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Report of the Workshop on Age Reading of Chub mackerel (*Scomber Colias*) (WKARCM)

2-6 November 2015

Lisbon, Portugal

ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

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Contents

1 Executive summary 1

2 Introduction 2

2.1 Terms of reference 2

2.2 Participants 2

3 Biology and life history of Chub Mackerel 7

4 Review information on age determination, otolith exchanges and validation techniques on this species (ToR a) 10

4.1 Background information on age determination, Otolith exchanges and Workshops on Age Reading of chub mackerel in European waters 10

4.2 Validation studies 10

5 Resume of the validation studies presented during the Workshop. 13

5.1 Bay of Biscay 13

5.2 Portugal 18

5.3 Mauritania 21

6 Standardization of material and methods and review of ageing techniques 22

6.1 Otoliths preparation techniques 22

6.2 Otolith image processing techniques 23

7 Estimate (relative) accuracy and precision of chub mackerel, age determination in the main European fishing areas (ToR b) 24

7.1 Exchange 2012–2013: Chub Mackerel (*Scomber colias*, Gmelin 1798) Otolith Exchange (Martins *et al*., 2014) 24

7.2 Exchange 2015: Small Exchange of *Scomber colias* Otoliths from Atlantic and Mediterranean Areas, March–June 2015 (Navarro *et al*., Working Document to WKARCM 2015). 26

7.3 WKARCM exercise 28

7.3.1 All readers 29

7.3.2 Intermediate readers 30

7.3.3 Trainee readers 30

7.3.4 Analysis by set of otoliths 30

7.4 Discussion and conclusions 30

8 Identify causes of age determination error and provide species-specific guidelines for the improvement of precision and reduction of bias between readers and laboratories (ToRc) 32

8.1 *Scomber colias* otoliths. Areas for interpreting the age. 32

8.2 Causes of age determination error identified during the WKARCM meeting 34
8.3 Effect of different magnification factor on age determination 36
8.4 Observation of otoliths fixed in resin versus otoliths immersed in seawater 37

9 Age reading protocol for Chub Mackerel (ToR d) 38

10 Otolith reference collection (ToR e) 40

11 WKARCM recommendations 50

12 References 51

Annex 1: WKARCM agenda 54

Annex 2: Contributions to the Workshop. Presentations and Working Documents. 57

Annex 3: Results of WKARCM otolith exchange 58

1 Executive summary

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS), meeting in February 2014, recommended the realization of a first Workshop on Age Reading of Chub Mackerel to discuss the results of a previous exchange and the development of validation studies in this species (ICES, 2014a). The Workshop on Age Reading of Chub Mackerel (WKARCM, chaired by Andreia Silva, Portugal, and Maria Rosario Navarro, Spain) has been held in Lisbon (Portugal) from the 2–6 November 2015. Three countries took part in this workshop (Portugal, Spain and Italy), with a total of 12 participants. The aim of this workshop was to review the information on age determination, discuss the results of the previous exchange (2012–2013), review the validation methods existing on these species, clarify the interpretation of annual rings, elaborate an age reading protocol and start a reference collection of well-defined otoliths.

This workshop was preceded by two otolith exchanges (2012–2013 and 2015). Three age validation studies, in three different areas (Bay of Biscay, Portugal and Mauritanian waters) were presented, as well as a compilation of age validation studies of this species in the literature. After the presentation of readings results (mean agreement percentage from 57.5%; mean CV from 29.6%) and the precision of age estimation, the participants identified the sources of bias in the interpretation of the Chub mackerel age. The large number of checks and the position of the first growth ring were identified as the most important problems.

After discussion, a new exercise was made. The precision increased to 60.6% and the mean CV increased to 45.6. Moreover, the number of participants that follow the same age reading criteria increased, although it is still necessary to continue to clarify the age reading interpretation. In consequence, the participants of WKARCM recommended studies on validation methods for *Scomber colias* in all the participating areas and the realization of a new otolith exchange in the following year (2016) to focus on the analysis of exchange results, validation studies and review the age reading protocol for *Scomber colias*.

2 Introduction

2.1 Terms of reference

The **Workshop on Age Reading of Chub Mackerel (*Scomber colias*)** (WKARCM), chaired by Andreia Silva, Portugal, and Maria Rosario Navarro, Spain, was held in Lisbon, Portugal 2–6 November of 2015, to:

a) Review the information on age determination, otolith exchanges and validation techniques on this species;

b) Estimate (relative) accuracy and precision of chub mackerel age determination in the main fishing areas of the European region;

c) Identify causes of age determination error and provide specific guidelines for the improvement of precision and reduction of bias between readers and laboratories;

d) Elaborate an age reading protocol;

e) Create a reference collection of otoliths and a database of images of otoliths;

f) Address the generic ToR's adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

WKARCM will report for the attention of SSGIEOM, WGBIOP, SCICOM and ACOM.

The agenda of the meeting is shown in Annex 1.

2.2 Participants

A total of eleven readers participated in the present Workshop, six from Portugal (IPMA, laboratories of Lisboa and Matosinhos), four from Spain (IEO, laboratories of Santander, Murcia and Canary Islands) and one from Italy (CIBM, Livorno) (Figure 2.2.1). A list of the participants with a summary about their experience in age estimation of chub mackerel is shown in the Table 2.2.1. The level of experience in chub mackerel reading was considered by number of otoliths (1st) and by years of experience in this species (2nd). Participants were ranged as Intermediate (more than 2000 otolith read) and Trainee (less than 2000 otoliths read). There was no Expert reader between participants (more than 10000 otoliths read).

Nine of WKARCM participants also took part in the last otolith exchange (2015). Four of them have also participated in the first otolith exchange of chub mackerel (2012–2013). The reader of COISPA (Italy) participated in the last otolith exchange (2015) but could not assist to the Workshop. However, the information about the age estimation of chub mackerel in COISPA laboratory has been included in this Report. Also, a set of otoliths of the reader's area of expertise (GSA18) was included in the workshop age reading exercise.

Figure 2.2.1: WKARCM participants; from left to right: Diana Feijó, Alba Jurado-Ruzafa, Andreia Silva, Dina Silva, Delfina Morais, Andrea Massaro, Charo Navarro, Miguel Vivas, Encarni García, Gina Correia, Ana Carolina, Eduardo Soares

Table 2.2.1: Summary of WKARCM participants and reading experience of chub mackerel otoliths.

Participants	Role	Level	Email	Address
Rosario (Charo) Navarro	Co-Chair /Reader 1	Intermediate	charo.navarro@st.ieo.es	Instituto Español de Oceanografía (IEO). C.O. Santander. Promontorio de San Martín, s/n. 39004 Santander (Cantabria). Spain
Begoña Villamor	Coordinator	-	begona.villamor@st.ieo.es	
Andreia V. Silva	Co-Chair /Reader 6	Trainee	avsilva@ipma.pt	Instituto Português do Mar e Atmosfera (IPMA). Avenida de Brasilia, 1449-006 Lisbon. Portugal
Alexandra Silva	Coordinator	-	asilva@ipma.pt	
Eduardo Soares	Coordinator /Reader 8	Trainee	esoares@ipma.pt	
Delfina Morais	Reader 5	Trainee	dmorais@ipma.pt	
Dina Silva	Reader 9	Trainee	dsilva@ipma.pt	
Georgina Correia	Reader 10	Trainee	gcorreia@ipma.pt	Instituto Português do Mar e Atmosfera (IPMA). Avenida General Norton de Matos 4, 4450-208 Matosinhos. Portugal
Diana Feijó	Reader 11	Trainee	dfeijo@ipma.pt	
Alba Jurado Ruzafa	Reader 2	Intermediate	alba.jurado@ca.ieo.es	Instituto Español de Oceanografía (IEO). C.O. Tenerife. Dársena Pesquera, Pcl. 8. 38180 S/C Tenerife (Canary Islands). Spain
Encarnación García	Reader 3	Intermediate	encarnacion.garcia@mu.ieo.es	Instituto Español de Oceanografía (IEO). C.O. Murcia. Calle Varadero, Nº1. 30740 San Pedro del Pinatar (Murcia). Spain.
Miguel Vivas	Reader 4	Intermediate	miguel.vivas@mu.ieo.es	
Andrea Massaro	Reader 7	Trainee	andreamassaro@live.it	Centro Interuniversiario di Biologia Marina ed Ecologia Applicata (CIBM). Vialen N. Sauro, 4. 57128 Livorno. Italy
Pierluigi Carbonara	Non presencial reader	Trainee	carbonara@coispa.it	COISPA Tecnologia & Ricerca - Stazione Sperimentale per lo Studio delle Risorse del Mare. Via dei Trulli 18/20. 70126 Bari - Torre a Mare. Italy

3 Biology and life history of Chub Mackerel

The Atlantic chub mackerel (*Scomber colias*, Gmelin 1789) is a pelagic fish which distributes in depths from 250 to 300 m in warm and temperate waters of the Atlantic Ocean and in the Mediterranean Sea (Collette, 1986). In the eastern Atlantic, this species occurs from the Bay of Biscay to South Africa including the Canary, Madeira, Azores and Saint Helena Islands and in several seamounts (Castro-Hernández and Santana-Ortega, 2000) (Figure 3.1). It may be considered the southern congener of the Atlantic mackerel *S. scombrus* (Villamor *et al*., 2004). Both species overlap in the Iberian Peninsula, with Atlantic mackerel being predominant to the north and chub mackerel to the south of Lisbon (Martins and Cardador, 1996). *S. colias* is now considered a separate species from the Indo-Pacific congener *Scomber japonicus*, based on mitochondrial and nuclear DNA analyses (Infante *et al*., 2007).

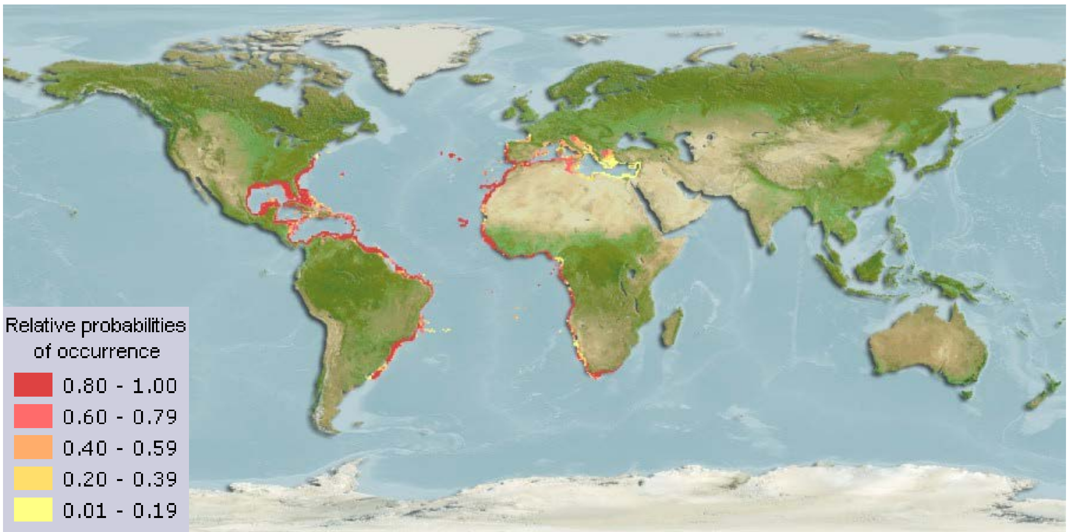


Figure 3.1: Distribution map for *Scomber colias* with relative probabilities of occurrence (adapted from Aquamaps 2015).

A gradient of spawning period is observed along East Atlantic Ocean when considering the literature (Table 3.1), being earlier in lower latitudes: from November-February in Canary Islands (Lorenzo *et al*., 1993), to February/March-May/June in Portugal waters (Martins, 1996), until March-June in the Bay of Biscay (Navarro *et al*., 2014b). This gradient could be related to temperature as the main spawning season of chub mackerel occurs when water temperature is at least 10ºC and most often when it is 15 to 20ºC (Castro-Hernández and Santana-Ortega, 2000), as occurs in other migratory species such as mackerel (ICES, 2014b). In Mauritanian waters, the spawning period occurs in winter according to some studies (Domanevsky, 1970; Weiss, 1981; FAO, 1986), and between December and June / March and June according to other studies (García, 1982 and García, 1986; respectively) (ought to the differences between authors, the spawning period in Mauritanian waters is not included in table 3.1).

Table 3.1: Summary of the spawning period of chub mackerel along East Atlantic collected from the literature (from Navarro *et al.*, 2014b).

Area	ICES Div.	Author	Years	Spawning period
Bay of Biscay	VIIIb, VIIIc	Lucio, P. (1997)	1989-1993, 1997	May-June*
Bay of Biscay	VIIIc, IXaN	Navarro et al. (2014)	2011-2013	March-June
Portugal Coast	IXa	Martins, M.M. (1996)	1986-1995	February/March - May/June
Azores Islands	X	Westhaus-Ekau (1982)	1980-1982	March-June**
Madera Islands		Vasconcelos, J. (2006)	2002-2005	January-May
Canary Islands		Lorenzo et al. (1993)	1988-1989	November-February

*Samples from only May and June **no samples in May and June

The spawning periods observed in the laboratories that participated in WKARCM are showed in Table 3.2. In Spanish Mediterranean waters spawning occurs between January-April, peaking in February-March. In Italian waters, spawning occurs between late spring-early summer, with a peak in June-July, which is similar of the spawning period observed in the Saros Bay (Turkey), between April and August with a peak in June (Cengiz, 2012).

Table 3.2: Chub mackerel spawning season/peak, length and age ranges and recruitment season observed in each area/institution (based in the information collected by the participants before the workshop).

Country	Lab.	Sample Origin	Spawning Season	Spawning Peak	Length Range	Age Range	Recruitment Season
Spain	IEO-Santander	ICES areas IXaN, VIIIc and VIIIb	March-June	April-June	12-48 cm	0-9	Sept.-Oct. (surveys data)
Portugal	IPMA	ICES area IXa	January-March (20-29cm) April-July (30-39cm)	April	20-39 cm	0-6+	Not known
Spain	IEO-Canarias	CECAF-Mauritania	Winter	January-February	13-45 cm	0-6+	Not known
Spain	IEO-Murcia	GSA06	Winter-Spring	January and May	20-32 cm	0-3	Not known
Italy	CIBM	GSA09	Late Spring-early Summer	Not known	-	-	-
Italy	COISPA	GSA10, GSA18 and GSA19	Spring-early Summer	June-July	10-42 cm	0-10	Sept.-Oct.

Both juveniles and adults mainly feed on zooplankton although the relative importance of larger organisms such as cephalopods, crustaceans and small pelagic fish increases with the size of individuals (Castro and Hernandéz-García, 1995). The ontogenetic change in diet is associated with a tendency for older individuals to be distributed more offshore (Baird, 1978).

Migrations across latitude and between coastal and offshore areas, related to seasonal cycles of spawning and feeding, have been described in several areas of the Atlantic Ocean (Castro-Hernández and Santana-Ortega, 2000). Within European Atlantic waters, spawning grounds and migrations are not well known.

Chub mackerel is a fast growing, early maturing species that can attain 62 cm total length and 20 years of age (Navarro *et al*., 2012), although usually it attain 50 cm total length and 13 years of age (Castro-Hernández and Santana-Ortega, 2000). Growth parameters studied by different authors and areas indicate that the maximum theoretical length ranged from 58.2 cm in Portugal mainland (Martins, 1996; Martins et al., 1983) to 37.8 cm in Bay of Biscay (Navarro *et al*., 2014a) (Figure 3.2). In Azores and Madeira these values are 57.2cm and 50.1cm (Carvalho *et al*., 2002; Vasconcelos *et al*., 2011). For the Gulf of Cadiz and Alboran Sea this species grows fast during the first year, reaching 50% of the asymptotic length in the Gulf of Cadiz and 59% of L∞ in Alboran Sea. This species grows rapidly during the first year of life and much more slowly after 3-4 years (Velasco *et al*., 2011; Lorenzo *et al*., 1995, Perrota *et al*., 2005).

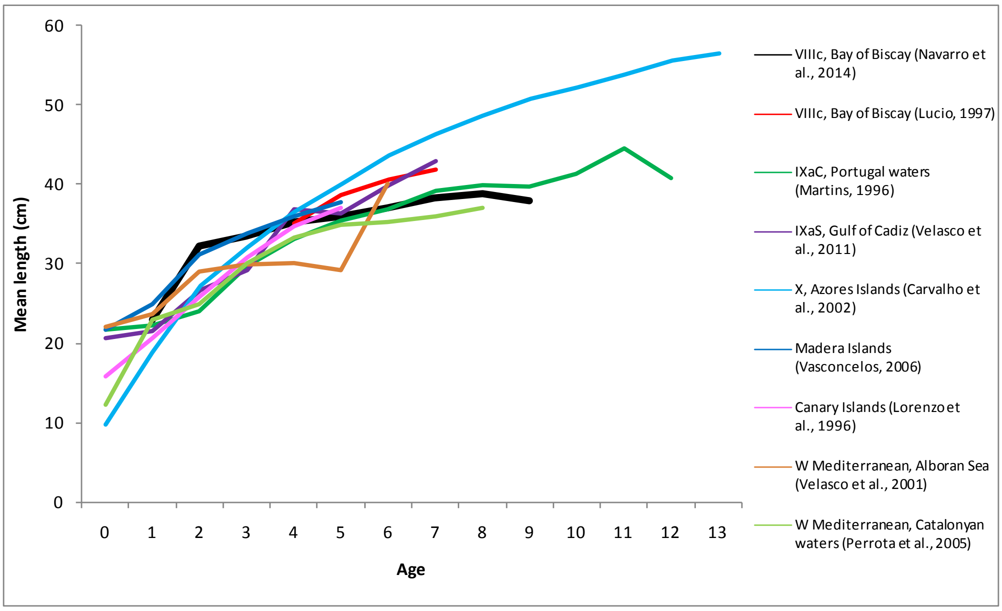


Figure 3.2: Chub mackerel growth curves of different areas (from Navarro *et al.*, 2014a).

4 Review information on age determination, otolith exchanges and validation techniques on this species (ToR a)

4.1 Background information on age determination, Otolith exchanges and Workshops on Age Reading of chub mackerel in European waters

Chub mackerel is not an assessed species in European waters. Until this moment, there was not an international age reading protocol, nor any consensual age reading criteria. For many years, there was only one expert reader in IPMA, Portugal (now retired), and occasional readers in some European countries. In November 2011, the expert reader met in Lisbon, Portugal, with two new readers (Spain and Portugal), for a few days of training sessions. Since 2011, new permanent readers have appeared in Portugal, Spain, Italy and Greece.

A first otolith exchange was carried out in 2012–2013, recommended by the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS) in 2011, to assess the difficulties in age reading, provide a first evaluation of the agreement, precision and accuracy of age determination (ICES 2011a). A total of 244 otoliths from ICES areas VIIIc and IXa and Western Mediterranean were examined. Five readers from Portugal (2) and Spain (3) participated in the exchange.

The PGCCDBS, meeting in February 2014, recommended the realization of the first Workshop on Age Reading of Chub Mackerel to discuss the results of this exchange and the development of validation studies in this species (ICES 2014a). This Workshop, WKARCM, chaired by Andreia Silva (Portugal) and Rosario Navarro (Spain), has been held in Lisbon, Portugal, the 2–6 November, 2015.

However, due to the time passed since the exchange took place and a renovation of the readers of this species (retirements and new incorporations), it was thought necessary to carry out a new otolith exchange before the start of the Workshop (March-June of 2015). A total of 125 images of chub mackerel otoliths from ICES areas VIIIc and IXa and Western Mediterranean were analysed via WebGR by 14 readers from Portugal (6), Spain (6) and Italy (2).

4.2 Validation studies

Until now, there has not been any validation study in the NE Atlantic Ocean and the Mediterranean Sea, using direct validation methods, although there have been some semi-direct and indirect validation studies in these areas. Semi-direct validation methods consist of observing the evolution of calcified structure marginal zones over time. Two types of semi-direct validation studies are possible: Marginal Increment Analysis (Quantitative) and Edge Zone Analysis (Qualitative) (Panfili *et al*., 2002). A summary of semi-direct validation studies of *S. colias* otoliths in different areas of NE Atlantic Ocean and Mediterranean Sea is shown in the Table 4.2.1.

Table 4.2.1: Summary of the semi-direct validation studies of Chub mackerel realized in NE Atlantic Ocean and Mediterranean Sea (from Villamor & Carbonara, 2015).

Area	Method	Time series	Age/size Range	References
Bay of Biscay	Quantitative / Qualitative	2011	All ages together / 19-42cm	Navarro et al., 2014
Portuguese Coast	Qualitative	1981-1982	All ages together	Martins et al., 1983
Azores Islands		1996-2002	All ages together / 9.6-53.5cm	Carvalho et al., 2002
Madeira Islands	Quantitative / Qualitative	2002-2004	All ages together / 19-41cm	Vasconcelos, 2006
Canary Islands	Qualitative	Mar. 1988-Jul. 1990	All ages together / 19.2-41.1cm	Lorenzo et al., 1995
Gulf of Cadiz		1977-1978	All ages together	Rodriguez-Rhoda, 1982
Gulf of Cadiz/ SW Mediterranean (Alboran Sea)		Oct. 2003-Sep. 2004	All ages together / 17-40cm	Velasco et al., 2011
NW Mediterranean (Catalan Coast)		April - July 1992 and Dec. 1997	All ages together	Perrota et al, 2005
Eastern Mediterranean (Hellenic Sea)	Quantitative / Qualitative	Jan.-Dec. 1996	Ages 1-3	Kiparissis et al., 2000

Marginal increment analysis consists of measuring the distances separating the latest marks at the edge of the calcified structure, and it is used for validating the periodicity of growth increment formation (Campana, 2001). There are two methods: the Absolute Marginal Distance (AMD=distance between the end of the last hyaline annulus and the edge) and the Relative Marginal Distance (RMD=Ratio of the AMD and $D_{i,i-1}$; being $D_{i,i-1}$ the distance between the last two hyaline annuli) (Panfili *et al*., 2002). The Edge Zone Analysis is suited for determining the month or season of formation of the opaque zone. Annulus formation and its changes in the seasonal timing of the marginal increment with age are different among areas. A summary of Opaque edge formation of *S. colias* otoliths in different areas of NE Atlantic Ocean and Mediterranean Sea, from the literature, are shown in Table 4.2.2.

Table 4.2.2: Summary of the opaque edges formation studies on Chub Mackerel in NE Atlantic Ocean and Mediterranean Sea (from Navarro *et al.*, 2014a).

	Area	Opaque edge	References
NE Atlantic Ocean	ICES, VIIIc. Bay of Biscay	June - November	Navarro et al., 2014
	ICES, IXa. Portugal waters	May - August	Martins et al., 1983
	ICES, IXaS. Bay of Cadiz	May - August	Rodriguez-Rhoda, 1982
	ICES, IXaS. Bay of Cadiz	Mar./Apr. - Sep./Oct.	Velasco et al., 2011
	ICES, X. Azores Islands	May - Sep./Oct.	Carvalho et al., 2002
	Madeira Islands	May - July (max.)	Vasconcelos, 2006
	Canary Islands	March - Sept.	Lorenzo et al., 1996
Mediterranean Sea	W Mediterranean Sea. Alboran Sea	Mar./Apr. - Sep./Oct.	Velasco et al., 2011
	W Mediterranean Sea. Catalonya waters	Spring - Summer (max. April - July)	Perrota et al., 2005
	E Mediterranean Sea. Hellenic Sea	March - Sept. (max. April)	Kipparissis et al., 2000
	E Mediterranean Sea. Turkey waters	Summer	Tuggac, 1957

Indirect validation methods are based on corroborative information that supports age interpretation but does not validate the periodicity of the calcified structure incremental growth patterns. The indirect validation method most used is the Length Frequency Analyses (Panfili *et al*., 2002). So far, there has been only one study of Length Frequency Analysis of chub mackerel in these areas, Vasconcelos (2006), for chub mackerel of Madeira Islands.

There are also many studies using back-calculation models. Although these methods are not considered true validation methods, they are used to estimate the past fish length and to estimate the growth parameters (Table 4.2.3).

Table 4.2.3: Summary of back-calculation studies on Chub Mackerel in NE Atlantic Ocean and Mediterranean Sea (from Villamor & Carbonara, 2015).

Area	Time series	Age/size Range	References
Madera Islands	2002-2004	Ages 1-4 / 20-40cm	Vasconcelos, 2006
Canary Islands	March 1988 - July 1990	Ages 1-7 / 19.2-41.1cm	Lorenzo et al., 1995
Gulf of Cadiz	1977-1978	Ages 0-2	Rodriguez-Rhoda, 1982

5 Resume of the validation studies presented during the Workshop.

5.1 Bay of Biscay

Annual growth pattern and age validation trials of *Scomber colias* in the Bay of Biscay using otoliths. By: Navarro, M.R.; Villamor, B.; Landa, J.; Hernández, C. Instituto Español de Oceanografía. C.O. de Santander. Spain. Presentation 4 to WKARCM Lisbon (Portugal), 2–6 November, 2015.

The age estimation criteria of *S. colias* are not still internationally standardized and have never been validated or corroborated in Iberian waters. This work presents the growth pattern in the Bay of Biscay (Cantabrian Sea, ICES Div. VIIIc) based on samples from a period of two years (2011, 2012). A semi-direct validation of the age estimation of this species in the area is performed, for the first time in this area, based on the nature of the edge and the otolith marginal increment analyses. A verification criterion is also performed; the consistency of the age interpretation is tested by the regularity of the increments formation.

A total of 2185 pairs of *sagitta* otoliths from samples collected from landings of commercial catches and in acoustic and trawl surveys during 2011 and 2012 (Cantabrian Sea, ICES Div. VIIIc) were aged. The nature of the edge (hyaline or opaque) was also recorded for all of them.

The diameter and radius of 343 otoliths were measured, as well as the radius of each annulus. These otoliths were selected in order to obtain a good representation of otoliths by month, sex and fish length. The absolute marginal distance (AMD=distance between the end of the last hyaline annulus and the edge) and the distance between the last two hyaline annuli ($D_{i,i-1}$) were also measured in 111 of those otoliths, for estimating the relative marginal distance (RMD=ratio of the AMD and $D_{i,i-1}$) (Panfili *et al.*, 2002) (Figure5.1.1). All these measures were obtained using a microscope connected to an image analyser (NIS-Element) and recorded in microns (μm).

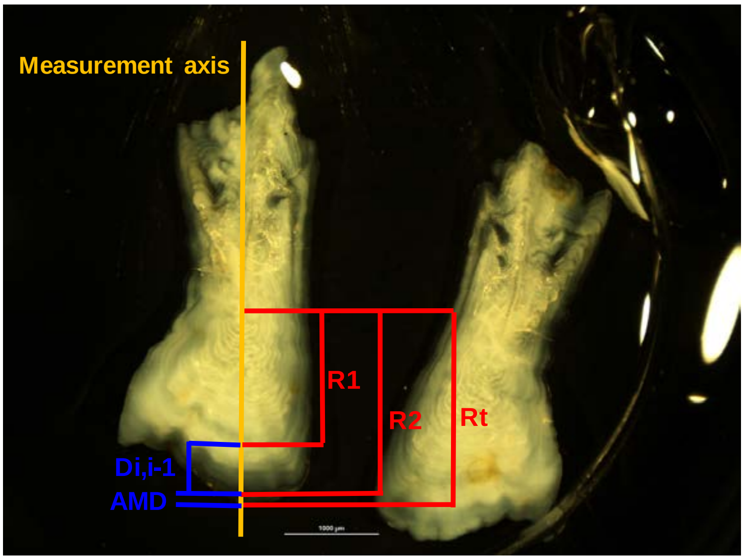


Figure 5.1.1: Measurement axis and measurements used in this study: Rt (otolith radius), R_i (annuli radius), $D_{i,i-1}$ (distance between the last two hyaline annuli) and AMD (distance between the end of last hyaline annulus and the edge) (Navarro *et al.*, Presentation 4 to WKARCM, 2015).

The relationship between otolith radius and fish length was significant and was expressed as a strong linear relationship (Figure 5.1.2).

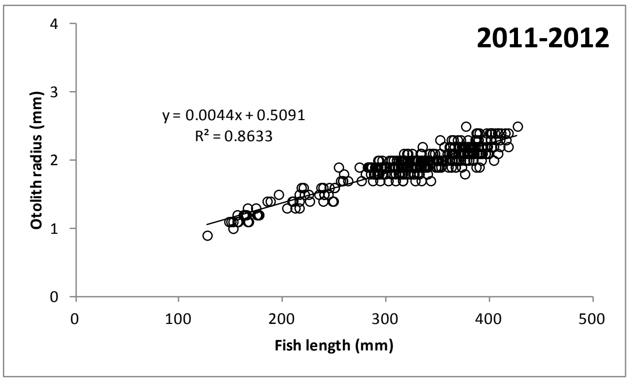


Figure 5.1.2: Otolith radius and fish length relationship of *S. colias* in the Bay of Biscay (2011-2012 combined).

The distribution of each annulus of *S. colias* had a normal distribution with a decreasing otolith growth rate with age (Figure 5.1.3). This linearly decreasing interval between increments is a verification criterion that forms the basis of age estimation (May, 1965).

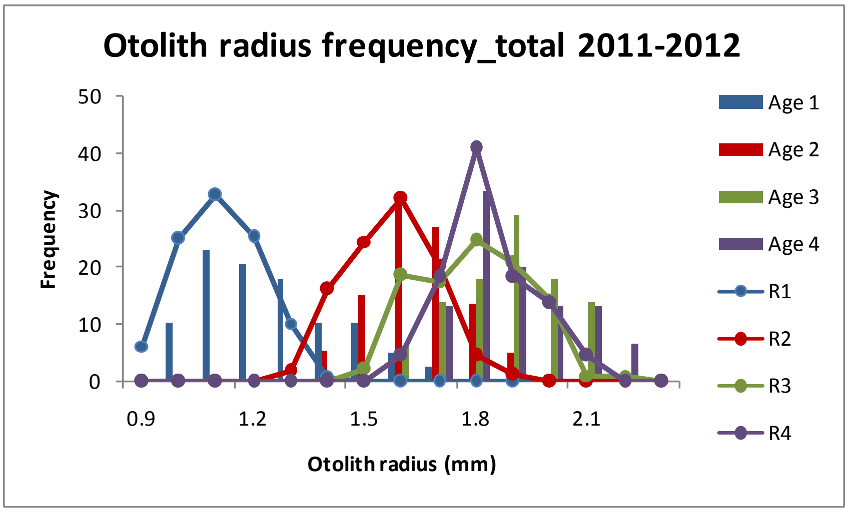


Figure 5.1.3: Annuli increment formation pattern in *S. colias* otoliths in the Bay of Biscay (2011–2012 combined).

The monthly proportion of edge type of *S. colias* indicated an annual periodicity in the formation of the hyaline and opaque annuli, appearing the opaque edge mainly from June to December. Winter (hyaline) annulus seems to be entirely formed in April. RMD also is higher in the second half of the year, between July and December, with higher values in July–August 2012 and November 2011 (Figure 5.1.4). The variability of these results can be explained by the low number of otoliths from which RMD could be measured (whole otoliths with opaque edge).

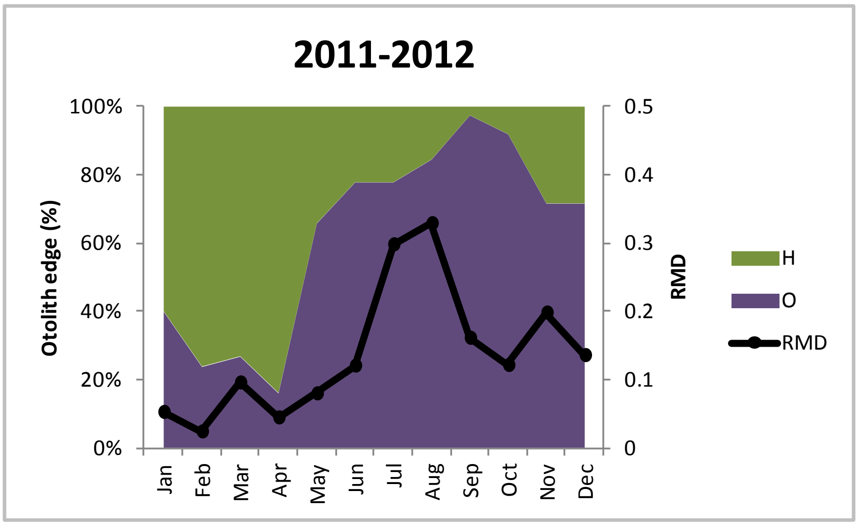


Figure 5.1.4: Monthly proportion of edge type and RMD analysis in *S. colias* otoliths of the Bay of Biscay (2011–2012 combined).

The commercial catches of chub mackerel in the Bay of Biscay is formed mainly of big individuals (34–40cm), which correspond to individuals from two years or more (Figure 5.1.5). Younger individuals are presented in the commercial catches in Galician waters (ICES div. IXaN). Otoliths from this area will be joined to this study shortly. A more completed length distribution of chub mackerel in the Bay of Biscay is obtained during the acoustic survey PELACUS, carry out in March-April every year (Figure 5.1.6).

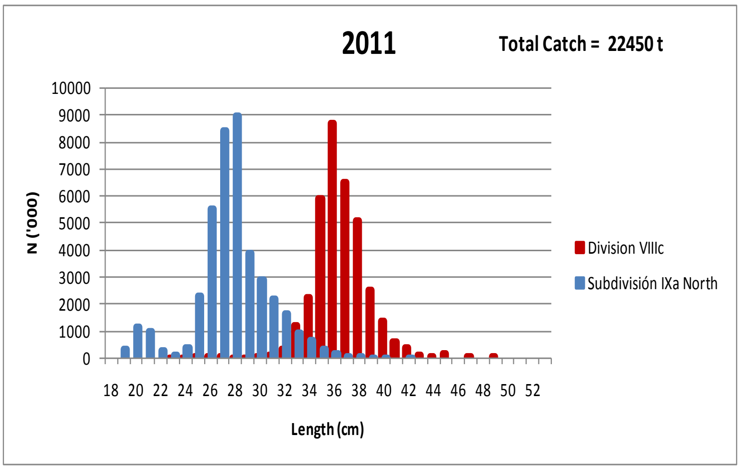


Figure 5.1.5: Length distribution of chub mackerel from commercial catches in the Bay of Biscay and Galician waters in 2011.

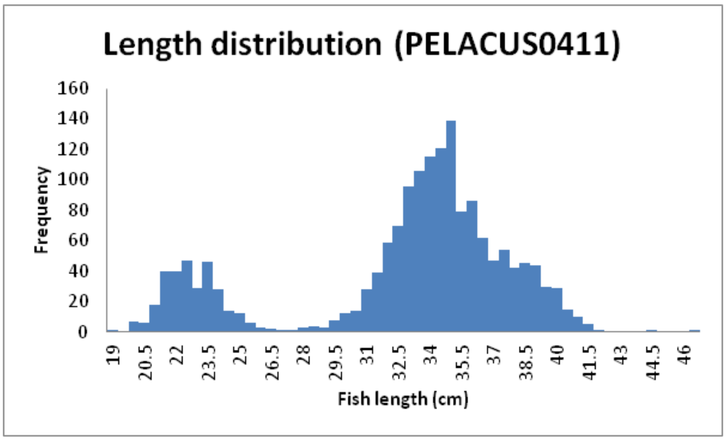


Figure 5.1.6: Length distribution of chub mackerel in the acoustic survey PELACUS0411 (ICES div. VIIIc)

Chub mackerel in the Bay of Biscay presents an exponential growth during early years. The growth slows down when the individuals reach 4 years (Figure 5.1.7).

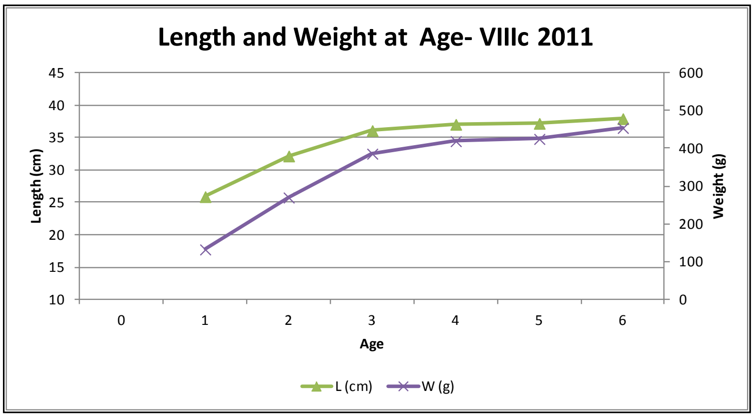


Figure 5.1.7: Length and weight at age relationship of chub mackerel in the Bay of Biscay in 2011.

Chub mackerel from the catch of 2011 in the Bay of Biscay shows a normal distribution of the length frequency by age (Figure 5.1.8), which gives consistency to the age estimation criteria in which this study is based.

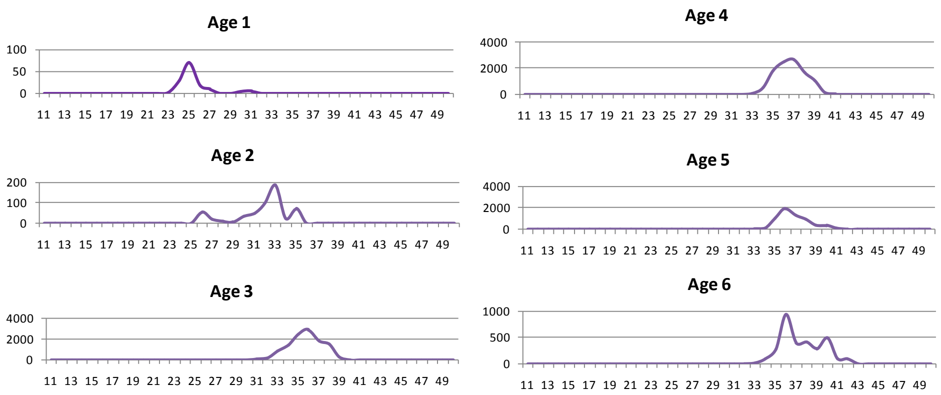


Figure 5.1.8: Length frequency by age of chub mackerel in the Bay of Biscay in 2011.

Typical chub mackerel otoliths from Bay of Biscay present a second annulus with a characteristic wide area. Some otoliths present as well marked false rings or checks around the middle of the second annulus that are usually well identifiable. Other otoliths presents one or more checks well marked which make more difficult the estimation of their age as can be mistaken with true rings. This is more frequent in otoliths from older individuals. This type of otoliths was excluded from this study.

The otolith selection for this study was made in order to obtain a good representation of otoliths by month, sex and length. Future steps in this study will include a more completed selection by age in order to include in the analysis individuals older than age 4. A good representation of otoliths by age, also will allow a study of the AMD to observe the differences in the edge formation by age. Also, a next step in this study will be the addition of the analysis of chub mackerel otoliths from Galician waters (ICES div. IXaN).

5.2 Portugal

Preliminary results of age validation study of chub mackerel (*Scomber colias*) of Portuguese waters. By: Ana Carolina Porfírio, Eduardo Soares, Cristina Nunes and Andreia V. Silva. Instituto Português do Mar e da Atmosfera, I.P. Lisboa. Portugal. Presentation 5 to WKARCM, Lisbon (Portugal), 2–6 November, 2015.

In Portugal chub mackerel (*Scomber colias*) is mainly captured by purse-seine fleet which targets sardine (*Sardina pilchardus*). Recently chub mackerel assumed an important role in the total Portuguese purse-seine landings (about 1/4 of the fish landed in Portuguese waters), in part likely because sardine abundance has decreased since 2006. Regarding this recent increased interest, recommendations were made by ICES concerning the need for an Iberian Chub mackerel stock assessment. In response to those recommendations this study attempts to validate the ages of chub mackerel in Portugal. The age of chub mackerel was determined from counts of opaque (transmitted light) annual growth zones in sagittal otoliths. Edge analysis (interpretation of whether the edge zone under formation is opaque or translucent) was performed in order to verify the existence of an annual growth pattern by examining the growing edge type of otoliths along time. Samples were collected bimonthly during 2012 in Peniche harbour. The progression of diameter frequency was also analysed in 170 otoliths to identify different age groups. Growth parameters were also estimated.

The length range of the chub mackerel analysed was 17–41cm with modes in 18 cm and 28 cm (Figure 5.2.1–a). The fish length/otolith diameter relationship explained 66% of the variance observed (Figure 5.2.1–b).

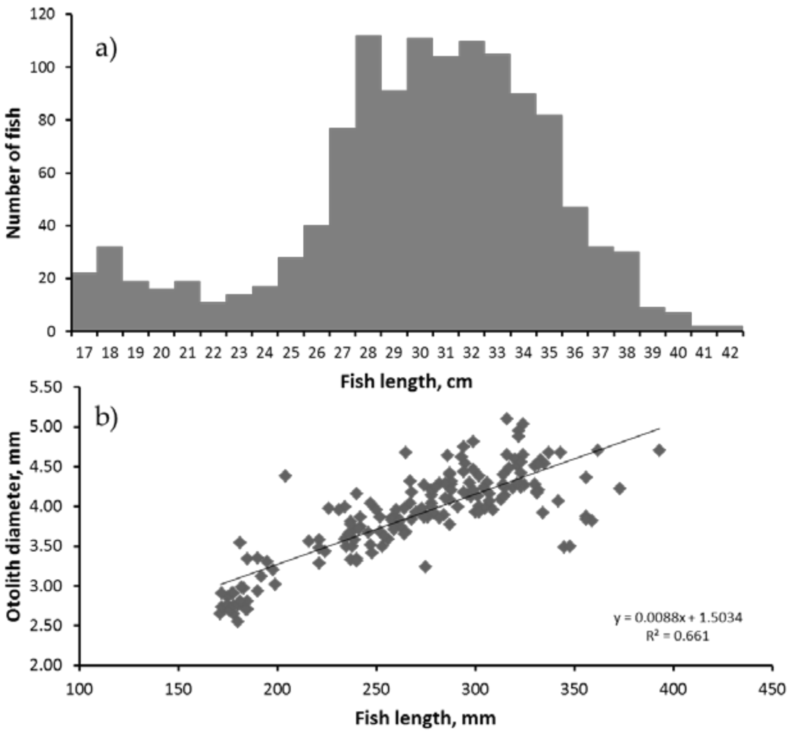


Figure 5.2.1: a) Fish length frequency and, b) Fish length/ otolith diameter relationship in Peniche harbour during 2012.

The otolith diameter frequency distribution (Figure 5.2.2) presents a "typical" pattern for a species with fast growth during the first year, and then a decline in the following ones. The otolith diameters at ages 0 and 1 appear almost completely separated from each other, and also more detached in relation to the other age classes. Otolith diameters for ages 2–4 are strongly overlapped, with no possible separation of age classes based on the diameter measurements.

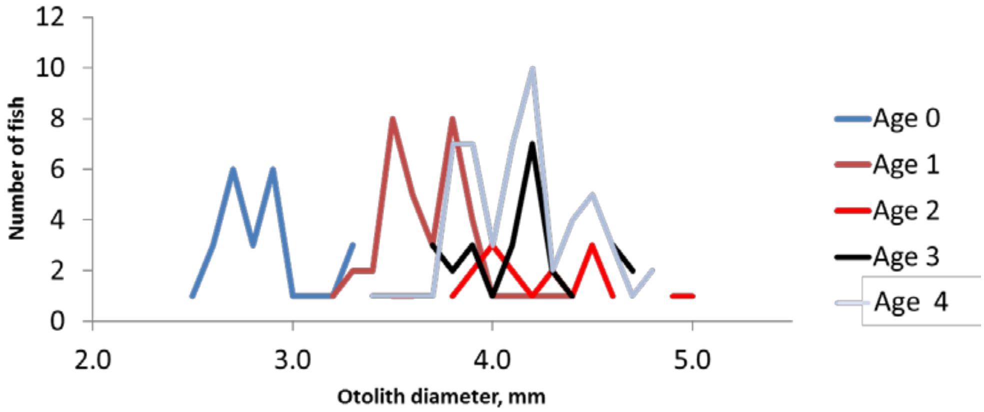


Figure 5.2.2: Otolith diameters of 2012 Peniche harbour.

Our results show that the opaque edge appeared between June and August, mostly in July, whereas the hyaline edge appeared in all months (Figure 5.2.3). These observations could indicate that the opaque edge is formed during summer; nevertheless, the overall number of otoliths with an opaque edge was very low, which raises questions on the apparent annual periodicity given by the edge analysis. A plausible reason behind this fact is that the number of age classes 0 and 1 available in the samples is relatively low, which constrained us to use all existing ages (0 to 4) for the edge analysis. But in older specimens it is difficult to distinguish an opaque edge, and thus the number of otoliths with an opaque edge may have been underestimated.

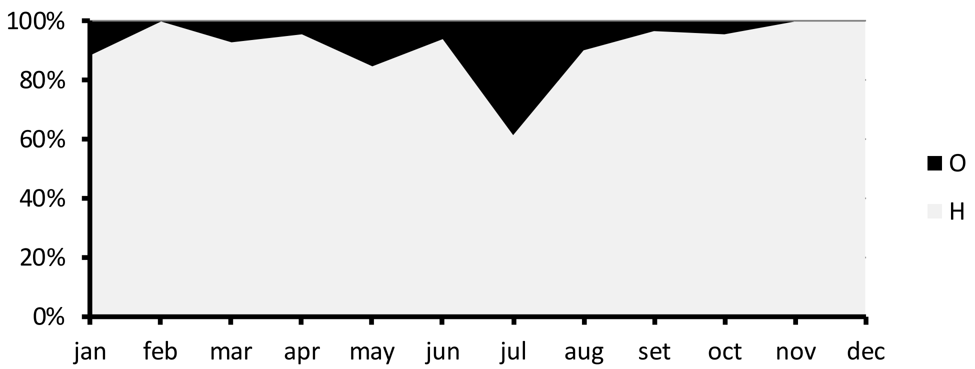


Figure 5.2.3: Average monthly percentage of otoliths of *S. colias* landed off Penicheharbour in 2012 with opaque (O) and hyaline (H) edges.

The von Bertalanffy fitted growth curve is shown in Figure 5.2.4. The growth parameters values obtained in the present study were: $L\infty$ =37.1cm, K=0.31 yr^{-1} and t_0= -2.36. The obtained growth parameters are reasonable in relation to estimates obtained in other studies (Navarro *et al*., 2014a; Martins *et al*., 1983). The theoretical maximum length value was close to the size of the largest fish sampled and the growth coefficient value (0.31) indicates a relatively rapid attainment of the maximum size. The values of growth parameters are very similar to those reported for the same species in Bay of Biscay (Table 5.2.1).

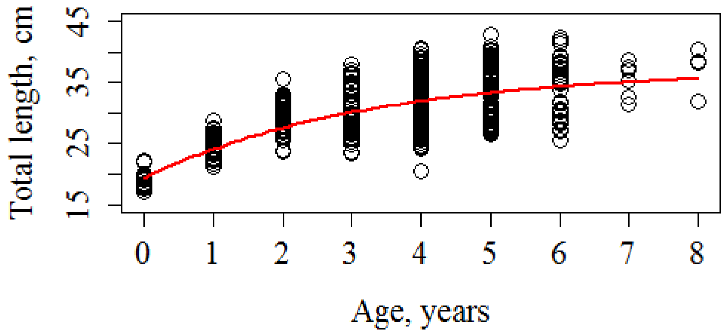


Figure 5.2.4: The von Bertalanffy growth curve of *S. colias* off Peniche harbour

Table 5.2.1: Values of the growth parameters for *Scomber colias* according to different studies

Studies	Area	L∞	K	T0
Present study	Peniche	37.1	0.31	-2.36
Martins, 1996	Continental portuguese coast	58.52	0.10	-3.68
Martins *et al.*, 1983	Continental portuguese coast	53.83	0.17	-2.04
Carvalho *et al.*, 2002	Azores	57.52	0.20	-1.09
Navarro *et al.*, 2014	Bay of Biscay	37.8	0.650	0.05

The chub mackerel growth shows an exponential growth similar to other studies which gives some consistency to the present age estimations.

These are preliminary results of an ongoing work that should obviously be carefully considered. The overall analysis will be improved and more data included. Other validation methods, such as progression of cohorts, measurements of the radius of each annulus, and Marginal Increment (MI) analysis, will be performed.

5.3 Mauritania

Ageing criteria validation of *Scomber colias* (Gmelin, 1789) from NW Africa. By: Jurado-Ruzafa, A.; E. Hernández and M.T.G. Santamaría. Instituto Español de Oceanografía – IEO. C.O. Canarias. Spain. Presentation 6 to WKARCM, Lisbon (Portugal), 2–6 November, 2015.

Through the EU-Data Collection Framework project, discards of small pelagic fish species (*Scomber colias*, *Trachurus* spp, *Sardina pilchardus*, *Sardinella aurita* and *Sardinella maderensis*) from the European pelagic freezer trawlers fleet operating off Mauritania are monitored. In addition to the monthly biological analyses, an age and growth study of *S. colias* was performed during 2005–2006. Due to the absence of small individuals, back calculation method was applied based on a total of 174 selected otoliths with high security in their age interpretation.

The partial radii of each annulus were used to assess the coherence of the age determination criteria used in the Canary Islands IEO Centre (May, 1965; Morales-Nin, 1992). In this laboratory, ageing criteria used are based on FAO (2002) in relation with the seasonal regularity of the growth pattern assumed for this species in close areas (Lorenzo, 1992).

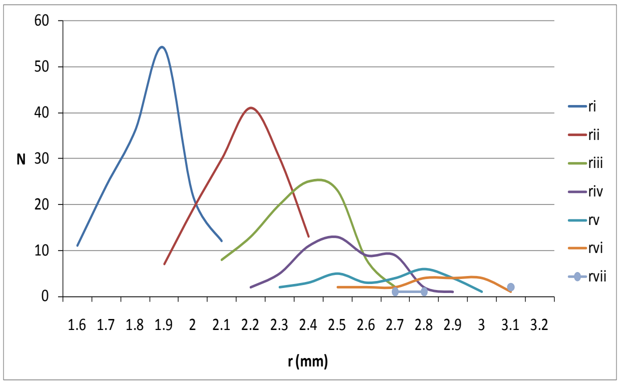


Figure 5.3.1: Frequency distributions of annuli radii (ri), in otoliths of *S. colias* from Mauritania

The linear decreasing interval between frequency distributions of annuli radii (Figure 5.3.1) verify the coherence of the ageing criteria used.

6 Standardization of material and methods and review of ageing techniques

6.1 Otoliths preparation techniques

All participating institutes to the present Workshop and the two previous Otolith Exchanges use otoliths for age estimation of *Scomber colias*. However, the preparation methods of these otoliths differ with the laboratory. A summary of the preparation techniques of each laboratory is shown in Table 6.1.1.

Table 6.1.1: Summary of otolith preparation techniques of chub mackerel otoliths of each participant laboratory (based on Silva *et al.*, presentation 3 to WKARCM 2015).

Country	Laboratory / Institution	Calcified structure	Preparation technique	Stored before preparation	Stored after preparation
Portugal	IPMA	Whole otoliths	Otoliths mounted in resin in black plastic plates	Cleared and dried in eppendorfs	Labelled in black plastic plates
Spain	IEO Santander	Whole otoliths	Otoliths mounted in resin in black plastic plates with cover	Loose and dry inside labelled plastic plates with cover, in an horizontal position	Fixed in resin in black plastic plates with cover; stored in cardboard boxes
	IEO Canarias	Whole otoliths	Otoliths mounted in clear resin in black plastic plates	Clean and dried, in labelled plastic containers	Labelled in black plastic plates
	IEO Murcia	Whole otoliths	Otoliths mounted in clear resin on custom plastic slides	Directly mounted in clear resin on custom plastic slides	In plastic bags
Italy	CIBM	Whole otoliths	Otoliths inmersed in sea water	Dried and stored in a pvc phial	Dried and stored in a pvc phial
	COISPA	Whole otoliths	Otoliths inmersed in clarification medium (sea water)	Dried in a plastic box	Dried and stored in a plastic box

In the laboratories of IPMA (Portugal) and IEO (Spain), chub mackerel otoliths are mounted in clear resin inside black plastic plates. The resin used is different in each laboratory: the resin used in IPMA (Portugal) is Entellan® (Merck, ref. HX807787); IEO laboratories of Santander and Canarias use Eukitt® and IEO laboratory of Murcia use NEO-Mount® (anhydrous mounting medium). All laboratories clean and dry the otoliths before being mounted. The laboratories of COISPA and CIBM (Italy) observe the otoliths immersed in seawater without a clarification phase before the age estimation. Age estimation of chub mackerel is performed in all laboratories by observing the otoliths with a binocular microscope with reflected light against a black background. Otoliths fixed in resin are stored in cardboard boxes or plastic bags while otoliths immersed in seawater are dried and stored in a PVC phial.

6.2 Otolith image processing techniques

All participant laboratories have a methodology to capture and analyse otoliths images. The type of capture/analysis software varies between laboratories, as well as the type of camera. Most laboratories use the otolith images for thesis and validation studies of chub mackerel and other species. Otoliths images can be available by all laboratories for otolith exchanges. The most frequent otolith measures are: otolith radius, annual ring radius and the distance between rings. JPEG and TIFF are the image format most commonly used. A summary of the otolith image processing techniques used by WKARCM participant laboratories is shown in Table 6.2.1.

Table 6.2.1: Summary of the otolith image processing techniques used by WKARCM participant laboratories (based on Silva *et al.*, presentation 3 to WKARCM 2015) (AMD - distance between the end of the last hyaline annulus and the edge; RMD - ratio of the AMD and the last two hyaline annuli).

Country	Laboratory / Institution	Image capture software	Type of camera	Image uses	Otoliths measures	Image format
Portugal	IPMA	Visilog 6.3 / TNPC 4	Sony digital camera DFY-SX910	Exchanges, thesis	Annual ring radius, otolith radius	TIFF
Spain	IEO Santander	NIS-Elements Viewer 4.0	Color Digital Camera NIKON Digital Sigth. DS-5M	Exchanges, thesis, validation studies, publications, IEO manuals, otolith images bank	Otolith diameter/radius, annual ring radius, AMD, RMD	LIM images, JPEG, TIFF, psp
	IEO Canarias	Nis-Elements and Image Pro	Nikon Digital Sight DS-U2	Exchanges, annual periodicity validation for one ring deposition, backcalculation	Distance between rings, otolith radius and annual ring radius	TIFF, JPEG
	IEO Murcia	Las ez as (capture) / Irfan view (analysis)	Digital camera	Exchanges	Distance between nucleus and first and second ring	JPEG
Italy	CIBM	Image pro premiere 9.1	Digital camera: color CCD sensor 1/3'', resolution 1295x960 pixel with 8/12 bit color (mod. C125)	Routine, exchanges, validation studies, thesis	Radius, length, distance between rings, annual ring radius	JPEG
	COISPA	Image J	BELL DVD1300 CMOS Camera	Exchanges, validation studies and formation a reference collection	Otolith total length, radius and ring radius	JPEG

7 Estimate (relative) accuracy and precision of chub mackerel, age determination in the main European fishing areas (ToR b)

The main results of the exchanges of 2013 and 2015 are presented in this part of the report. A closer examination of the results of a new exercise realized during the Workshop is also presented

The spreadsheet (Eltink, 2000) was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink *et al*. (2000). Modal ages were calculated for each otolith read, with percentage agreement, mean age and precision coefficient of variation as a definition (for each otolith):

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

7.1 Exchange 2012–2013: Chub Mackerel (*Scomber colias*, Gmelin 1798) Otolith Exchange (Martins *et al.*, 2014).

Following a recommendation of PGCCDBS in 2011, an exchange of chub mackerel otoliths was carried out in 2012–2013 to assess difficulties in age reading and provide a first evaluation of the agreement, precision and accuracy of age determination. Five age readers from Portugal and Spain, with variable degrees of experience as otolith readers of chub mackerel and other pelagic species participated in the exchange. A total of 244 otoliths were examined, from fish with 17.8–40.6 cm total length collected in 2011 off the ICES areas VIIIc (Bay of Biscay) and IXa (Portugal waters) and in the Western Mediterranean waters (GSA06). Age readings were analysed for the whole otoliths set and separately for Atlantic and Mediterranean waters using Eltink Workbook on Age Reading comparisons (Eltink *et al*., 2000). Two options were used to set a Reference Age, one where it corresponded to age readings of the most experienced reader on this species and another where it corresponded to the modal age of three readers with variable experience on chub mackerel but long experience on mackerel or other pelagic species.

Age readings ranged from age 0–9. The two reference age options gave similar overall results (Table 7.1.1). Considering the three readers modal age, the average level of agreement was 60.4%, the CV was 22.7% and there was evidence of bias especially for ages ≥4 years. Ages 0 and 1 showed high agreement (99 and 93%, respectively), low CV (10.2 and 15.4%) and no signs of bias in relation to the modal age, suggesting that the identification of the first annual ring was not an issue in this species. The percentage of agreement dropped substantially at ages 2 (62%) and 3 (59%) and from age 3 onwards, agreement was generally below 50%. Age 2 showed particularly low precision (CV=27%) compared to neighbour ages (15% at age 1 and 22% at ages 3-5) possibly due to the frequency of false rings. Bias increases substantially with age but this effect is mainly due to underestimation of ages >4 years by Readers 3 and 4 (experienced in Mediterranean otoliths).

Table 7.1.1: Percentage of agreement and CV from the two reference age options.

	OPTION 1	OPTION 2
% Agreement	59.5	60.4
CV	22.7	22.7

The results suggested that readers could be divided in two groups: those with experience in Atlantic species and those with experience in Mediterranean species. The later showed good precision and no bias in otoliths of younger individuals (until age 2) probably because they were used to identify false rings. On the other hand, their results on older individuals were poor. Some readers with experience in Atlantic species had participated in a training meeting with the expert reader before the exchange and therefore had a more similar interpretation of chub mackerel otoliths. Agreement between Atlantic and Mediterranean readers was generally poorer (34–47%) than agreement among readers of the same group, 84.4% for Mediterranean readers and 53.4–61.4% for Atlantic readers.

The exchange indicated that chub mackerel age determination could be done with acceptable precision and accuracy in younger individuals (up to ages 3–4 years). The major difficulties were the frequency of false rings in young individuals (until age 3), otolith edge interpretation and the assignment of ages older than 7 years.

The realization of a workshop was recommended to discuss the results of this exchange. Moreover, to improve the age determination in this species, the group recommended that otolith exchanges were carried out regularly between all readers.

The use of a quality scale for readings was also recommended, as follow: 1–EASY (75–100% reliability); 2-DIFFICULT (25–75% reliability); 3-ILLEGIBLE (0–25% reliability).

As chub mackerel otoliths readings had never been validated in the IXa and VIIIc ICES areas, it was recommended to perform validation studies for these areas.

7.2 Exchange 2015: Small Exchange of *Scomber colias* Otoliths from Atlantic and Mediterranean Areas, March–June 2015 (Navarro *et al.*, Working Document to WKARCM 2015).

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting in February 2014, recommended a Workshop to discuss the results of the otolith exchange carried out in 2012–2013. However, due to the time passed since the exchange took place and a renovation of the readers of this species (retirements and new incorporations), it was thought necessary to carry out a new otolith exchange before the start of the Workshop. As the time available to carry out the new exchange was so short, it was decided to use a selection of 125 otoliths images from the previous exchange. This Small Exchange was hold via WebGR between March–June 2015, and organized by IEO-Santander (Spain) and IPMA (Portugal). A total of 14 readers from six laboratories of three European countries (Portugal, Spain and Italy) participated in this exchange. Readers were ranked Intermediate and Trainee level considering the reader experience (in number of otoliths and in years) with this species. It was not considered any Expert reader (experience with more than 10000 otoliths).

Age readings results were analysed using the GussEltink spreadsheet (Eltink, 2000). The analysis was performed for the total of areas and for all readers; and also considering only the intermediate readers, and with only the trainee readers. Additional analyses were performed by set of otoliths: Bay of Biscay set, Portugal set and Mediterranean set. It was also analysed the chub mackerel growth pattern using the length-at-age per area and reader using R software 2.15.0 (R Development Core Team 2008).

The overall agreement was 57.3% for all readers, 53.3% for intermediate readers and slightly higher, 63.7%, for trainee readers. The results of all readers showed modal ages from 0 to 6. For all age readers analysis, the best agreements were reached for age 1 (74%), for ages 2 and 3 agreements were 59% each, for age 0 agreement was 57%, for ages 5 and 6 agreements were only 50%, being the lowest agreement for age 4 (49%). Overall CV was 29.6% (Table 7.2.1).

Table 7.2.1: Percentage of agreement, CV and bias results of the analyses of all readers, intermediate readers and trainee readers.

ALL OTOLITHS	All readers	Intermediate readers	Trainee readers
% Agreement	57.3	53.3	63.7
CV	29.6	31.0	25.4
Bias	0.18	-0.02	0.03

By area, the overall agreement was 53.5% for the Bay of Biscay set, 55.3% for the Portugal set and 62.1% for the Mediterranean set (Table 7.2.2).

Table 7.2.2: Percentage of Agreement, CV and bias results of the analyses of the Bay of Biscay set, Portugal set and Mediterranean set.

ALL READERS	Bay of Biscay set	Portugal set	Mediterranean set
% Agreement	53.5	55.3	62.1
CV	27.4	22.8	35.2
Bias	0.25	0.12	0.14

There had been a small decrease in the level of agreement compared to the previous exchange results (Table 7.2.3).

Table 7.2.3: Comparison between the % Agreement and CV results of the otoliths exchanges of 2013 and 2015.

ALL READERS	2013	2015
% Agreement	60.4	57.3
CV	22.7	29.6

Four readers from the previous exchange participated also in this exchange, three of them as Intermediate readers this time. However, the results of this group analysis showed big differences between them.

Six readers did not have any experience with chub mackerel otoliths before this exchange. However, the results of the trainee readers were slightly better than the results of the intermediate readers.

The results of the growth pattern analysis showed that the growth pattern of Mediterranean and Portugal sets seemed to represent a standard growth pattern of Chub mackerel, which is characterized by high growth rate in small age groups. The Mediterranean and Portugal sets seemed to have higher growths at ages 0–1 and 1–2 and a drastic drop in growth from ages 4 and onwards. Most readers did not reflect a constant and consistent growth in Bay of Biscay set.

There seemed to be four different groups of readers with similar age reading criteria, which in turn differed from the other groups' criteria. Thereby, readers 1, 6, 13 and 14 showed an underestimation in older ages regarding the Modal age. Also, this group of readers showed no bias in the inter-reader bias test (with the exception of the test between readers 6 and 13), and have similarities in the growth pattern. This could be explained due that readers 1 and 6 were trained in 2011 by the expert reader (now retired) and, in turn, readers 13 and 14 were trained by reader 1. This way this group of readers had similar age reading criteria for chub mackerel age estimation.

A second group of readers seemed to be formed by readers 3 and 4. Both readers showed an overestimation in most ages regarding the Modal age. Also, both readers showed no bias in the inter-reader bias test and have similar growth patterns. Both readers belonged to the same laboratory, which can explain the similar age reading criteria between them.

A third group of readers seemed to be formed by readers 8, 10, 11 and 12. This group showed a better estimation regarding the Modal age. Readers 8, 11 and 12 showed no bias with the Modal age in the reader against Modal age bias test. Readers 8, 11 and 12 showed no bias between them in the inter-reader bias test. Reader 11 also showed no bias against reader 10 in the inter-reader bias test. It draws attention the fact that even when readers 10, 11 and 12 had no experience reading chub mackerel otoliths before this exchange they showed the best estimation regarding the Modal age, showing similar age reading criteria between them.

Only 14 otoliths from the 125 otoliths of the exchange had an agreement of more than 80%. From these, only 2 otoliths had 100% of agreement. For the other 12 otoliths with more than 80% of agreement, the differences between all readers' age estimations were of only one year.

The otolith with the lowest agreement was otolith number 7 (29%), which was aged 3–8. Otoliths with low agreement usually coincided with otoliths with false rings (checks), which were not well identified by some readers. Also, the first annulus was not well identified by some readers, especially in the Bay of Biscay set.

7.3 WKARCM exercise

A total of 11 readers participated in the Workshop (Table 2.1.1). Some of those participants also took part in the 2015 otolith exchange and in the first otolith exchange of chub mackerel (2012–2013).

After the problems and age difficulties interpretation of last exchange were discussed together on a live screen and the first points of the reading criteria were established between all WKARCM participants, a new exercise was realized.

Like the previous exchange, the WebGR tool was used to this exchange and each image was uploaded to WebGR (http://webgr.azti.es/ce/search/myce).

All areas used whole otoliths and its number and preparation method per area was:

- 25 images from Bay of Biscay, otoliths fixed with Eukitt
- 25 images from North Portugal waters, otoliths fixed with Entelan
- 25 images from Mauritanian waters, otoliths fixed with Eukitt
- 25 images from Western Mediterranean, otoliths fixed with Neo-mount
- 23 images from Ligurian Sea, otoliths immersed in seawater
- 26 images from Ionian Sea, otoliths immersed in seawater

The analysis was performed for the total of areas and all readers and intermediate and trainee readers separately. Additional analyses were performed by set of otoliths. A summary with the overall % agreement, CV, bias and age range of all analyses are shown in Table 7.3.1. The Figures and Tables showing the results of each analysis are presented in Annex 3.

Table 7.3.1: Summary of the % Agreement, CV, bias and age range obtained in the analyses of chub mackerel readings of WKARCM exercise.

Analysis	% Agreement	CV	Bias	Age range
All readers	60.6	45.6	0.01	0-7
Intermediate readers	64.1	39.6	-0.11	0-7
Trainee readers	67.5	39.6	0.07	0-6
Bay Biscay	66.7	36.2	0.07	0-5
North Portugal	55.6	37.3	-0.15	0-4
Mauritania	60.2	41.6	0.04	0-7
Western Mediterranean	65.3	29.3	0.05	0-4
Ligurian Sea	46.4	64.6	0.05	0-4
Ionian Sea	68.2	65.8	0.12	0-4

7.3.1 All readers

From the total of 149 images of chub mackerel otoliths 1 reader estimated the age of all images; 2 readers estimated the age of 143 images; 1 reader estimated the age of 140 images; 1 reader estimated the age of 139 images; 1 reader estimated the age of 130 images; 1 reader estimated the age of 117 images; 1 reader estimated the age of 107 images and 1 reader estimated the age of 90 images. The results of all readers showed modal ages from 0–7.

Overall age reading results of all readers are shown in Annex 3 (Figures A3.1.1.a, b).

The overall agreement for all readers was 60.6%. The best agreements were reached for age 0 (79%), for ages 1 and 2 agreements were 63% and 64% respectively, for age 3 agreement was 58%, for ages 4, 5 and 7 agreements were only 40%, 52% and 27% respectively. (Table A3.1.1, Annex 3).

The analysis including all readers revealed a coefficient of variation (CV) of 45.6%. CV peaked at 107.7% for modal age 0, which was due mostly to the difficulty that the formula shows when analysing different values for modal age 0 (age 0 of some readers opposite to age 1 of the other readers). Lowest CV was revealed for modal age 5 (19.0%) (Table A3.1.1). The overall relative bias was low (0.01) (Table 7.3.1).

The results of the inter-reader bias test show a group of readers with no bias between their readings clearly defined: a group with readers 1, 2, 5, 8 and 10; a second group with readers 3 and 4 and, a third group with readers 6 and 9, whose reading criteria are very similar to the first group. Reader 7 showed bias with all readers, as well as with the modal age (Table A3.1.2).

Figure A3.1.2 shows age bias plots with the mean age recorded and the standard deviation of each reader and all readers combined plotted against the modal age. Readers 1 and 2 showed the better estimation regarding the modal age. Readers 3 and 4 showed underestimation in most ages regarding the modal age. Readers 6, 9 and 11 showed an underestimation of older ages, regarding the modal age. Reader 7 showed overestimation in most ages and readers 5, 8 and 10 showed overestimation in younger ages and underestimation in older ages, regarding the modal age. The standard deviation showed a decreasing of its values with the age for all readers combined (Figure A3.1.3).

7.3.2 Intermediate readers

The overall agreement for intermediate readers was 64.1% and the best agreements were reached at ages 0 (88%), 1 (75%) and 2 (70%). The lowest agreement was reached at age 7 (50%) (Table A3.2.1).

The CV was 39.1% being this value influenced by the age class 0 that increase CV to more than 100%. Table A3.2.2 shows that all intermediate readers had certainty of bias between their readings and the modal age. It is also noted that there are two distinct groups of readers with no bias between them; readers 1 and 2 and readers 3 and 4.

7.3.3 Trainee readers

The overall agreement for trainee readers was 67.5% and the best agreements were reached at ages 0 (78%), 2 (71%), 5 (69%) and 2 (68%). The lowest agreement was reached at age 6 (38%) (Table A3.3.1).

The CV was 39.6% being this value influenced by the age class 0 that increase CV to more than 100%. Table A3.3.2 shows that readers 6, 8 and 10 do not present bias between their readings and the modal age. Readers 5, 8 and 10 seemed to have the same age reading criteria. Readers 6 and 9; and 9 and 11 also showed no bias between them. Reader 7 seemed to follow different criteria from the other readers.

7.3.4 Analysis by set of otoliths

The sets with best agreements were the sets from Ionian Sea (68.2%), Bay of Biscay (66.7%) and Western Mediterranean (65.3%) (Table 7.3.1). The Ligurian Sea set and North Portugal set had the lowest agreement (46.4% and 55.6%, respectively). Tables and Figures with the results of each set analysis are shown in Annex 3.

7.4 Discussion and conclusions

When comparing the results of the exchange of WKARCM meeting with the previous exchange, there has been a small increase in the level of agreement between all readers (57.7% to 60.6%). The CV increased from 29.6% to 45.6%. This could probably be due to the elevate number of otoliths with age zero in the sample. The formula has difficulties when analysing different values for modal age 0 (age 0 of some readers opposite to age 1 to the other readers).

Nevertheless, it should be noted the effort made by all the readers to adopt similar criteria. Readers that showed bias between them in the previous exchange, showed no bias in the WKARCM exercise, like readers 1, 2, 5 and 8. Also, trainee readers 6 and 9 presented no bias between them, nor against the modal age. .

Readers 3 and 4 agreed between each other but like the 2015 exchange they still do not agree with the rest of the participants. Also, both readers showed no bias in the inter-reader bias test and have similar growth patterns (Figures A3.10.1; A3.10.2; A3.10.3). Both readers belong to the same laboratory, which can explain the similar age reading criteria between them. Unlike the previous exchange both readers showed an underestimation in most ages regarding the Modal age. This could be due to the effort made by these readers to readjust their age reading criteria after the discussion of the criteria during the Workshop, as in the previous exchange both readers showed overestimation in most ages regarding the modal age. Also, it was the first time that readers 3 and 4 estimated the age of chub mackerel otoliths without knowing the fish length.

Reader 7 seemed to follow a different age reading criteria. This reader showed an over-estimation in most ages regarding the Modal age. This may be because reader 7 is used to read chub mackerel otoliths immersed in seawater. In the exchange most of the otoliths images were taken from otoliths fixed in transparent resin, which can explain the difference in the age estimation.

Readers 11, 1, 10 and 2 were the readers with more otoliths rejected (59, 42, 32 and 19, respectively), which could have influenced some results compared with the other readers, with less than 10 otoliths rejected.

Most of the readers had difficulty in the age interpretation of the Ligurian Sea set, possibly due to difficulties in the identification of rings, as these images corresponded to otoliths immersed in seawater, which most of the readers were not familiar with. The better results obtained in the Ionian Sea set, with otoliths also immersed in seawater, could be explained due that most otoliths corresponded to age 0, and therefore, there were no annuli to identify by readers. The north Portugal otoliths showed a large number of checks, which made age reading interpretation difficult for most of the readers in the exchange.

A total of 32 otoliths from the 149 otoliths of the exchange had an agreement of more than 80%. From these, 12 otoliths had 100% of agreement. By set of otoliths, 7 otoliths from the Bay of Biscay set had more than 80% of agreement, 3 from the Portugal set, 8 from the Mauritanian set, 2 from the Western Mediterranean set and 11 from the Ionian set. By age, 13 otoliths of age 0, 5 otoliths of age 1, 11 otoliths of age 2 and 3 otoliths of age 3 had more than 80% of agreement. Otoliths of 4 years onwards were not yet well identified by most readers due to the difficulty in discriminate true annulus from false rings in otoliths from older individuals.

Some readers found confusing that the images of all otoliths were not taken with the same magnification. Also, the use of the 1st of January as the birth date for the Ligurian and Ionian Sea sets created confusion between most readers, when ageing some otoliths of younger specimens.

8 Identify causes of age determination error and provide species-specific guidelines for the improvement of precision and reduction of bias between readers and laboratories (ToRc)

8.1 *Scomber colias* otoliths. Areas for interpreting the age.

Chub mackerel otoliths have an irregular shape (Figure 8.1.1), which is more accentuated in otoliths of older individuals. This shape differs slightly between individuals.

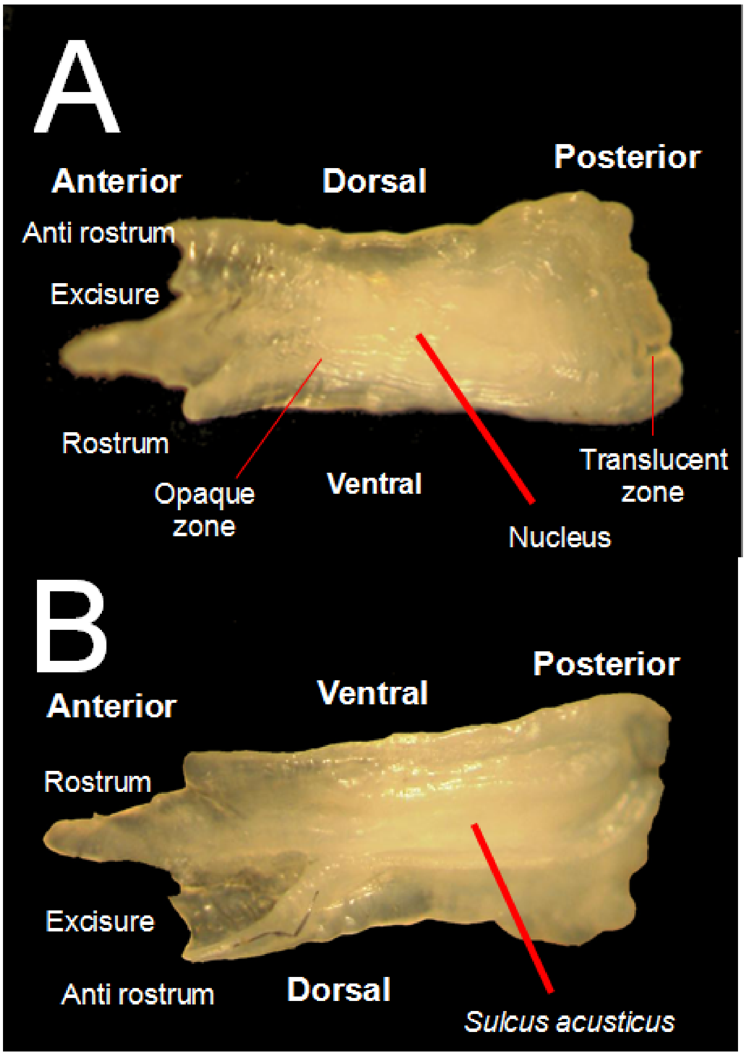


Figure 8.1.1: View of chub mackerel otolith: identification of main structural areas: A–dorsal face; B–Ventral face

For age reading, the otoliths are orientated with the distal surface turned up and the proximal surface (*sulcus acusticus*) turned down. Annuli are more clearly observed in the post-rostrum and the edge near the rostrum areas. Unlike Atlantic mackerel otoliths, the rostrum offers most times little help to the age estimation in chub mackerel otoliths, especially in older individuals, whose annuli usually are not very clear in this area (Figure 8.1.2).

Figure 8.1.2: *S. colias* otoliths. In green, areas where the annuli are best observed; in red, areas where the annuli are less clear.

During the discussion of the exchange results it was recommended to take a whole observation of the otoliths before rejecting them. Some otoliths with high presence of false rings could present an area where the interpretation is possible, even when in other areas it is not (Figure 8.1.3).

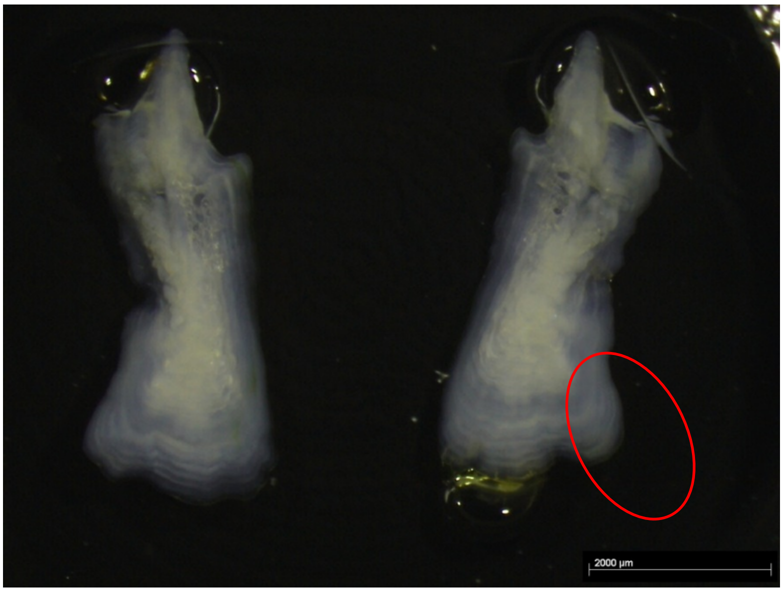


Figure 8.1.3: Otolith of S. colias. High presence of false rings should drive to reject the otolith, but the circle shows a readable area (ICES Area IXa, LT 34.0 cm, Female, Catch date: June)

8.2 Causes of age determination error identified during the WKARCM meeting

The main otoliths interpretation difficulties for *S. colias* are linked to the identification of the two first annuli, due to the high presence of false rings, frequently double rings (Figures 8.2.1 and 8.2.2).

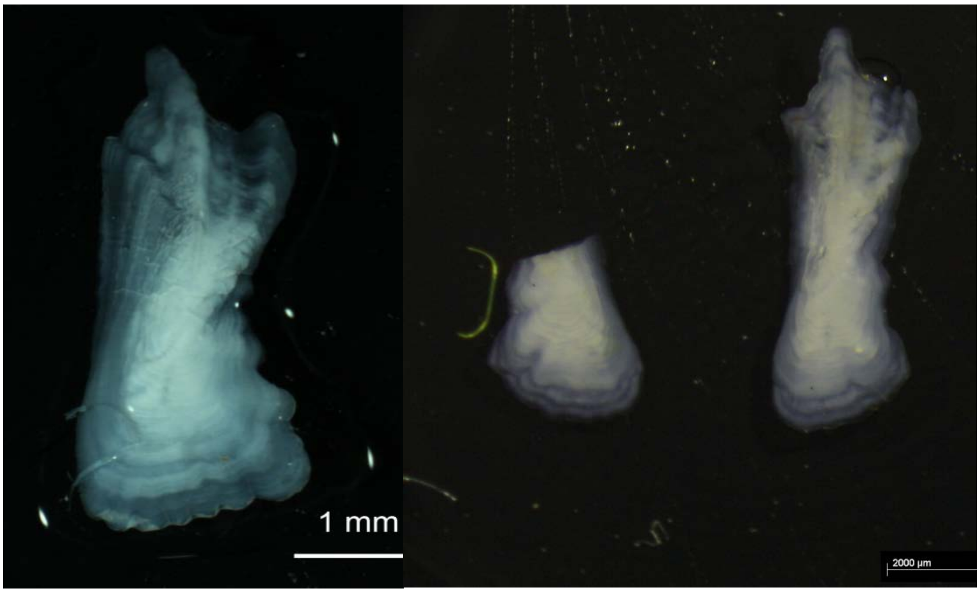


Figure 8.2.1: Otolith of *S. colias*. Left: CECAF-MAURITANIA (Total Length=28.4 cm, Female, Catch date: May) Right: Portugal waters (ICES Area IXa, Total Length= 26.2 cm, Male, Catch date: April)

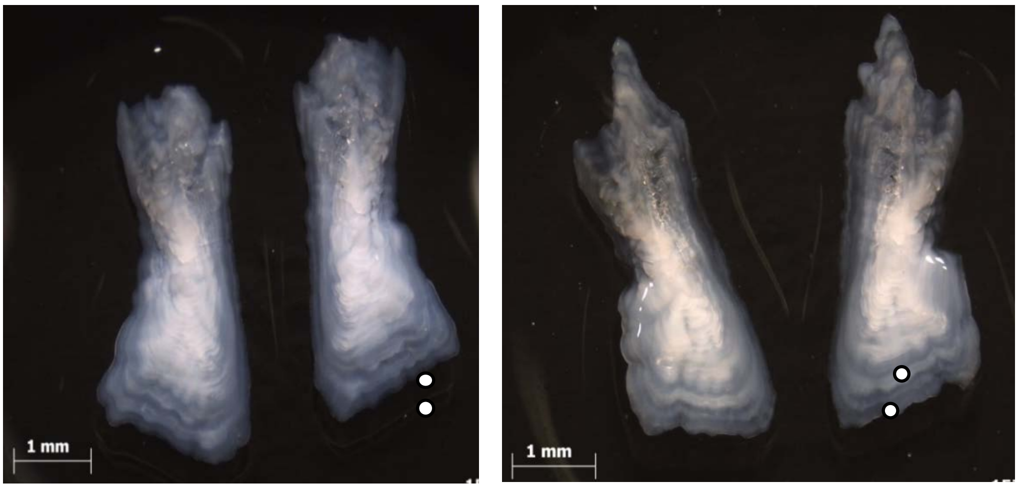


Figure 8.2.2: *S. colias* otoliths from the western Mediterranean (GSA 6), double rings are deposited in the two first annuli (white dots), (Left: Total Length=26.8 cm, Male, Catch date: May; Right: Total length=27.9 cm, Female, Catch date: July).

Otoliths of *S. colias* present a characteristic growth pattern with a large first annulus and the following annuli with a decreasing width until the third annulus (when the growth rate also decreases). But this pattern does not occur in some otoliths (Figure 8.2.3), that should be rejected. Following annuli overlap each other. In this sense, readers proposed to use mean annuli radii as a useful reference in order to allocate the first two annuli. However, it is noticeable that the mean radius of each annulus varies among areas. Therefore, it is recommended to perform studies to obtain the mean radius of the two first annuli in all areas.

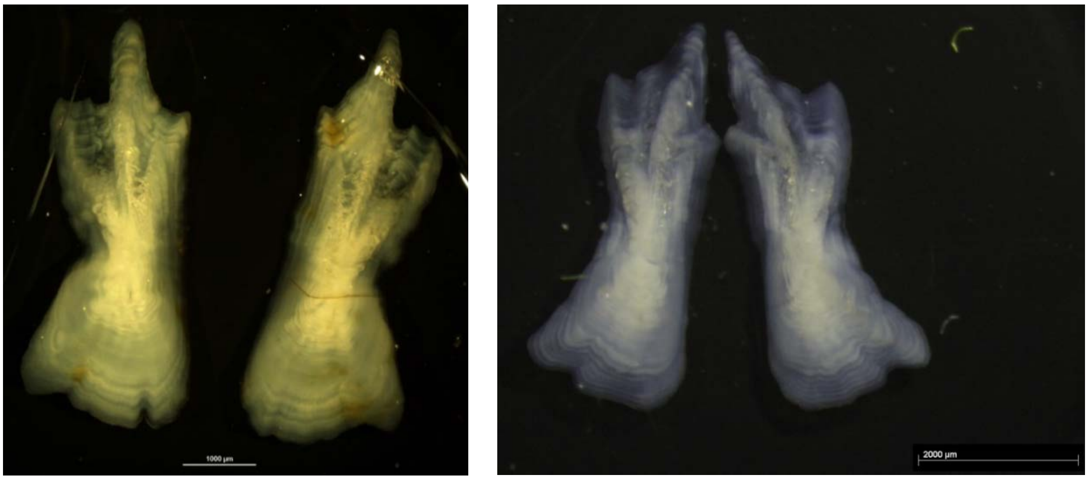


Figure 8.2.3: Otolith of *S. colias* discarded due to not expected pattern deposition. Left: Bay of Biscay (ICES Area VIIIc, Total Length= 41.1 cm, Female, Catch date: December) (1st annulus too much large, no decreasing growth pattern in the following annuli) Right: Portugal waters (ICES Area IXa, Total Length= 31.8 cm, Male, Catch date: October)

Otoliths of specimens of 4 years old onward are difficult to interpret due to the difficulty in discriminate true annuli from false rings (Figure 8.1.4). When this occurs, otoliths should be rejected.

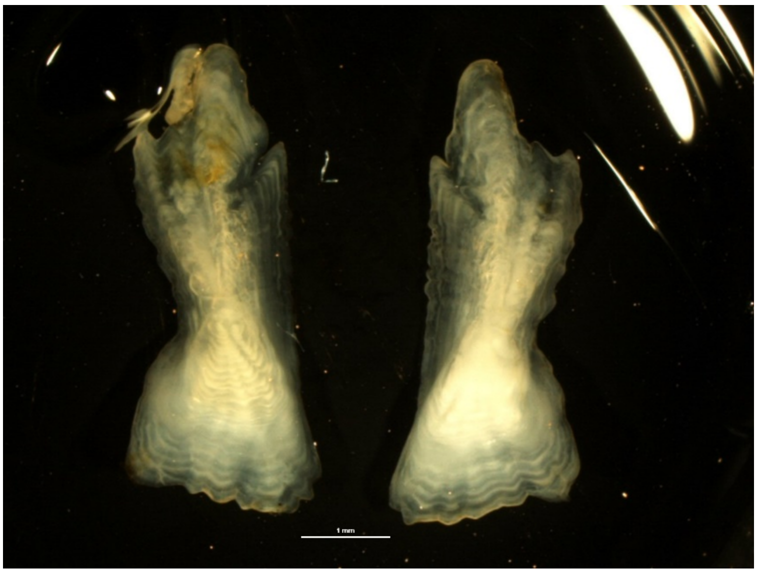


Figure 8.2.4: *S. colias* otolith rejected. False rings cannot be discriminate from true annulus (ICES Area VIIIc, Total Length= 35.9 cm, Catch date: August)

On the other hand, the overlapping of translucent rings on the margin produces confusion in the edge type identification, what is another source of disagreement among readers (Figure 8.2.5). Also, the difficulty in the edge type identification increases with the age of the otolith, which could influence the age estimation.

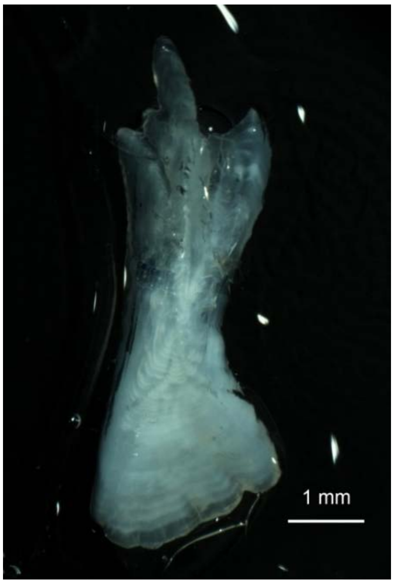


Figure 8.2.5: Otolith of *S. Colias*. This otolith should be rejected because of the edge (CECAF- MAURITANIA, Total Length= 43.5 cm, Female, Catch date: May)

8.3 Effect of different magnification factor on age determination

During the exchanges, some readers found confusing the use of different magnification in photos corresponding to the same set, mistaking true annuli as false rings in photos with less magnification. Thus, some readers underestimated the age in these large otoliths (Figure 8.3.1).

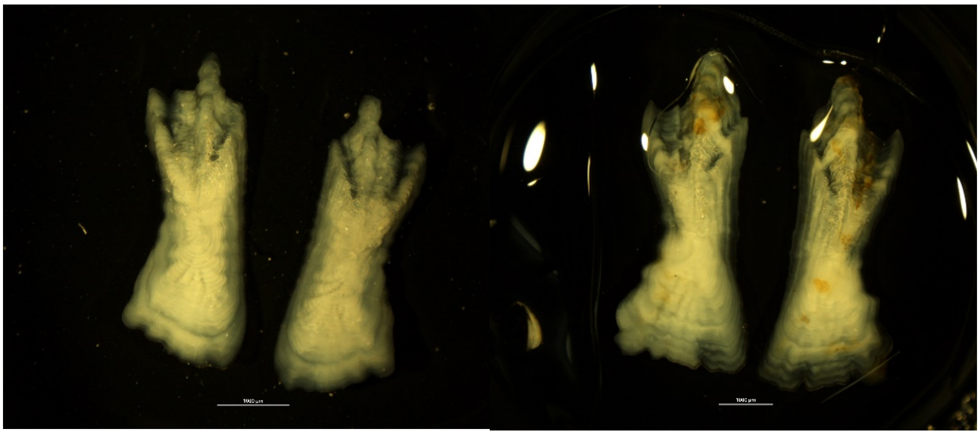


Figure 8.3.1: Otoliths of *S. colias* Left: (ICES Area VIIIc, Total Length= 25.4 cm, Male, Catch date: March; Magnification 40x; Modal Age Class: 2; Agreement: 100%) Right: (ICES Area VIIIc, Total Length=46.9 cm, Female, Catch date: June; Magnification 30x; Modal Age Class: 5; Agreement: 64%)

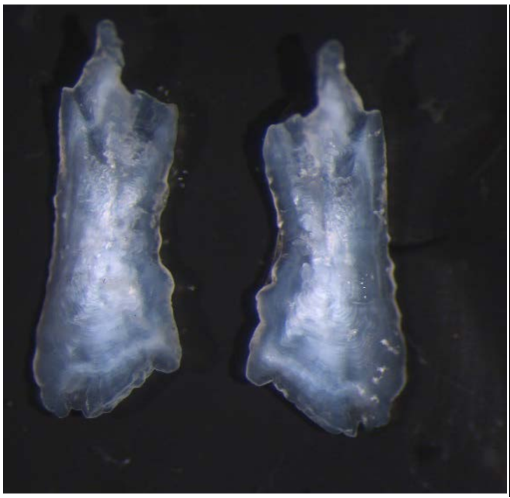
As a result, it was recommended to use the same magnification in the otoliths images of the same set in future exchanges.

8.4 Observation of otoliths fixed in resin versus otoliths immersed in seawater

During the WKARCM exercise, it was used for the first time otoliths images of two different techniques of otoliths preparation: otoliths fixed in resin (four sets of otoliths) and otoliths immersed in seawater (two sets of otoliths). Differences in the appreciation of the annuli in both kinds of otolith images were evident by the readers. Most of them found more difficult the age estimation in the otoliths immersed in seawater.

After the WKARCM exercise, it was decided to make a comparison between images of the same otolith fixed in resin and immersed in seawater, both in a black background with reflected light. One otolith from the Ligurian set (immerse in seawater) was then fixed in resin and both images were compared (Figure 8.4.1). Although there were differences in contrast and definition between both images, in this case there were not many differences in the annuli identification. This was only tested for one otolith; further analysis should be done for a large number of otoliths of all areas to be presented during the next workshop.

A)



B)

Figure 8.4.1: Same pair of otoliths of *S. colias*, a) immersed in seawater and b) fixed in resin

9 Age reading protocol for Chub Mackerel (ToR d)

1) One annulus is formed by one opaque zone and one hyaline zone (annulus), this being considered a year.

2) As a first step, the reading should be performed without knowing the fish length. The only information at the beginning of the reading should be the date of capture.

3) Priority areas: the first area for the interpretation of age should be the posterior area, followed by the edge next to the rostrum area (Figure 8.1.2).

4) Annuli width decreases with age, being more evident in the first three years of life. It should be taken into account the frequent presence of checks or false rings during the first years, which can be identified following this pattern of width decrease (checks does not follow the pattern).

5) The adopted birth date is 1st January for all Atlantic areas and Western Mediterranean areas. As a result, when a translucent ring is observed at the edge of the otolith at the first semester of the year, it is counted as an annulus. However, when a translucent ring is observed at the edge of the otolith in the second quarter of the year (April to June**), it should be carefully assessed by the reader, based on the width of this increment. It has to be determined whether this translucent ring corresponds to the finalization of the annulus of the previous year, or to the new translucent ring of the year (ICES, 2015). It can be found often opaque edge during March, April, May in otoliths of young specimens that is not counted. When a translucent ring is observed at the edge of the otolith at the second semester of the year, it should not be counted as an annulus (Figure 9.1).

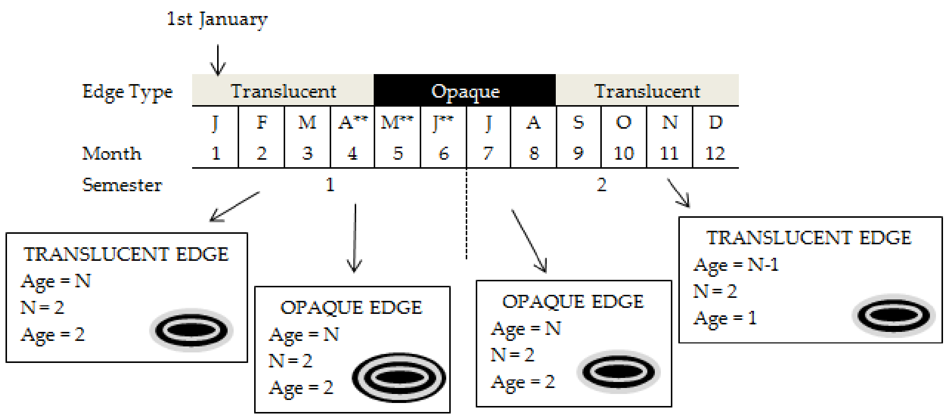


Figure 9.1: Approach of Chub Mackerel age from otoliths reading in Atlantic and Mediterranean areas. N is a number of translucent areas. Conventionally, the birth date is fixed at the 1st January as the birth date for all individuals (**) explained in the main text).

6) In the Ligurian Sea and Adriatic Sea, the adopted birth date criteria is 1st July, as occurs in Northern Aegean Sea (Cengiz, 2012), as the spawning period occurs at a similar time. In these areas, if a translucent ring is observed at the edge of the otolith at the first semester of the year, then it should not be counted as an annulus. In contrary, if a translucent ring is observed at the edge of the otolith at the second semester of the year, then it should be

counted as an annulus. It can be found opaque edge during October, November, and December in otoliths of young specimens that is not counted (Figure 9.2).

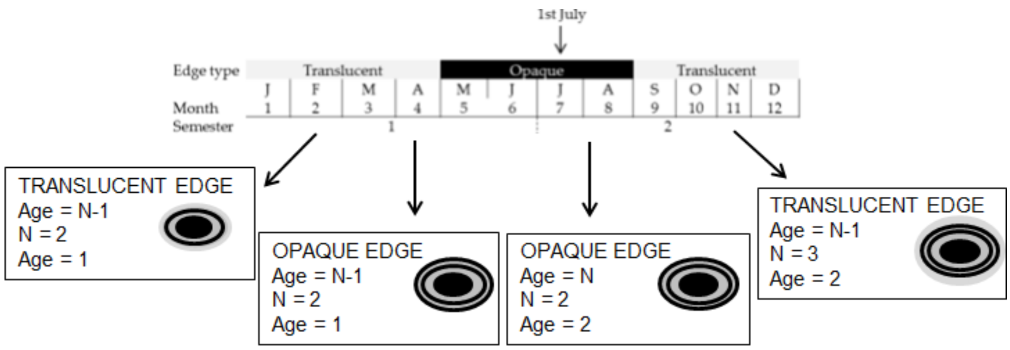


Figure 9.2: Approach of Chub Mackerel age from otoliths reading in Ligurian and Adriatic Seas. N is a number of translucent areas. Conventionally, the birth date is fixed at the 1st July as the birth date for all individuals.

7) The mean radius of the first annuli (distance between the nucleus and the first ring) for Bay of Biscay otoliths is 1.2 mm (Navarro *et al*., presentation to WKARCM 2015). These measurements are in study in the other areas.

8) Special presence of false rings or checks:

- Bay of Biscay otoliths: Common false ring in the second annulus. Common presence of various false rings from the fourth annulus above.
- Western Mediterranean otoliths: in some years, presence of false rings in the two first annuli depending on environmental conditions (especially in summer).

9) Discard otoliths in a bad state and when there is a succession of annuli, where the readers cannot be sure if they are true annuli or checks.

10) In addition to the age estimation, the quality (or credibility) of each estimation should be also assigned according to the "3 point grading system" recommended by WKNARC-1 (ICES, 2011b), where three possible results of age quality (AQ) are distinguished:

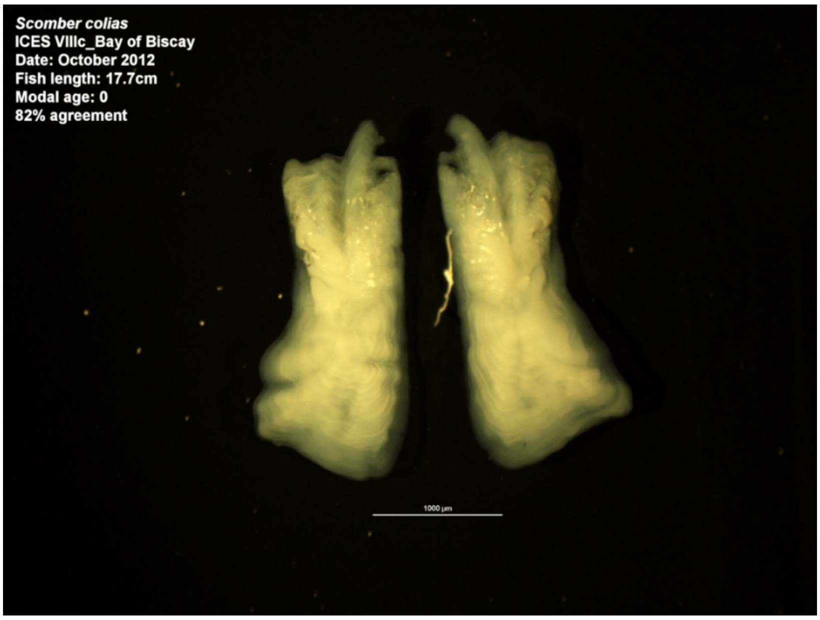
- AQ 1. Otoliths easy to age whose estimated age is assigned without any doubt at the first reading. The estimated age is considered as the final age for that individual.
- AQ 2. Otoliths difficult to age, whose estimated age is assigned with certain doubts at the first reading and are examined a second time. If doubts between the two estimations still remains, the otoliths is read a third time, and the most frequent age of the three values is assigned as the final age.
- AQ 3. Otoliths practically unreadable or very difficult to age, with doubts among three or more possible age values. These otoliths should be rejected.

10 Otolith reference collection (ToR e)

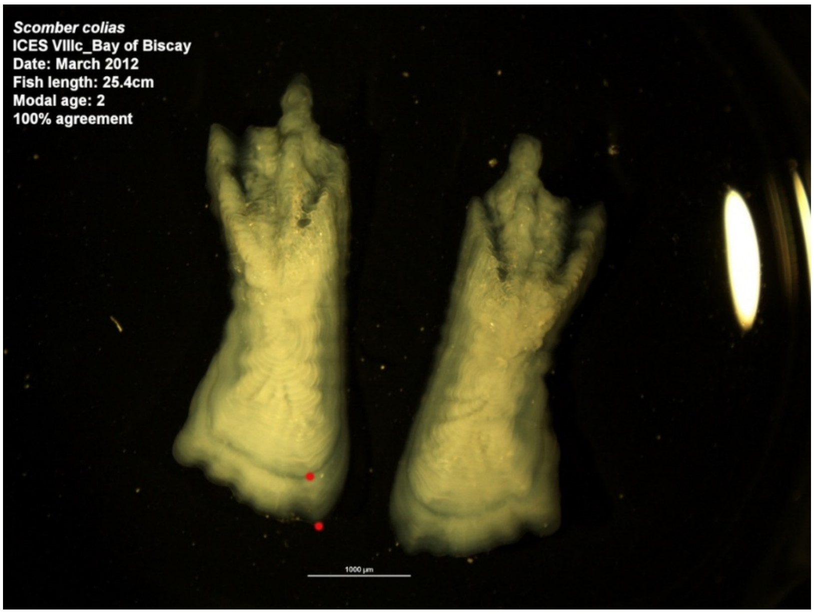
This first reference collection of chub mackerel otoliths has been elaborated by a selection of otoliths images with more than 80% agreement from the last two otolith exchanges (Small Exchange 2015 and WKARCM exercise). When possible, one otolith of each age (and semester) has been selected from each set.

ICES div. VIIIc - BAY OF BISCAY

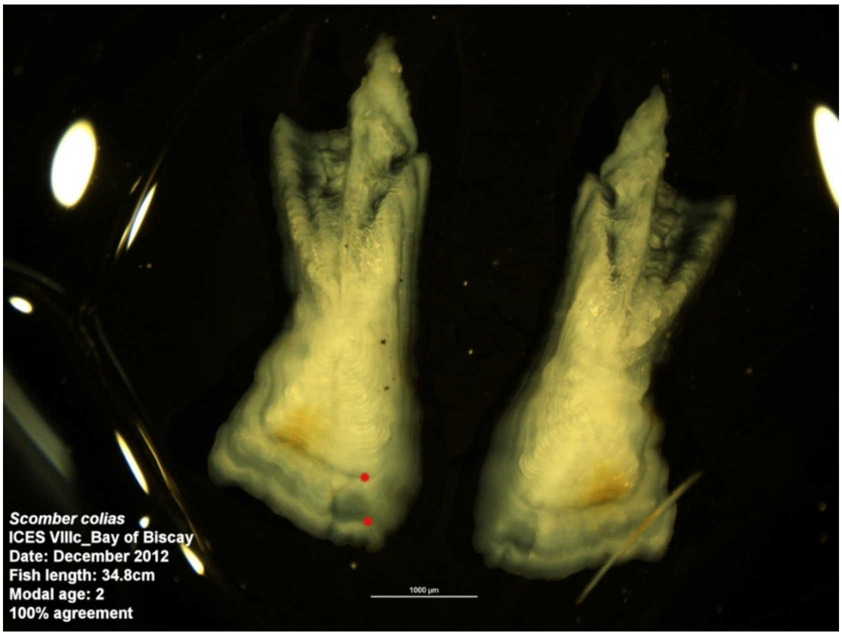
Age 0 (2nd semester)



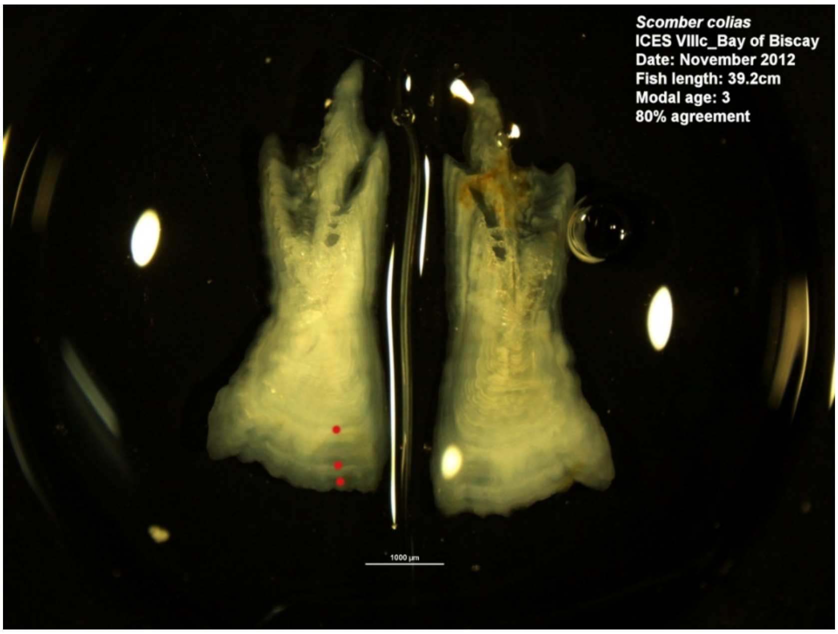
Age 2 (1st semester)



Age 2 (2nd semester)

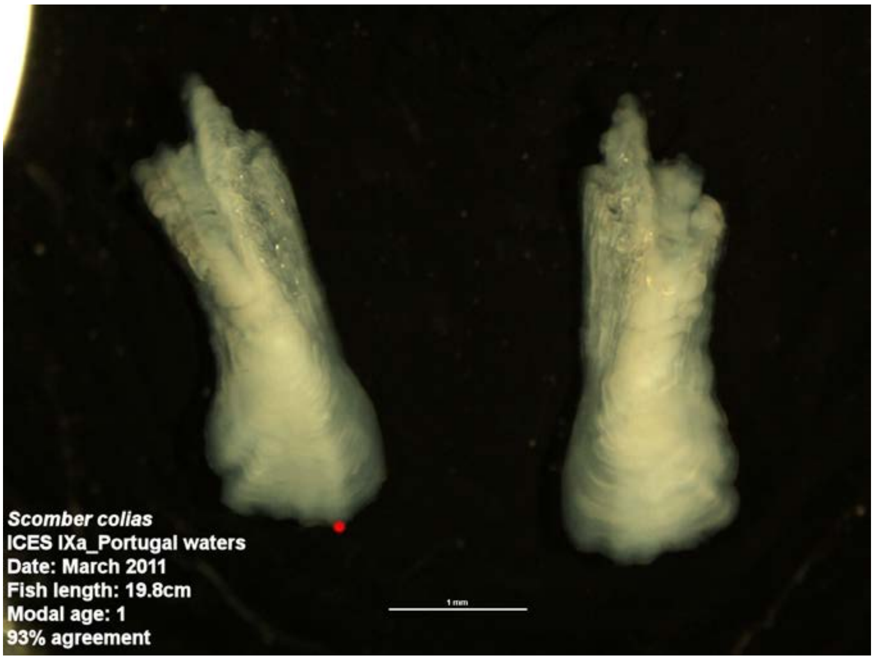


Age 3 (2nd semester)



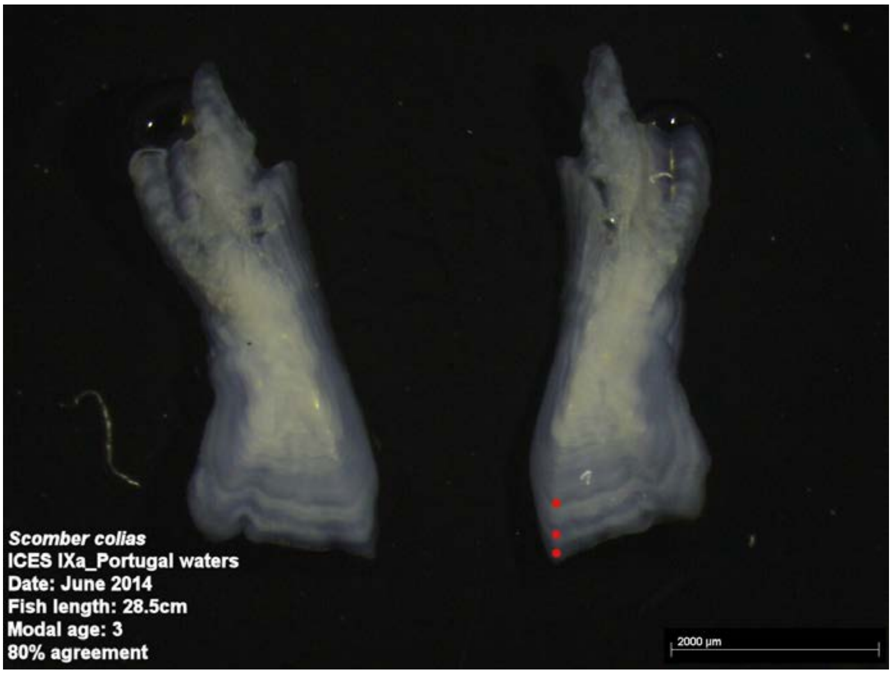
ICES div. IXa – PORTUGAL WATERS

Age 1 (1st semester)



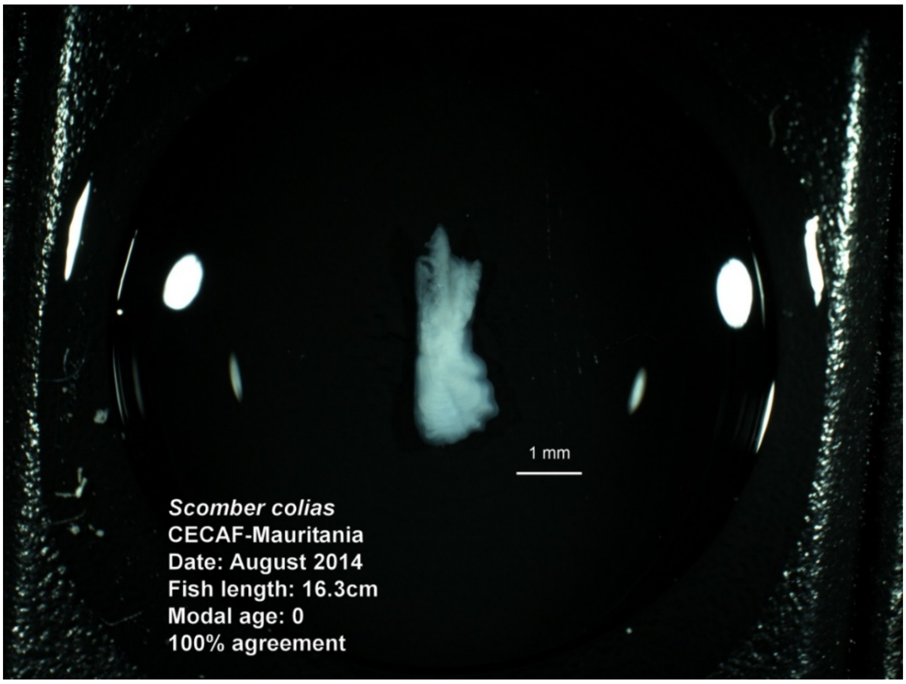
Age 2 (1st semester)

Age 3 (1st semester)

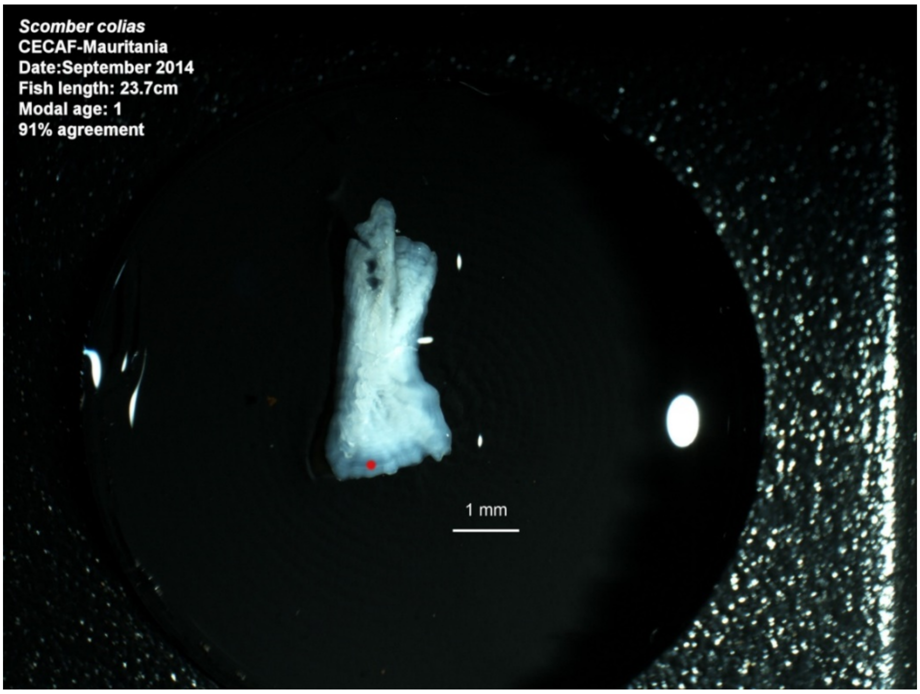


CECAF-MAURITANIA

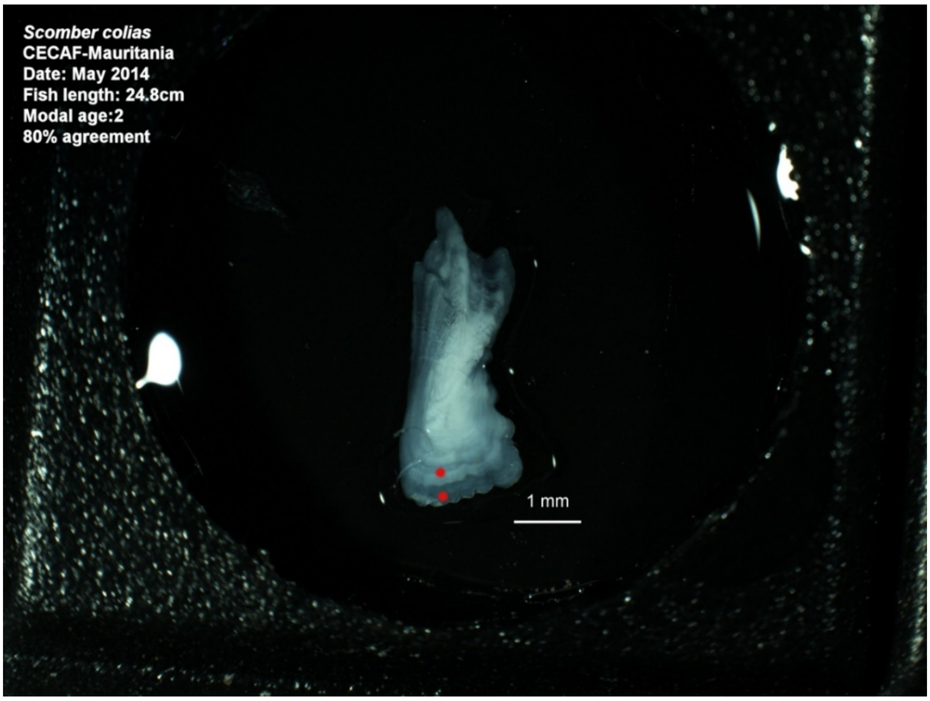
Age 0 (2nd semester)



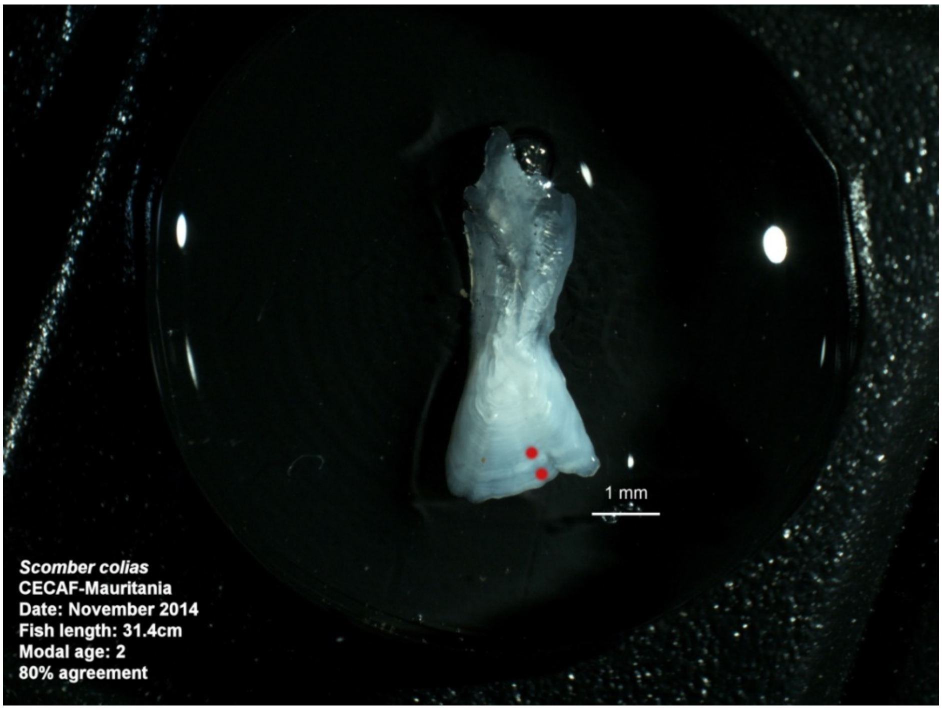
Age 1 (2nd semester)



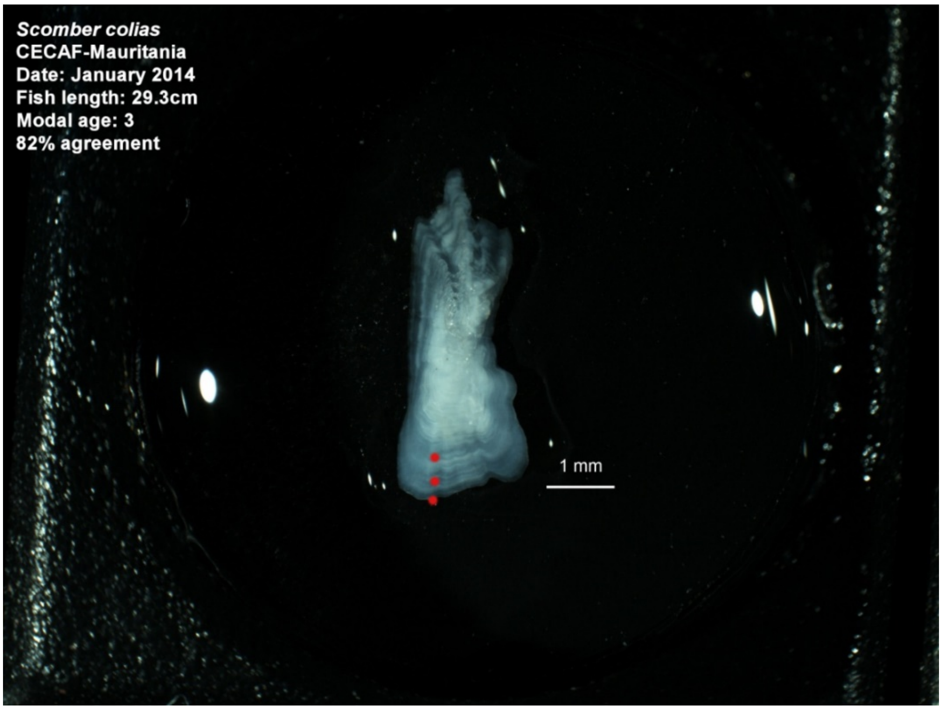
Age 2 (1st semester)



Age 2 (2nd semester)

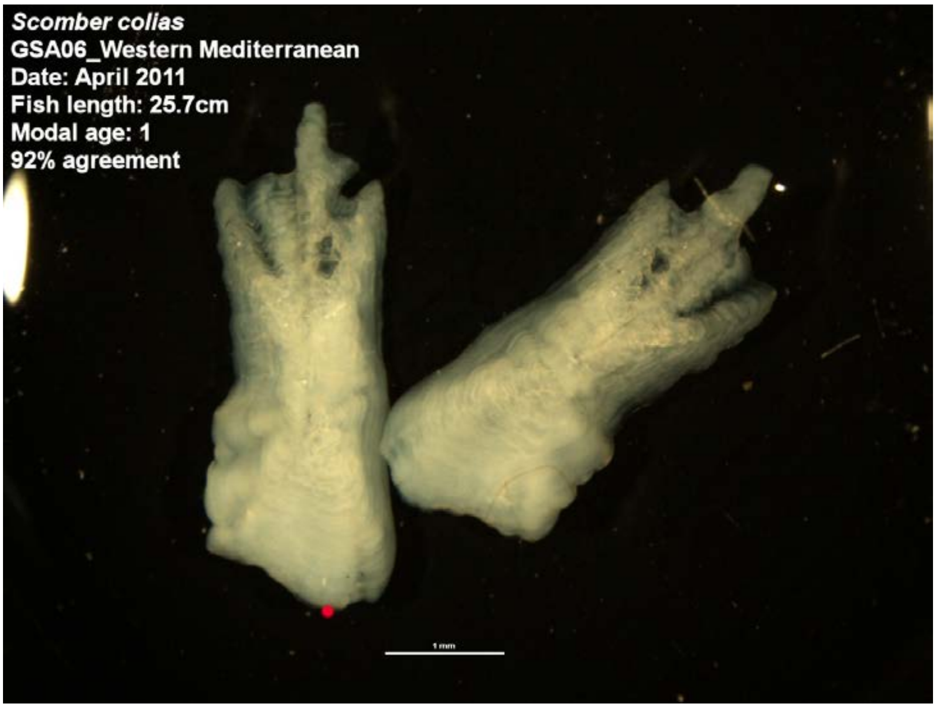


Age 3 (1st semester)

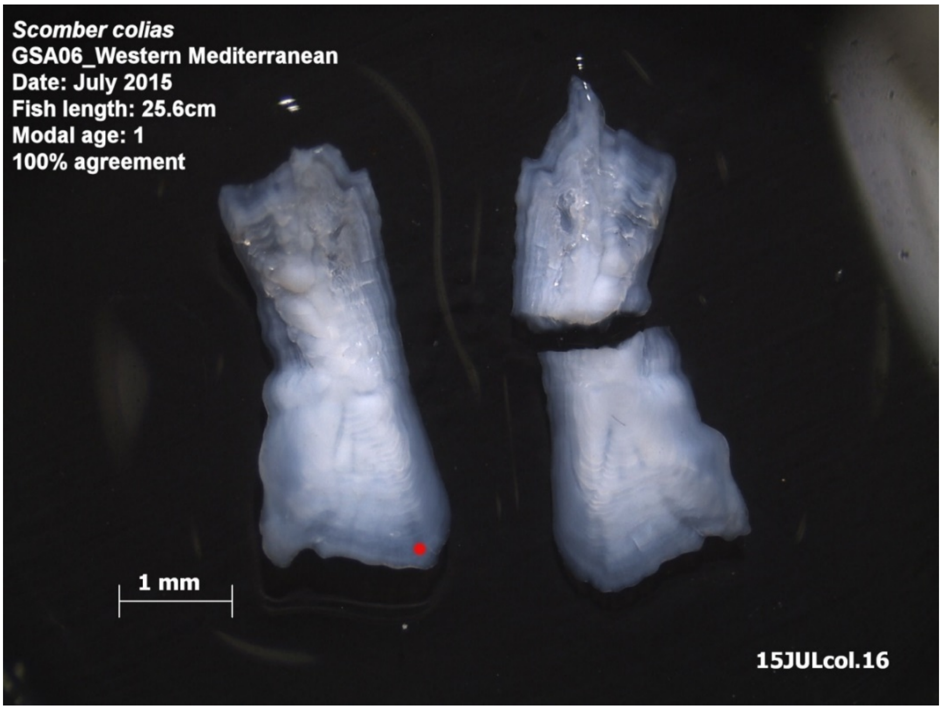


GSA06 – WESTERN MEDITERRANEAN

Age 1 (1st semester)



Age 1 (2nd semester)

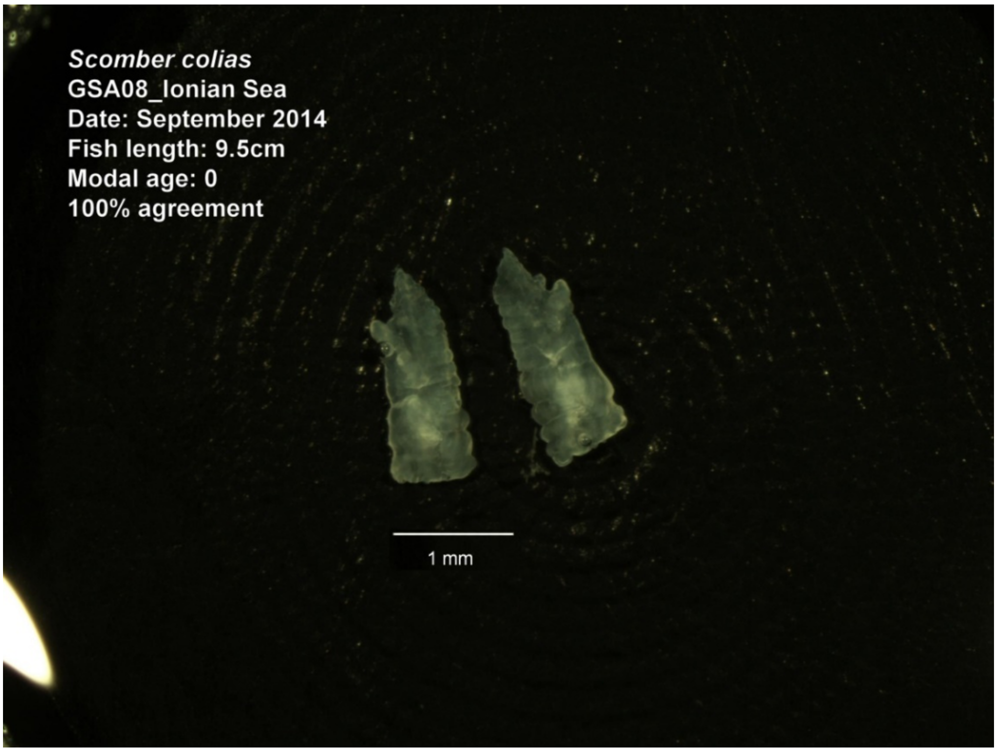


Age 2 (2nd semester)

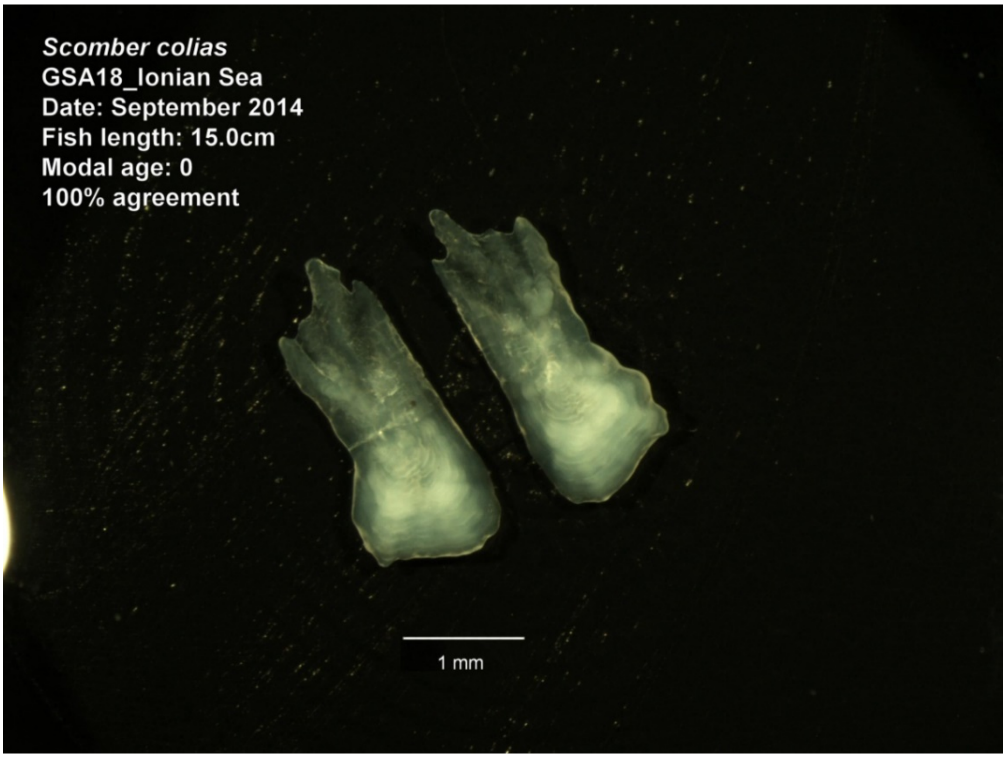
Age 3 (1st semester)

GSA18 – IONIAN SEA*

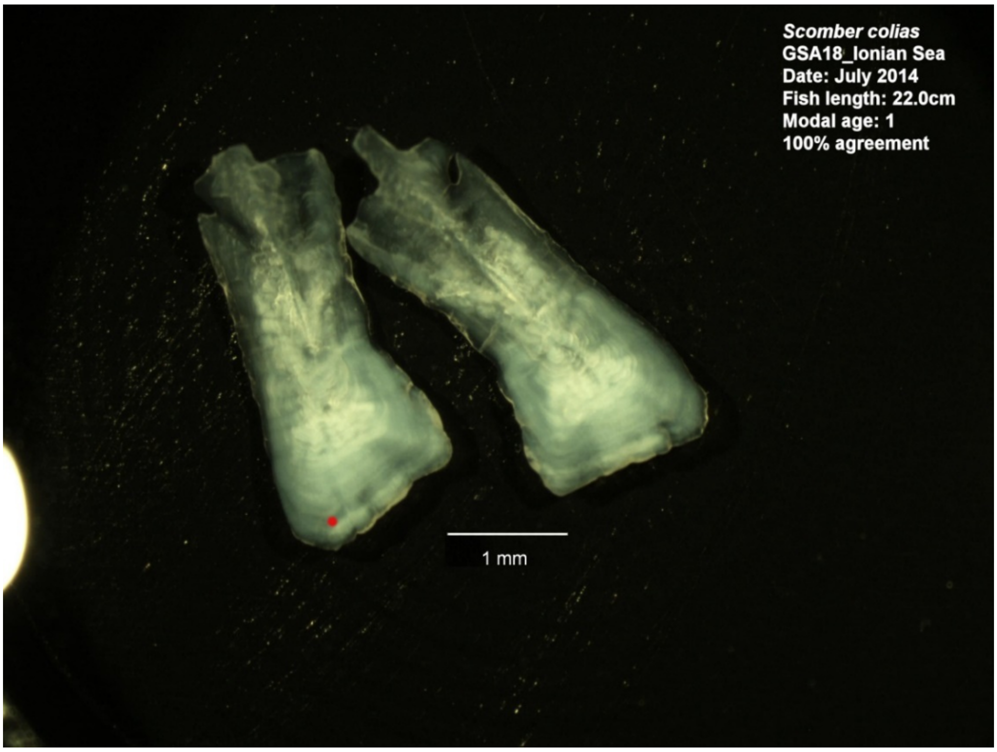
Age 0 (2nd semester)



Age 0 (2nd semester)



Age 1 (2nd semester)



*In this otolith images it was considered the date of birth of 1st January.

11 WKARCM recommendations

- It is recommended the realization of a new exchange to be carried out during the following year (2016) in order to see if the new criteria have been adopted by all readers and to see if the accuracy and precision continue to improve. A possibly date for the exchange would be from late spring to late summer of 2016.
- It is recommended to use the same magnification to obtain the images of each set of otoliths for the next and other future exchanges. The largest otolith of the set should be used as reference to choose the magnification.
- Sample selection: otoliths should be randomly selected by areas and length range and, when more than one area is studied by a laboratory, otoliths of all these areas should be included in the exchanges. It is recommended that all laboratories use the same otolith preparation method (otoliths fixed in transparent resin). Also, all images should include bar of calibration.
- It is recommended to keep using the 1st January as the date of birth for all Atlantic and Western Mediterranean areas, and 1st July for Italian areas.
- It is recommended to estimate the age of chub mackerel otoliths without knowing the fish length beforehand.
- It is recommended the realization of studies of otolith radius growth pattern in all areas to be presented during the next Workshop, in order to know the length of the first ring, growth pattern and other useful information. For the realization of these studies of otolith radius growth pattern it was proposed to use the axis that results by placing the otolith on a vertical axis over the ventral side of the otolith (Figure 5.1.1), that has been already used in the study of the Bay of Biscay area (Navarro *et al*., Presentation to WKARCM 2015).
- It was recommended the realization of a verification study of the age interpretation criteria of chub mackerel in the Western Mediterranean area to be presented in the next Workshop.

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Annex 1: WKARCM agenda

The Workshop on Age Reading of Chub Mackerel Otoliths has taken place in Lisbon (Portugal), the 2-6 November, 2015. The agenda of the meeting was the following:

	Monday, November 2nd, 2015
09.30-10.15	Opening of the meeting; presentation of the agenda and participants; local and network arrangements; brief overview of ToRs
10.15-11.15	Review information on age determination, otolith exchanges and validation techniques of this species done so far (ToR a)
11.15-11.30	*Coffee break*
11.30-13.00	Presentation and discussion of the otolith exchanges results, comparison of precision against modal age and bias; evaluation of levels of agreement among readers and institutes (ToRs a and b)
13.00-14.30	*Lunch break*
14.30-15.00	Summary of the different techniques of chub mackerel otoliths preparation by laboratory
15.00-15.45	Validation study of the age estimation of chub mackerel (Scomber colias) in the Bay of Biscay (ICES div. VIIIc); preliminary results (ToR a)
15.45-16.00	*Coffee break*
16.00-16.45	Validation study of the age estimation of chub mackerel (Scomber colias) in Portugal waters (ICES div. Ixa); preliminary results (ToR a)
16.45-18.00	Validation study of the age estimation of chub mackerel (Scomber colias) in Mauritanian waters; preliminary results (ToR a)

	Tuesday, November 3rd, 2015
09.00-11.00	Identification of problems and difficulties in age estimation of chub mackerel, including on-screen discussion of relevant otolith readings from the exchange (ToR c)
11.00-11.15	*Coffee break*
11.15-13.00	Identification of problems and difficulties in age estimation of chub mackerel, including on-screen discussion of relevant otolith readings from the exchange (ToR c)
13.00-14.30	*Lunch break*
14.30-16.00	Elaboration of age reading criteria based on the validation studies results and the discussion of relevant otolith readings from the exchange (ToR d)
16.00-16.15	*Coffee break*
16.15-18.00	Elaboration of age reading criteria based on the validation studies results and the discussion of relevant otolith readings from the exchange (ToR d)

	Wednesday, November 4th, 2015
09.00-11.00	Workshop age reading exercise via WebGR (physical otoliths available)
11.00-11.15	*Coffee break*
11.15-13.00	Workshop age reading exercise via WebGR (physical otoliths available)
13.00-14.30	*Lunch break*
14.30-16.00	Workshop age reading exercise via WebGR (physical otoliths available) / Final Report structure and assignment of responsabilities among participants
16.00-16.15	*Coffee break*
16.15-18.00	Final Report draft eleboration

	Thursday, November 5th, 2015
09.00-11.00	Presentation of the results from the Workshop age reading exercise; comparison of presision against modal age and bias; evaluation of levels of agreement among readers and institutes (ToRs a and b)
11.00-11.15	*Coffee break*
11.15-13.00	On-screen discussion of relevant otolith readings from the Workshop age reading exercise; Identification of persistent problems and difficulties in age estimation of chub mackerel otoliths (ToR c)
13.00-14.30	*Lunch break*
14.30-16.00	On-screen discussion of relevant otolith readings from the Workshop age reading exercise; Identification of persistent problems and difficulties in age estimation of chub mackerel otoliths (ToR c)
16.00-16.15	*Lunch break*
16.15-18.00	Creation of a reference collection (ToR e)

	Friday, November 6th, 2015
09.00-11.00	Creation of a reference collection (ToR e) / Recommendations based on the Workshop results
11.00-11.15	*Coffee break*
11.15-13.00	Recommendations based on the Workshop results / Planning of future activities for enhancing quality in chub mackerel age determination
13.00	*End of meeting*

Annex 2: Contributions to the Workshop. Presentations and Working Documents.

During the workshop a total of 7 presentations were performed. The list of presentations is the following:

Presentation 1: **Review information on age estimation, otolith exchanges and validation techniques of Chub mackerel (ToR a).** By: Navarro, M.R.; Silva, A.V.; Villamor, B. Instituto Español de Oceanografía (IEO). C.O. Santander. Spain. Presentation to WKARMC, Lisbon (Portugal), 2–6 November, 2015. Presented by María Rosario Navarro.

Presentation 2: **Chub mackerel (*Scomber colias, Gmelin 1798*) otolith exchange (2012-2013). Small Exchange of *Scomber colias* Otoliths from Atlantic and Mediterranean Areas (2015). (ToRs a and b).** By: Navarro, M.R.; Silva, A.V.; Villamor, B.; Silva, A. Instituto Español de Oceanografía (IEO). C.O. Santander. Spain. Presentation to WKARCM, Lisbon (Portugal), 2–6 November, 2015. Presented by María Rosario Navarro.

Presentation 3: **Summary of the different techniques of chub mackerel otoliths preparation by laboratory.** By: Silva, A.V.; Navarro, M.R.; Villamor, B.; Soares, E.; Silva, A. Instituto Português do Mar e da Atmosfera (IPMA). Portugal. Presentation to WKARCM, Lisbon (Portugal), 2–6 November, 2015. Presented by Andreia V. Silva.

Presentation 4: **Annual growth pattern and age validation trials of *Scomber colias* in the Bay of Biscay using otoliths.** By: Navarro, M.R.; Villamor, B.; Landa, J.; Hernández, C. Instituto Español de Oceanografía (IEO). C.O. Santander. Spain. Presentation to WKARCM, Lisbon (Portugal), 2–6 November, 2015. Presented by: María Rosario Navarro.

Presentation 5: **Validation study of the age estimation of chub mackerel (*Scomber colias*) in Portugal waters, preliminary results. ToR a.** By: Porfírio, A.C.; Silva, A.V.; Soares,E.; Silva, A. Instituto Português do Mar e da Atmosfera (IPMA). Portugal. Presentation to WKARCM, Lisbon (Portugal), 2-6 November, 2015. Presented by Andreia V. Silva.

Presentation 6: **Ageing criteria validation of *Scomber colias* Gmelin, 1789 from NW Africa.** By: Jurado-Ruzafa, A.; Hernández, E.; Santamaría, M.T.G. Instituto Español de Oceanografía (IEO), C.O. Canarias. Spain. Presentation to WKARCM, Lisbon (Portugal), 2-6 November, 2015. Presented by Alba Jurado-Ruzafa.

Presentation 7: **Problem identification in ageing of otoliths of chub mackerel (*Scomber colias*) ToR c.** By: Silva, A.V.; Navarro, M.R.; Villamor, V.; Soares, E.; Silva, A. Instituto Português do Mar e da Atmosfera (IPMA). Portugal. Presentation to WKARCM, Lisbon (Portugal). 2-6 November, 2015. Presented by: Andreia V. Silva.

Also, a Working Document was presented:

Navarro, M.R.; Silva, A.V.; Villamor,, B.; Silva, A.; Soares, E. 2015. Report of the Small Exchange of *Scomber colias* Otoliths from Atlantic and Mediterranean Areas (March – June 2015). Working Document to the WKARCM, Lisbon (Portugal), 2–6 November, 2015.

Annex 3: Results of WKARCM otolith exchange

All readers

Stratum	Fish no	Fish length	Landin month	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	MODAL age	Percent agreem	Precision CV
VIIIc	1	38.4	1	3	3	2	2	3	3	6	3	3	3	-	3	70%	36%
VIIIc	2	30.9	1	2	2	2	2	2	2	3	2	2	2	-	2	90%	15%
VIIIc	3	39.0	2	4	6	-	-	5	4	5	5	5	4	5	5	56%	14%
VIIIc	4	37.9	2	3	3	2	3	3	3	5	3	3	4	2	3	64%	27%
VIIIc	5	25.4	3	2	2	2	2	2	2	2	2	2	2	-	2	100%	0%
VIIIc	6	30.3	3	2	3	2	2	3	2	3	3	2	3	-	2	50%	21%
VIIIc	7	36.6	4	3	3	2	2	3	3	4	3	3	3	-	3	70%	20%
VIIIc	8	30.8	4	2	2	2	2	3	2	4	2	2	3	2	2	73%	29%
VIIIc	9	31.4	5	2	3	2	2	3	3	5	3	2	3	-	3	50%	33%
VIIIc	10	32.7	5	2	2	1	2	3	2	3	3	3	3	-	3	50%	29%
VIIIc	11	38.1	6	3	4	3	2	3	3	4	4	3	3	2	3	55%	23%
VIIIc	12	46.9	6	5	5	3	3	5	4	5	5	5	4	5	5	64%	18%
VIIIc	13	35.3	7	2	2	2	2	2	2	4	2	2	3	-	2	80%	29%
VIIIc	14	33.2	7	2	2	2	2	2	2	2	2	2	3	-	2	90%	15%
VIIIc	15	38.4	8	4	3	2	2	3	3	5	3	3	4	3	3	55%	27%
VIIIc	16	38.5	8	2	2	2	1	2	2	5	2	2	3	3	2	64%	43%
VIIIc	17	40.5	9	3	2	1	2	3	2	-	2	2	-	-	2	63%	30%
VIIIc	18	41.4	9	-	-	1	2	3	3	6	4	4	3	6	3	33%	47%
VIIIc	19	17.7	10	0	0	0	0	0	0	0	1	0	1	0	0	82%	222%
VIIIc	20	15.6	10	0	0	1	1	0	0	1	1	0	1	0	0	55%	115%
VIIIc	21	38.3	10	3	3	1	3	3	2	4	3	3	3	-	3	70%	28%
VIIIc	22	39.2	11	3	3	1	2	3	3	3	3	3	3	-	3	80%	25%
VIIIc	23	37.6	11	2	3	2	2	2	2	2	3	2	2	3	2	73%	21%
VIIIc	24	34.8	12	2	2	2	2	2	2	2	2	2	2	-	2	100%	0%
VIIIc	25	41.1	12	-	-	2	1	3	3	5	4	4	3	4	3	33%	37%
IXa	1	25.7	12	1	3	1	1	2	2	3	2	2	2	3	2	45%	39%
IXa	2	27.7	12	2	3	1	1	2	2	3	2	2	2	2	2	64%	32%
IXa	3	28.8	12	2	-	1	1	2	2	2	2	2	2	2	2	80%	23%
IXa	4	27.5	5	2	2	1	1	1	2	3	2	2	2	2	2	64%	33%
IXa	5	29.6	5	3	3	2	1	2	2	4	3	2	3	3	3	45%	32%
IXa	6	31.8	5	2	2	2	1	2	2	2	2	2	2	2	2	91%	16%
IXa	7	32.1	5	2	3	2	1	3	3	3	3	3	3	2	3	64%	27%
IXa	8	28.4	8	-	2	1	1	2	1	4	2	1	2	2	2	50%	51%
IXa	9	30.1	8	-	-	1	1	3	2	4	3	-	3	-	3	43%	47%
IXa	10	31.8	8	2	2	2	1	2	2	5	2	2	2	1	2	73%	50%
IXa	11	32.7	8	2	2	2	-	3	2	3	3	3	3	-	3	56%	21%
IXa	12	32.2	7	2	2	1	1	3	2	3	2	2	2	-	2	60%	33%
IXa	13	33.5	7	-	2	-	-	3	4	5	3	3	3	-	3	57%	29%
IXa	14	33.2	7	2	2	2	2	3	3	3	2	3	2	3	2	55%	21%
IXa	15	34.0	7	-	-	-	1	4	3	5	3	3	3	3	3	63%	36%
IXa	16	35.1	7	-	3	0	1	3	-	-	3	-	-	-	3	60%	71%
IXa	17	28.5	6	-	3	2	1	3	3	3	3	3	3	3	3	80%	25%
IXa	18	34.0	6	-	3	1	-	6	4	6	4	3	4	-	4	38%	42%
IXa	19	35.2	11	-	4	1	1	6	4	6	5	4	5	-	4	33%	47%
IXa	20	31.8	10	-	-	1	1	5	4	7	6	2	4	-	1	25%	60%
IXa	21	25.1	4	-	2	3	1	2	1	3	2	2	2	1	2	50%	39%
IXa	22	26.1	4	2	2	1	1	2	2	2	2	2	2	1	2	73%	27%
IXa	23	26.5	4	-	2	1	1	3	2	3	2	3	3	-	3	44%	38%
IXa	24	26.2	4	2	2	1	1	2	2	4	1	3	3	2	2	45%	45%
IXa	25	27.7	4	-	2	1	1	3	2	4	3	5	3	-	3	33%	50%
CECAF	1	34.3	5	4	4	2	3	3	3	3	3	3	3	2	3	64%	21%
CECAF	2	25.5	2	2	3	2	2	2	2	2	2	2	2	1	2	82%	22%
CECAF	3	24.8	5	-	3	2	2	2	3	2	2	2	2	2	2	80%	19%
CECAF	4	45.8	5	6	6	3	6	5	5	7	5	4	4	5	5	36%	22%
CECAF	5	22.2	9	0	0	1	0	0	0	1	0	0	-	0	0	80%	211%
CECAF	6	33.6	6	4	4	3	2	4	3	4	3	2	3	2	4	36%	27%
CECAF	7	23.7	9	1	1	1	1	1	1	0	1	1	1	1	1	91%	33%
CECAF	8	32.4	11	2	2	2	1	2	3	3	3	3	3	2	2	45%	29%
CECAF	9	43.2	5	5	5	2	3	4	4	5	4	2	-	4	4	40%	30%
CECAF	10	31.4	11	2	2	1	1	2	2	2	2	2	2	-	2	80%	23%
CECAF	11	42.1	1	7	7	2	4	6	5	7	6	6	5	5	7	27%	28%
CECAF	12	41.1	1	-	5	3	3	4	-	5	4	5	4	-	5	38%	20%
CECAF	13	16.5	3	1	0	-	0	1	0	0	1	0	1	0	0	60%	129%
CECAF	14	38.4	11	3	3	2	2	3	3	3	4	3	3	3	3	73%	19%
CECAF	15	23.7	9	1	1	1	1	0	1	1	1	1	1	1	1	91%	33%
CECAF	16	25.5	9	1	2	1	0	0	0	1	1	0	-	0	0	50%	117%
CECAF	17	43.4	1	6	6	3	3	5	5	5	5	5	5	5	5	64%	20%
CECAF	18	16.3	8	0	0	0	0	0	0	0	0	0	-	0	0	100%	0%
CECAF	19	43.5	5	-	6	4	2	5	5	6	4	5	4	4	4	40%	26%
CECAF	20	39.5	5	4	4	2	2	4	4	5	3	4	3	3	4	45%	27%
CECAF	21	29.3	1	3	3	3	3	3	3	4	3	3	3	2	3	82%	15%
CECAF	22	38.7	11	-	2	1	1	2	-	3	2	0	-	-	2	43%	62%
CECAF	23	26.1	5	1	1	1	1	2	2	2	2	1	2	1	1	55%	36%
CECAF	24	32.5	1	-	4	2	3	4	3	3	3	3	3	3	3	70%	18%
CECAF	25	31.6	6	-	2	1	1	3	2	4	4	1	3	-	1	33%	52%

Figure A3.1.1.a: WKARCM overall reading results (otoliths from Atlantic areas).

Stratum	Fish no	Fish length	Landin month	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	MODAL age	Percent agreem	Precision CV
GSA06	1	24.0	3	-	1	1	1	2	1	1	3	2	1	1	1	70%	50%
GSA06	2	26.6	3	-	3	3	2	3	3	3	3	3	2	2	3	70%	18%
GSA06	3	30.5	3	4	3	2	2	4	4	4	4	2	3	3	4	45%	27%
GSA06	4	30.4	4	3	3	2	3	3	5	4	3	3	3	-	3	70%	25%
GSA06	5	33.2	4	3	3	2	2	3	5	4	3	3	-	-	3	56%	30%
GSA06	6	25.3	4	-	2	0	1	1	1	2	2	1	2	1	1	50%	52%
GSA06	7	27.5	5	3	3	2	2	4	3	4	3	4	3	-	3	50%	24%
GSA06	8	26.8	5	-	2	2	2	3	3	2	2	2	2	-	2	78%	20%
GSA06	9	27.4	5	3	3	2	2	4	4	4	4	4	3	-	4	50%	25%
GSA06	10	35.3	6	-	4	2	1	3	3	3	3	3	3	-	3	67%	30%
GSA06	11	27.1	6	-	2	1	1	2	2	3	2	2	2	2	2	70%	30%
GSA06	12	24.4	6	-	2	2	1	2	2	3	2	3	-	1	2	56%	35%
GSA06	13	27.9	7	2	2	2	1	2	3	3	3	3	2	2	2	55%	28%
GSA06	14	25.6	7	1	1	1	1	1	1	1	1	1	-	-	1	100%	0%
GSA06	15	31.0	7	2	2	2	2	2	2	2	3	2	2	-	2	90%	15%
GSA06	16	29.8	8	-	2	1	1	2	2	1	1	1	-	1	1	67%	38%
GSA06	17	27.8	8	3	3	1	1	3	3	3	2	3	2	-	3	60%	35%
GSA06	18	30.3	8	2	2	2	1	2	2	4	2	3	2	-	2	70%	36%
GSA06	19	34.2	9	3	3	1	2	3	3	3	3	2	3	-	3	70%	27%
GSA06	20	28.9	9	1	1	1	1	1	2	2	1	1	-	-	1	78%	36%
GSA06	21	30.2	9	-	2	-	-	4	3	4	4	3	4	3	4	50%	22%
GSA06	22	29.7	9	2	2	1	1	3	1	3	2	2	2	-	2	50%	39%
GSA06	23	30.0	10	-	2	2	1	2	2	2	3	2	-	-	2	75%	27%
GSA06	24	30.3	10	-	1	1	1	2	2	1	2	1	-	-	1	63%	38%
GSA06	25	30.5	10	-	2	1	2	2	2	3	2	2	-	-	2	75%	27%
GSA09	1	32.5	7	-	-	0	1	3	-	1	2	-	3	-	1	33%	73%
GSA09	2	33.5	7	3	-	0	1	5	-	-	2	-	-	-	2	0%	87%
GSA09	3	33.5	7	-	2	1	2	3	3	3	-	2	-	2	2	50%	31%
GSA09	4	31.0	7	2	2	-	2	4	1	3	1	2	-	-	2	50%	47%
GSA09	5	34.0	7	2	2	2	1	3	3	3	1	2	-	-	2	44%	37%
GSA09	6	33.0	7	-	2	1	1	3	3	3	2	3	3	1	3	50%	42%
GSA09	7	33.0	7	4	-	-	2	4	4	4	2	3	3	1	4	44%	37%
GSA09	8	32.0	7	-	2	-	1	3	2	3	2	1	3	2	2	44%	37%
GSA09	9	32.0	7	-	-	0	0	3	3	-	-	2	-	-	0	40%	95%
GSA09	10	42.0	7	4	2	1	1	4	1	5	4	2	4	2	4	36%	55%
GSA09	11	29.0	7	2	1	1	1	2	1	3	1	1	3	-	1	60%	53%
GSA09	12	31.5	7	3	3	1	2	2	3	4	2	2	-	2	2	50%	35%
GSA09	13	29.5	7	3	-	1	1	3	2	4	2	2	0	2	2	40%	58%
GSA09	14	33.0	7	2	-	1	1	2	1	-	1	1	-	-	1	71%	38%
GSA09	15	34.5	7	-	-	0	-	3	-	5	3	1	3	-	3	50%	70%
GSA09	16	36.5	7	5	4	2	3	4	4	5	3	2	4	3	4	36%	29%
GSA09	17	32.0	7	3	-	-	2	3	2	4	2	2	3	-	2	50%	28%
GSA09	18	37.5	7	4	-	1	3	5	3	6	3	-	-	-	3	43%	45%
GSA09	19	13.5	4	1	-	0	0	0	1	1	1	-	0	0	0	56%	119%
GSA09	20	23.5	4	1	1	0	0	0	1	1	-	-	-	0	1	50%	107%
GSA09	21	14.0	4	1	1	0	0	0	1	1	-	-	0	0	0	56%	119%
GSA09	22	15.5	4	1	1	0	0	0	1	1	-	-	0	0	0	56%	119%
GSA09	23	15.0	4	1	1	0	0	0	1	-	-	-	-	0	0	57%	125%
GSA18	1	27.0	7	1	1	1	1	1	0	2	0	1	-	1	1	70%	63%
GSA18	2	24.0	7	1	1	1	1	0	1	2	2	2	2	-	1	50%	52%
GSA18	3	14.0	10	0	0	0	0	0	0	0	0	0	1	0	0	91%	332%
GSA18	4	10.5	9	0	0	0	0	0	0	0	0	0	-	0	0	100%	0%
GSA18	5	32.5	7	2	2	1	1	3	2	3	2	1	3	-	2	40%	41%
GSA18	6	14.5	10	0	0	0	0	0	0	0	0	0	-	0	0	100%	0%
GSA18	7	18.0	10	0	0	0	1	0	0	0	0	0	2	0	0	82%	237%
GSA18	8	9.5	9	0	0	0	0	0	0	0	0	0	0	0	0	100%	0%
GSA18	9	15.0	9	0	0	0	0	0	0	0	0	0	-	0	0	100%	0%
GSA18	10	37.5	7	-	-	0	1	4	4	5	3	2	3	-	4	25%	61%
GSA18	11	29.0	7	2	2	1	1	2	1	2	1	1	-	-	1	56%	36%
GSA18	12	38.0	8	-	4	1	2	4	3	5	3	3	3	-	3	44%	38%
GSA18	13	40.0	8	-	-	1	1	7	4	5	5	3	4	-	1	25%	55%
GSA18	14	27.0	8	2	2	1	1	2	1	2	1	1	3	1	1	55%	44%
GSA18	15	35.0	7	3	-	1	1	3	2	-	3	4	3	1	3	44%	48%
GSA18	16	20.0	7	1	1	0	1	1	0	2	2	0	2	0	1	36%	91%
GSA18	17	20.5	7	1	1	1	1	1	0	1	1	1	-	0	1	80%	53%
GSA18	18	25.5	11	2	3	1	1	2	1	3	2	2	2	1	2	45%	41%
GSA18	19	22.0	7	1	1	1	1	1	1	1	1	1	1	1	1	100%	0%
GSA18	20	17.0	11	0	0	1	1	0	0	1	1	0	1	0	0	55%	115%
GSA18	21	29.0	7	-	2	1	-	2	2	2	1	1	-	1	2	50%	36%
GSA18	22	16.0	10	0	0	0	-	0	0	0	0	0	1	0	0	90%	316%
GSA18	23	8.5	10	0	0	0	0	0	0	0	0	0	0	0	0	100%	0%
GSA18	24	31.0	7	2	2	1	1	3	3	4	3	2	3	2	2	36%	39%
GSA18	25	12.0	9	0	0	0	0	0	0	0	0	0	0	0	0	100%	0%
GSA18	26	11.5	9	0	0	0	0	0	0	0	0	0	0	0	0	100%	0%

Figure A3.1.1.b: WKARCM overall reading results (otoliths from Mediterranean areas)

Table A3.1.1: Summary of the average percentage of agreement, CV and relative bias by age for all readers.

Modal Age	Otolith No	% Agreement	CV	Bias
0	22	79%	107.7%	0.24
1	23	63%	47.5%	0.42
2	48	64%	31.0%	0.08
3	37	58%	32.7%	-0.17
4	13	40%	35.0%	-0.57
5	5	52%	19.0%	-0.32
6	-	-	-	-
7	1	27%	-	-1.55
Total	149	60.6%	45.6%	0.01

Table A3.1.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for all readers (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	**	**									
Reader 4	**	**	–								
Reader 5	–	–	**	**							
Reader 6	*	–	**	**	**						
Reader 7	**	**	**	**	**	**					
Reader 8	–	–	**	**	*	*	**				
Reader 9	*	**	**	**	**	–	**	**			
Reader 10	–	–	**	**	–	*	**	–	**		
Reader 11	**	**	**	**	**	**	**	**	–	**	
MODAL age	*	*	**	**	**	–	**	*	–	*	**

Table A3.1.3: Mean length-at-age of all readers

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	**ALL**
0	14.6	14.7	19.8	16.2	16.2	16.4	14.6	15.0	16.8	14.3	15.9	**16.2**
1	21.7	23.1	29.9	29.4	23.7	25.0	22.2	24.7	28.0	19.0	27.2	**26.7**
2	30.8	30.8	32.8	33.3	29.6	30.7	28.3	29.8	31.2	28.0	31.0	**30.7**
3	-	31.8	36.7	37.6	33.2	33.4	31.0	33.2	33.1	33.1	33.8	**33.1**
4	36.4	35.9	43.5	42.1	34.8	36.3	31.3	37.1	36.6	39.0	42.6	**35.7**
5	42.2	43.7	-	-	40.2	39.7	38.4	41.7	40.3	40.2	43.4	**40.3**
6	44.6	42.9	-	45.8	37.1	-	38.3	37.0	42.1	-	41.4	**40.3**
7	42.1	42.1	-	-	40.0	-	39.9	-	-	-	-	**40.7**

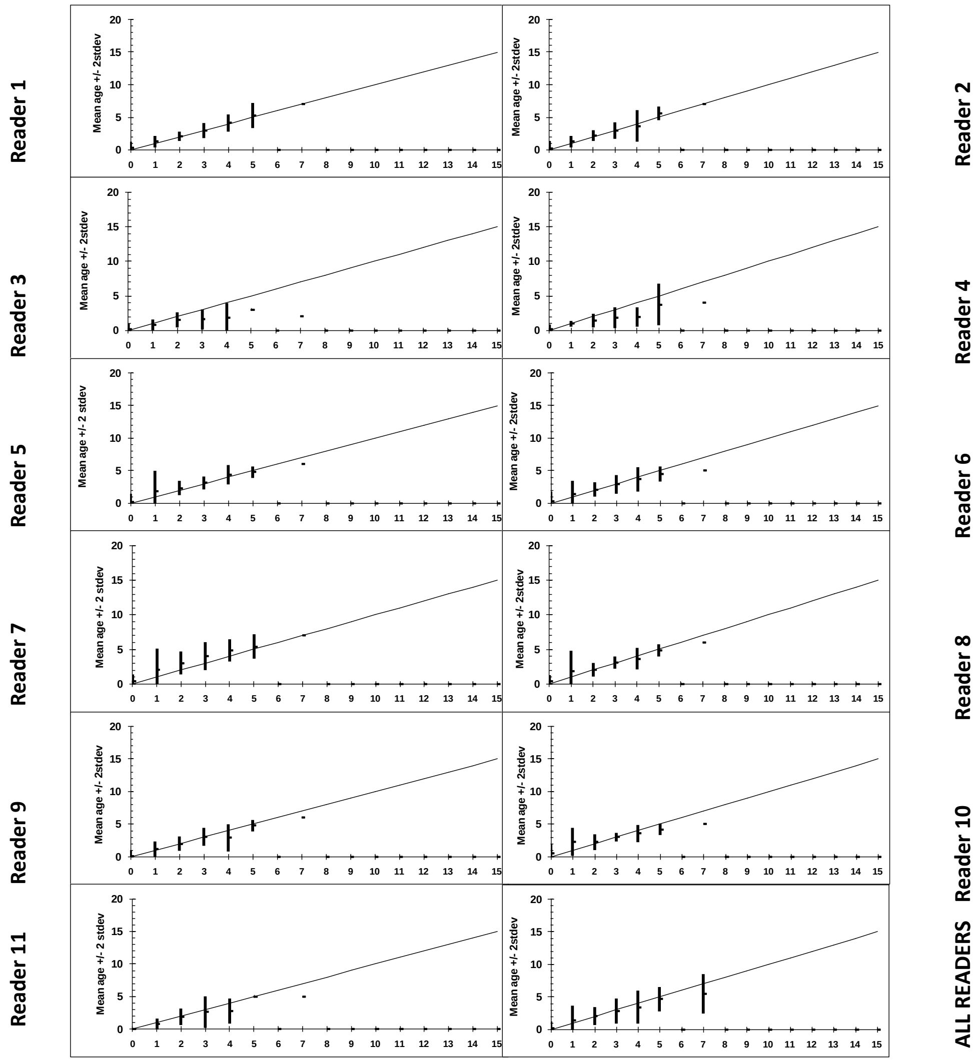

Figure A3.1.2: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (all readers).

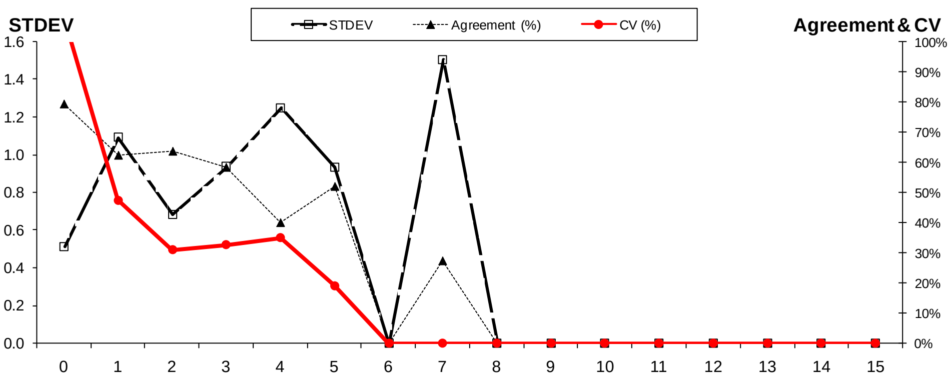

Figure A3.1.3: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for all readers combined.

Intermediate readers

Table A3.2.1: Summary of the average percentage of agreement, CV and relative bias by age for Intermediate readers.

Modal Age	Otolith No	% Agreement	CV	Bias
0	19	88%	51.4%	0.12
1	44	75%	41.4%	0.16
2	53	70%	25.3%	-0.12
3	22	56%	30.2%	-0.41
4	5	47%	36.9%	-0.67
5	3	40%	32.4%	-0.90
6	2	63%	33.5%	-1.13
7	1	50%	-	-2.00
Total	149	64.1%	39.1%	-0.11

Table A3.2.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for Intermediate readers (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4
Reader 1				
Reader 2	–			
Reader 3	**	**		
Reader 4	**	**	–	
MODAL age	**	**	**	**

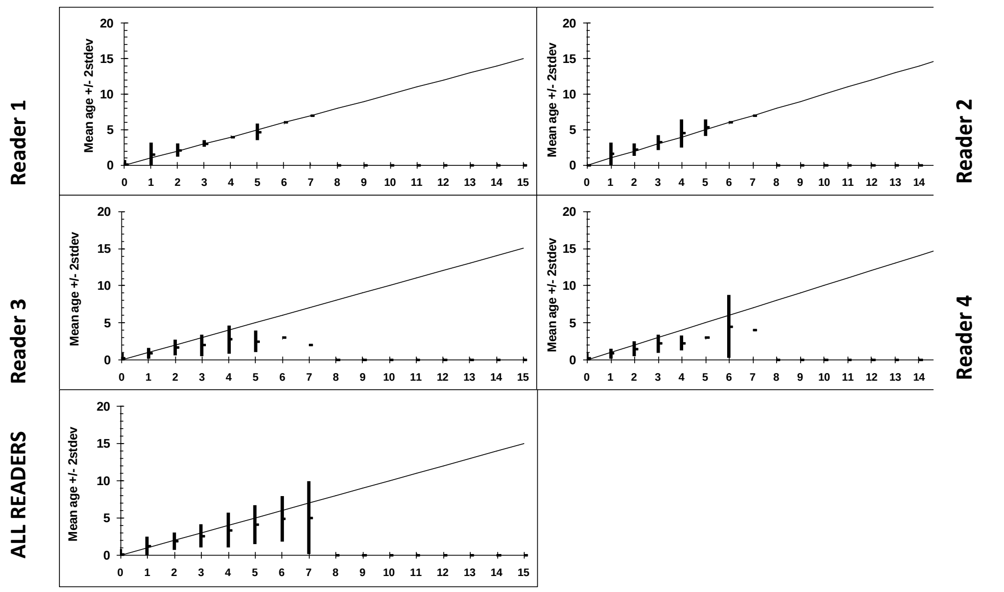

Figure A3.2.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Intermediate readers).

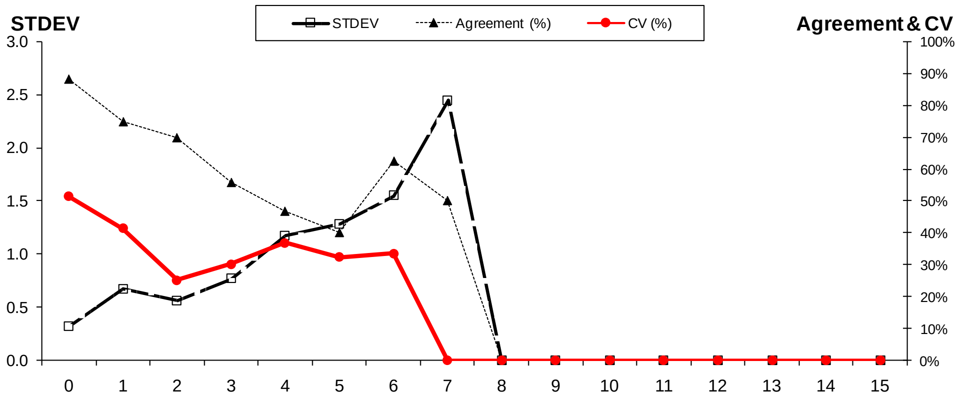

Figure A3.2.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Intermediate readers.

Trainee readers

Table A3.3.1: Summary of the average percentage of agreement, CV and relative bias by age for Trainee readers.

Modal Age	Otolith No	% Agreement	CV	Bias
0	23	78%	109.2%	0.24
1	15	68%	44.2%	0.27
2	41	71%	27.6%	0.14
3	48	67%	22.5%	0.01
4	15	49%	25.6%	-0.22
5	5	69%	11.3%	-0.17
6	2	38%	15.6%	-0.62
Total	149	67.5%	39.6%	0.07

Table A3.3.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for Trainee readers (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 5							
Reader 6	**						
Reader 7	**	**					
Reader 8	*	*	**				
Reader 9	**	–	**	**			
Reader 10	–	*	**	–	**		
Reader 11	**	**	**	**	–	**	
MODAL age	**	–	**	–	**	–	**

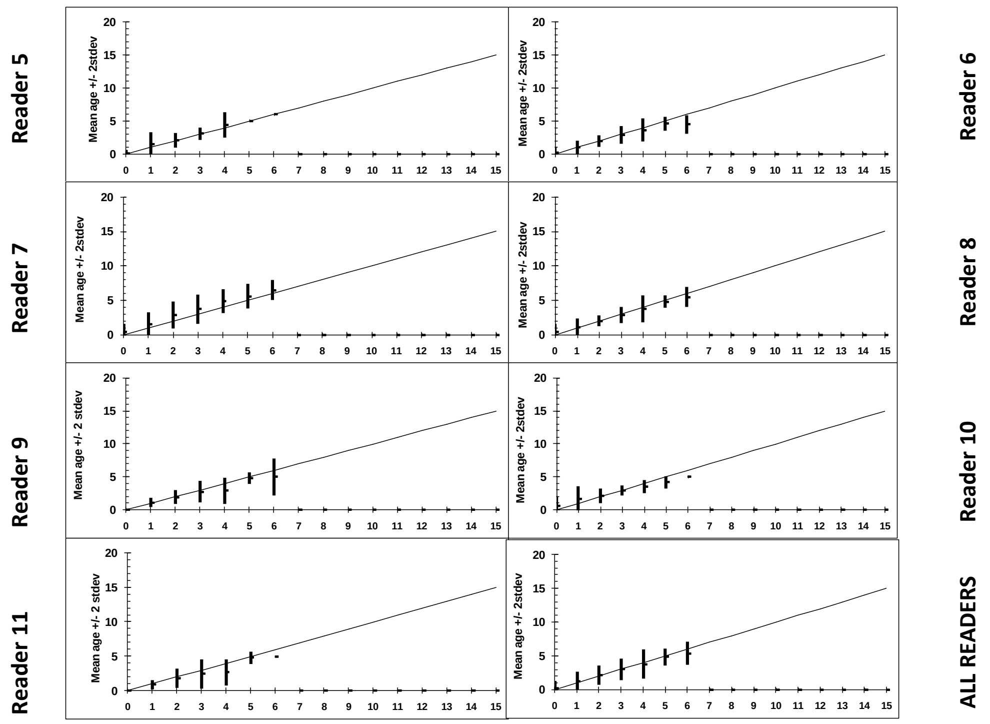

Figure A3.3.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Trainee readers).

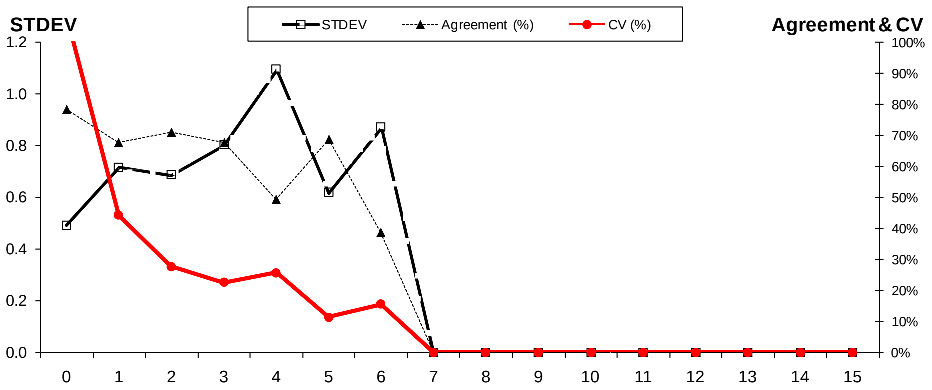

Figure A3.3.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Trainee readers.

Bay of Biscay set

Table A3.4.1: Summary of the average percentage of agreement, CV and relative bias by age for the Bay of Biscay set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	2	68%	168.7%	0.32
1	-	-	-	-
2	10	78%	20.3%	0.22
3	11	58%	30.1%	-0.02
4	-	-	-	-
5	2	60%	16.2%	-0.40
Total	25	66.7%	36.2%	0.07

Table A3.4.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the Bay of Biscay set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	*	**									
Reader 4	*	*	–								
Reader 5	–	–	**	**							
Reader 6	–	*	**	*	*						
Reader 7	**	**	**	**	**	**					
Reader 8	–	–	**	**	–	**	**				
Reader 9	–	–	**	*	–	–	**	*			
Reader 10	*	–	**	**	–	**	**	–	–		
Reader 11	–	–	–	–	–	–	*	–	–	–	
MODAL age	–	–	**	**	–	–	**	*	–	–	–

Table A3.4.3: Mean length-at-age of the Bay of Biscay set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	ALL
0	16.7	16.7	17.7	17.7	16.7	16.7	17.7	-	16.7	-	16.7	**16.9**
1	-	-	34.6	31.7	-	-	15.6	16.7	-	16.7	-	**27.5**
2	32.8	33.6	34.7	35.0	33.7	34.0	32.8	33.7	33.5	32.2	35.6	**33.9**
3	38.4	36.5	42.5	41.0	36.8	38.1	33.3	36.1	37.5	36.1	38.2	**37.1**
4	38.7	38.1	-	-	-	43.0	35.8	40.2	41.3	40.6	41.1	**39.3**
5	46.9	46.9	-	-	43.0	-	39.0	43.0	43.0	-	43.0	**41.8**
6	-	39.0	-	-	-	-	39.9	-	-	-	41.4	**40.1**

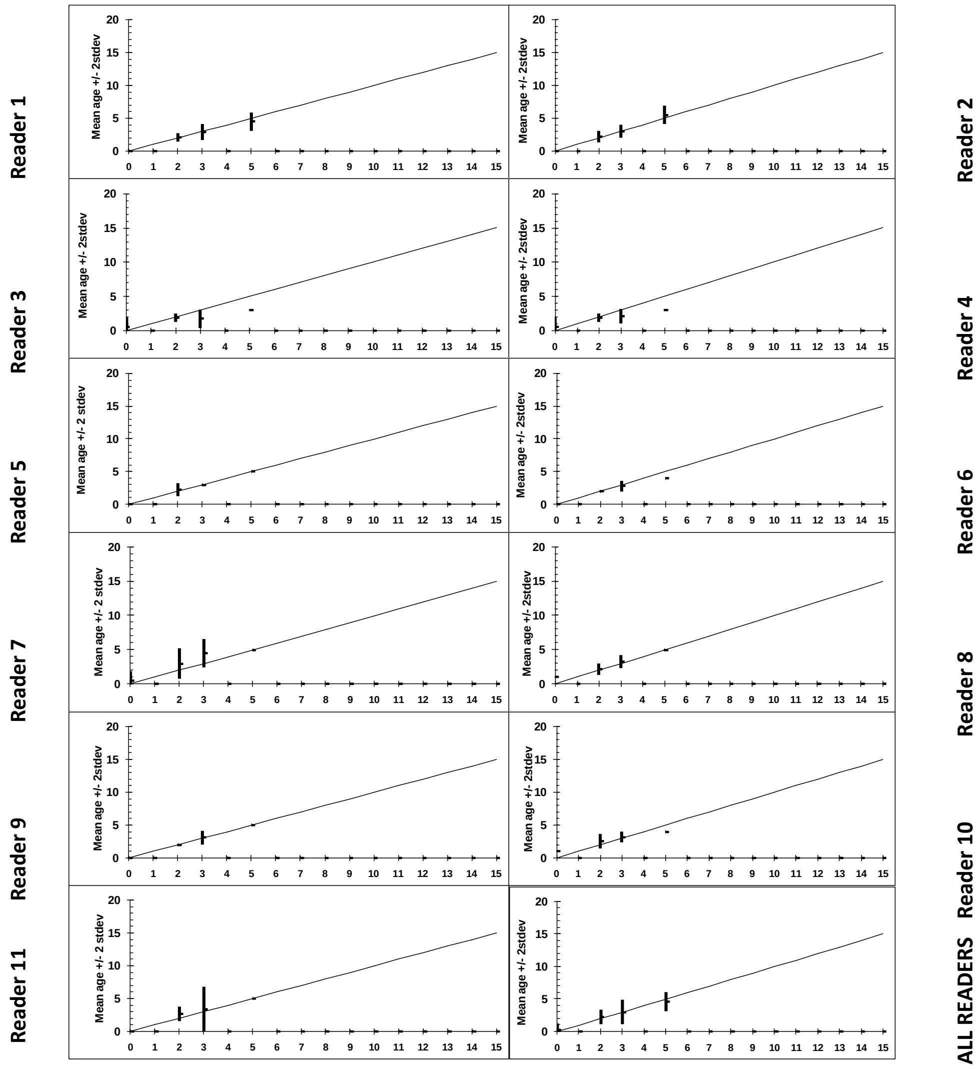

Figure A3.4.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Bay of Biscay set).

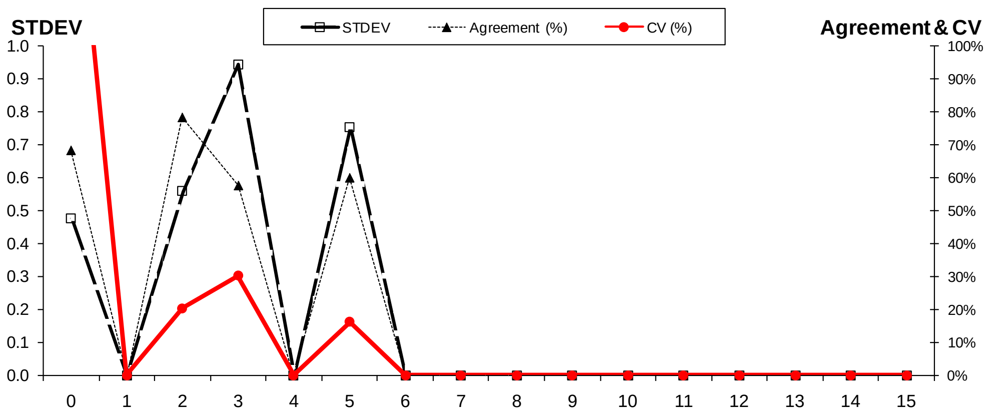

Figure A3.4.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Bay of Biscay set of otoliths.

Portugal waters set

Table A3.5.1: Summary of the average percentage of agreement, CV and relative bias by age for the North Portugal set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	-	-	-	-
1	1	-	-	-
2	12	63%	34.1%	-0.03
3	10	55%	37.4%	-0.38
4	2	-	-	-
Total	25	55.6%	37.3%	-0.15

Table A3.5.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the North Portugal set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	*	**									
Reader 4	**	**	–								
Reader 5	–	–	**	**							
Reader 6	–	–	**	**	*						
Reader 7	**	**	**	**	**	**					
Reader 8	–	–	**	**	–	–	**				
Reader 9	–	–	**	**	–	–	**	–			
Reader 10	–	–	**	**	–	–	**	–	–		
Reader 11	–	–	–	**	–	–	**	–	–	–	
MODAL age	–	–	**	**	–	–	**	–	–	–	–

Table A3.5.3: Mean length-at-age of the Portugal waters set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	ALL
0	-	-	35.1	-	-	-	-	-	-	-	-	**35.1**
1	25.7	-	29.1	29.6	27.5	26.8	-	26.2	28.4	-	27.7	**29.0**
2	30.0	29.4	31.4	33.2	28.1	28.9	28.9	28.7	28.9	28.9	28.9	**29.2**
3	29.6	30.4	25.1	-	31.2	32.0	29.1	31.5	31.2	30.1	30.2	**30.5**
4	-	35.2	-	-	34.0	33.6	28.4	34.0	35.2	32.9	-	**32.0**
5	-	-	-	-	31.8	-	33.1	35.2	27.7	35.2	-	**32.7**
6	-	-	-	-	34.6	-	34.6	31.8	-	-	-	**34.0**
7	-	-	-	-	-	-	31.8	-	-	-	-	**31.8**

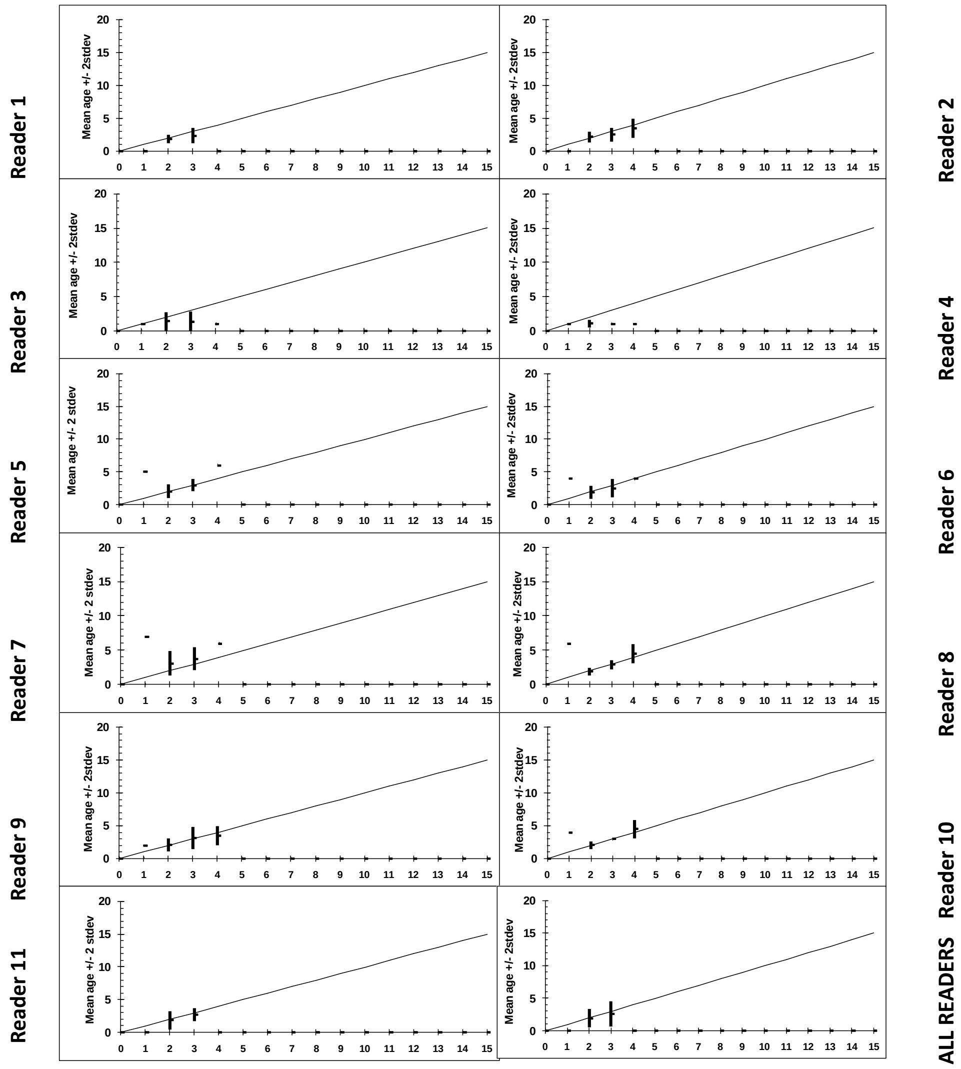

Figure A3.5.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Portugal waters set).

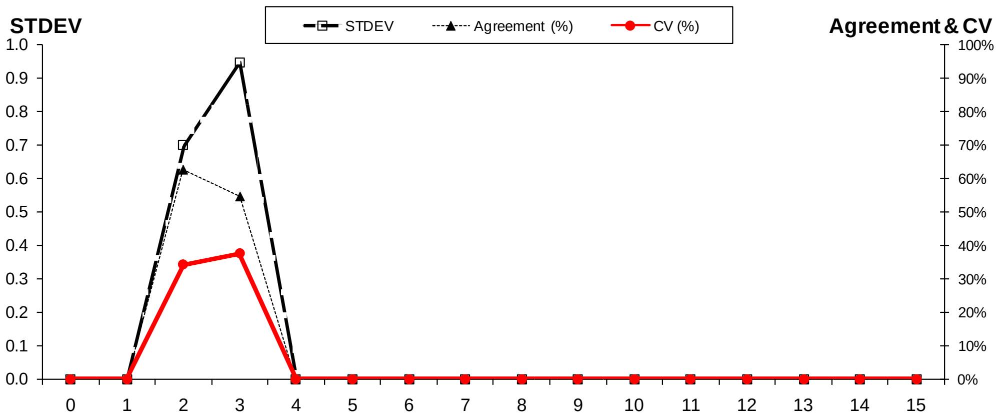

Figure A3.5.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Portugal waters set of otoliths.

Mauritanian waters set

Table A3.6.1: Summary of the average percentage of agreement, CV and relative bias by age for the Mauritanian waters set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	4	73%	114.1%	0.30
1	4	69%	38.7%	0.36
2	5	67%	31.1%	0.02
3	4	72%	18.2%	0.00
4	4	40%	27.5%	-0.31
5	3	47%	21.0%	-0.27
6	-	-	-	-
7	1	27%	-	-1.55
Total	25	60.2%	41.6%	-0.04

Table A3.6.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the Mauritanian waters set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	*	**									
Reader 4	**	**	–								
Reader 5	–	*	**	**							
Reader 6	–	*	**	**	–						
Reader 7	–	–	**	**	*	*					
Reader 8	–	–	**	**	–	–	*				
Reader 9	*	**	*	–	–	–	**	–			
Reader 10	–	–	**	*	–	–	*	–	–		
Reader 11	**	**	–	–	*	**	**	**	–	–	
MODAL age	*	**	**	**	–	–	**	–	–	–	*

Table A3.6.3: Mean length-at-age of the Mauritanian waters set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	ALL
0	19.3	18.3	16.3	20.1	21.9	20.1	18.8	19.3	23.8	-	20.1	**20.4**
1	23.1	24.5	27.9	29.7	20.1	23.7	23.8	22.4	26.3	21.3	24.8	**25.2**
2	29.8	31.9	34.7	34.2	29.8	28.7	27.0	29.3	31.7	27.0	30.9	**31.0**
3	33.9	29.5	38.6	37.3	33.4	32.2	35.3	33.6	33.4	34.0	36.8	**34.3**
4	35.8	35.0	43.5	42.1	38.0	41.4	31.5	39.6	42.7	43.5	43.4	**38.7**
5	43.2	42.2	-	-	44.2	43.7	41.8	44.6	42.7	42.8	43.8	**43.2**
6	44.6	44.2	-	45.8	42.1	-	43.5	42.1	42.1	-	-	**43.8**
7	42.1	42.1	-	-	-	-	44.0	-	-	-	-	**43.0**

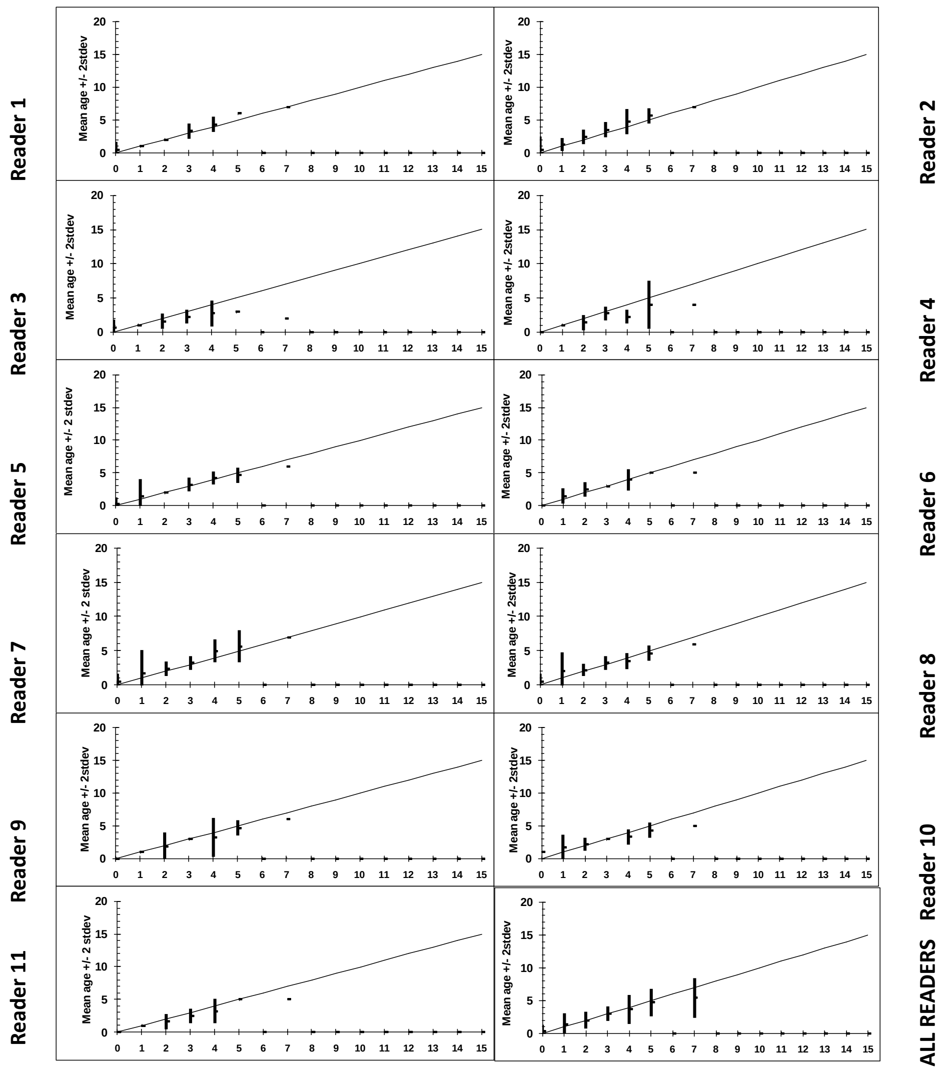

Figure A3.6.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Mauritanian waters set).

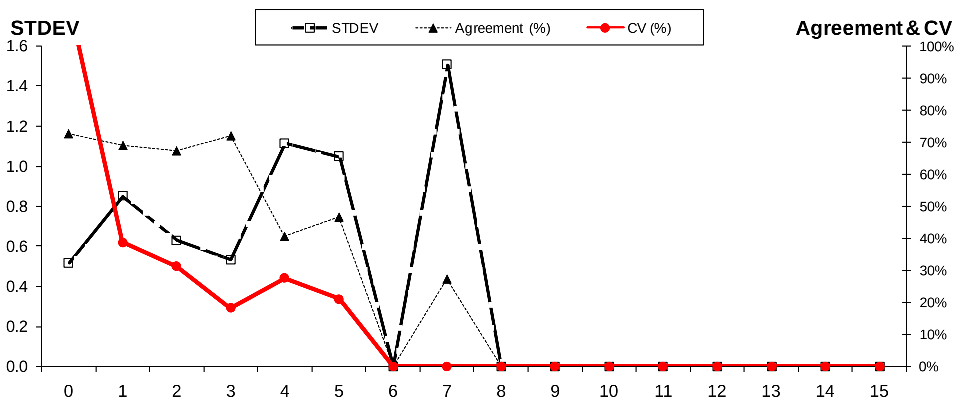

Figure A3.6.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Mauritanian waters set of otoliths.

Western Mediterranean set

Table A3.7.1: Summary of the average percentage of agreement, CV and relative bias by age for the Western Mediterranean set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	4	-	-	-
1	4	71%	35.5%	0.27
2	4	68%	28.5%	0.07
3	6	63%	26.9%	-0.16
4	4	48%	24.8%	-0.72
5	2	-	-	-
6	1	-	-	-
Total	25	65.3%	29.3%	-0.05

Table A3.7.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the Western Mediterranean set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	*	**									
Reader 4	**	**	–								
Reader 5	–	–	**	**							
Reader 6	–	–	**	**	–						
Reader 7	*	**	**	**	–	–					
Reader 8	–	–	**	**	–	–	–				
Reader 9	–	–	**	**	–	–	**	–			
Reader 10	–	–	*	**	*	–	**	*	–		
Reader 11	–	–	–	–	*	*	*	*	–	–	
MODAL age	–	–	**	**	*	–	**	–	–	–	–

Table A3.7.3: Mean length-at-age of the Western Mediterranean set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	ALL
0	-	-	25.3	-	-	-	-	-	-	-	-	**25.3**
1	27.3	27.2	28.8	28.3	26.6	26.2	27.4	28.1	28.0	24.0	25.9	**27.7**
2	29.7	28.6	29.6	29.7	28.5	29.1	28.4	28.0	29.3	28.1	27.2	**28.8**
3	30.1	29.7	26.6	30.4	30.5	29.5	29.3	30.0	29.6	30.9	30.4	**29.9**
4	30.5	35.3	-	-	28.9	29.0	29.9	29.4	27.5	30.2	-	**29.6**
5	-	-	-	-	-	31.8	-	-	-	-	-	**31.8**

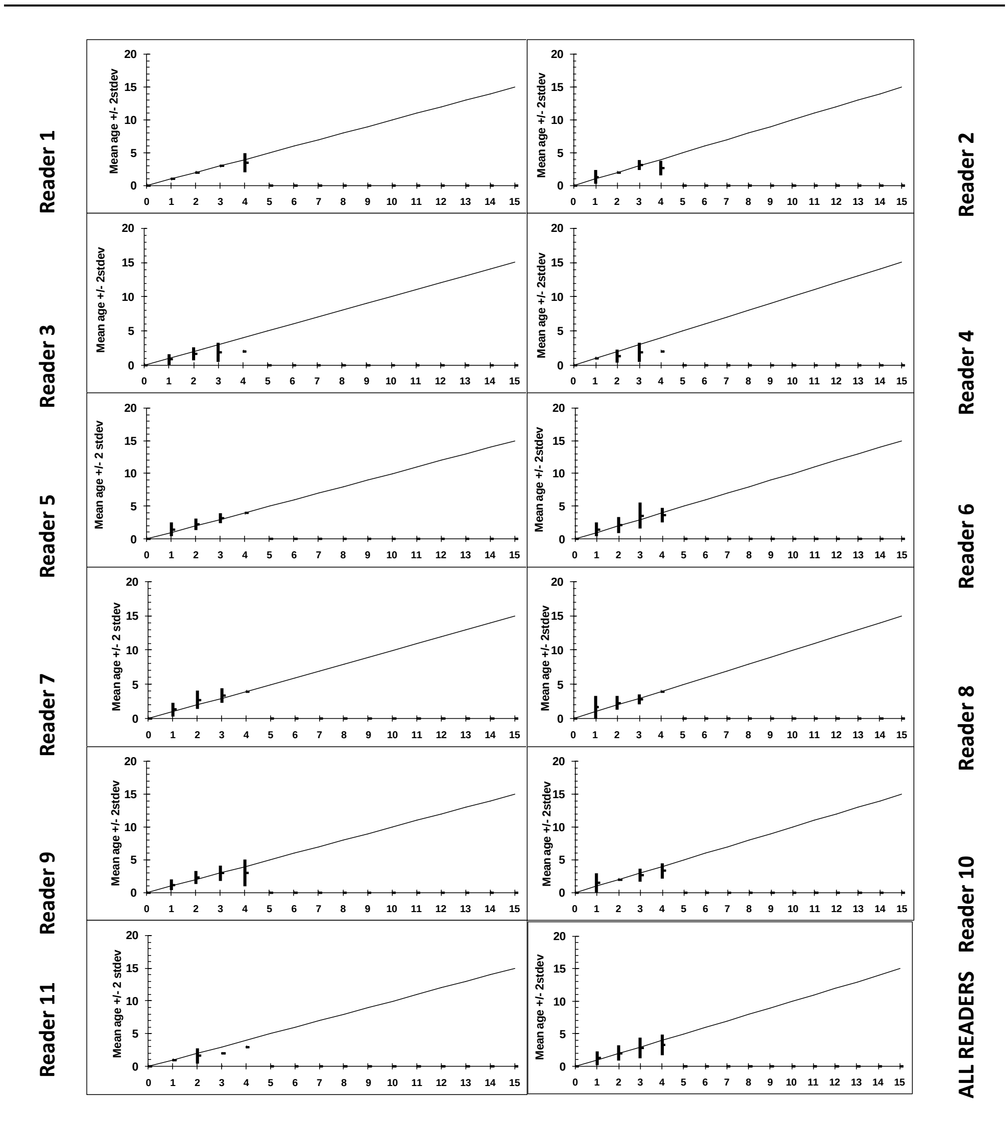

Figure A3.7.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Western Mediterranean set).

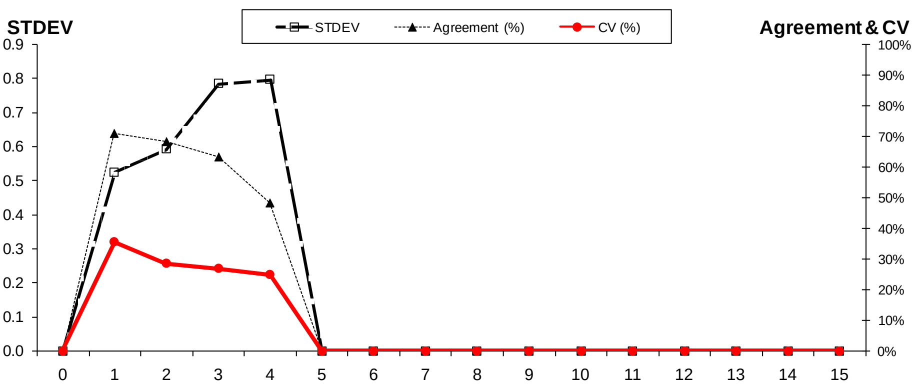

Figure A3.7.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Western Mediterranean set of otoliths.

Ligurian Sea set

Table A3.8.1: Summary of the average percentage of agreement, CV and relative bias by age for the Ligurian Sea set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	4	54%	115.1%	0.59
1	2	55%	67.6%	0.26
2	5	47%	39.0%	0.23
3	5	48%	-	-0.3
4	4	39%	40.4%	-0.9
5	2	-	-	-
6	1	-	-	-
Total	23	46.4%	64.6%	0.05

Table A3.8.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the Ligurian Sea set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2	–										
Reader 3	**	*									
Reader 4	**	*	–								
Reader 5	–	–	**	**							
Reader 6	–	–	**	**	–						
Reader 7	*	*	**	**	–	*					
Reader 8	**	–	*	–	**	–	**				
Reader 9	*	–	*	–	**	–	**	–			
Reader 10	–	–	–	–	*	–	–	–	–		
Reader 11	*	*	–	–	*	*	**	–	–	–	
MODAL age	**	–	**	*	*	–	**	–	–	–	–

Table A3.8.3: Mean length-at-age of the Ligurian Sea set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	ALL
0	-	-	23.8	18.9	16.3	-	-	-	-	18.1	16.3	**19.4**
1	16.3	19.4	33.6	33.2	-	24.1	19.8	28.1	32.1	-	33.0	**26.9**
2	31.8	34.3	35.3	32.2	31.2	31.2	-	32.1	33.6	-	33.7	**32.9**
3	31.6	31.5	-	37.0	32.6	33.6	32.1	36.2	33.0	32.3	36.5	**33.1**
4	37.5	36.5	-	-	35.6	34.8	31.5	42.0	-	39.3	-	**35.7**
5	36.5	-	-	-	35.5	-	37.7	-	-	-	-	**36.8**
6	-	-	-	-	-	-	37.5	-	-	-	-	**37.5**

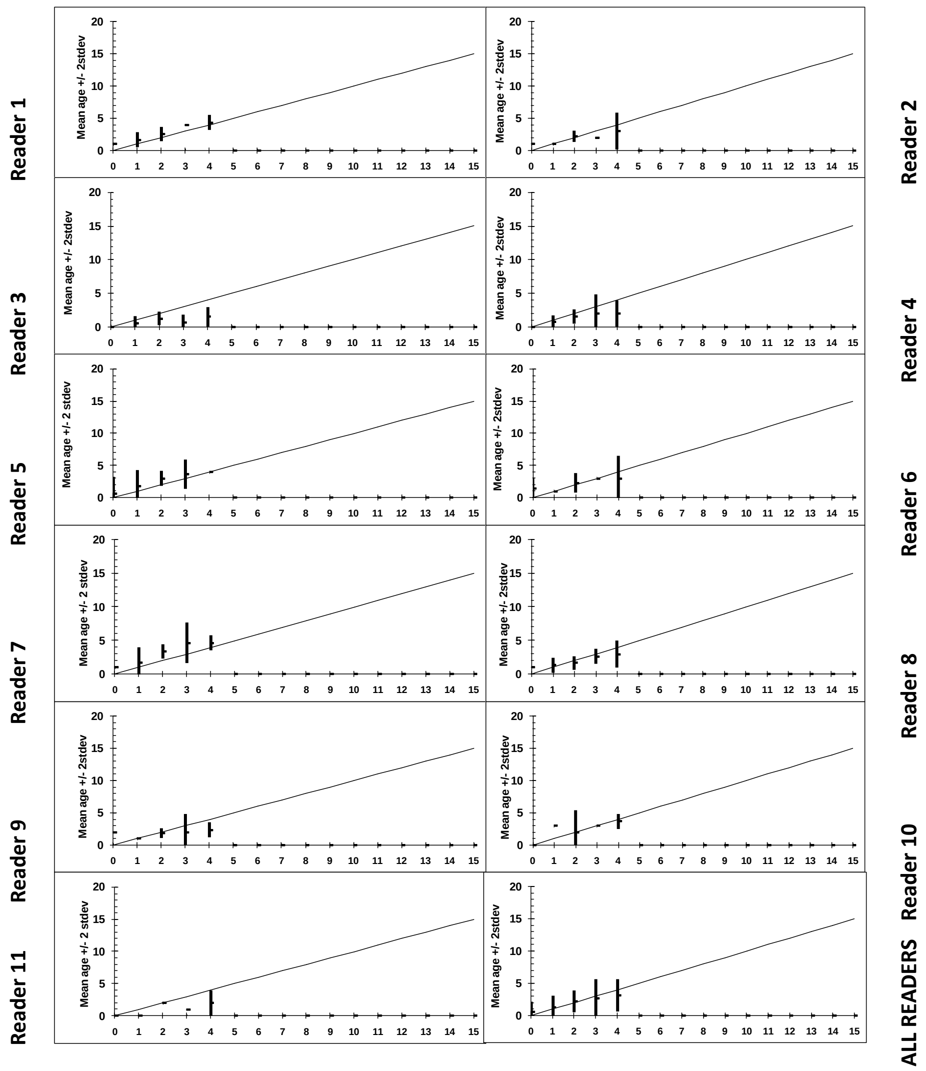

Figure A3.8.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Ligurian Sea set).

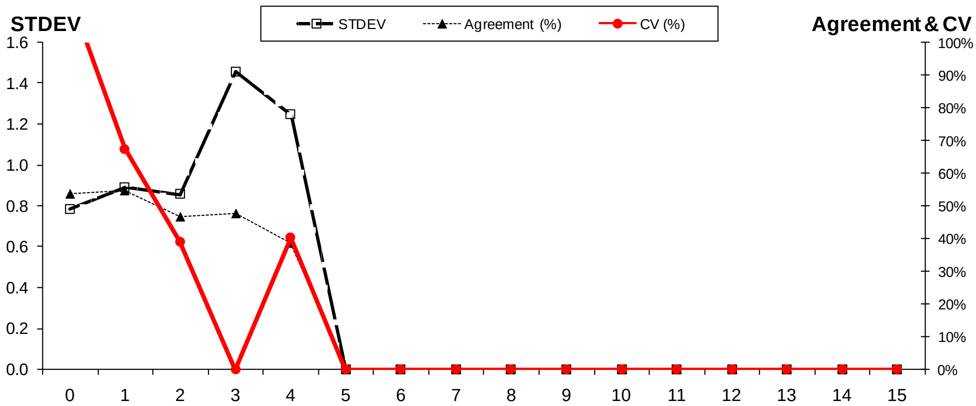

Figure A3.8.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Ligurian Sea set of otoliths.

Ionian Sea set

Table A3.9.1: Summary of the average percentage of agreement, CV and relative bias by age for the Ionian Sea set of otoliths.

Modal Age	Otolith No	% Agreement	CV	Bias
0	6	92%	90.9%	0.09
1	2	60%	49.4%	0.39
2	5	41%	42.3%	-0.10
3	6	44%	-	-0.28
4	4	-	-	-
5	2	-	-	-
6	1	-	-	-
Total	26	68.2%	65.8%	0.12

Table A3.9.2: Inter-reader bias test and reader against modal age bias test of *Scomber colias* otoliths for the Ionian Sea set of otoliths (-: no sign of bias (p>0.05); *: possibility of bias (0.01<p<0.05); **: certainty of bias (p<0.01)).

	Sp CN Reader 1	Sp AJ Reader 2	Sp EG Reader 3	Sp MV Reader 4	Pt DM Reader 5	Pt AS Reader 6	It AM Reader 7	Pt ES Reader 8	Pt DS Reader 9	Pt GC Reader 10	Pt DF Reader 11
Reader 1											
Reader 2											
Reader 3	–	–									
Reader 4	–	–	–								
Reader 5	*	–	*	–							
Reader 6	*	*	–	–	*						
Reader 7	*	*	**	*	–	**					
Reader 8	–	–	*	–	–	–	*				
Reader 9	–	–	–	–	–	–	**	–			
Reader 10	*	–	**	**	–	*	–	–	*		
Reader 11	–	–	–	–	*	–	*	–	–	*	
MODAL age	–	–	–	–	–	–	**	–	–	*	–

Table A3.9.3: Mean length-at-age of the Ionian Sea set of otoliths.

Age	Sp CN R1	Sp AJ R2	Sp EG R3	Sp MV R4	Pt DM R5	Pt AS R6	It AM R7	Pt ES R8	Pt DS R9	Pt GC R10	Pt DF R11	**ALL**
0	13.3	13.3	15.6	11.9	14.2	15.3	13.0	14.2	13.9	10.4	14.4	**14.1**
1	22.7	22.7	28.4	27.1	22.4	25.5	19.8	24.1	26.7	17.3	27.6	**25.3**
2	29.0	29.7	-	38.0	27.6	32.2	26.0	25.5	29.5	21.9	31.0	**27.9**
3	35.0	25.5	-	-	32.8	34.5	29.0	35.4	39.0	33.5	-	**33.6**
4	-	38.0	-	-	37.8	38.8	31.0	-	35.0	40.0	-	**37.1**
5	-	-	-	-	-	-	38.5	40.0	-	-	-	**38.9**
6	-	-	-	-	-	-	-	-	-	-	-	**-**
7	-	-	-	-	40.0	-	-	-	-	-	-	**40.0**

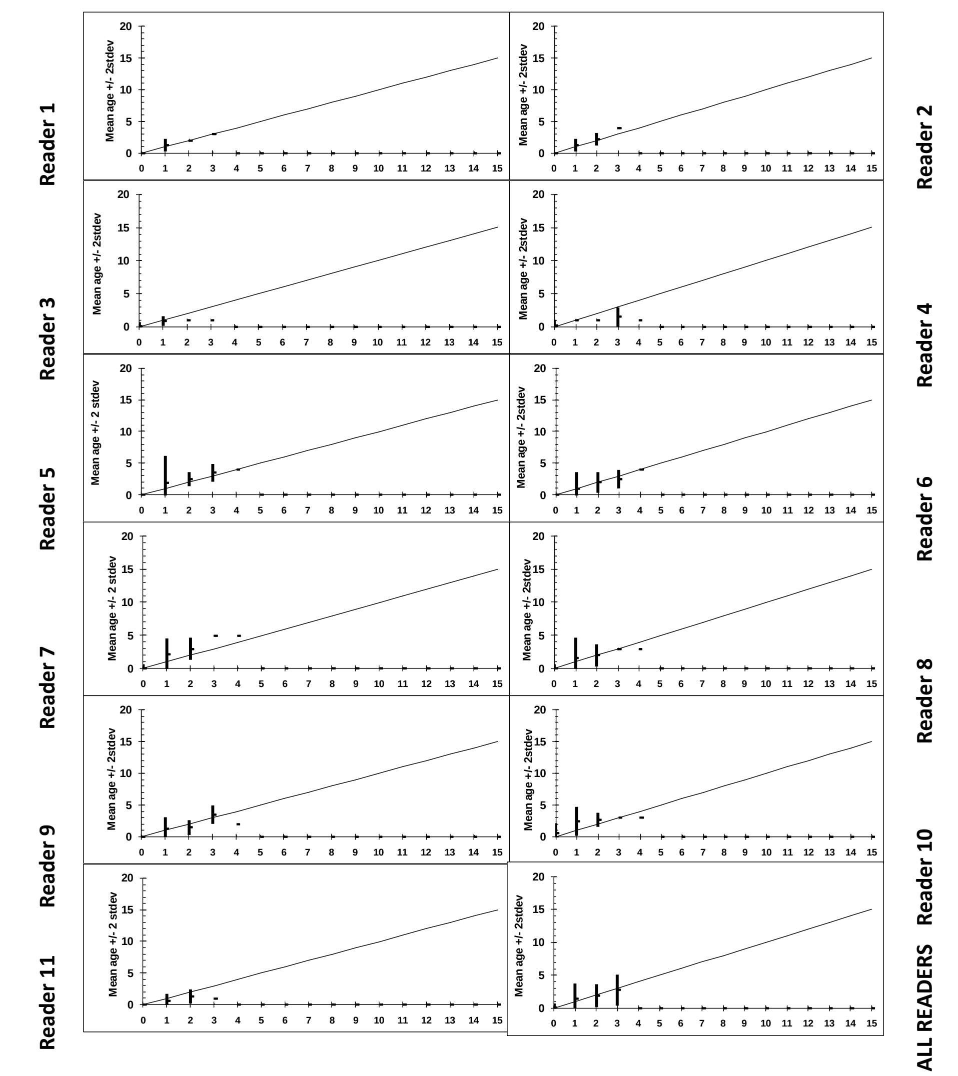

Figure A3.9.1: Age bias plots with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Ionian Sea set).

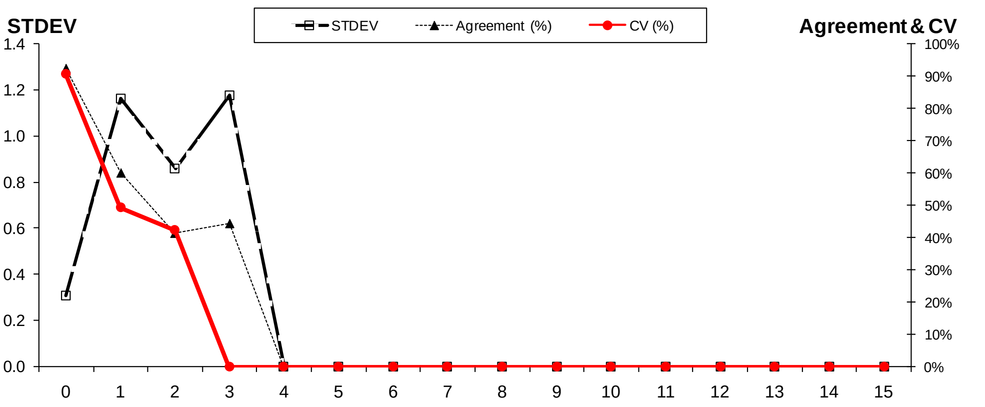

Figure A3.9.2: Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age for Ionian Sea set of otoliths.

Growth Patterns

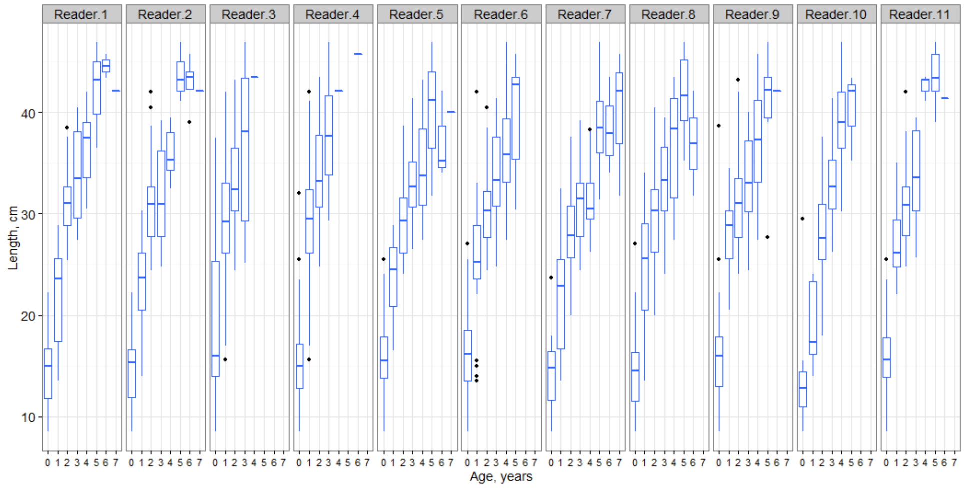

Figure A3.10.1: Chub Mackerel growth patterns by reader.

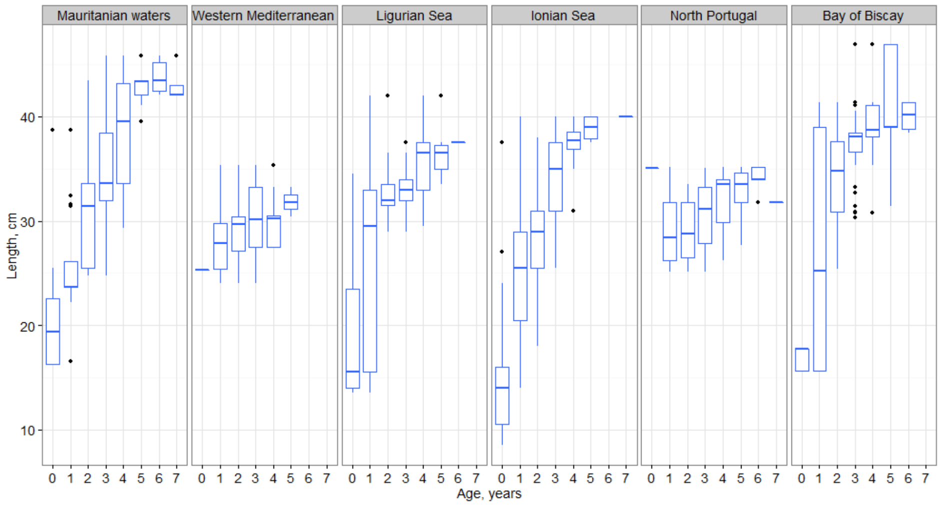

Figure A3.10.2: Chub Mackerel growth patterns by area given by the average age of all readers.

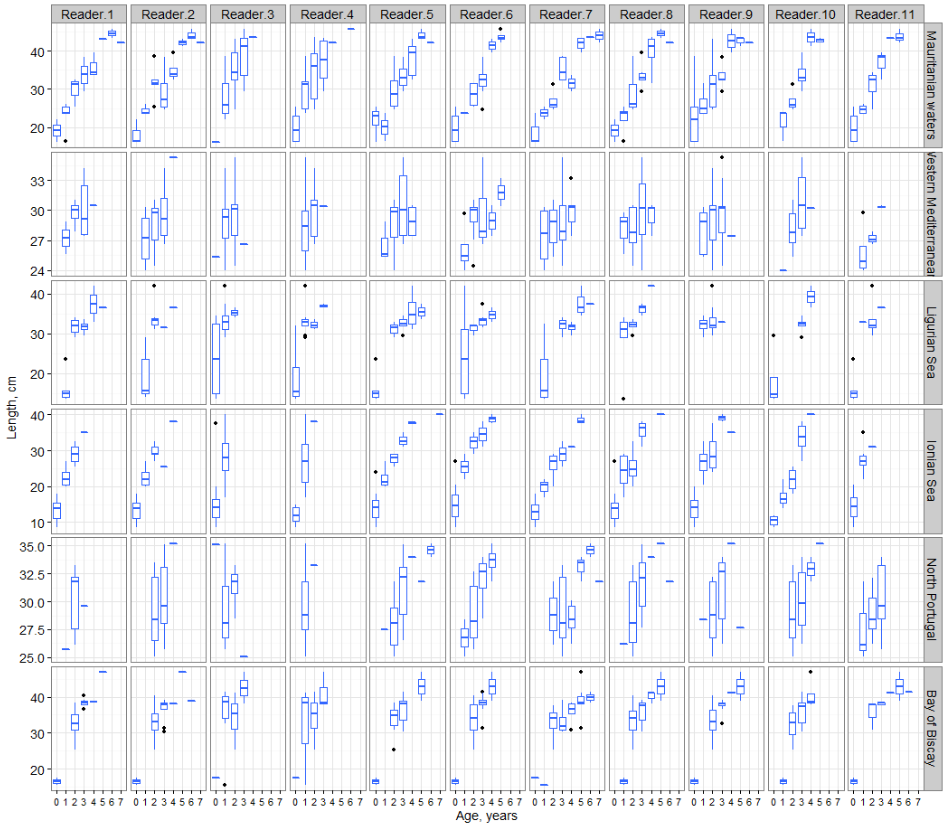

Figure A3.10.3: Chub Mackerel growth patterns by reader and by area.