50	75	-	25	25	0	-	25	50	50	0	75	50	75	-	50	25	50	50	0	25	75	0	75	75	**43**
11	25	67	50	50	0	50	75	0	-	0	67	100	25	25	0	75	0	75	25	50	50	0	75	100	0	75	25	**44**
12	40	50	60	40	0	20	60	60	100	60	40	40	40	40	60	60	0	40	0	60	80	0	60	60	0	60	60	**46**
13	57	14	43	57	100	57	14	43	-	14	17	14	14	29	43	57	100	43	0	43	86	0	29	29	0	43	71	**36**
14	50	60	0	33	-	50	33	0	0	67	33	33	0	33	83	67	25	50	0	67	0	0	33	20	0	17	100	**35**
15	0	-	-	100	0	0	0	0	-	0	-	0	0	0	100	0	-	100	0	100	0	0	0	0	-	100	0	**24**
16	57	57	0	57	50	50	43	57	100	29	33	29	0	14	67	29	0	40	0	43	50	0	57	57	-	71	71	**40**
17	50	0	0	0	50	67	0	25	-	50	0	50	25	50	25	50	0	25	0	25	25	0	50	33	0	50	25	**27**
18	12	33	0	25	50	50	12	25	-	12	0	14	0	88	50	62	0	43	0	62	38	0	50	14	-	50	50	**31**
19	0	100	0	100	0	100	0	0	-	0	-	100	0	0	0	100	-	-	0	0	100	0	100	0	-	100	0	**36**
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
21	100	100	0	0	-	0	100	100	-	0	-	0	0	100	0	0	-	100	0	0	0	0	100	100	-	100	100	**45**
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**

25	0	0	-	0	-	0	0	0	-	0	-	-	-	0	0	0	-	0	0	0	100	-	0	100	-	100	100	**22**
Weighted Mean	**54**	**52**	**45**	**53**	**36**	**52**	**41**	**37**	**67**	**32**	**56**	**56**	**30**	**55**	**53**	**57**	**29**	**56**	**34**	**56**	**50**	**10**	**49**	**59**	**6**	**62**	**71**	**47**

The relative bias is the difference between the mean age (per modal age per reader) and modal age. As for the previous tables, a combined bias for all readers and weighted means are calculated.

Table 5.20. Relative bias table of all readers combined per modal age and a weighted mean of the relative bias per reader. A rank is also assigned to each reader.

Modal age	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	R18 ES	R22 ES	R24 IT	R26 IT	R28 PT	R30 FR	R32 GR	R34 ES	R38 PT	R42 NL	R46 GR	R48 ES
3	0.60	0.50	0.33	0.67	-	-0.20	0.67	-0.50	-	2.17	0.33	0.00	0.00	0.67	0.33	1.17	-	0.67	0.33	0.17
4	0.00	0.15	0.09	0.23	-	-0.15	0.23	0.46	-	1.85	0.00	0.23	0.15	-0.08	0.15	0.08	-	0.38	0.00	0.08
5	-0.33	-0.17	0.14	0.43	-	-0.17	0.00	0.57	-	1.57	0.17	-0.14	0.40	-0.17	-0.14	0.29	1.00	0.86	-0.14	-0.29
6	1.25	1.50	0.00	0.75	-	0.25	1.75	1.00	-	1.25	0.00	0.00	0.00	1.25	0.50	0.75	-	0.50	0.00	1.25
7	0.40	0.60	-0.25	0.40	-	-0.50	1.20	0.00	-	2.00	-0.60	-0.20	-0.60	0.40	0.60	0.40	-	-0.20	-0.80	0.40
8	0.80	0.20	-0.60	0.00	-	-0.40	0.40	0.20	-	0.40	-0.20	-0.20	-0.60	0.20	0.40	0.60	-	-0.20	-0.60	0.20
9	0.17	-0.17	-1.00	-0.17	0.00	-0.33	0.83	0.33	-	0.17	0.00	0.17	-1.67	0.33	1.17	0.50	0.00	0.00	-0.83	0.33
10	0.75	0.25	-1.00	0.25	-	0.25	1.00	0.75	-	1.25	-0.50	-0.50	0.00	0.25	0.75	0.50	-	-0.50	-1.50	-0.50
11	1.50	1.00	-0.25	0.75	0.00	0.00	1.00	1.50	-	1.00	-0.33	0.00	-0.75	1.25	1.50	0.50	0.00	0.75	-2.00	1.25
12	0.20	0.00	-0.40	0.00	-0.50	0.00	0.60	0.00	0.00	1.60	-0.80	0.20	-0.20	-0.20	0.40	0.40	-2.00	-0.60	-2.80	-0.20
13	0.14	-0.14	-0.71	-0.29	0.00	-0.71	1.00	0.86	-	2.29	-2.00	-0.71	-2.43	-0.29	0.57	-0.14	0.00	-0.14	-2.71	0.57
14	0.50	0.80	-2.00	-0.17	-	-0.17	1.00	0.17	-2.00	0.33	-1.17	-1.00	-1.67	0.83	-0.17	-0.50	-1.25	0.33	-4.00	0.00
15	2.00	-	-	0.00	2.00	-1.00	1.00	-1.00	-	1.00	-	-3.00	-4.00	1.00	0.00	-2.00	-	0.00	-3.00	0.00
16	0.71	-0.14	-1.86	0.29	2.00	0.00	1.43	1.14	0.00	2.29	-1.17	-0.71	-2.71	0.57	0.33	0.43	-3.00	-0.40	-3.71	0.57
17	0.75	1.00	-2.50	-2.50	1.00	1.00	2.00	1.50	-	2.00	-4.00	-0.50	-2.00	1.00	0.50	0.75	2.00	0.50	-5.25	1.00
18	1.12	-0.33	-3.57	-1.62	0.50	0.75	2.12	1.25	-	1.38	-1.50	-1.43	-3.75	-0.12	0.25	-0.38	-3.00	0.29	-5.50	0.25
19	2.00	0.00	-2.00	0.00	-1.00	0.00	3.00	2.00	-	3.00	-	0.00	-2.00	-1.00	2.00	0.00	-	-	-5.00	1.00
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	0.00	0.00	-6.00	-1.00	-	-1.00	0.00	0.00	-	-1.00	-	-4.00	-4.00	0.00	-1.00	-2.00	-	0.00	-6.00	1.00
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	-1.00	-2.00	-	-6.00	-	-3.00	1.00	-2.00	-	-1.00	-	-	-	-1.00	1.00	-3.00	-	-4.00	-10.00	1.00
Weighted Mean	**0.51**	**0.20**	**-0.97**	**-0.14**	**0.50**	**-0.11**	**0.96**	**0.56**	**-0.67**	**1.45**	**-0.56**	**-0.37**	**-1.20**	**0.28**	**0.41**	**0.20**	**-0.71**	**0.13**	**-2.07**	**0.31**

For each pair that is being compared, the differences between the readings per image are found and the frequency of each occurring difference is obtained.

Table 5.21. Inter reader bias test (i.e. probability of bias between readers and with modal age). - = no sign of bias (p>0.05), * = possibility of bias (0.01<p<0.05), * * = certainty of bias (p<0.01)

Comparison	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	R18 ES	R22 ES	R24 IT	R26 IT	R28 PT	R30 FR	R32 GR	R34 ES	R38 PT	R42 NL	R46 GR	R48 ES	R52 NO	R56 GR	R58 IE	R62 IT	R66 IT	R70 FR	R72 NO
R02 NO	-	*	**	**	-	**	**	-	-	**	**	**	**	*	-	**	*	*	**	-	-	**	*	**	**	**	-
R04 NL	*	-	**	-	-	*	**	*	-	**	**	**	**	-	-	-	*	-	**	-	**	**	-	**	**	-	-
R06 PT	**	**	-	**	*	**	**	**	-	**	-	**	-	**	**	**	-	**	**	**	**	**	**	**	**	**	**
R08 MA	**	-	**	-	-	-	**	**	-	**	**	**	**	*	*	*	-	-	**	*	**	**	*	*	**	*	**
R10 ES	-	-	*	-	-	-	*	-	-	*	*	-	**	-	*	-	-	-	**	-	-	**	-	-	-	-	-
R12 NL	**	*	**	-	-	-	**	**	-	**	-	-	**	**	**	-	-	-	**	*	**	**	*	-	**	*	**
R14 IE	**	**	**	**	*	**	-	*	-	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
R16 ES	-	*	**	**	-	**	*	-	-	**	**	**	**	*	-	**	*	*	**	-	-	**	*	**	**	**	*
R18 ES	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
R22 ES	**	**	**	**	*	**	*	**	-	-	**	**	**	**	**	**	*	**	**	**	**	**	**	**	**	**	**
R24 IT	**	**	-	**	*	-	**	**	-	**	-	**	-	**	**	**	-	**	**	**	**	**	**	*	**	**	**
R26 IT	**	**	**	**	-	-	**	**	-	**	**	-	**	**	**	**	-	**	**	**	**	**	**	-	**	**	**
R28 PT	**	**	-	**	**	**	**	**	-	**	-	**	-	**	**	**	-	**	**	**	**	**	**	**	**	**	**
R30 FR	*	-	**	*	-	**	**	*	-	**	**	**	**	-	-	-	*	-	**	-	**	**	-	**	**	-	-
R32 GR	-	-	**	*	*	**	**	-	-	**	**	**	**	-	-	-	*	-	**	-	-	**	-	**	**	-	-
R34 ES	**	-	**	*	-	-	**	**	-	**	**	**	**	-	-	-	*	-	**	-	*	**	-	**	**	-	-
R38 PT	*	*	-	-	-	-	**	*	-	*	-	-	-	*	*	*	-	-	**	*	**	**	-	-	*	-	**
R42 NL	*	-	**	-	-	-	**	*	-	**	**	**	**	-	-	-	-	-	**	-	*	**	-	**	**	-	-
R46 GR	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**	**	**
R48 ES	-	-	**	*	-	*	**	-	-	**	**	**	**	-	-	-	*	-	**	-	-	**	-	**	**	-	-
R52 DE	-	**	**	**	-	**	**	-	-	**	**	**	**	**	-	*	**	*	**	-	-	**	*	**	**	**	*
R56 GR	**	**	**	**	**	**	**	**	-	**	**	**	**	**	**	**	**	**	**	**	**	-	**	**	**	**	**
R58 IE	*	-	**	*	-	*	**	*	-	**	**	**	**	-	-	-	-	-	**	-	*	**	-	**	**	-	-
R62 IT	**	**	**	*	-	-	**	**	-	**	*	-	**	**	**	**	-	**	**	**	**	**	**	-	**	**	**
R66 IT	**	**	**	**	-	**	**	**	-	**	**	**	**	**	**	**	*	**	**	**	**	**	**	**	-	**	**
R70 FR	**	-	**	*	-	*	**	**	-	**	**	**	**	-	-	-	-	-	**	-	**	**	-	**	**	-	-
R72 NO	-	-	**	**	-	**	**	*	-	**	**	**	**	-	-	-	**	-	**	-	*	**	-	**	**	-	-
Modal age	**	-	**	-	-	-	**	**	-	**	**	**	**	*	**	-	-	-	**	*	**	**	*	**	**	*	**

4.3.2 Advanced readers

Table 5.22. Coefficient of Variation (CV, %) of all advanced readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	all
3	17	0	0	31	-	0	17	33	**19**
4	0	13	17	16	-	16	9	40	**19**
5	9	9	8	19	-	14	8	13	**13**
6	0	17	0	24	-	22	9	0	**13**
7	7	13	0	7	-	18	15	0	**12**
8	10	5	13	5	-	7	10	11	**11**
9	5	6	5	8	-	8	6	5	**7**
10	0	6	16	6	-	19	6	15	**10**
11	8	0	8	9	13	14	10	4	**9**
12	6	7	28	18	-	12	7	10	**14**
13	7	16	12	9	-	8	7	7	**10**
14	9	9	11	14	-	8	6	4	**11**
15	0	5	12	5	-	5	5	5	**9**
16	7	3	12	7	-	8	12	7	**10**
17	0	-	0	10	-	-	11	8	**14**
18	3	3	4	10	-	5	8	5	**12**
19	5	8	7	9	3	4	8	4	**10**
20	-	-	-	-	-	-	-	-	**12**
21	11	15	5	16	-	-	0	0	**13**
22	-	-	-	-	-	-	-	-	**-**
23	-	-	-	-	-	-	-	-	**10**
Weighted Mean	**5**	**9**	**11**	**12**	**9**	**11**	**9**	**14**	**13**

Table 5.23. Percentage agreement (PA, %) of all advanced readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	all
3	67	100	100	67	-	100	67	33	**76**
4	100	71	53	71	-	75	82	41	**70**
5	80	80	83	50	-	60	83	33	**67**
6	100	33	100	33	-	67	67	100	**71**
7	75	75	100	75	-	67	25	100	**72**
8	67	83	33	83	-	33	67	33	**57**
9	80	60	20	60	-	60	40	80	**57**
10	100	67	50	67	0	0	67	33	**52**
11	50	100	67	50	0	33	67	33	**50**
12	60	40	60	40	-	60	80	40	**54**
13	50	0	50	62	100	88	25	50	**47**
14	80	67	0	0	0	60	40	60	**42**
15	100	50	0	50	-	50	50	50	**50**
16	57	71	14	43	100	43	43	57	**48**
17	100	0	0	0	0	100	0	50	**31**
18	50	75	0	25	0	50	50	50	**43**
19	60	50	0	20	0	60	60	40	**37**
20	100	100	0	0	-	0	100	100	**57**
21	50	50	0	0	0	0	100	100	**43**
22	-	-	-	-	-	-	-	-	**-**
23	0	100	-	0	-	0	0	100	**33**

Weighted Mean	73	63	43	49	14	58	59	51	56

Table 5.24. Relative bias table of all advanced readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R02 NO	R04 NL	R06 PT	R08 MA	R10 ES	R12 NL	R14 IE	R16 ES	all
3	0.33	0.00	0.00	0.67	-	0.00	0.33	0.00	**-**
4	0.00	0.18	0.07	0.24	-	-0.31	0.18	0.00	**-**
5	-0.20	-0.20	0.17	0.33	-	0.00	0.17	0.83	**-**
6	0.00	0.00	0.00	0.33	-	-0.67	0.33	0.00	**-**
7	0.25	0.50	0.00	0.25	-	-0.67	1.25	0.00	**-**
8	0.50	0.17	-1.00	-0.17	-	-0.67	0.50	0.00	**-**
9	0.20	-0.40	-0.80	0.00	-	0.00	0.60	0.20	**-**
10	0.00	-0.33	-1.00	-0.33	-1.00	-1.00	0.33	0.33	**-0.38**
11	0.83	0.00	-0.50	0.33	0.25	0.00	0.67	0.67	**0.28**
12	0.00	0.20	-2.00	-1.00	-	-0.80	0.40	-0.40	**-**
13	0.38	0.50	-0.62	0.12	0.00	-0.38	1.12	0.88	**0.25**
14	0.60	-0.67	-2.25	-0.80	3.00	-0.80	0.80	-0.40	**-0.06**
15	0.00	-0.50	-3.00	-0.50	-	-0.50	0.50	0.50	**-**
16	0.86	-0.29	-1.57	0.14	0.00	0.29	1.57	0.86	**0.23**
17	0.00	1.00	-3.00	-3.00	2.00	0.00	2.50	1.00	**0.06**
18	0.50	-0.25	-4.67	-2.25	1.00	0.00	1.25	0.75	**-0.46**
19	0.60	-1.00	-3.60	-1.80	-1.33	0.00	1.20	0.80	**-0.64**
20	0.00	0.00	-5.00	-4.00	-	1.00	0.00	0.00	**-**
21	-1.50	-2.00	-6.50	-3.00	-1.00	-1.00	0.00	0.00	**-1.88**
22	-	-	-	-	-	-	-	-	**-**
23	1.00	0.00	-	-4.00	-	-1.00	3.00	0.00	**-**
Weighted Mean	**0.27**	**-0.05**	**-1.25**	**-0.38**	**0.07**	**-0.30**	**0.72**	**0.32**	**-0.11**

4.4 Trachurus mediterraneus

Table 5.25. Overview of samples used for the *Trachurus mediterraneus* otoliths exchange.

Year	ICES area	Quarter	Number of samples	Modal age range	Length range (mm)
2012	27.8.c	2	4	5-10	430-480
2013	27.8.c	3	1	3	245
2014	27.8.c	2	1	4	300
2014	27.8.c	3	2	5	380-410
2014	27.8.c	4	3	4-5	370-380
2015	11.1	2	6	1-2	125-175
2015	11.1	3	9	1-4	185-315
2015	11.1	4	2	1	110-135
2015	27.8.c	3	1	4	335
2015	27.8.c	4	1	3	300
2016	11.1	1	1	1	140
2016	27.8.c	2	6	3-7	280-375
2017	27.8.c	1	2	6-7	415-440
2017	27.8.c	2	4	2-5	185-295
2018	27.8.c	2	7	3-4	255-320
2018	9	2	5	1-2	130-155
2018	9	3	9	0-5	65-330
2018	9	4	1	2	315
2019	11.1	1	2	2	265-280
2019	22	2	5	1-7	135-315
2019	9	4	8	0-4	95-230
2020	11.1	3	10	1-3	120-305
2020	22	2	10	2-4	150-265
2020	22	3	8	2-4	175-250
2020	22	4	7	1-5	100-275
2020	9	1	4	1-2	105-120
2020	9	2	12	1-3	100-325
2020	9	3	3	1	115-135
2021	9	2	6	1-3	160-235
2021	9	3	9	1-4	75-290
2021	9	4	1	1	155

Table 5.26. Readers overview.

Reader code	Expertise	SmartUser
R04 IT	Advanced	Casciaro
R08 GR	Advanced	Sioulas
R14 IT	Basic	Bellodi
R16 PT	Basic	Correia
R40 GR	Basic	Legaki
R42 ES	Basic	LopezE
R54 GR	Basic	Nikiforidou
R60 IT	Basic	Pesci
R64 IT	Basic	RussoL

4.4.1 All readers

The weighted average percentage agreement based on modal ages for all readers is 61 %, with the weighted average CV of 36 % and APE of 24 %.

Table 5.27. Coefficient of Variation (CV, %) of all readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R04 IT	R08 GR	R14 IT	R16 PT	R40 GR	R42 ES	R54 GR	R60 IT	R64 IT	all
0	-	-	-	-	-	-	-	-	-	**-**
1	0	46	43	41	107	52	59	34	24	**50**
2	40	29	23	34	56	48	37	25	32	**37**
3	20	32	18	29	33	35	28	21	19	**29**
4	24	29	21	38	29	29	18	16	29	**31**
5	17	27	8	28	24	25	12	14	24	**28**
6	11	0	13	16	20	6	28	11	-	**33**
7	0	7	8	7	0	16	22	7	20	**18**
8	-	-	-	-	-	-	-	-	-	**-**
9	-	-	-	-	-	-	-	-	-	**-**
10	-	-	-	-	-	-	-	-	-	**32**
Weighted Mean	**22**	**32**	**24**	**34**	**54**	**40**	**35**	**23**	**26**	**36**

The percentage agreement per reader per modal age tells how large part of the readings that are equal to the modal age. The weighted mean including at the bottom of the table is weighted according to number of age readings.

Table 5.28. Percentage agreement (PA, %) of all readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R04 IT	R08 GR	R14 IT	R16 PT	R40 GR	R42 ES	R54 GR	R60 IT	R64 IT	all
0	100	100	100	60	100	0	100	80	20	**73**
1	100	66	79	54	46	86	70	79	93	**75**
2	45	78	77	78	52	49	68	73	60	**65**
3	74	64	69	56	62	41	53	50	69	**60**
4	40	57	70	33	25	14	76	76	35	**48**
5	36	36	82	27	45	9	70	45	20	**41**
6	50	100	50	0	50	0	50	50	-	**44**
7	100	75	25	75	100	0	25	75	0	**54**
8	-	-	-	-	-	-	-	-	-	**-**
9	-	-	-	-	-	-	-	-	-	**-**
10	0	-	-	100	100	0	0	0	0	**29**
Weighted Mean	**63**	**68**	**74**	**58**	**52**	**43**	**67**	**68**	**59**	**61**

The relative bias is the difference between the mean age (per modal age per reader) and modal age. As for the previous tables, a combined bias for all readers and weighted means are calculated.

Table 5.29. Relative bias of all readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R04 IT	R08 GR	R14 IT	R16 PT	R40 GR	R42 ES	R54 GR	R60 IT	R64 IT	all
0	0.00	0.00	0.00	0.40	0.00	1.00	0.00	0.20	0.80	**0.27**
1	0.00	0.21	0.07	0.54	-0.29	-0.03	-0.07	0.21	0.07	**0.08**
2	-0.20	0.27	0.20	0.15	0.10	0.26	0.25	0.20	0.02	**0.14**
3	0.13	0.24	0.27	-0.36	0.43	0.62	-0.06	0.23	-0.23	**0.14**
4	0.70	0.90	0.10	-0.39	0.75	2.10	0.14	0.00	-0.47	**0.43**
5	1.00	0.27	0.18	0.00	1.36	2.82	-0.10	0.18	-0.90	**0.54**
6	0.50	0.00	-0.50	-1.50	1.00	5.50	-1.00	0.50	-	**-**
7	0.00	0.25	-0.75	-0.25	0.00	2.00	-1.25	0.25	-2.00	**-**
										0.19
8	-	-	-	-	-	-	-	-	-	**-**
9	-	-	-	-	-	-	-	-	-	**-**
10	-1.00	-	-	0.00	0.00	3.00	-3.00	-4.00	-5.00	**-**

For each pair that is being compared, the differences between the readings per image are found and the frequency of each occurring difference is obtained.

Table 5.30. Inter reader bias test (i.e. probability of bias between readers and with modal age). - = no sign of bias (p>0.05), * = possibility of bias (0.01<p<0.05), * * = certainty of bias (p<0.01)

Comparison	R04 IT	R08 GR	R14 IT	R16 PT	R40 GR	R42 ES	R54 GR	R60 IT	R64 IT
R04 IT	-	*	-	-	-	**	*	-	**
R08 GR	*	-	*	*	-	**	**	-	**
R14 IT	-	*	-	-	-	**	-	-	**
R16 PT	-	*	-	-	-	**	-	-	*
R40 GR	-	-	-	-	-	**	*	-	**
R42 ES	**	**	**	**	**	-	**	**	**
R54 GR	*	**	-	-	*	**	-	*	*
R60 IT	-	-	-	-	-	**	*	-	**
R64 IT	**	**	**	*	**	**	*	**	-
Modal age	-	**	*	-	*	**	-	**	*

4.4.2 Advanced readers

Table 5.31. Coefficient of Variation (CV, %) of all advanced readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R04 IT	R08 GR	all
0	-	-	**-**
1	0	33	**35**
2	29	22	**26**
3	7	27	**21**
4	21	13	**18**
5	14	7	**12**
6	7	18	**15**
7	6	7	**6**
8	-	-	**-**
9	-	-	**-**
Weighted Mean	**12**	**23**	**25**

Table 5.32. Percentage agreement (PA, %) of all advanced readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R04 IT	R08 GR	all
0	71	100	**86**
1	100	49	**74**
2	77	79	**78**
3	95	71	**84**
4	53	81	**68**
5	57	86	**71**
6	80	50	**64**
7	83	67	**75**
8	-	-	**-**
9	100	-	**100**
Weighted Mean	**83**	**69**	**76**

Table 5.33. Relative bias of all advanced readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R04 IT	R08 GR	all
0	0.29	0.00	**0.14**
1	0.00	0.51	**0.26**
2	0.31	0.24	**0.28**
3	0.05	0.48	**0.26**
4	0.74	0.24	**0.49**
5	0.57	0.14	**0.36**
6	0.20	1.00	**0.60**
7	0.17	0.33	**0.25**
8	-	-	**-**
9	0.00	-	**-**
Weighted Mean	**0.23**	**0.37**	**0.31**

4.5 Trachurus picturatus

Table 5.34. Overview of samples used for the *T. picturatus* otoliths exchange.

Year	Area	Strata	Quarter	Number of samples	Modal age range	Length range (mm)
2015	GSA 9	Q2	2	2	0	80-95
2016	GSA 9	Q2	2	12	1-4	165-300
2016	GSA 9	Q3	3	13	0-5	85-410
2018	27.10.a	Q4	4	5	2-4	255-295
2018	GSA 9	Q4	4	12	2-4	330-425
2019	27.10.a	Q4	4	3	2	220-240
2020	27.10.a	Q1	1	5	4-6	305-375
2020	27.10.a	Q4	4	16	1-7	110-395
2020	GSA 9	Q4	4	10	3-5	365-425
2021	34.1.2	Q2	2	21	0-3	130-210
2021	34.1.2	Q3	3	18	0-2	130-195
2021	GSA 9	Q3	3	2	2-3	380-455

Table 5.35. Readers overview.

Reader code	Expertise	SmartUser
R02 ES	Advanced	RuzafaA
R04 PT	Basic	Correira
R06 PT	Basic	CruzR
R08 PT	Basic	Felicio
R12 ES	Basic	RodriguezE

4.5.1 All readers

The weighted average percentage agreement based on modal ages for all readers is 64 %, with the weighted average CV of 40 % and APE of 30 %.

Table 5.36. Coefficient of Variation (CV, %) of all readers combined per modal age and a weighted mean of the CV per reader.

Modal age	R02 ES	R04 PT	R06 PT	R08 PT	R12 ES	all
0	-	-	-	-	-	**-**
1	52	65	29	91	35	**62**
2	26	25	28	63	31	**41**
3	33	18	9	35	41	**33**
4	0	19	13	21	22	**20**
5	-	19	13	0	17	**18**
6	-	-	-	-	-	**22**
7	-	-	-	-	-	**9**
Weighted Mean	**38**	**32**	**21**	**54**	**32**	**40**

The percentage agreement per reader per modal age tells how large part of the readings that are equal to the modal age. The weighted mean including at the bottom of the table is weighted according to number of age readings.

Table 5.37. Percentage agreement (PA, %) of all readers combined per modal age and a weighted mean of the PA per reader.

Modal age	R02 ES	R04 PT	R06 PT	R08 PT	R12 ES	all
0	100	88	0	100	78	**76**
1	74	83	4	76	78	**62**
2	38	73	56	48	68	**60**
3	38	84	92	38	45	**65**
4	100	67	75	55	46	**63**
5	100	50	0	100	80	**62**
6	-	0	-	100	100	**67**
7	-	100	-	0	-	**50**
Weighted Mean	**69**	**76**	**50**	**61**	**65**	**64**

The relative bias is the difference between the mean age (per modal age per reader) and modal age. As for the previous tables, a combined bias for all readers and weighted means are calculated.

Table 5.38. Relative bias table of all readers combined per modal age and a weighted mean of the relative bias per reader.

Modal age	R02 ES	R04 PT	R06 PT	R08 PT	R12 ES	all
0	0.00	0.12	1.75	0.00	0.22	**0.42**
1	0.21	0.22	1.52	0.10	0.22	**0.45**
2	0.75	0.23	0.56	0.63	0.12	**0.46**
3	1.12	-0.04	0.00	0.86	0.80	**0.55**
4	0.00	-0.13	-0.08	0.73	0.38	**0.18**
5	0.00	-0.67	-1.25	0.00	0.40	**-0.30**
6	-	-2.00	-	0.00	0.00	**-**
7	-	0.00	-	1.00	-	**-**
Weighted Mean	**0.39**	**0.04**	**0.58**	**0.47**	**0.34**	**0.40**

For each pair that is being compared, the differences between the readings per image are found and the frequency of each occurring difference is obtained.

Table 5.39. Inter reader bias test (i.e. probability of bias between readers and with modal age). - = no sign of bias (p>0.05), * = possibility of bias (0.01<p<0.05), * * = certainty of bias (p<0.01)

Comparison	R02 ES	R04 PT	R06 PT	R08 PT	R12 ES
R02 ES	-	**	**	**	-
R04 PT	**	-	**	**	*
R06 PT	**	**	-	-	*
R08 PT	**	**	-	-	-
R12 ES	-	*	*	-	-
Modal age	**	-	**	**	**

4.6 SmartDots experience

The SmartDots program functioned reasonably well for viewing and annotating otolith images. However, several issues raised regarding the SmartDots experience. As with other ageing groups, the WKARHOM4 participants discussed around the use of SmartDots and their feedback had identified several points:

The WKARHOM4 participants consider that it was too difficult to use one common reference axis. It would be preferable to set a limited area or zone of reading to have the comparable distance between different reading axes created by the reader.

The WKARHOM4 participants would like to be able to rotate the images.

The WKARHOM4 participants would like to identify their identification number when reviewing the exchanges reports. A unique annotation colour for each reader participating in several events during the same workshop would be very appreciated.

The WKARHOM4 chairs suggest simplifying file and image management:

ability to rename images directly on website.

delete images and files by introducing a check-box for each sample

possibility for delegates to change the size and colours of the readers' annotation or alternatively using same mark size for all readers and different colours for each reader.

for each sample, given the number of marks and edge type, to be able to apply an ageing scheme (or other) automatically to all readings without working on the R script.

These improvement recommendations are a development of a general recommendation included in section 8.

5 Update age reading protocols for *Trachurus* species

5.1 Horse mackerel (*Trachurus trachurus*)

Otoliths preparation

Protocols agreed during the subgroup session for whole and sliced otoliths were:

- For whole otoliths:

  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. 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 (Fig. 6.1).

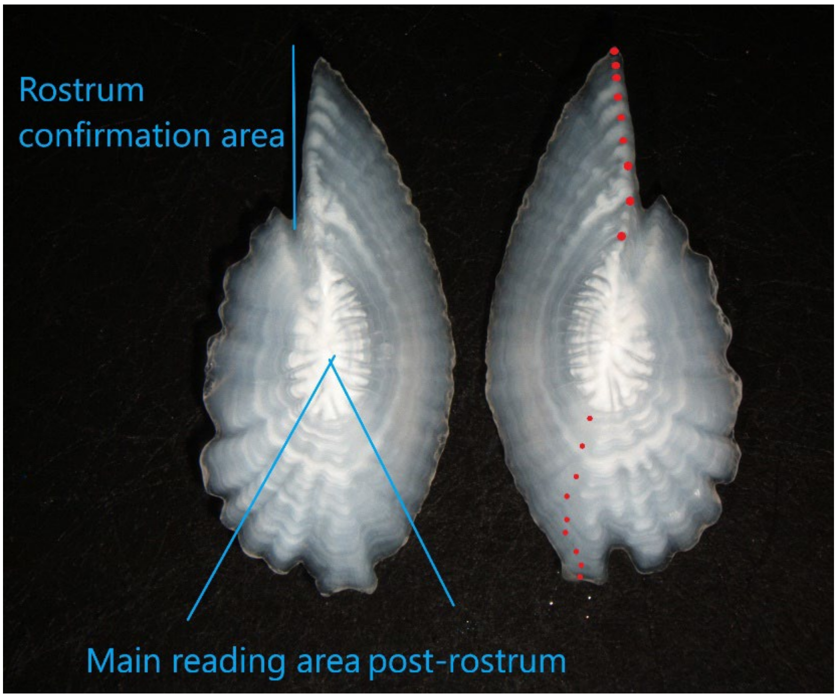


Figure 6.1. Preferred reading areas in *T. trachurus* whole otoliths

For sliced otoliths:

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. In this way the translucent/dark rings (winter) can be counted (Fig. 6.2).

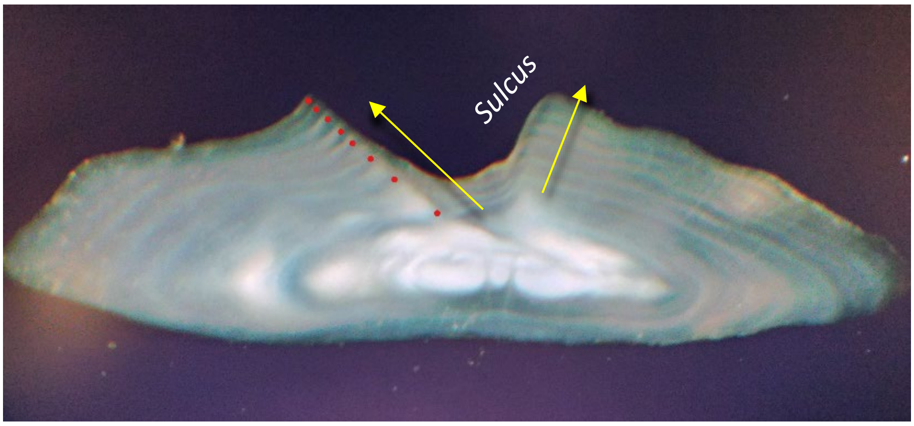


Figure 6.2. Preferred reading areas in *T. trachurus* sliced otoliths

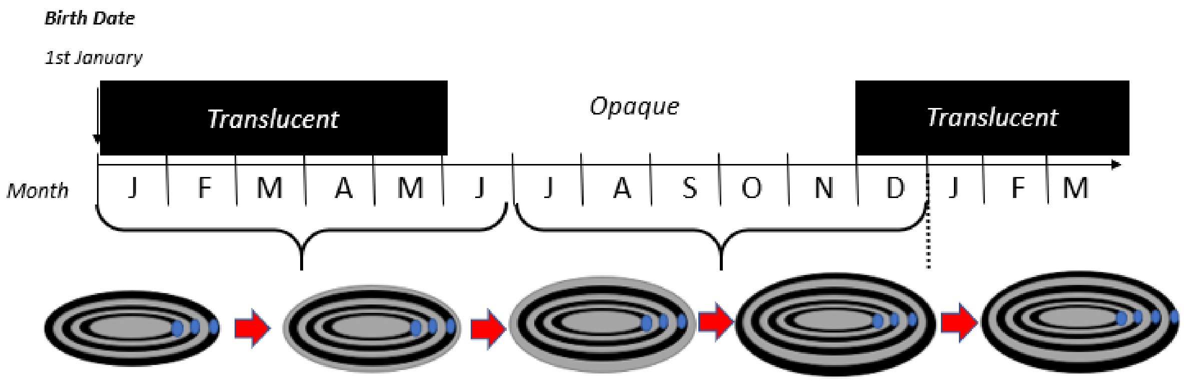
Changing the focus can get rid of false or split rings, resulting in the true rings becoming more visible. Adjusting the light intensity and direction can also be helpful.

The protocols used by the institutions participating in the WKARHOM4 exchanges are summarized in Table 6.1.

Table 6.1. Protocols used in *T. trachurus* otoliths by the institutions participating in the WKARHOM4.

Participant Institutions	Area (ICES, GSA)	Otolith preparation method
Norway (IMR)	ICES 27.4a	Sliced (0.8 mm thick) and glued on glass with epoxy (5-6 otoliths per slide)
Ireland (Marine Institute)	ICES 27.6 and 27.7	Sliced (0.6-0.65 mm thick) and glued on glass slide with histokitt (10 otoliths per slide)
Netherlands (WMR)	ICES 27.4, 27.6, 27.7 and 27.8	Sliced (0.55-0.6 mm thick) and glued to glass slides (containing 25 otoliths). Age is determined using SmartDots
Germany (Thünen-Institut)	ICES 27.4, 27.7	Sliced (0.5-0.6 mm) glued on glass slide, brushed with baby oil.
France (IFREMER)	ICES 27.7, 27.8	Immersed in fresh water, fish >3 years are sliced in resin (0.5-0.6mm thick). Photos of all otoliths, and aged using SmartDots
Greece (FRI, HCMR)	GSA 22 GSA 20	FRI: Otoliths pair immersed in solution of fresh-water and 70% alcohol HCMR: Otoliths immersed in fresh water
Italy (COISPA, CIBM, Univ. Cagliari)	GSA 18, 19 GSA 09, 10, 11	Whole otoliths immersed in sea water
Portugal (IPMA)	ICES 27.9a	Small (<26cm): immersed whole in distilled water and observed over a black background immersed in baby oil Big (>27cm): sliced (0.5 mm) and and glued on glass slide
Spain (IEO- CSIC, AZTI)	ICES 27.8b, 27.8c and 27.9a north (AZTI, IEO-Santander and IEO-Vigo) GSA 01 (IEO-Málaga)	AZTI: one broken and burnt, mounted in black plastiline and both submerged in 70% alcohol (whole otolith) IEO-Málaga: whole otoliths submerged in seawater IEO: whole otoliths submerged in tap water and over a black background.
Morocco (INRH)	FAO 34.1.1	Sliced (0.4-0.5 mm) then polished down to 0.2-0.3 mm; drop of cedar oil added to the slice when reading.

Diagram of otoliths interpretation



Grey growth ring: opaque zone // Black growth ring: translucent zone

The assignment of the age class to the counted rings depends both on the birth date (which is 1st January in all the cases) and the catch date (considering the quarter or the semester), following the next ageing schemes:

Catch date (quarter)	1		2		3		4	
Edge of otolith	T	O	T	O	T	O	T	O
Age	N	N+1	N	N	N	N	N-1	N

Catch date (semester)	Edge of otolith	Age
1	Opaque (Jan-Mar) Translucent New opaque (May-Jun)	N+1 N N
2	Opaque Translucent (Nov-Dec)	N N-1

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. Do not use transmitted light.
- 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 1 mm scale bar, pretreatments of images could induce bias due to different size of otoliths) 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).

- For whole otoliths, the first ring should be visible at the start of the rostrum and continues along both sides of the excisura (the area between the rostrum and anti-rostrum). As a guideline, the minimum distance of the 1st annulus is ca. 2 mm.
- First four growth annuli: on whole otolith, it is possible to follow them all around the entire otolith. 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.
- Catch date is vital depending on the quarter. Extra data such as fish length, sex, maturity and catch area may also be considered when assigning an age.

Other recommendations

The readers recommend to only use calibrated images, with 1 mm scale bar and standardized magnification for both whole and sliced images; as well as a standardized image size for all submissions to otolith exchanges. For whole otoliths both otoliths should be present on the image, decalcified or damaged otoliths should be excluded from an exchange.

It was suggested at WKARHOM3 that otoliths extracted from fish >26 cm (or older than 4 years) should be sliced. However, this indication was not followed by all the laboratories participating in the previous and *adhoc* WKARHOM4 otolith exchanges, and this suggestion should be adopted. In addition, for sliced otoliths a standard thickness of 0.5-0.6 mm is recommended.

5.2 Mediterranean horse mackerel (*Trachurus mediterraneus*)

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, 1984) 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 an attempt to reach agreement on a common method of annuli interpretation (Eltink, 1985; ICSEAF, 1986; Marecos, 1986; Borges, 1989; Eltink and Kuiter, 1989; ICES, 1991). 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 annuli (Karlou-Riga, 2000), indeed, the characteristic of the detection of a ring around the otolith also on the rostrum zone is not always helpful.

Storage

After the extraction, otoliths are washed in order to remove the organic material, then dried and stored in Eppendorf.

Preparation and interpretation

The otoliths are analyzed 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 (Fig. 6.3). 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 an axis from to core to the posterior edge of distal face (Fig. 6.4). Usually, the otoliths of *T. mediterraneus* need a clarification phase before the age analysis and are immersed in seawater. Time depends on the otolith size and on laboratories approach (from 1 to 4 hours). To increase contrast between dark and opaque rings, otoliths can be toasted in an oven for minimum 24 hours and 190-200°C of temperature, depending on the size of the samples, to achieve the best growth rings alternation (Fig. 6.5).

For the biggest specimens (>26-27 cm) to increase the readability of the translucent rings is suggested as preparation thin section, following the same protocol of *T. trachurus.*

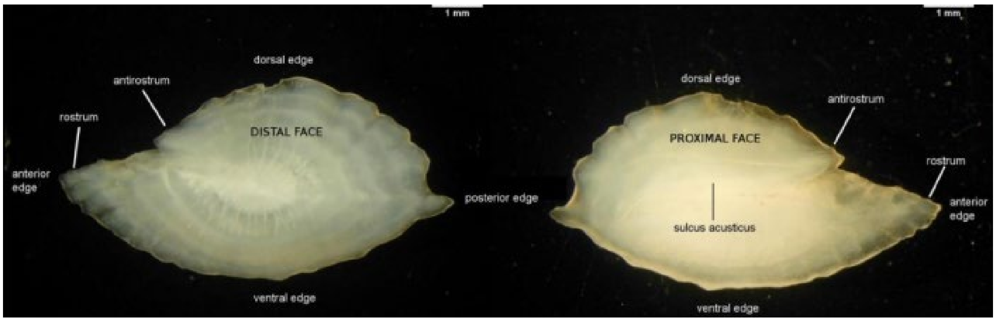


Figure 6.3. Proximal and distal face of *T. mediterraneus* otolith.

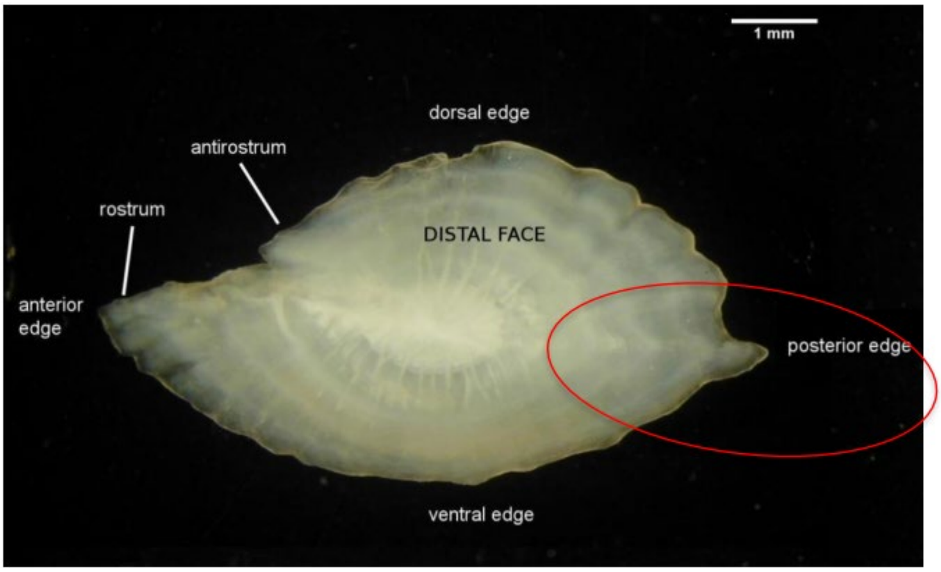


Figure 6.4. Preferred area to age *T. mediterraneus* otoliths. In addition, post-rostrum area is used to calculate distance from the core to each ring.

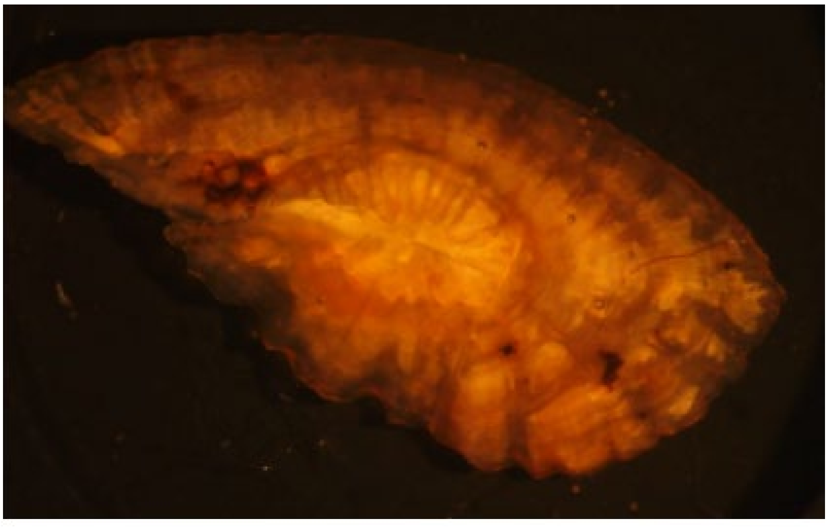


Figure 6.5. Toasted otolith of *T. mediterraneus*.

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, 2000).

The scheme to age the otoliths reported in Table 6.2. considering the time of annulus formation (generally translucent ring (T) during winter -spring months; opaque ring (O) during -autumn months), the capture date (based on quarters), the otolith edge nature and the spawning period. For specimens caught during the third quarter, if a translucent ring is observed at the edge in July and August (capture date), the suggestion age is N because the opaque ring has not started yet to be formed. While, in September, a T edge could be interpreted as an early deposition of translucent and the age is N-1. In the fourth quarter, for the otolith with the translucent edge, the age is N-1. For the specimens with O edge, caught in third and fourth quarter, the age is N. If a translucent 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 quarter, the age corresponds to the number of the T rings, though the translucent ring at the edge is not still formed. For the specimens caught in the second quarter with the O edge, the age corresponds to N-1. Indeed, in that period otoliths start a new deposition of opaque material, but the birthday has not passed (Fig. 6.6).

Table 6.2. The age scheme to age the otoliths of *T. mediterraneus*. Birth date: 1st July. N is the number of the translucent rings.

Catch date (quarter)	1		2		3		4	
Edge of otolith	T	O	T	O	T	O	T	O
Age	N-1	N	N-1	N-1	N (July-Aug) N-1 (Sept)	N	N-1	N

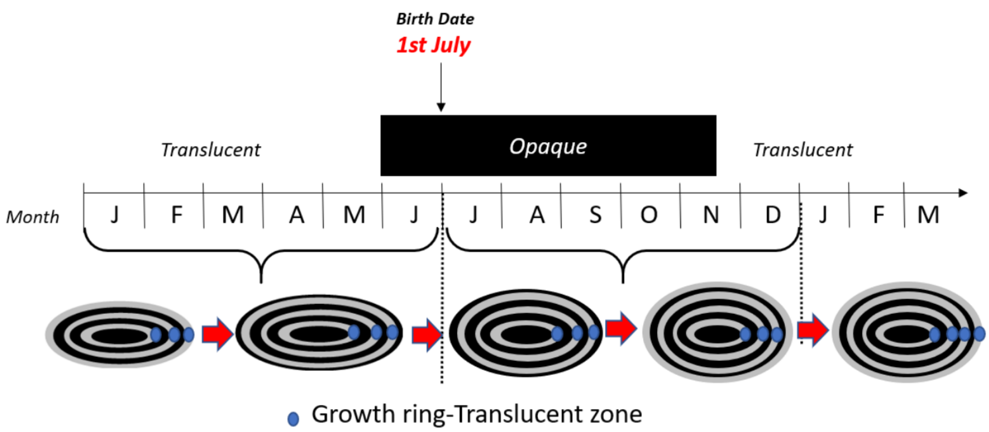


Figure 6.6. Scheme age for *T. mediterraneus*. Birth date: 1st July

Due to the lack of knowledge about the spawning pattern for the Atlantic area, it is suggested to follow an age scheme based on 1st January as birth date like in *T. trachurus*.

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 translucent edge (Figure 6.7). This is a false ring probably laid down when the juveniles changed the environment and the diet.

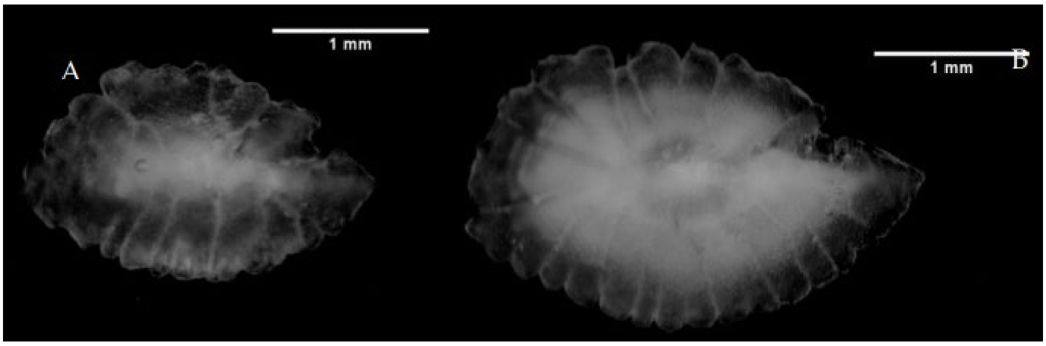


Figure 6.7. Otoliths from small specimens of *T. mediterraneus* (A, 5 cm and B, 7.5 cm total length), caught (A) during the summer (29/07/2011) and (B) the autumn (06/10/2011), respectively.

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 6.8).

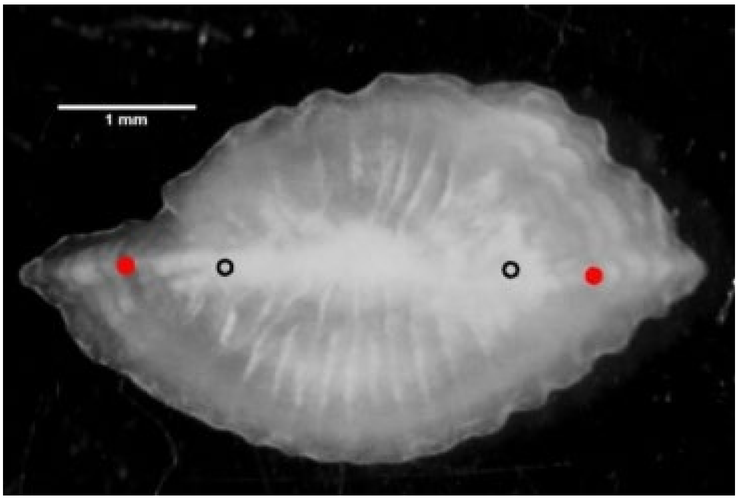


Figure 1.8. Specimen of *T. mediterraneus* with 14.5 cm of total length caught in the summer (28/07/2011). The open black circle is a false ring the red one the first winter ring

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 translucent 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 6.9).

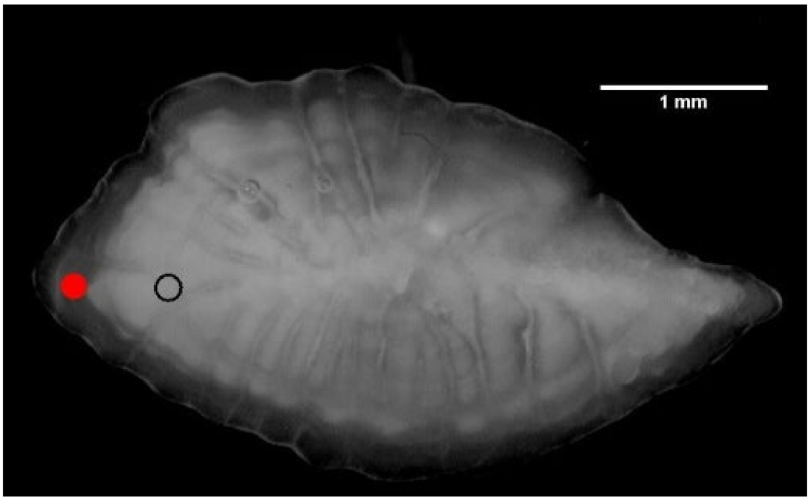


Figure 6.9. Otolith of *T. mediterraneus* of specimen with 12.5 cm of total length caught in the early spring (12/05/2011). The open black circle is a false ring the red one the first winter ring.

Sometimes the first true ring appears not exclusively as a single ring. Indeed, Karlou-Riga (2000) distinguished 4 types of otoliths based on the morphology of the first winter ring (Figure 6.10).

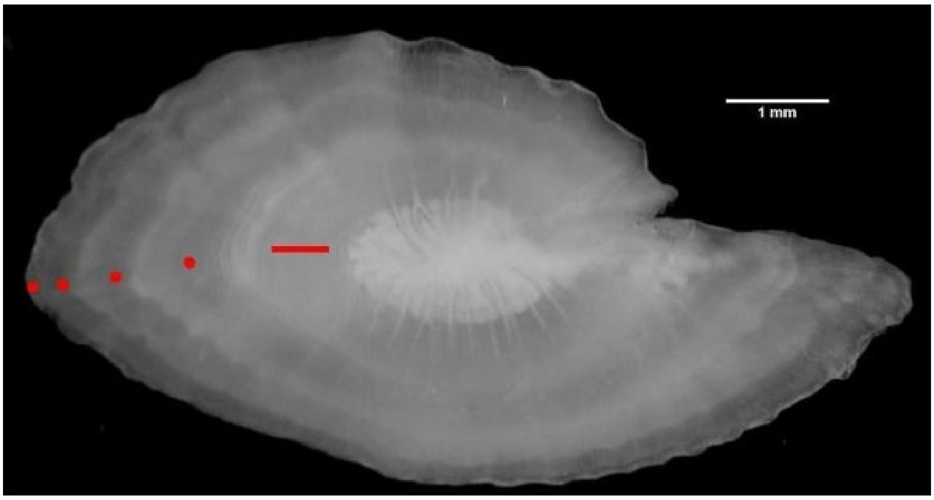


Figure 6.10. The specimens (female 29 cm total length caught on the 15/05/2011) with first winter ring as a translucent zone because the false rings are jointed with the first true ring. The red spot represents the winter ring; the red line represents the first winter.

After the first winter ring, other false ring could be laid down during the second year of life (Figure 6.11). 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.

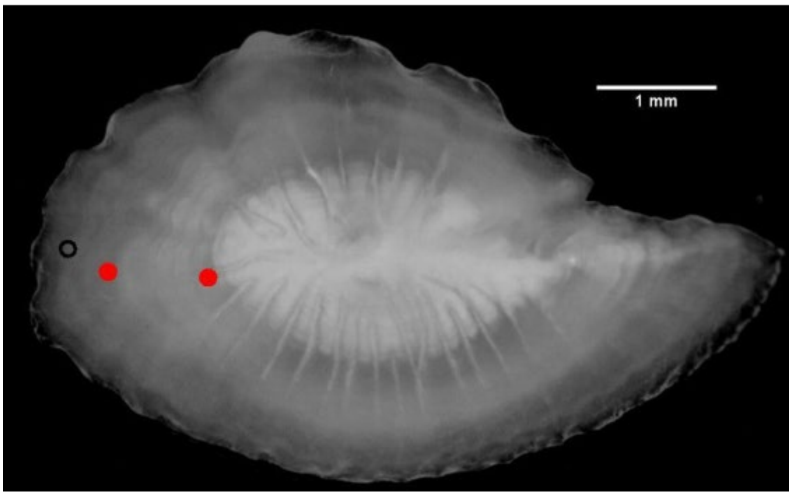


Figure 6.11. Otolith of a female with 20.5 cm of total length and the gonads in a post reproductive stage caught during

After the second winter ring, the deposition pattern of the winter band (translucent – black one) appear regular with a reduction of its distance (Figure 6.12).

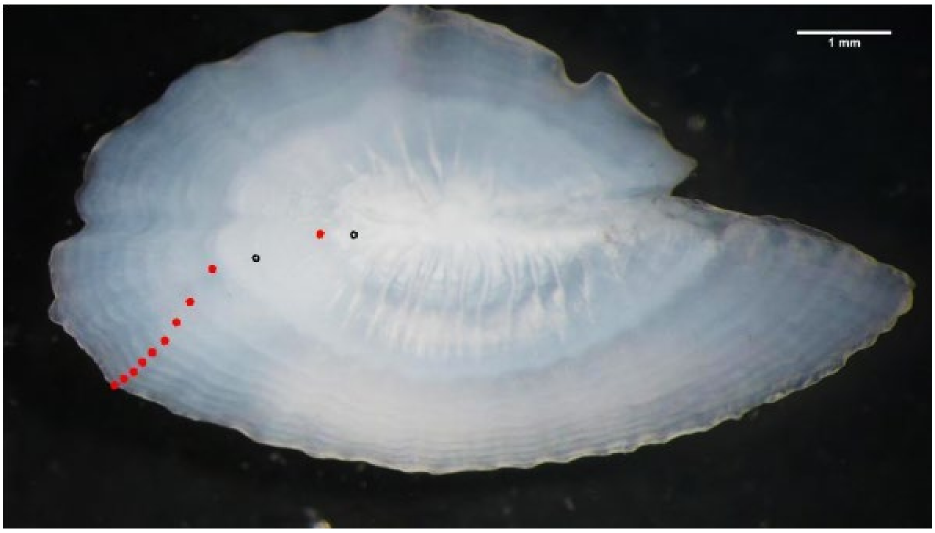


Figure 6.12. Otolith of *T. mediterraneus* male with 35.5 cm caught in the winter (24/03/2011). The open black circles represent the false rings while the red dots represent the true winter rings.

5.3 Blue jack mackerel (*Trachurus picturatus*)

Introduction

General information about the species, including geographical distribution and biological aspects, was presented in the WKARHOM2 (ICES, 2015). FAO catches statistics of the species continue very unrealistic with a great peak in their total landings in 2016, which keep them uncertain and not reliable (FAO, 2021). However, national official statistics in the areas where *T. picturatus* is a historically important fishing resource (i.e. Azores, Madeira and the Canary Islands), and in Portugal mainland, are more reliable (INE, 2022; RCG-LDF, 2022) (Fig. 6.13). Although landings seem to be quite variable between years in the archipelagos, blue jack mackerel remains an important species in the region and is becoming more important northwards.

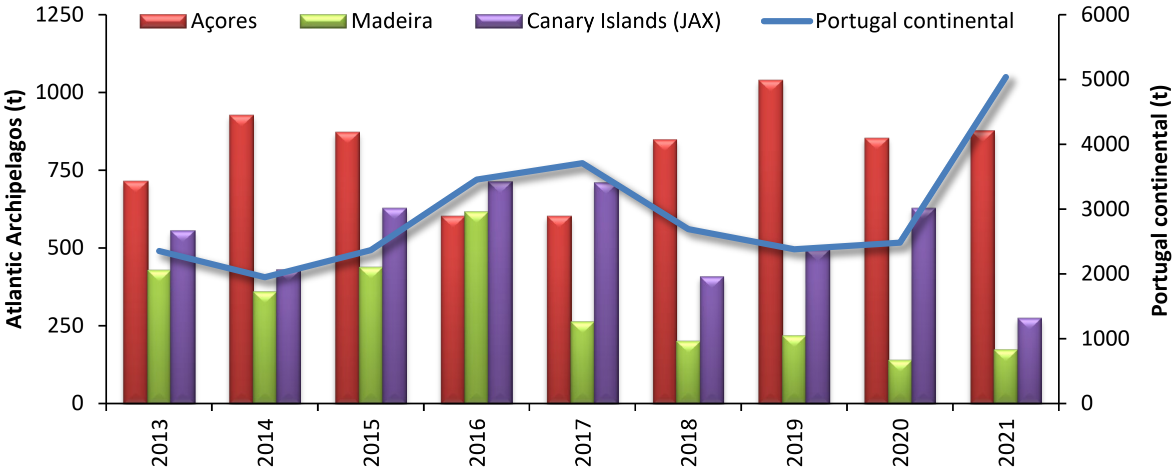


Figure 6.13. Blue jack mackerel total landings (sources: INE, 2022; RCG-LDF, 2022)

Regarding the age and growth knowledge of the species, some advances have been performed (Table 6.3).

Finally, several studies have aimed to describe the population structure of the species, including the Central and North-east Atlantic and the Mediterranean Sea, reflecting the interest of this topic. However, those studies do not cover the same geographical area and conclusive results about the identity of stocks units nor mixing among them are not available at present.

Table 6.3. Summary of the age and growth studies on *T. picturatus*. ML: from the paired values mean length per age (age-length keys); DR: from the total paired values of the direct readings; BC: from the paired values of mean back-calculated length per age; LF: from length frequencies evolution. New studies appear grey-shaded.

Area	Reference	Method	N	Length	Growth parameters				
					L_{inf} (cm)	*k* (years-1)	t_0 (years)	R^2	ϕ'
Azores	Isidro (1990)	ML	516	FL	52.9	0.2	-0.23	n.a.	2.75
	Westhaus-Ekau and Ekau (1982)	DR	n.a.	TL	51.1	0.144	-1.584	n.a.	2.57
	Garcia et al. (2015)	DR	1420	FL	58.3	0.09	-2.67	n.a.	2.48
		BC	796	FL	52.9	0.11	-2.45	n.a.	2.49
	Santos et al. (2022)	LF	104299	FL	55.9	0.08	n.a.	n.a.	2.39
Portugal mainland	Neves et al. (2022)	DR	575	TL	63.3	0.07	-3.37	n.a.	2.03
		BC	376	TL	46.7	0.15	-2.04	n.a.	2.41
Madeira	Jesus (1992)	ML	489	TL	44.3	0.316	n.d.	0.93	2.79
	Pereira (1993)	ML	877	TL	43.8	0.23	-0.57	0.97	2.64
	Vasconcelos et al. (2006)	DR	578	TL	42.3	0.161	-2.563	0.74	2.46
		BC	229	TL	48.3	0.135	-2.898	0.99	2.50
	Vasconcelos et al. (2008)	ML	889	TL	36.0	0.254	-1.445	0.99	2.52
Mediterranean Sea	Casaponsa (1993)	n.a.	n.a.	TL	32.2	0.228	-1.469	n.d.	2.37
	Cefali et al. (2004)	n.a.	875	FL	38.8	0.22	-1.79	n.d.	2.52
Canary Islands	Jurado-Ruzafa and Santamaría (2018)	DR	913	TL	34.9	0.214	-2.545	0.87	2.42

Otoliths preparations

Otoliths' protocols by laboratory are summarized in Table 6.4.

Table 6.4. Summary of the otolith's preparation of *T. picturatus* by laboratory participating in the WKARHOM4.

Country	Institutions	Area	Otolith preparation
Italy	COISPA	GSA 18, 19	Whole otoliths immersed in sea water (24h) Birth date: **1st January**.
	APLYSIA - CIBM	GSA 09	
	Univ. Cagliari	GSA 11	
Portugal	Gov. dos Açores (resuming activity)	Açores (ICES 27.10)	Immersed whole in distilled water and over a black background
	DSIDRP (no current reading)	Madeira (FAO 34.1.2)	Immersed whole in distilled water and over a black background
	IPMA (beginning 2021)	Portugal mainland (ICES 27.9a)	**≤26cm**, immersed whole in distilled water (24h) and over a black background, immersed in "baby oil". **>27cm**, sliced otoliths (0.5 mm) (IPMA-protocol)
Spain	IEO-CSIC	Canary Islands (FAO 34.1.2)	Immersed whole in distilled water and over a black background

Annulus identification

When the age determination technique for *T. picturatus* is carried out on whole otoliths, annuli are counted preferentially on the *nucleus* to the *postrostrum* area (Fig. 6.14). 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.

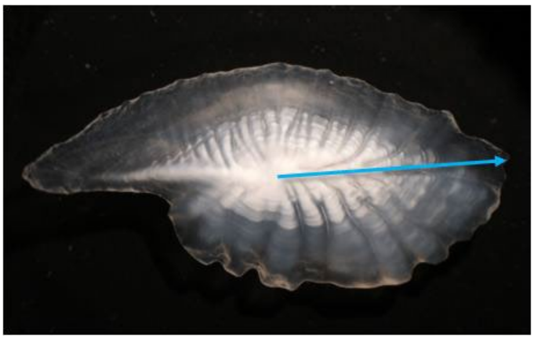


Figure 6.14. Preferred reading area in otoliths of *T. picturatus*

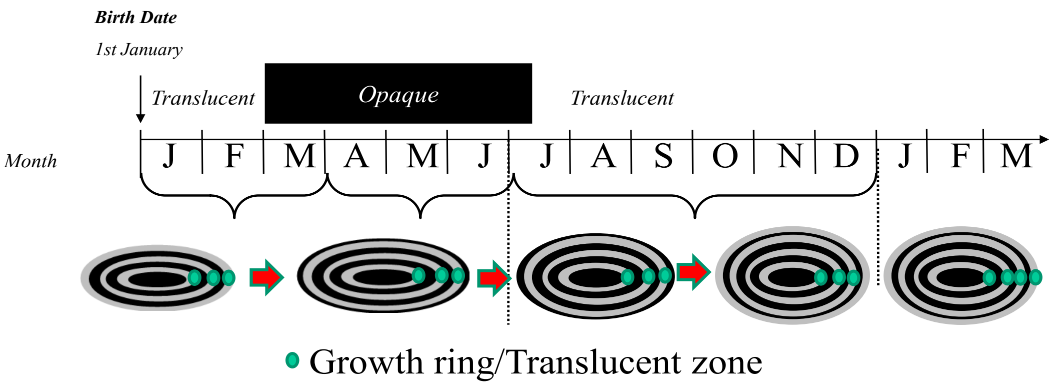
However, big specimens are more common in landings at Portugal mainland than in the other areas. Therefore, small specimens (<26cm) are immersed whole in distilled water (24h) and observed over a black background, immersed in baby oil. While the bigger ones (>27cm) are sliced and glued on glass slide (0.5 mm), following the protocol in Annex 3.

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; Garcia et al., 2015; Jurado-Ruzafa and Santamaría, 2013).
- 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.

However, the annuli deposition pattern in otoliths of *T. picturatus* only have been specifically studied in waters of **Madeira** (Vasconcelos, 2017) **and the Canary Islands** (Jurado-Ruzafa and Santamaría, 2018):

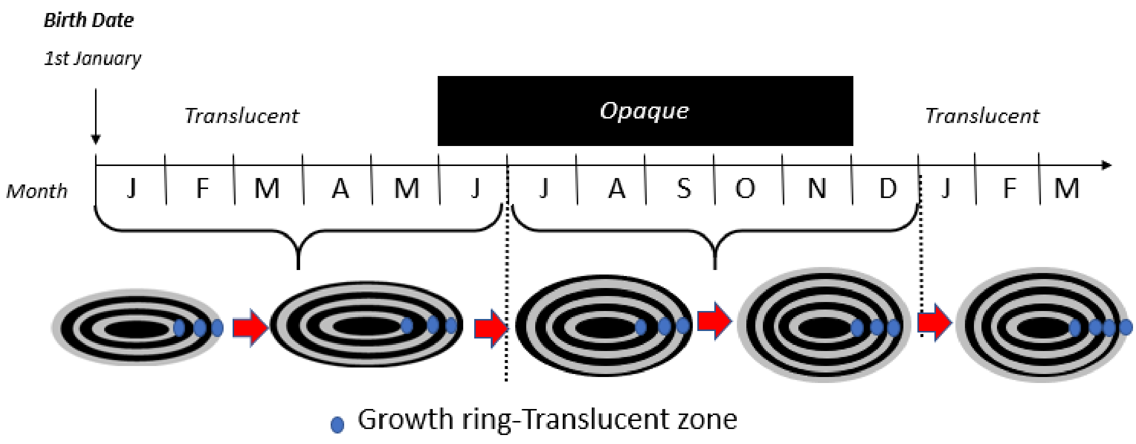


The age scheme assumed in the area to assign ages to counted annuli (N) is:

Catch date (quarter)	Edge of otolith	Age
1	Translucent	N
	Opaque	N-1
2	Translucent Opaque	**N-1***
3-4	Translucent Opaque	N-1

- 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.
- 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.

Due to a lack of data and growth studies, **in the Mediterranean Sea and NE Atlantic waters**, the implied laboratories assume the deposition pattern observed in other *Trachurus* species, which is practically the contrary than in the Macaronesian archipelagos. Although it is not proven in the Atlantic waters, in the Mediterranean Sea, *T. picturatus* has been described as a "summer spawner" (Lloris and Moreno, 1995), what in fact is the contrary than in northern Macaronesian archipelagos, where the species spawns from January to April-May (Faria and Vasconcelos, 2008; Garcia et al., 2015; Jurado-Ruzafa and Santamaría, 2013):



The related age scheme is:

Catch date (quarter)	1		2		3		4	
Edge of otolith	T	O	T	O	T	O	T	O
Age	N	N+1	N	N	N	N	N-1	N

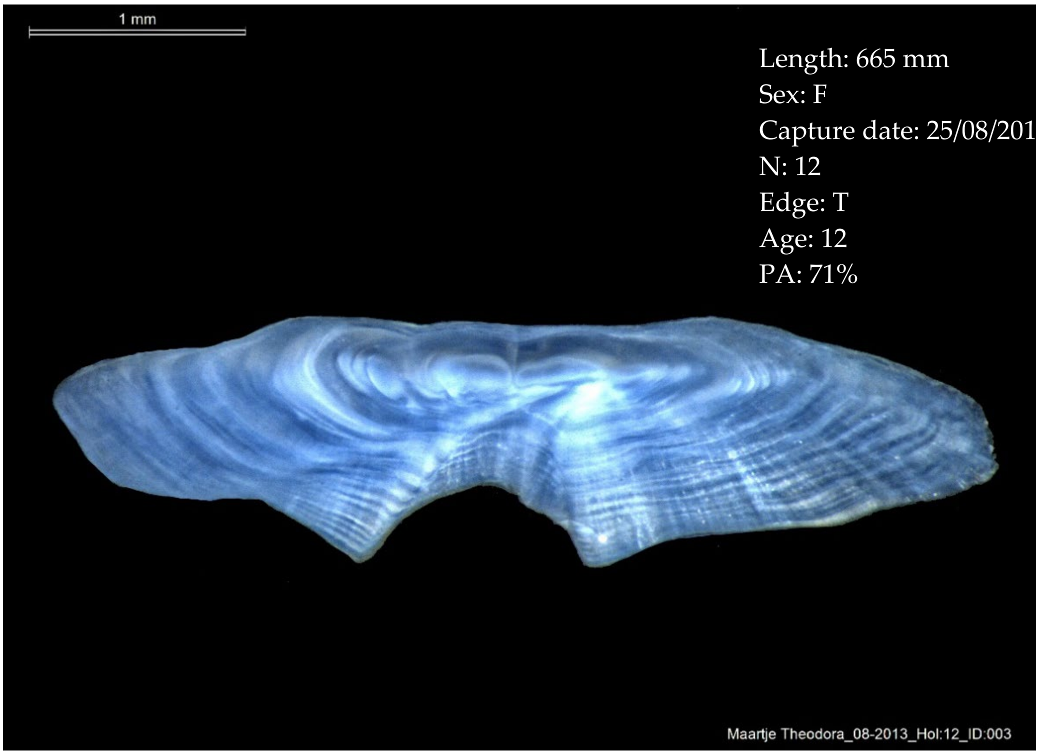
N: total annual (translucent) rings

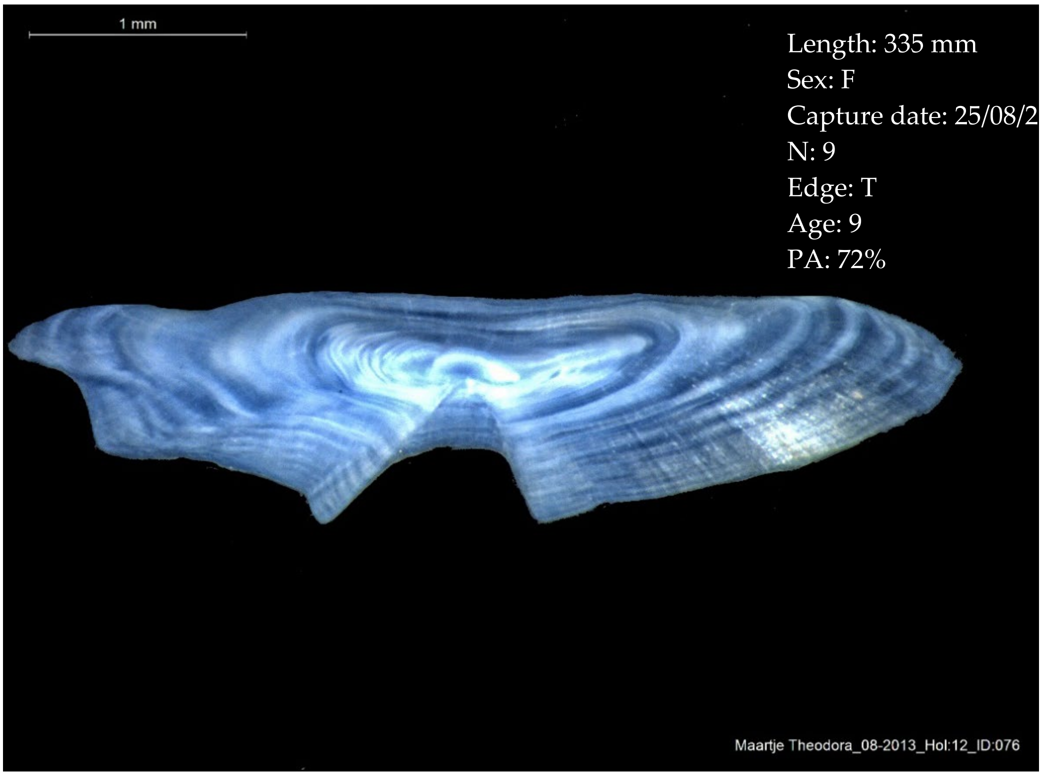
Age determination coherency

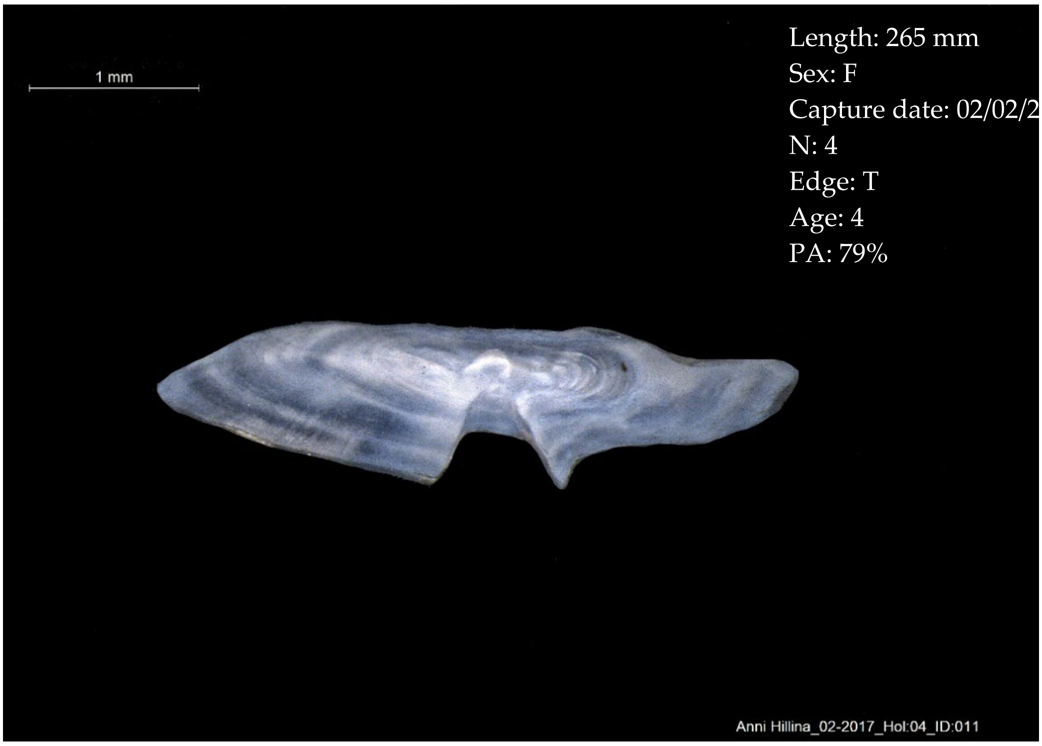
A recent study performed in the *T. picturatus* age and growth in waters of Portugal mainland, assessed the age determination coherency has been assessed through radii measurement analysis (Neves et al., 2022). Previously, this technique had been used in studies carried out for the species inhabiting waters of Madeira (Vasconcelos et al., 2008) and the Canary Islands (Jurado-Ruzafa and Santamaría, 2018). In all the cases, results agree with the seasonal regularity of the growth pattern considered for the species.

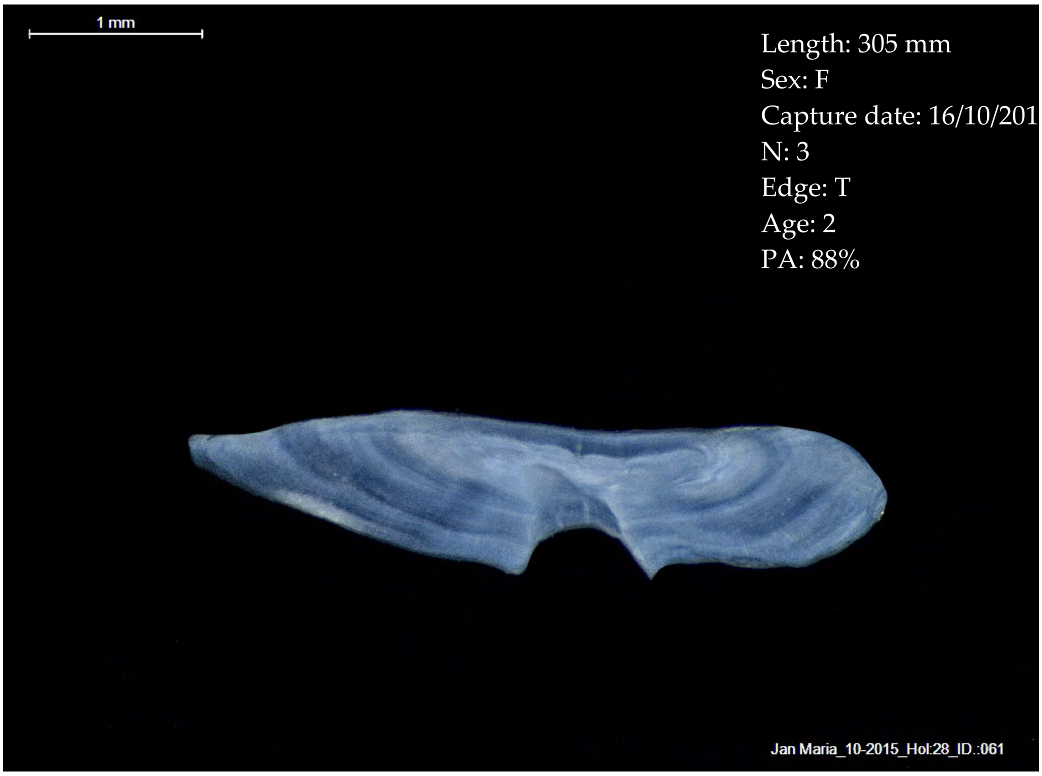
6 Update otoliths reference collections

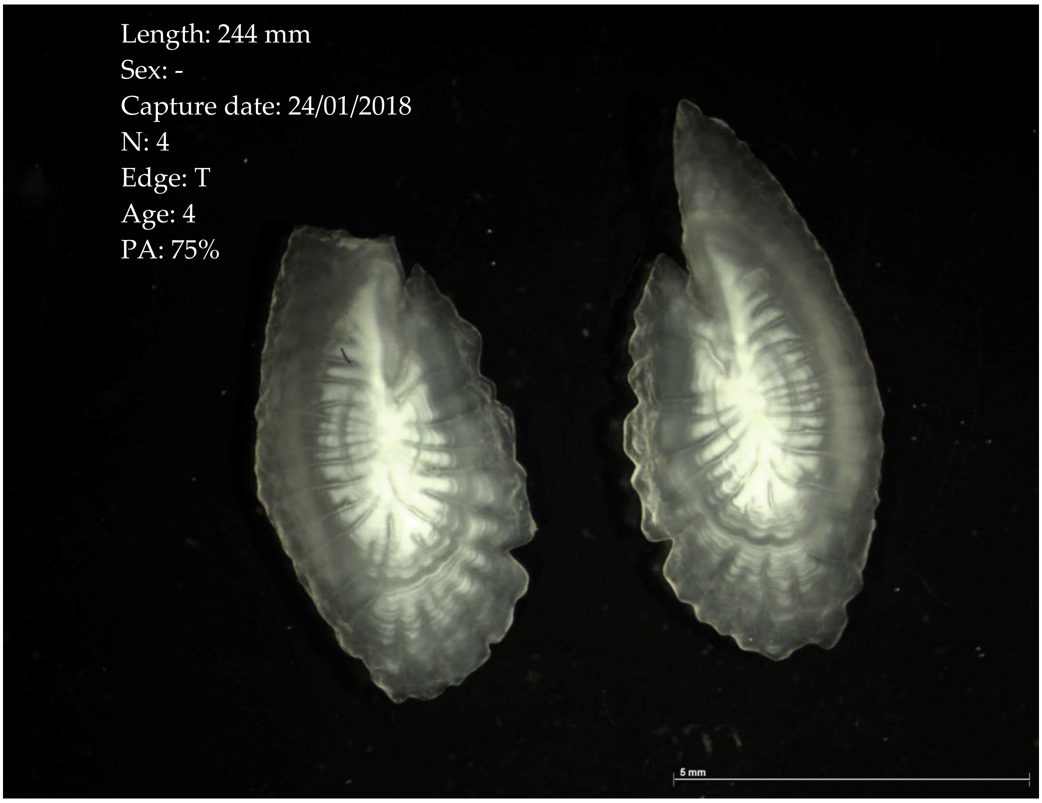
6.1 Reference otoliths of horse mackerel (*Trachurus trachurus*)





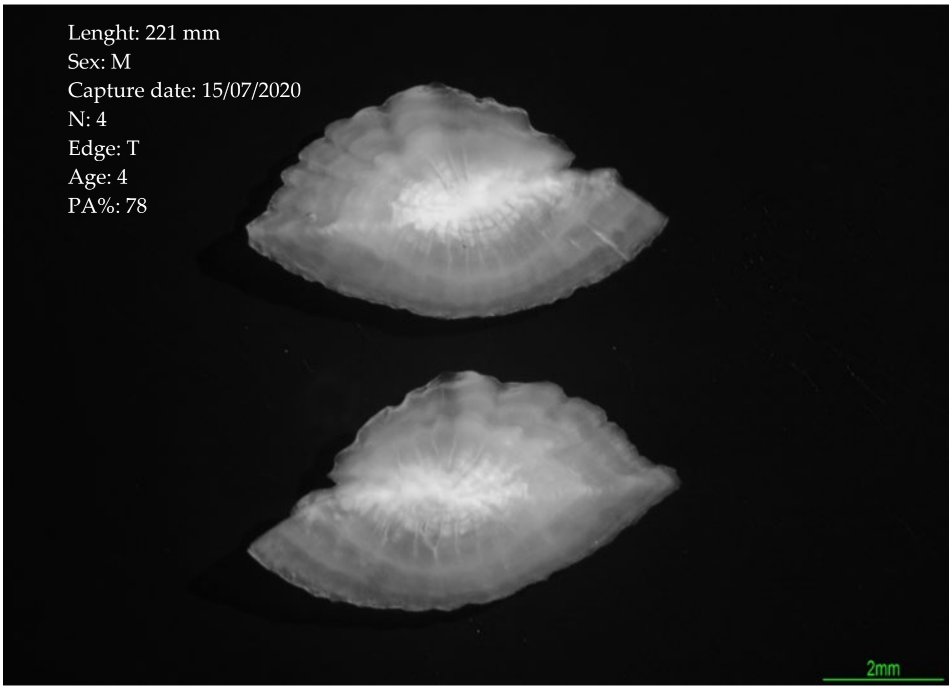

1 mm
Length: 265 mm
Sex: F
Capture date: 02/02/2
N: 4
Edge: T
Age: 4
PA: 79%
Anni Hillina_02-2017_Hol:04_ID:011

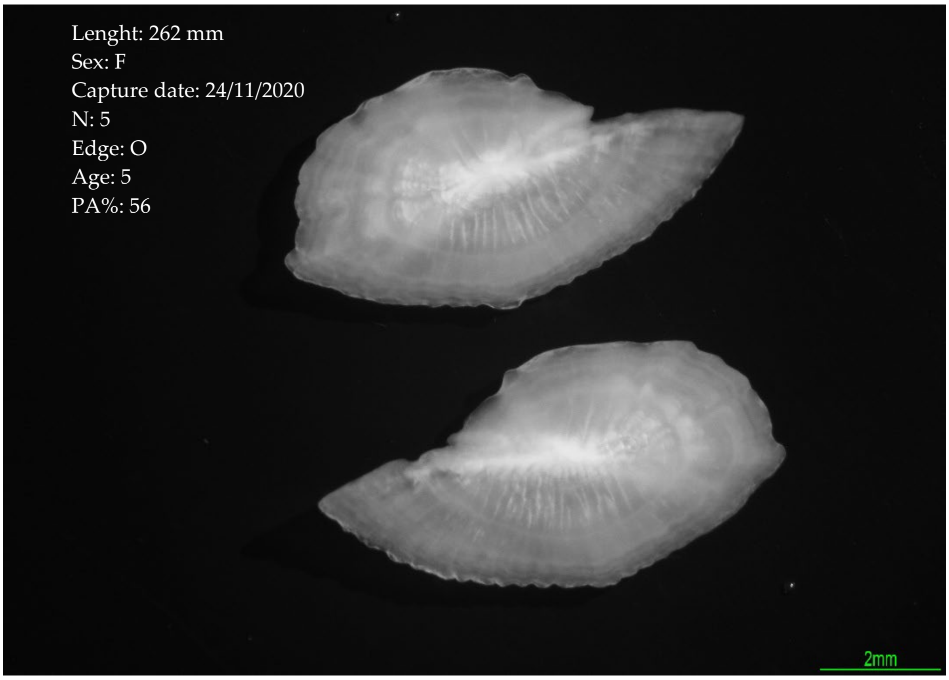

1 mm
Length: 305 mm
Sex: F
Capture date: 16/10/201
N: 3
Edge: T
Age: 2
PA: 88%
Jan Maria_10-2015_Hol:28_ID.:061

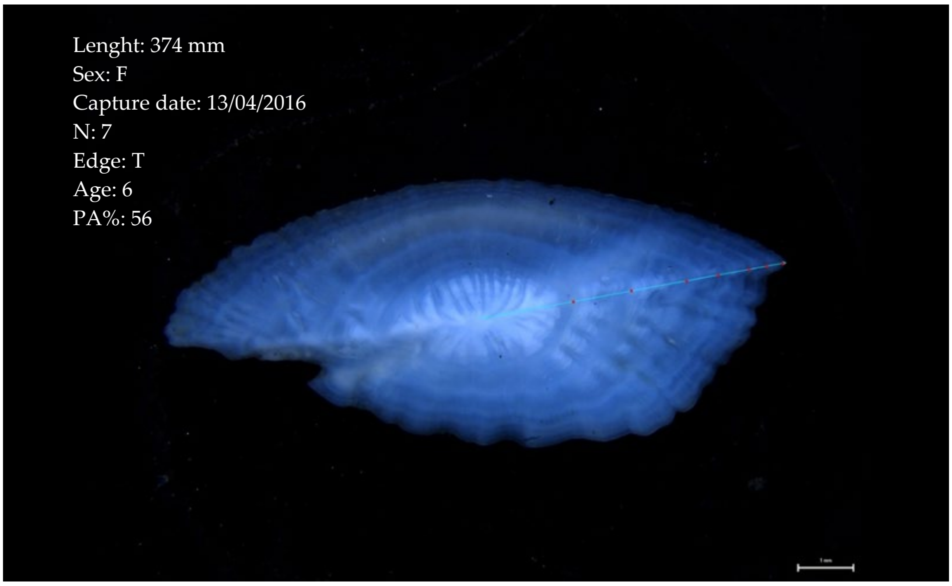

Length: 244 mm
Sex: -
Capture date: 24/01/2018
N: 4
Edge: T
Age: 4
PA: 75%
5 mm


Length: 242 mm
Sex: -
Capture date: 24/01/2
N: 4
Edge: T
Age: 4
PA: 79%

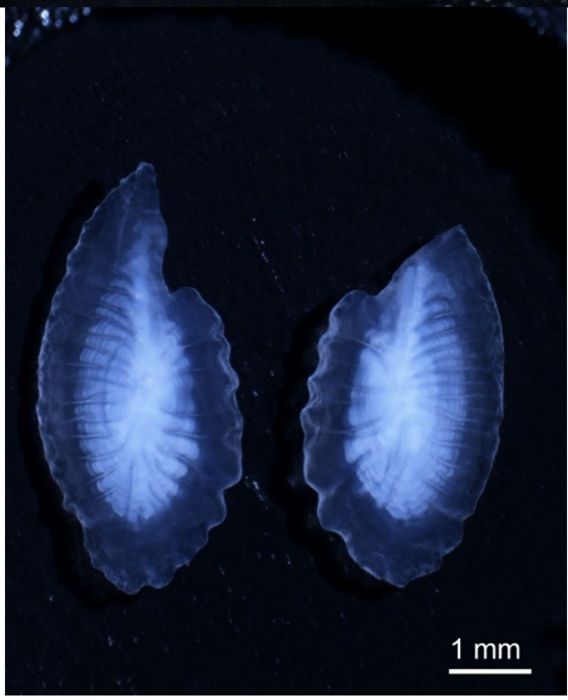
6.2 Reference otoliths of Mediterranean horse mackerel (*Trachurus mediterraneus*)




Lenght: 262 mm
Sex: F
Capture date: 24/11/2020
N: 5
Edge: O
Age: 5
PA%: 56
2mm

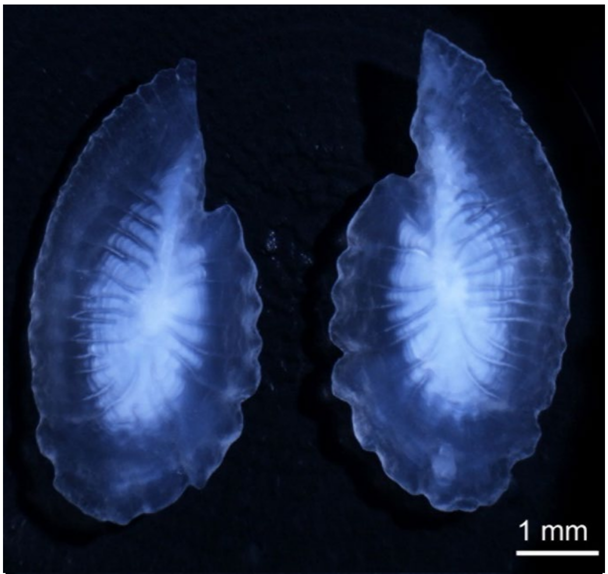

Lenght: 374 mm
Sex: F
Capture date: 13/04/2016
N: 7
Edge: T
Age: 6
PA%: 56

6.3 Reference otoliths of blue jack mackerel (*Trachurus picturatus*)



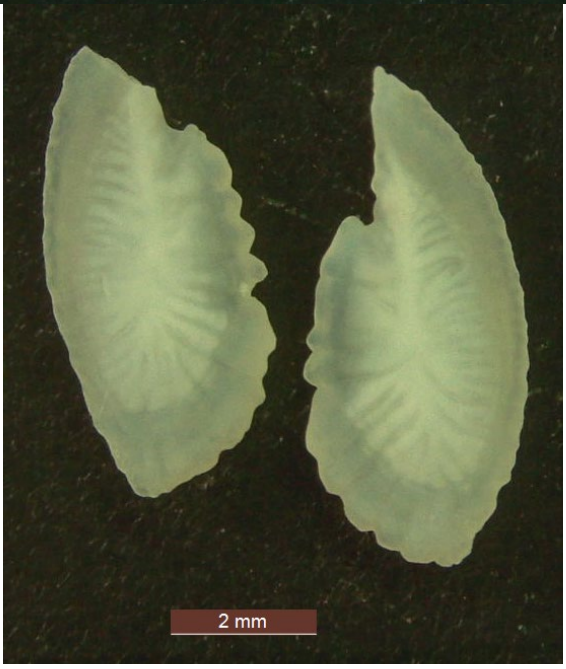
Canary Islands (Image: JAA-2021-4)
TL 20.6 cm; Finished annuli: 2
PA%: 100
CV%:0
APE%:0
Readings: 5/5

Canary Islands (Image: JAA-2021-22)
TL 15 cm; Finished annuli: 2
PA%: 100
CV%: 0
APE%: 0
Readings: 4/5



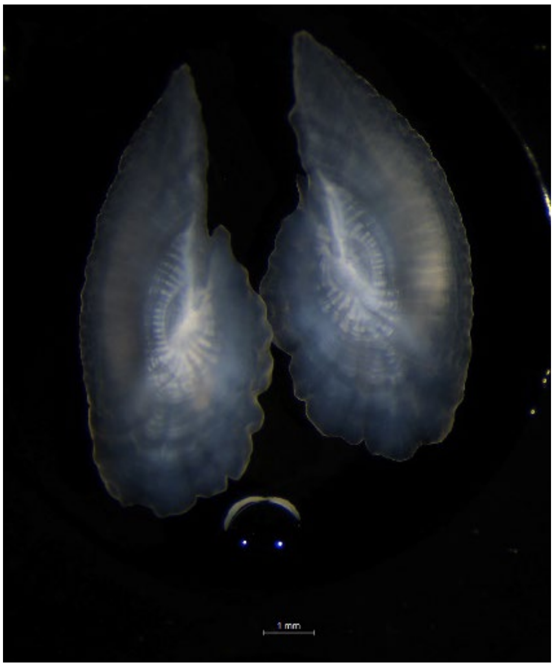
Canary Islands (Image: JAA-2021-28)
TL 18.6 cm; Finished annuli: 2
PA%: 80
CV%: 20
APE%: 15
Readings: 5/5

Canary Islands (Image: JAA-2021-38)
TL 16.5 cm; Finished annuli: 2
PA%: 80
CV%: 25
APE%: 18
Readings: 5/5



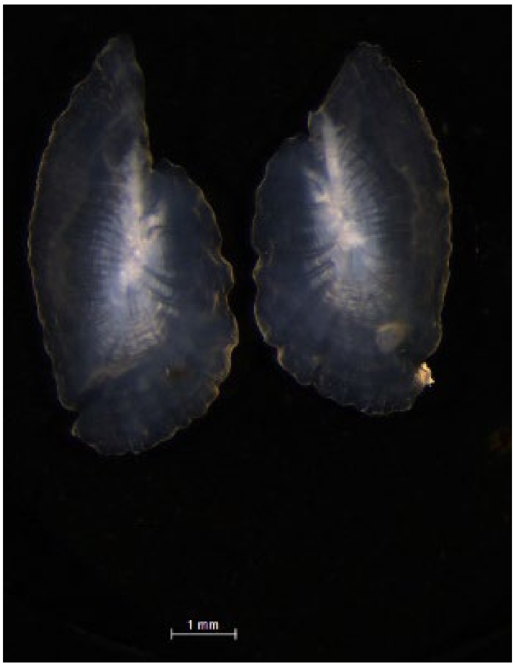
Açores (Image: 4_TRAPIC_10_2S)
TL 25.5 cm; Finished annuli: 3
PA%: 100
CV%: 0
APE%: 0
Readings: 4/5

Açores (Image: 7_TRAPIC_10_2S)
TL 22.0 cm; Finished annuli: 2
PA%: 80
CV%: 56
APE%: 40
Readings: 4/5



Mediterranean GSA9 (Image: GSA9_TP_WHOLE_22)
TL 40.5 cm; Finished annuli: 4
PA%: 80
CV%: 11
APE%: 8
Readings: 5/5

Mediterranean GSA9 (Image: GSA9_TP_WHOLE_38)
TL 8.0 cm; Finished annuli: 0
PA%: 100
CV%: 0
APE%: 0
Readings: 4/5



Mediterranean GSA9 (Image: GSA9_TP_WHOLE_42)
TL 26.5 cm; Finished annuli: 3
PA%: 100
CV%: 0
APE%: 0
Readings: 4/5

Mediterranean GSA9 (Image: GSA9_TP_WHOLE_43)
TL 30.0 cm; Finished annuli: 3
PA%: 60
CV%: 24
APE%: 13
Readings: 5/5

7 Recommendations for future Otolith Exchanges on *Trachurus* species

The WKARHOM4 participants agreed the following recommendations for the next otolith Exchange:

- To include no more than 240 otoliths of *Trachurus trachurus* for the next exchange, including Atlantic Ocean and the Mediterranean Sea:
    - ICES Areas 27.8 and 27.9: 20 whole otoliths (≤ 26 cm total length), 20 sliced (>27 cm fish length)
    - ICES Areas 27.6 and 27.7: 40 sliced otoliths
    - GSA 20: 20 whole otoliths
    - GSA 22: 10 whole otoliths and 10 sliced otoliths
    - GSA 9, 10, 18, and 19: 20 whole otoliths (≤ 26 cm fish length), 20 sliced (>27 cm fish length)
    - Moroccan waters: 40 sliced otoliths
- To include no more than 100 otoliths of *Trachurus mediterraneus* for the next exchange, including Atlantic Ocean and the Mediterranean Sea:
    - GSA 20: 20 whole otoliths
    - GSA 22: 10 whole otoliths and 10 sliced otoliths
    - GSA 9, 10, 18, and 19: 20 whole otoliths (≤ 26 cm fish length), 20 sliced (>27 cm fish length)
    - ICES Areas 27.8 and 27.9: 20 whole otoliths
- To include no more than 120 otoliths of *Trachurus picturatus* for the next exchange, including Atlantic Ocean and the Mediterranean Sea:
    - ICES Area 27.9: 30 whole otoliths
    - ICES Area 27.10a: 30 whole otoliths
    - ICES Area 34.1.2: 30 whole otoliths
    - GSA 9: 30 whole otoliths

Recommendations regarding the samples and the images:

- Otoliths from each area, half should correspond to the first semester and the other half, to the second semester.
- Otoliths should be selected including all the fish length range analysed in each sampling area (information as sex 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, depending on the species and the size.
- The magnification should be a set value for all submitted images regardless of otolith size
- Only high-quality images (without bubbles or light reflections) should be submitted to the exchange.
- Size of the images are recommended to be kept as the original (without re-size).
- Only true annual rings (translucent rings) should be marked (annotated).
- To interpret otolith corresponding to specimens bigger than 26 cm of total length, using otoliths sliced is strongly recommended to avoid underestimation in the age assignment.
- To annotate each annulus just at the end of the translucent ring in order to implement validation/corroboration studies, and to allow comparing annuli measurements.
- To simplify the name of the images to make easier the uploading process to the SmartDots app.

Recommendations for the delegates:

- To create different separated events for *T. trachurus*, i.e. for sliced and whole otoliths. Be aware about the probable different expertise level of the readers for each preparation technique.

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Annex 1: Agenda

Monday 14 Nov 2022

10:00-12:30 Welcome, participants introduction, meeting opening and agenda approval. Presentation "WKARHOM background".

12:30-14:00 *Lunch*

14:00-16:00 Review information on age determination, previous otolith exchanges and validation study on these species.

16:00-16:30 *Coffee break*

16:30-17:30 (Cont.) Review information on age determination, previous otolith exchanges and validation study on these species

Tuesday 15 Nov 2022

09:30-10:30 Presentation "Otolith exchanges 2022 results".

10:30-11:00 *Coffee break*

11:00-12:30 (Cont.) Otolith exchanges 2022 results and discussion (Results and SmartDots).

12:30-14:00 *Lunch*

14:00-16:00

16:00-16:30 *Coffee break*

16:30-18:00 Revise and agree the ageing schemes for the three species (sub-groups work)

Wednesday 16 Nov 2022

09:00-10:30 Presentation of the *ad hoc* Reading exercise

10:30-11:00 *Coffee break*

11:00-12:30 (Cont.) Reading exercise (individual work)

12:30-14:00 *Lunch*

14:00-16:30 (Cont.) Reading exercise (individual work)

16:00-16:30 *Coffee break*

16:30-17:30 (Cont.) Reading exercise (individual work)

Thursday 17 Nov 2022

09:00-10:30 Revise and agree the ageing schemes for the three species (sub-groups work)

10:30-11:00 *Coffee break*

11:00-12:30 Revise and agree the ageing schemes for the three species (sub-groups work)

12:30-14:00 *Lunch*

14:00-16:30 Plenary: presentation and discussion of the guidelines and common ageing criteria agreed (by subgroups' leaders: *T. trachurus*, *T. mediterraneus*, *T. picturatus*)

16:00-16:30 *Coffee break*

16:30-18:00 Plenary: Presentation and Discussion of the Reading exercise results. Develop existing reference collections of otoliths by species

Friday 18 Nov 2022

09:00-10:30 Plenary: Recommendations and conclusions

10:30-11:00 *Coffee break*

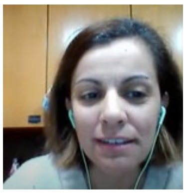
11:00-13:00 Plenary: Planning of future work and meeting closure

Annex 2: List of participants

Name Email * Co-chair	Institution (Station), country	In WKARHOM3 (2018)	*Trachurus trachurus*	*Trachurus mediterraneus*	*Trachurus picturatus*	Stock assessment reader	In Otolith Exchange 2021-2022	*Ad hoc* reading exercise
In person								
Alba Jurado Ruzafa* alba.jurado@ieo.csic.es	IEO-CSIC (Canarias), Spain	Yes	no	no	whole	no	X	X
André Dijkman-Dulkes Andre.Dijkman@wur.nl	WUR, Netherlands	Yes	sliced	no	no	2a, 3a, 4a-c, 5b, 6a, 7a-k, 8	X	X
Andrea Massaro* andrea.massaro@aplysia.it	Aplysia, Italy	Yes	whole	whole	whole	GSA9	X	
Clara Dueñas Liaño clara.duenas@ieo.csic.es	IEO-CSIC (Santander), Spain	Yes	whole	no	no	8c- 9a N	X	X
Eugene Mullins eugene.mullins@marine.ie	Marine Institute, Ireland	Yes	sliced	no	no	yes	X	X
Geoffrey Bled Defruit Geoffrey.Bled.Defruit@ifremer.fr	IFREMER, France.	Yes	whole sliced	no	no	no	X	X
Giannis Dimitriadis gdimitriadis@inale.gr	FRI, Greece	Yes	whole	whole	no	no	X	X
Hammou El Habouz helhabouz@yahoo.fr	INRH, Morocco	No	Sliced	no	no	no	X	X
Iria Maneiro Moreira iria.maneiro@ieo.csic.es	IEO-CSIC (Santander), Spain	No	whole	no	no	9a N	X	X
Justine Diaz justine.diaz@hi.no	IMR, Norway	Yes	sliced	no	no	yes	X	X
Loredana Russo loredanars7@gmail.com	CIBM, Italy	No	whole	whole	whole	GSA9	X	X
Maria João Ferreira mjferreira@ipma.pt	IPMA (Algés), Portugal	Yes	whole sliced	no	whole sliced	9a	X	X
Pierluigi Carbonara carbonara@coispa.it	COISPA, Italy	Yes				no		
Renato Cruz renato.es.Cruz@azores.gov.pt	Governo dos Açores, Portugal	No	no	no	whole	no	X	X
Seán O' Connor sean.oconnor@marine.ie	Marine Institute, Ireland	No	sliced	no	no	no	X	X
Solene Telliez Solene.Telliez@ifremer.fr	IFREMER, France	No	whole sliced	no	no	no	X	X
Susanne Tonheim susanne.tonheim@hi.no	IMR, Norway	No	sliced	no	no	no	X	X
Thanasis Sioulas sioulas@inale.gr	FRI, Greece	No	whole	whole	no	no	X	X

Name Email	Institution (Station), country	In WKARHOM3 (2018)	*Trachurus trachurus*	*Trachurus mediterraneus*	*Trachurus picturatus*	Stock assessment reader	In Otolith Exchange 2021-2022	*Ad hoc* reading exercise
Online								
Aglaia Legaki aglegaki@hcmr.gr	HCMR, Greece	No	whole	whole	no	no	X	X
Ainhoa Arévalo aarevalo@azti.es	Azti, Spain	No	whole burnt	no	no	8c-8b	X	X
Andrea Bellodi abellodi@unica.it	University of Cagliari, Italy	No	whole	whole	no	no	X	X
Eduardo López eduardo.lopez@ieo.csic.es	IEO-CSIC, Spain	Yes	whole	whole	no	9aN	X	X
Eva Hernández eva.hernandez@ieo.csic.es	IEO-CSIC (Canarias), Spain	No	no	no	whole	no	X	X
Georgina Correira gcorreia@ipma.pt	IPMA (Matosinhos), Portugal	No	whole sliced	no	whole sliced	9a	X	X
Gitta Hemken gitta.hemken@thuenen.de	Thuenen Institute, Germany	Yes	sliced	no	no	4, 7d	X	-
Iñaki Rico irico@azti.es	Azti, Spain	No	whole burnt	no	no	8c-8b	X	X
Manuel Jesús Acosta García jesus.acosta@ieo.csic.es	IEO-CSIC (Málaga), Spain	No	whole	Whole	no	no	X	X
Loredana Casciaro casciaro@coispa.eu	COISPA, Italy	Yes	whole	whole	no	no	X	X
Mónica Felício mfelicio@ipma.pt	IPMA (Matosinhos), Portugal	No	whole sliced	no	whole sliced	9a	X	X
Norie van der Meeren Norie.Vandermeeren@wur.nl	WUR, Netherlands	No	sliced	no	no	2a, 3a, 4a-c, 5b, 6a, 7a-k, 8		X
Paola Pesci ppesci@unica.it	University of Cagliari, Italy	No	whole	whole	no	no	X	X
Pedro Torres Cutillas pedro.torres@ieo.csic.es	IEO-CSIC (Málaga), Spain	No				no		-
Romain Ellebode romain.elleboode@ifremer.fr	IFREMER, France	No	no	no	no	no		-
Tim Huijer Tim.Huijer@wur.nl	WUR, Netherlands	No	sliced	no	no	2a, 3a, 4a-c, 5b, 6a, 7a-k, 8		X
Timo Meissner timo.meissner@hi.no	IMR, Norway	No	sliced	no	no	no	X	X
Ulrika Beier Ulrika.Beier@wur.nl	WUR, Netherlands	No	no	no	no	no		-
Vasiliki Nikiforidou v.nikiforidou@hcmr.gr	HCMR, Greece	No	whole	whole	no	no	X	X

From the top to the bottom, from left to right: Sean O'Connor, Andre Dijkman, Andrea Massaro, Pierluigi Carbonara, Renato Cruz, Goeffrey Bled-Defruit, Eugene Mullins, Justine Diaz, Susanne Tonheim, Giannis Dimitriadis, Iria Maneiro, Solenne Telliez, Loredana Russo, Clara Dueñas, Maria Joao Ferreira, Alba Jurado-Ruzafa, Thanasis Sioulas, Hammou El Habouz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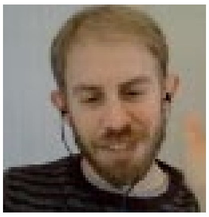
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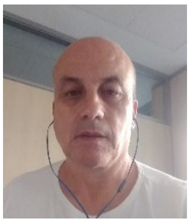
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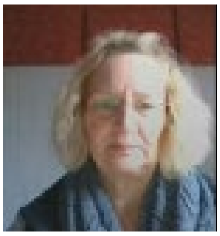
Georgina Correia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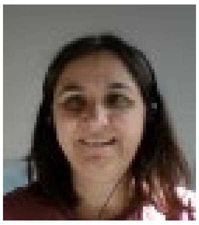
Norie van der Meeren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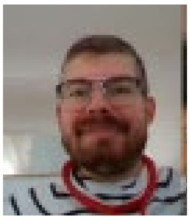
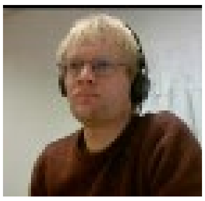
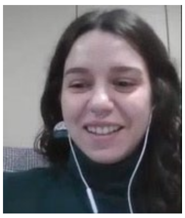

Romain Elleboode


Tim Huijer


Timo Meissner


Ulrika Beier


Vasiliki Nikiforidou

Annex 3: Working documents

Presentations - abstracts

P01: IFREMER (France) - *Trachurus trachurus* otolith protocol.

Authors: Bled-Defruit, G. and S. Telliez.

The otolith preparation of *Trachurus trachurus:*

- For all fishes:
  1- Put tap water in tube where the otoliths are and let soak in it during 24 hours
  2- Take picture under stereomicroscope on black background and reflected light
- For old fishes (more than 3 years) or for difficult otoliths interpretation:
  1- Put the otoliths in Epoxy resin
  2- Cut it transversally at 0.5 mm-0.6 mm
  3- Take picture under stereomicroscope on black background and reflected light

Pictures are taken with *Icy software*, and the age estimation is realised on Smartdots.

P02: Age determination methodology of Horse mackerel (*Trachurus trachurus*) in the Cantabrian Sea and Galician waters (ICES Division 27.8.c and Subdivision 27.9.a.n).

Authors: Dueñas-Liaño, C. [1*], Maneiro, I. [1], Hernández, C. [1] López, E. [2] and Valtierra, J. [2]

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Horse mackerel is a long-lived species, reaching up to 40 years of age, with rapid growth until the three years to slow down later (Abaunza et al., 2003). They are multiple spawning fishes with a long spawning season (up to 8 months) between January and June in Galician waters and between February and July in Cantabrian Sea. Length at first maturity is between 16 and 25 cm, most commonly around 21 cm. Males mature at a slightly smaller length than females. Age at first maturity for females has been estimated to range from 2 to 4 years, depending on the geography. Horse mackerels of length about 4.4 cm and age 0 are found in these areas by mid-August, reaching about 12 cm at the end of the year. They reached an average length of 20 cm at 2 years.

The two study areas, the Cantabrian Sea and Galician waters (ICES div.27.8.c and subdv.27.9.a.n) belong to the western horse mackerel stock (ICES subarea 8 and divisions 2.a, 4.a, 5.b, 6.a, 7.a-c, e-k) and the southern stock (ICES Division 9a) respectively, both under ICES stock annual assessment. Biological data of horse mackerel were collected by the IEO since 1982, samples come from commercial landings and acoustic (March-April) and bottom-trawl (September-October) surveys.

For age determination whole otoliths are placed in a black container and immersed in water to be observed in a binocular microscope under reflected light. The magnification used is between 15x and 50x. The reading axes are preferably in the anterior (rostrum) and posterior (post-rostrum) parts of the otoliths.

The age estimation criteria were established by ICES (1999 and 2012), based on direct validation studies (Kerstan and Waldrom, 1995) and indirect validation (ICES, 1999; Waldron and Kerstan, 2001; Abaunza et al, 2003). Those recommended in ICES (1999; 2012; 2015; 2018) are followed. Birthday is considered January 1st. Translucent zones are used to determine the age, at the end of the year these zones should not be counted as a fully developed ring until the 1st January. An annulus should be traceable on the whole otolith, with the exception of the dorso-medial surface of the rostrum.

Otoliths are read independently of the fish length. Otolith edge and quality (or credibility) of each age estimation (AQ1, AQ2 & AQ3) are registered since 2013. Each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, the reader takes in count the length and finally the lecture appears with quality lecture AQ2. IEO-CSIC readers have been participating in all international age exchanges and workshops from 1999.

The guideline that was indicated in the last WKARHOM3 (ICES, 2018): "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" in the study area is observed as a feature that the 1st translucent ring is thinner than the 2nd, 3rd and 4th ring (Fig. 1a, b).

Figure 1. ***Trachurus trachurus*** **otoliths: (left) 214mm and (right) 245mm, fish length.**

In order to identify properly the first annual ring, it should be visible on both sides of the excisura, rostrum and post-rostrum. All the rings which are not reaching the excisura must be considered checks, avoiding count it as a first winter ring (Fig. 2).

Figure 2. ***Trachurus trachurus*** **fish length 231mm.**

Some differences are observed between the otoliths from the two study areas, otoliths from subdivision 27. 9.a.n have less defined rings and they mark many multi-rings even in the opaque zone of growth.

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P03: IMR Norway Updated Otolith Protocol - *Trachurus trachurus*.

Authors: Diaz, J. and S. Tonheim.

Until 2019, otoliths at the Institute of Marine Research (IMR) in Norway were broken, polished and burned. For a more precise age analysis, we have adopted the slice method as used in Ireland and the Netherlands.

Otoliths are extracted from the first 30 individuals and cleaned in freshwater for any residue. Each pair is left to dry for at least 24 hours. One otolith is used for slicing, usually the right otolith, and the other stays in dry storage for other types of analyses or secondary slicing if necessary. A line is drawn transverse across the otolith through the nucleus with a pencil.

One layer of black epoxy is prepared 3-4 hours prior to mounting the otoliths in the epoxy. A laser line is used to ensure that the cut will be through the otolith's nucleus. Ten otoliths are placed per column, then left for about an hour for the air to escape from under the sulcus. The second layer of black epoxy is then prepared and carefully poured over the otoliths. The epoxy block is left to harden for one week.

Slices are 0.8 mm thick and mounted to microscope slides using a clear epoxy. After 24 hours of drying, they can be age read using a stereomicroscope with reflected light. After discussing with the other institutes, the slices will be 0.5 mm thick from 2023 and onwards.

Currently there is only one age reader at the IMR, there are two additional readers in training. Due to the small Norwegian fishery of horse mackerel, only 10-20 samples are processed annually.

P05: Life history and otolith preparation and interpretation for horse mackerel, Mediterranean horse mackerel and blue jack mackerel (*T. trachurus, T. mediterraneus* and *T. picturatus*), in GSA9, Ligurian and northern Thyrrenian Sea.

Authors: Massaro, A. and L. Russo.

In terms of landings, horse mackerel and Mediterranean horse mackerel represent one of the most important species in GSA9; they are caught mostly by purse seines, bottom trawls, and gill nets. During the last years, landings of both species have decreased (DCF data).

Analyzing data from international bottom trawl surveys (MEDITS) in GSA9 from 1994 to 2021, a positive trend in terms of biomass and density for *T. trachurus* and *T. mediterraneus* has been observed.

Trachurus trachurus shows a spawning period between November and June with a peak from January to April and length at first maturity is 16.6 cm; for *Trachurus mediterraneus* spawning period occurs between April and October, with a peak from April to June; length at first maturity is 16.0 cm.

Trachurus picturatus is not a target species for GSA 9 under Data Collection Framework; otoliths and biological parameters are collected for growth studies.

For these species, otolith is extracted through a transversal cut of skull, rinsed in water to remove organic material, dried and stored in Eppendorf. Otoliths are placed in seawater for 24 hours before analysis and whole otoliths are photographed using stereomicroscope connected to camera, in sea water with the distal side up, reflected light and a black background.

Age assignment follow ageing scheme proposed during WKARHOM 3, using 1st January as date of birth for *T. trachurus* and 1st July for *T. mediterraneus.*

P06: Hellenic Center for Marine Research (HCMR) protocols for age determination for *Trachurus trachurus* and *Trachurus mediterraneus*.

Authors: Nikiforidou V., Legaki A. and Anastasopoulou A.

Sampling

In the Hellenic Center for Marine Research (HCMR) the two *Trachurus* species, *Trachurus trachurus* and *Trachurus mediterraneus*, are collected in the Framework of the National Data Collection Programme all year round. Samples are derived from commercial bottom trawl and purse-seine and MEDITS bottom trawl surveys in GSA 20 (Eastern Ionian Sea) and part of GSA 22 (South Aegean Sea) (Fig. 1). After the collection of the samples, individual total length (TL) is measured to the nearest millimeters (mm), weighted in grams (g) and sex is determined macroscopically. The size of the samples ranges mainly between 50-290 mm TL for *Trachurus trachurus* and 40-350 mm TL for *Trachurus mediterraneus*. Samples are taken in length classes of 10 mm.

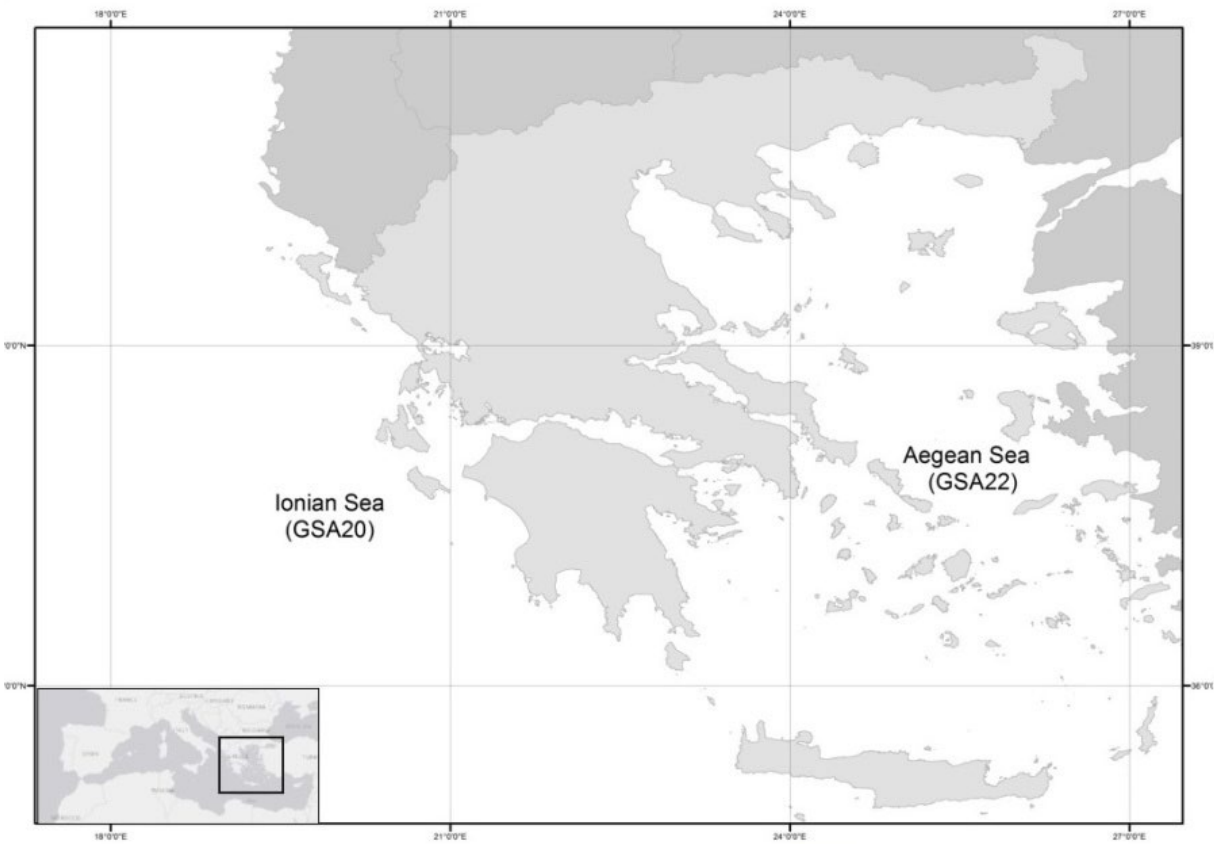

Figure 1: Map of the sampling areas in the E. Ionian Sea (GSA20) and S. Aegean Sea (GSA22).

Storage of otoliths

For each specimen, both right and left sagittal otoliths are removed from the cranial cavity and cleaned of any remaining tissues or fluids with fresh water. Otoliths are dried before storage, stored in plastic bags, and archived for later use (Fig. 2).

Figure 2: Extraction, cleaning and storage of *Trachurus* sp. otoliths.

Equipment & digital images of the otoliths

Each otolith is located in a glass Petri dish filled with water for observation with a stereoscope. A digital camera (SONY ExwaveHAD COLOR VIDEO) is connected to a stereoscope (Wild Heerbrugg M420 Makroskop 1.25x) and a computer monitor. Each otolith is observed under transmitted light against a black background. A digital Image-Pro Plus software system is used to acquire calibrated digital images of the otoliths (Fig.3).

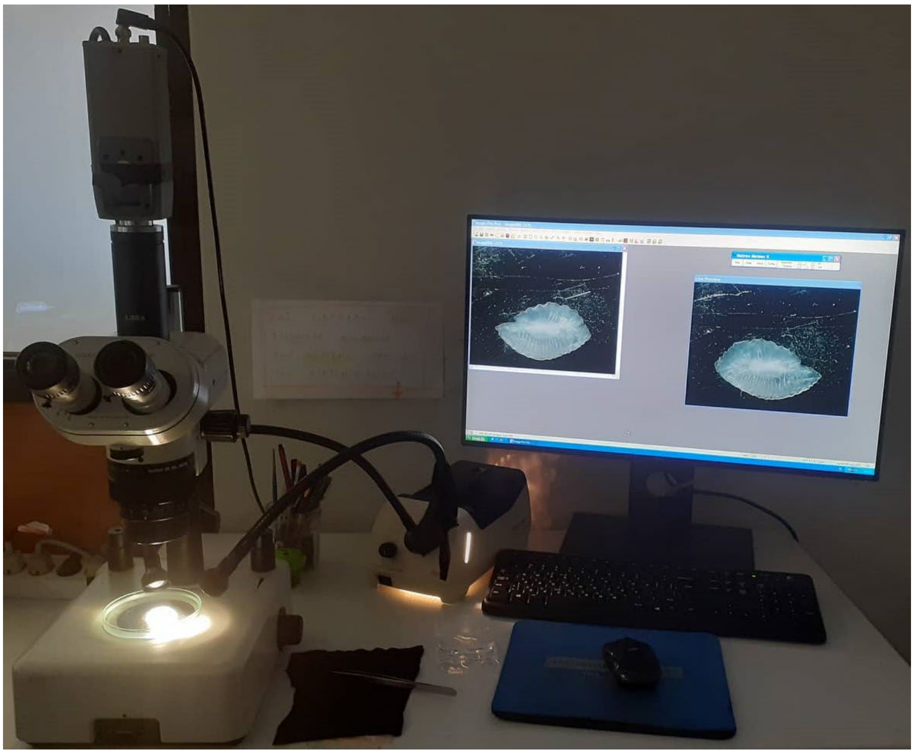

Figure 3: Digitalization system used in the laboratories of HCMR.

Age reading

Age estimation is based on counting the annual growth rings (the alternating opaque and translucent zones) macroscopically using the left sagittal otolith. The right otolith is used in case the left otolith is broken or damaged. The number of annual rings corresponds to the number of translucent zones excluding the first translucent zone which is considered as false ring. The otoliths of *Trachurus trachurus* are photographed under the magnification 1x8 (Fig. 4) while, the otoliths of *Trachurus mediterraneus* are photographed under the magnification 1x10 (Fig. 5).

Figure 4: Otolith of *Trachurus trachurus* with three annual growth rings (red dots); date of capture June; TL: 254 mm.

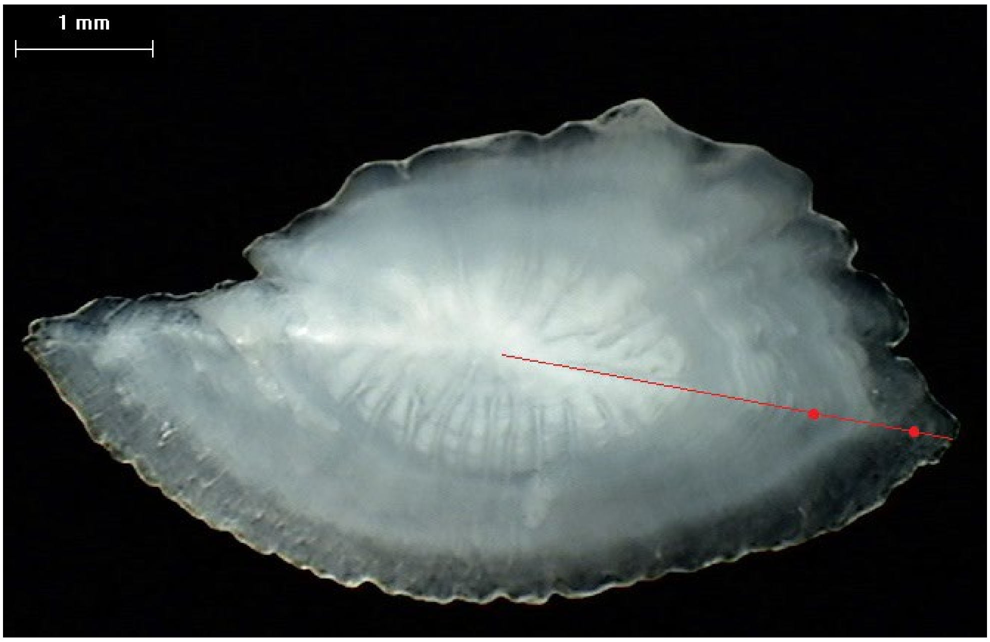

Figure 5: Otolith of *Trachurus mediterraneus* with two annual growth rings (red dots); date of capture July; TL: 206 mm.

Interpretation

The 1st of January is considered as the date of birth for all the individuals of *Trachurus* species independently of their spawning period (ICES, 2018; NOAA, 2020). So, for the estimation of age, the number of annual rings was calculated, and the date of birth and the date of capture are considered for the interpretation of the age. If a translucent ring is recognized at the otolith's margin during the first semester of the year, it is considered as annual ring, while if a translucent ring is seen at the edge of the otolith in specimens collected in the second half of the year, this is not considered as annual ring.

Quality control

The otoliths are read by two readers. To compare the age readings between the different readers, the formulas of percent agreement (PA %), coefficient of variation (CV %), and average percent error (APE %) are calculated. When readings differed, a re-reading is made to obtain a common decision or to exclude the otolith in case of disagreement.

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NOAA (National Oceanic and Atmospheric Administration Fisheries) 2020. Age and Growth. https://www.fisheries.noaa.gov/national/science-data/age-and-growth. Accessed online 25 October 2022.

P07: Review on *Trachurus picturatus* in East Atlantic waters: achievements and challenges.

Author: Jurado-Ruzafa, A.

The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) is a benthopelagic species, widely distributed in the CE Atlantic, including the Macaronesian archipelagos of Azores, Madeira and the Canary Islands, and eastwards into the Mediterranean Sea. Regarding the global captures (FAO, 2021), no data are available previous to 1999, and the huge landings registered in 2016 may reflect a problem of misidentification or unreported landings, meaning the absence of reliable time series. However, checking data from national statistics, landings are locally important.

As other small pelagic fish, they are commonly fished by artisanal purse-seiners at night. Bigger specimens are caught in deeper waters, by bottom longline off Azores and Madeira archipelagos, and even with pelagic trawls in NW African waters. So, each fishing gear affects to different fractions of the population.

Concerning its life history traits, few new studies are available since the last WK (ICES, 2018). Among the updated information, a noticeable decrease of 4 cm has been observed in the size at first maturity for the species in Madeira (Vasconcelos, 2017) and the Canary Islands (Jurado-Ruzafa et al., 2021). During the intersession, Neves et al. (2022) have publish the first age and growth study of *T. picturatus* from mainland Portuguese waters, which includes a new indirect validation and/or corroboration of the ageing criteria used to age the species, which is in accordance to previous information.

Finally, interesting information has been published on the species, using otolith shape analyses. Firstly, Tuset et al. (2019) distinguished three different morphotypes (phenotypes or contingents) in the Canary Islands, based on a high resolute sample comprising one year-round. The most probable explanation for the variation of the proportions of each morphotype between seasons is that these groups behave differently along their ontogeny. These phenological (and potentially, genetically) diversity, could mean higher plasticity and potential adaptability to the species. Secondly, Vasconcelos et al. (2021) found that the same phenotypes of blue jack mackerel are present in other close areas whose fisheries are considered to impact on different stock of *T. picturatus* (i.e. the Canary Islands, Madeira and mainland Portugal). The frequencies of these phenotypes also varied between seasons, but with totally different patterns among origins. Again, the presence of intra-specific diversity in a region would imply more probabilities of successful adaptation to changes in the environment, what means a clear advantage to face the current global warming scenario. For these reasons, it is important that readers pay attention on potential differences in *Trachurus'* otoliths within their areas. Although these findings should have direct implications in the management of the species, other conclusions should be considered regarding the age based on otoliths interpretation. Indeed, different growth patterns were linked to each morphotype, what strongly support the importance on performing 'blind readings' when interpreting otoliths, because the knowledge of the fish size could lead to bias the age interpretation, resulting in unrealistic age structured populations.

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P09: IPMA (Portugal) - *Protocol for collection and preparation of Trachurus trachurus and Trachurus picturatus otoliths* for age determination.

Authors: Ferreira, M. J., G. Correia and M. Felício

The main fishing harbours for *Trachurus trachurus* are Matosinhos, Figueira da Foz, Aveiro, Nazaré, Sesimbra, Peniche, Portimão and Olhão. Samples are collected at all these fishing harbour and in scientific surveys

The collection of otoliths for age determination is based on commercial size category (SC) sampling. (ICES, 2017) Since the sampling plan aims to characterize the length composition of the commercial size categories of horse mackerel (large fish – SC1 to small fish – SC7). Is selected randomly 5 pairs of otoliths per category commercial in length and zone.

The main fishing harbours for *Trachurus picturatus* are Matosinhos, Peniche, Sesimbra and Portimão. Samples are collected at Matosinhos harbour and in scientific surveys.

The collection of otoliths for age determination, 10 individuals by length class are selected for complete biological sampling in the main Matosinhos.

For the two species after collected, the otoliths are washed with fresh water and cleaned to remove any remains of organic tissue that could make it difficult to see them.

Each pair of otoliths collected is stored, after washing and cleaning, in a paper bag duly identified with the sample number, the place and date of collection, the number of the sampled specimen, the length of the fish, the sex and the state of maturation.

The otoliths selected for reading with total length ≤26cm are immersed whole in distilled water for a period of about 24 hours or a little longer, depending on size and to enhance the rings., posteriorly, are observed on a black background, immersed in "baby oil".

The horse mackerel otoliths with total length > 27cm are cleaned with alcohol and marked with a pencil line on the respective nucleus.

The next step is the assembly in epoxy resin (Epoxy Resin SP106®). For this, a mold is used to which a non-stick substance is applied to the interior surfaces, in order to facilitate the subsequent removal of the resin blocks.

The positioning of the otoliths to be mounted on each line of the mold follows the registered order, this step is carried out with the help of camera connected to a monitor that allows viewing the correct positioning of the otolith

It is extremely important that, when assembling, the nucleus is perfectly aligned and centred on each line in the mold, in order to guarantee that the section to be obtained will pass through it.

After drying and hardening, the resulting resin blocks are sectioned in a cutting device. (*Mechatone T330®*), equipped with a high rotation disc hardened with diamond powder.

Each block is placed in the machine and cut separately, obtaining 0.5mm thickness sections, at the level of the core of the otoliths (3 cuts per block to ensure coverage of the nucleus of all otoliths)

The sections thus obtained are then glued with *Mounting Medium for substitutes of xylene for clinical diagnosis®* (https://www.itwreagents.com) on 26x76mm glass slides, properly identified, with the information to which block it corresponds, such as the species and year of capture.

The entire and the sectioned otoliths are observed in a binocular magnifying glass under reflected light, opaque areas appear white and hyaline areas appear dark.

P10: Otolith preparation and interpretation for horse mackerel ageing process in GSA 11.

Authors: Bellodi, A., P. Pesci and M.C. Follesa

In GSA 11, Sardinian Seas (Italy), Mediterranean Horse Mackerel (*Trachurus mediterraneus*) and Horse Mackerel (*T. trachurus*) are both collected for DCF, both from commercial catches (bottom otter trawl) and scientific survey (Medits). Occasionally, in sampling can be also found *T. picturatus.* This species, however, is not a target species for ageing data for GSA11 so, while otoliths are usually collected anyway, is not routinely aged in the region.

Otoliths are removed from individuals, washed to get rid of all organic material, dried and stored in plastic tubes with a univocal code. Then otoliths are analyzed: whole, under a stereomicroscope, in sea-water (clarification medium), with reflected light, against a black background (Fig. 1), with the distal surface side up and the proximal surface (*sulcus acusticus*) turned down. If images are needed, we often quickly post-produce them increasing their contrast and sharpness following Campana (2014) (Fig. 2).

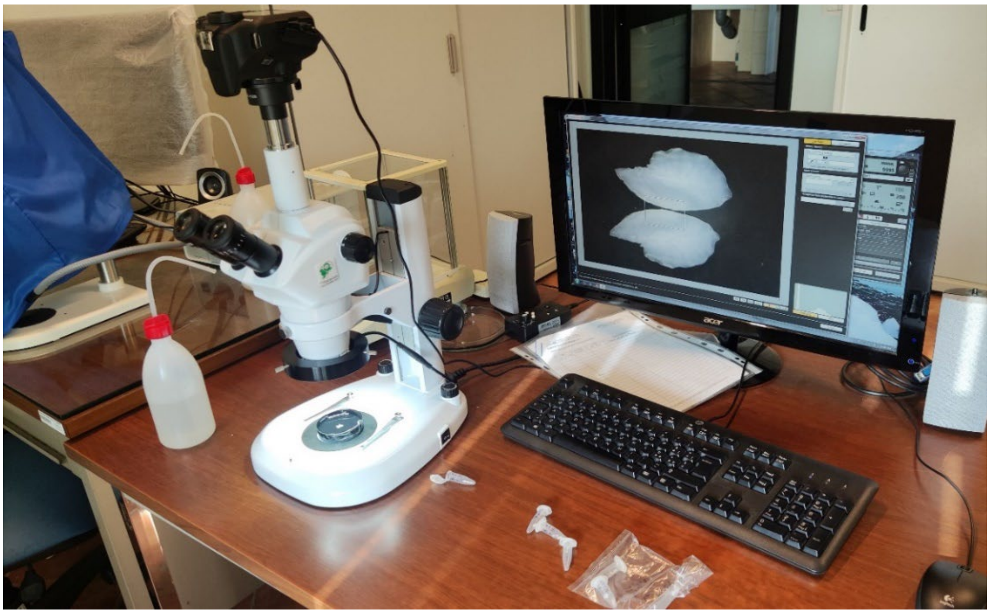


Figure 2. Stereomicroscope and digitalization system used un GSA11 laboratory.

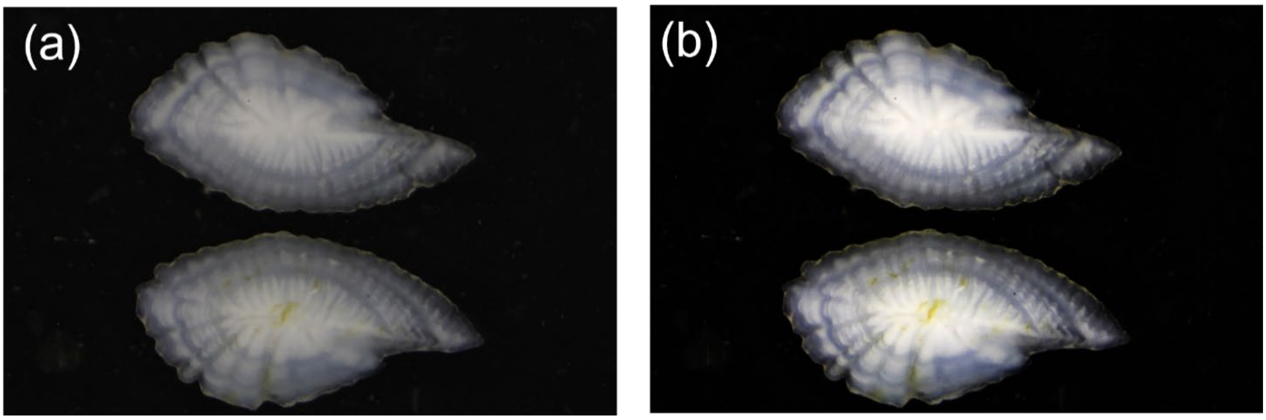


Figure 3. *T. trachurus* otoliths before (a) and after the post production process (b).

A fast clarification phase before the age analysis is done for the two species, not more than 30 minutes, due to the small dimension of individuals usually caught (< 25 cm).

The ageing criteria used are those from the the "Handbook for Fish Age Determination" (Carbonara and Follesa, 2019). Birthdate is set to the 1st January for *T.trachurus* and 1st July for *T. mediterraneus*, taking into consideration their different spawning period. For each otolith, the number of hyaline rings is counted, and the otolith edge is recorded. Age is assigned with a resolution of 0.5 year. The yearly growth band deposition pattern is also investigated for both species (Fig. 3). The otoliths are read by two readers independently, in case of different interpretations the sample is observed again by both readers together in order to obtain either a common decision or to exclude the otolith in case of disagreement.

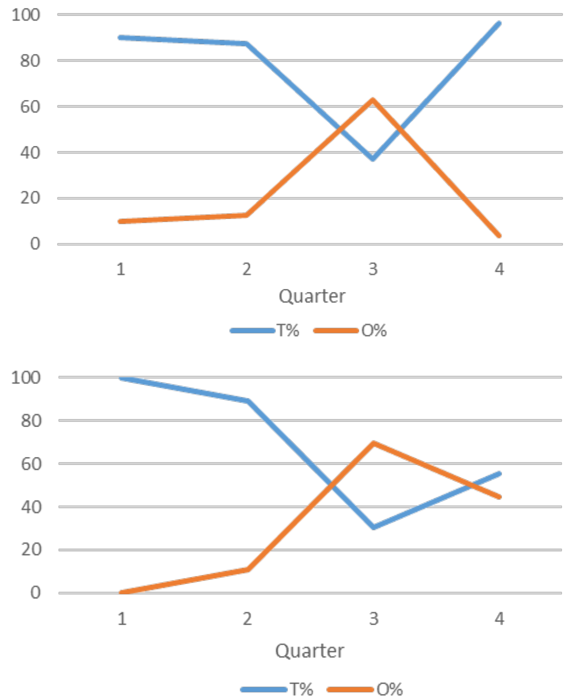


Fig. 3. Temporal evolution of the edge by quarters in *Trachurus trachurus* (left) and *T. mediterraneus* (right) (T% percentage of translucent edge, O% percentage of opaque edge).

<u>References</u>

Campana S.E. (2014). Age determination of elasmobranchs, with special reference to Mediterranean species: a technical manual. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 94. Rome, FAO 2014. 38 p.

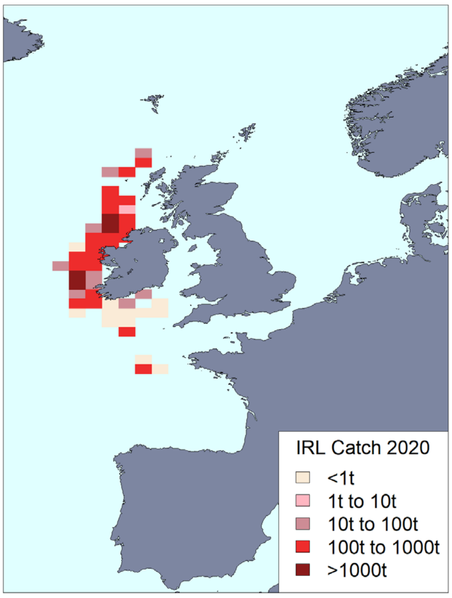
Carbonara, P., Follesa M.C. eds. (2019). Handbook on fish age determination: a Mediterranean experience. Studies and Reviews. No. 98. Rome, FAO. 98: 180 pp.

P12: Marine Institute Ireland: Sampling and ageing protocols for Horse mackerel *Trachurus trachurus.*

Authors: Mullins, E. and S. O'Connor.

Biology

Horse mackerel is a widely-distributed pelagic species, sometimes regarded as a semi pelagic as they are found near the seabed. They are a relatively long-lived species that can live for more than 26 years. Large fluctuations in year class strength are a feature of horse mackerel stocks. Maturity is reached between the ages of 2 and 4. The western stock spawns in the Celtic sea in June, followed by a northerly feeding migration. The stock may enter the North Sea from the north with abundance associated with the influx of Atlantic water. The return migration takes place in late autumn. Juveniles are pelagic feeders while larger individuals feed closer to the bottom, mainly on 0-group fish.



<u>Irish landings and value of TAC</u>

On average 24,000 tonnes of Horse mackerel were caught and landed by Irish vessels between 2018 and 2020 mainly from sub areas 6a and 7b. In 2020 landings were worth approximately €10 million for Irish vessels.

Age determination procedures

<u>Collection and storage of otoliths</u>

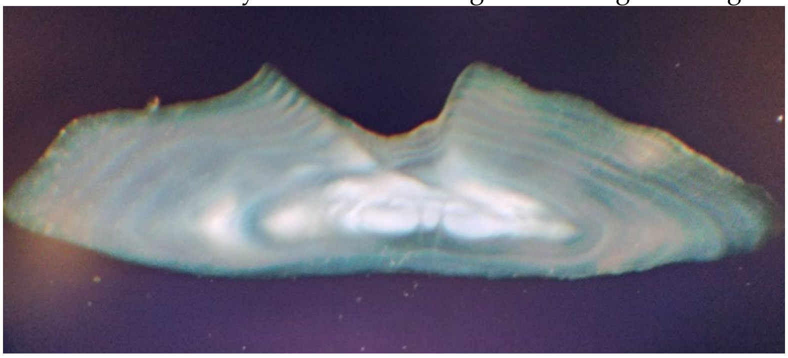
- Horse mackerel are sampled in port labs from commercial fishery and on survey vessels for length, weight, sex, maturity and age.
- Both otoliths are removed from the fish
- Otoliths are cleaned, dried and stored in plastic boxes
- Otoliths are then sent to the age prep lab at the Marine Institute, Galway.

<u>Sectioning of otoliths</u>

- Otoliths are sectioned and mounted onto glass slides according to the protocol outlined from our otolith preparation team in WKARHOM 2015 report.

Ageing of otoliths

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



Preferred orientation of otolith images for reading

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 Control

- 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 2^{nd} and 3^{rd} 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

P13: Ageing analysis of *Trachurus mediterraneus* and *Trachurus trachurus* in the GSAs 18 and 19 (South Adriatic Sea and Western Ionian Sea)

Authors: Casciaro, L. and P. Carbonara

The adopted ageing criteria are that presented in the "Handbook on Fish Age Determination" (Carbonara and Follesa, 2019). These criteria are in line with which listened in the report of the previous workshop on age reading of mackerels (ICES, 2018).

The first step is the otoliths extraction, carried out through a transversal section slice. After the extraction, the otoliths are cleaned of any residual organic tissue and then dried with paper, coded and stored in plastic tubes.

The otoliths were analyzed whole: by the binocular microscope, rinsed with sea-water, with reflected light against a black background, with the distal surface turned up and the proximal surface (sulcus acusticus) turned down, annuli are counted on the posterior part of the otolith (post-rostrum).

T. mediterraneus otoliths do not need a long clarification phase before the analysis, except for the bigger specimens (> 30 cm) where a very short permanence in the sea water (5-10 minutes) could be necessary. For *T. trachurus* the time of the immersion depending on the size of the fish: one or two minutes for the otoliths of juveniles' specimens (< 20 cm TL), no more of two hours for specimens between 20 and 30 cm Total Length (TL).

For *T. mediterraneus*, the theoretical birthday is the 1st July, because in the considered area, the higher percentages of the mature females occur from April to August.

The criteria to recognize the true winter ring are that translucent true rings should be visible more or less around the whole otolith in order to be considered as annual rings and the increments between the consecutive annuli should be decreasing with age. As reported by Karlou-Riga (2000), before the first winter ring some false rings are laid down. Indeed, the small specimens born during the spring-summer and caught during summer-autumn months, present a translucent edge. This is a false ring probably laid down when the juveniles changed the environment and the diet (from the pelagic to the bentho-pelagic phase). The false rings are visible also in the otoliths of older specimens at about 1 mm from the core. The adopted ageing scheme uses the quarter as capture date and the age is given with a precision of half year.

For *T. trachurus* theoretical birthday is the 1st January according with the spawning period that is extended almost all year with a peak during the winter month (Carbonara et al., 2012). The otoliths can be analyzed whole or sectioned for the specimens with total length greater than 28 - 30 cm. The otoliths are included in resin and transverse sections, 0.4-0.6 mm thick through core, were obtained by low speed saw. The sections are analyzed under reflected or under transmitted light. The age is assigned with a resolution of 0.5 years, counting the translucent (winter) ring.

References

Carbonara P., Casciaro L., Bitetto I., Spedicato M.T. 2012. Reproductive cycle and length at first maturity of *Trachurus trachurus* in the Central-Western Mediterranean seas. Biol. Mar. Medit., 19 (1): 204-205.

Carbonara P., Follesa M.C. (eds.). 2019. Handbook on fish age determination: a Mediterranean experience. Studies and reviews. No. 98. Rome, FAO. 2019. 180 pp

ICES. 2018. Report of the Workshop on Age Reading of horse mackerel (*Trachurus trachurus*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and blue jack mackerel (*Trachurus picturatus*) (WKARHOM3), 5-9 November 2018, Livorno, Italy. ICES CM 2018/EOSG:28.186 pp

Karlou-Riga C. 2000. Otolith morphology and age and growth of *Trachurus mediterraneus* (Steindachner) in the Eastern Mediterranean. Fisheries Research, 46: 69-82

P13: Biology sampling methodology for *Trachurus trachurus* and *Trachurus mediterraneus* (Greek DCF).

Authors: Dimitriadis, G. and T. Sioulas.

Sampling

Greece has classified Horse Mackerel (*Trachurus trachurus*) and Mediterranean Horse Mackerel (*Trachurus mediterraneus*) as priority species for the National DCF. Therefore, Greece monitoring the biological characteristics of these species in annual basis though an annual sample. The sample is representative of the Greek GSA areas and the fishing gears operating in these areas. The annual sample goal is 600 and 800 specimens for *T. trachurus* and *T. mediterraneus* respectively from GSA 22 (Aegean Sea) 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 two coordinators of National DCF. 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, MEDIAS etc.) and rarely from small scale fisheries. Therefore, the entire sample is correlated with a fishing trip and the trip's catch. 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 fresh-water and 70% alcohol. The otoliths are photographed as pair by a Nikon SMZ18 stereomicroscope (Figure 1) using direct intensive light against a dark background.

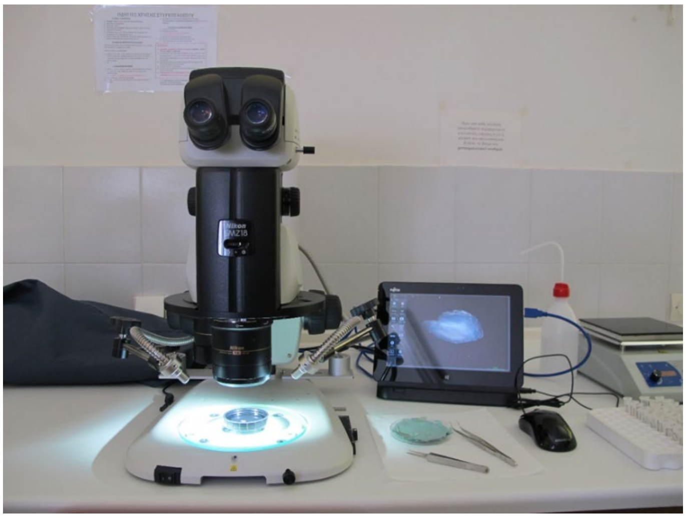


Figure 1. The Nikon SMZ18 stereomicroscope used to take pictures in FRI

Interpretation

FRI is exclusively responsible for the age reading of the both species. The images are imported in Image J, an open-source software. The orientation of the pair 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 2 & 3). It has to be noted that for these species the translucent growth zone represents 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 reports. FRI is adopted all the suggested guidelines of WHARHOM 3 for reading schemes and observed patterns. Therefore, 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 and the lengths of the annual rings are imported into the database.

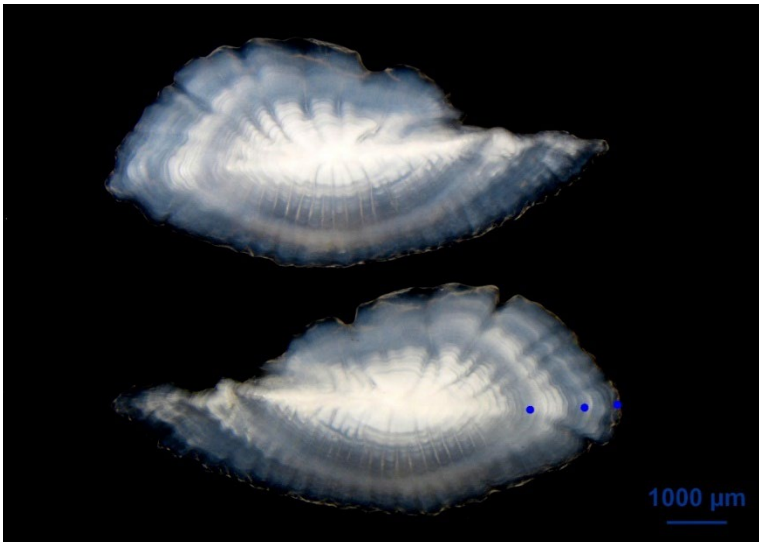


Figure 2. Imported image into Image J Software of a T. trachurus specimen (TL: 231mm, modal age: 3)

Figure 3. Imported image of T. mediterraneus otoliths (TL: 201mm, modal age: 1)

Quality Control

FRI has two readers for each species with high experience. The otolith sets are read by the readers and their results are analysed and discussed. The data are extracted from the database and are used for a series of statistical analysis and tests by a third person. The statistical analysis though growth parameters, curves and age length keys give the required feedback back to two readers. FRI doesn't apply any method for age validation, but is planning to adopt in near future. Finally, it is considered that Greece has only one stock per species.

P14: Contribution to the development of a methodology for reading the age of the European horse mackerel *Trachurus trachurus* (Linnaeus, 1758) from thin sections of "sagitta" otoliths.

Author: El Habouz, H.

This study is focused on the growth and reproduction biology of the species *Trachurus trachurus* (Linnaeus, 1758) in the Moroccan Atlantic Centre

Linear growth of horse mackerel was studied through age-reading data from thin sections of otolith sagitta.

The otoliths to be cut are sandwiched in a resin pin (Fig. 1). For this, an aluminum mold is used whose dimensions are adapted to the characteristics of the isomet 4000 positioning and precision system. Thin sections are from 0.4mm to 0.5mm Ø are obtained (Fig.2)

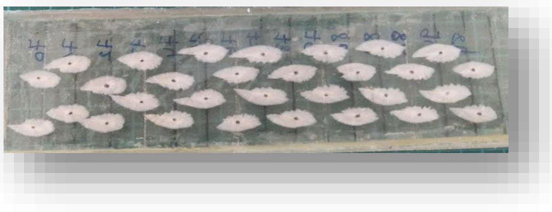


Fig. 1: Fixing the otoliths sandwiched in a resin pin

Fig. 2: Thin section of the otolith (0.4 to 0.5 mm in Ø) on a resin slide

After the thin section of the otolith, polishing is carried out to visualize the different growth rings. Thin section thickness should be reduced to 0.2~0.3 mm Ø. The thin section of the otolith after polishing presents a smooth surface where the different rings can be distinguished by observation with a stereoscopic magnifying glass (Fig. 3)

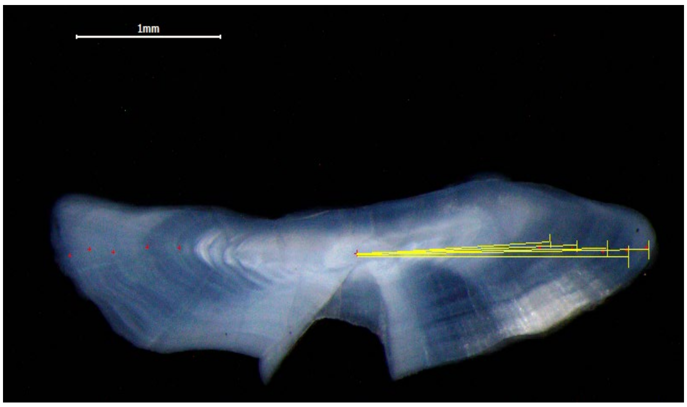


Fig. 3: Image of an otolith section with measurement of the different rings.

For the sample of horse mackerel analyzed and whose total length of the individuals is between 21 and 41 cm, the age increases proportionally according to the size and the weight of the "sagitta" otoliths. Indeed, the linear regression calculated between the age and the size of the otolith on the one hand and its weight on the other hand, shows a good correlation (R≥0.8). The equations of the regression lines and their graphical representations are shown in Figure 4.

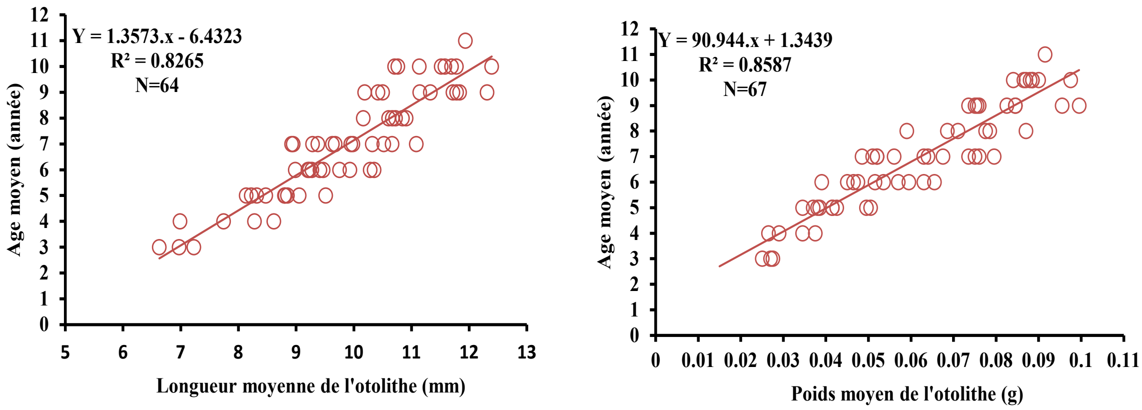


Fig. 4: Evolution of age in European horse mackerel according to the average length (left) and the average weight (right) of the "sagitta" otolith.

From the calculation of the average marginal increment (AM) per season, (Fig. 5), it was noted that the value close to zero of the marginal increment (AM=0) is frequently observed in winter (Fig.5). This approach allowed us to fix the deposition of the hyaline ring in winter and of the opaque ring in summer. It therefore confirms the identification of hyaline rings and their use as annual marks.

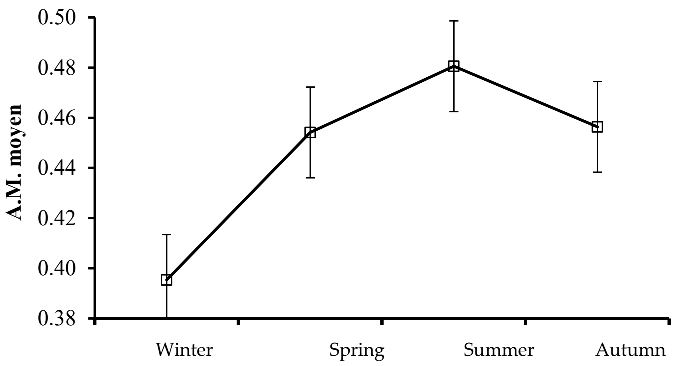


Fig. 5: Seasonal variation in the marginal-translucent increment of sagitta otoliths in horse mackerel

The parameters of Von Bertalanffy's equation for linear growth were estimated by back calculation (Table 1)

Table 1: Parameters of the Von Bertalanffy equation for the linear growth of *Trachurus trachurus*. M: Male; F: Female

Sex	L∞ (mm)	K	t0	Number
Male and female	506.559	0.135	-0.517	82
Female	506.5757	0.1415	-0.2152	50
Male	416.87	0.171	-1.334	32

The graphic representation of the Von Bertalanffy equations for the linear growth of horse mackerel with or without distinction of sex is presented in figure 6. The latter shows that for ages less than or equal to five years, the total lengths at age in males are higher than those of females. At the age of six years, both sexes have the same total length. Beyond six years and for equal ages, females are larger than males.

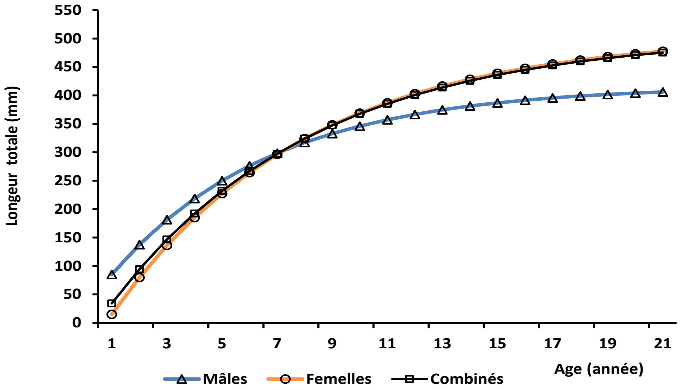


Fig. 6: Von Bertalanffy curves for the linear growth of *Trachurus trachurus* in the Moroccan Atlantic Centre

Protocols updated

AZTI – Horse mackerel age reading method

The AZTI institute has sampling during last year about 300 otolith, 50 specimens from each sample.

We take, from each fish date of capture, length, weight, sex, maturity stage and otolith.

Otoliths are washed immediately after collection in order to remove the organic material from the surface. They are stored dry in envelopes with the sample code.

One otolith of the pair is broken though the nucleus. The broken otolith is burnt. The burnt half otolith is mounted in black plasticine and submerged in 70% alcohol, together with the untreated whole otolith. Both are read using reflected light.

The translucent bands are counted.

Quality control

AZTI 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/workshops.

We don’t have a reference collection.

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

Biological information

Within the German sampling scheme, only otoliths from commercial catches and surveys of the horse mackerel species *Trachurus trachurus* are sampled and read. *Trachurus trachurus* is a highly migratory schooling species which is mainly caught with pelagic nets close to the bottom. The species is commonly found from West Africa/Cape Verde to Iceland and the Norwegian Sea, and also occurs in the Mediterranean and Black Seas.

The distribution area in European waters in the Northeast Atlantic relevant for the German sampling is shown in Fig 1. Within this distribution area three *Trachurus trachurus* stocks are defined: the Western Stock, the Southern stock and the North Sea stock. Germany is sampling only the North Sea stock, mostly in quarter 4 in 7d, and the Western stock in ICES area 7 (7j, 7h).

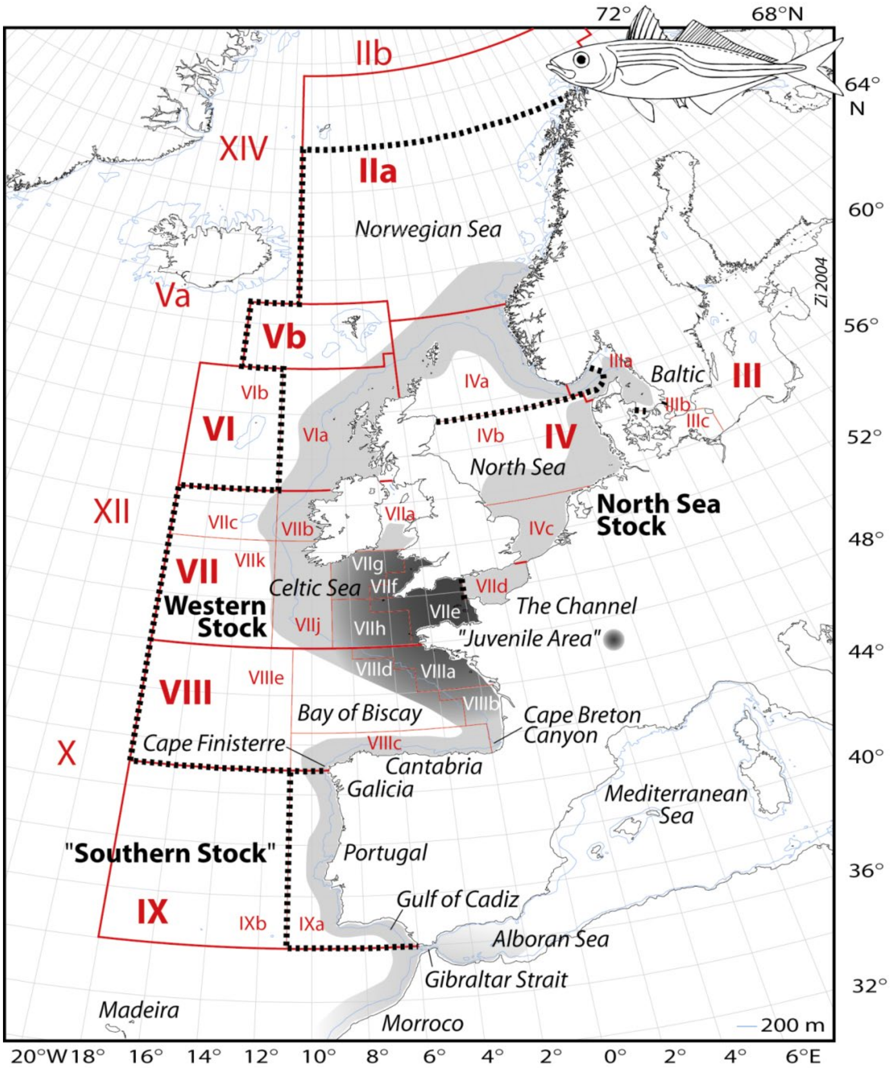


Fig 1 Distribution of Horse Mackerel in the Northeast-Atlantic: Stock definitions as used by the 2004 WG MHSA. Note that the "Juvenile Area" is currently only defined for the Western Stock distribution area – juveniles do also occur in other areas (like in Div. 7.d). Map source: GEBCO, polar projection, 200 m depth contour drawn. (Source: ICES. 2021. Working Group on Widely Distributed Stocks (WGWIDE). ICES Scientific Reports. 3:95. 874 pp. http://doi.org/10.17895/ices.pub.8298)

Sampling and storing of calcified structures

Horse mackerel from commercial catches are sampled by the Institute of Sea Fisheries within the EU data collection framework. Otolith collected on surveys are marginal. 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 (Fig. 2). The block is then half-automatically cut by a wet abrasive cut-off machine producing 0.5mm thick slices through the nucleus of the otoliths (Fig. 3).

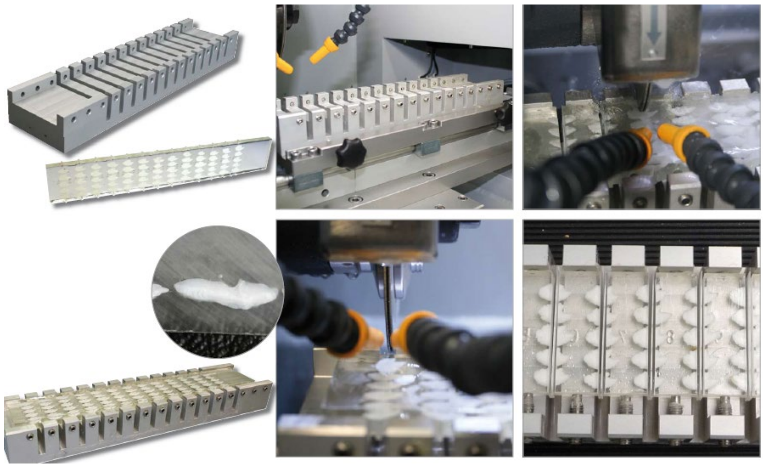


Figure 2. Device for series cuts of otoliths (Picture by ATM GmbH)

Figure 3. 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. 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.

Review of procedures for otolith preparation and analysis in *Trachurus trachurus* for CN IEO –CSIC (Oceanographic Centre of Santander, Spain)

Dueñas-Liaño, C.[1*], Maneiro, I.[1], Hernández, C.[1]

[1]*Instituto Español de Oceanografía (IEO), C. O. Santander. Av. Severiano Ballesteros n. 16, 39004 Santander, Spain.*

*Email: clara.duenas@ieo.csic.es

Sampling

Horse mackerel from commercial landings and acoustic/bottom-trawl surveys are sampled by IEO-CSIC within the EU data collection framework (ICES div.27.8.c and subdv.27.9.a.n). The otoliths are taken by a simple random sampling of 100 individuals by month/quarter.

Otolith preparation and interpretation

In order to preserve the correct organization, sagitta otoliths are stocked dried in Eppendorf tubes, and each samples collection is kept in a separate square cardboard box.

Previous to the age determination, the otoliths are hydrated with fresh water less than an hour. The whole otoliths are placed at the bottom of a black dish, and cover with fresh water. We observe them under the binocular reflected light. Magnification used in our laboratory is between 15x and 50x.

At the end of the reading, we leave all the tubes opened under an extraction hood (around 24h) to get it completely dry in order to have the better conditions to storage it for unknown time.

Ageing criteria described in ICES Workshops are followed (ICES, 1999, 2012, 2015 and 2018) .

Within the recommended area for reading (Fig.1), we usually use the axis marked as green, having the red lines as confirmation zone.

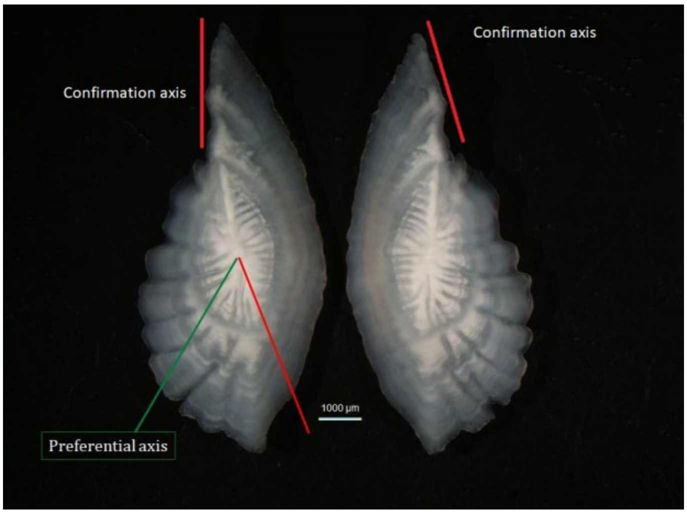


Figure 1. Recommended areas for otolith reading

Both otoliths are analysed on the different faces concave and convex, this give us more information, we can compare them and recognize the true hyaline rings (Fig. 2).

Figure 2. Otoliths extracted from *Trachurus trachurus* (left) 248mm and (right) 269mm, fish length, false rings in green.

With this first view, normally we could have a clear idea about where may be the first annual ring, in this way we can detect false rings, they usually appear as thin lines and they are not firmly detected in the convex otolith face. The first rings that are formed by multi-rings can be distinguished, as a single compact ring on the convex face.

Quality control

Otoliths are read independently of fish length, each otolith is read twice, in two separate occasions. The age estimation of a given otolith is accepted only if both estimations match. When there is a discrepancy between them, the reader takes in count the length and finally the lecture appears with quality lecture AQ2.

Otolith edge and quality (or credibility) of each age estimation (AQ1, AQ2 & AQ3) are registered since 2013.

To assure the concordance in the age precision IEO-CSIC readers have been participating in all international age exchanges and workshops.

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: TIFF files (*.tif), JPEG files (*.jpg), Also jp2 (jpeg2000) files, JASC PAINT SHOP PRO (*.psp).

Annex 4: Resolution

WKARHOM 4 participants agreed to convene a new full otolith exchange in 2025, co-chaired by Justine Diaz (Norway), Andrea Massaro (Italy) and Alba Jurado-Ruzafa (Spain). Results will be presented to the following ICES WGBIOP, whose members will determine the necessity (or not) of a new WKARHOM meeting.

Annex 5: Terms of Reference

The low Percentage of Agreement achieved during the previous otolith exchange (Massaro and Jurado-Ruzafa, 2022) led to the following WGBIOP resolution (ICES, 2020, 2021):

2021/WK/DSTSG07 Workshop 4 on age reading of Horse Mackerel, Mediterranean Horse Mackerel, and Blue Jack Mackerel (*T. trachurus*, *T. mediterraneus*, and *T. picturatus*) (WKARHOM4), chaired by Andrea Massaro, Italy, and Alba Jurado-Ruzafa, Spain, will be established and meet in Lisbon, Portugal, 14– 18 November 2022 to:

a) Review information on age determination, otolith exchanges (the last one to be performed during October 2021), and validation work done so far on these species; (Science Plan codes: 5.1, 5.2);

b) Revise and agree the ageing schemes for each species; (Science Plan codes: 5.1, 5.2);

c) Evaluate the effect of different ageing schemes related to different date of birth; (Science Plan codes: 5.1, 5.2);

d) Update guideline and reference images by species for the ageing analysis; (Science Plan codes: 5.1, 5.2);

e) Address the generic ToRs adopted for workshops on age calibration (see: WGBIOP Guidelines for Workshops on Age Calibration) (Science Plan codes: 5.1, 5.2).

The meeting was organized to allow attendance both in person and online. The agenda and the list of participants are presented in Annex 1 and Annex 2, respectively.